Animal Models of Drug Addiction
Series Preface

Under the guidance of its founders Alan Boulton and Glen Baker, the Neuromethods series by Humana Press has been very successful since the first volume appeared in 1985. In about 17 years, 37 volumes have been published. In 2006, Springer Science + Business Media made a renewed commitment to this series. The new program will focus on methods that are either unique to the nervous system and excitable cells or which need special consideration to be applied to the neurosciences. The program will strike a balance between recent and exciting developments like those concerning new animal models of disease, imaging, in vivo methods, and more established techniques. These include immunocytochemistry and electrophysiological technologies. New trainees in neurosciences still need a sound footing in these older methods in order to apply a critical approach to their results. The careful application of methods is probably the most important step in the process of scientific inquiry. In the past, new methodologies led the way in developing new disciplines in the biological and medical sciences. For example, physiology emerged out of anatomy in the nineteenth century by harnessing new methods based on the newly discovered phenomenon of electricity. Nowadays, the relationships between disciplines and methods are more complex. Methods are now widely shared between disciplines and research areas. New developments in electronic publishing also make it possible for scientists to download chapters or protocols selectively within a very short time of encountering them. This new approach has been taken into account in the design of individual volumes and chapters in this series.

Wolfgang Walz
Preface

The study of drug addiction integrates research from a wide range of disciplines including psychiatry, psychology, sociology, neuroscience, pharmacology and genetics. Preclinical studies with behaving animals have been a critical part of this endeavour for close to 100 years. The rise of animal models in drug addiction research can be traced to the growth of pharmacology as an academic discipline in North America during the 1920s. The work was advanced in 1928 when the National Research Council of the USA appointed a Committee on Drug Addiction to seek a non-addicting substitute for morphine. This directive led to the establishment of a behavioural pharmacology laboratory at the University of Michigan under the supervision of Charles W. Edmunds and Nathan Eddy. Early investigations by these researchers tested whether newly developed compounds could reduce withdrawal symptoms in a variety of animals following chronic administration of morphine. Addiction was viewed as “drug use that caused considerable harm to the individual”[1], with both researchers and clinicians equating the treatment of addiction with the treatment of withdrawal.

Our understanding of addiction and how it is treated has advanced remarkably since that time, with much of the progress related directly to animal research. This is true for both the behavioural aspects of drug use as well as the biological underpinnings of the disorder. Clinicians and researchers alike rely on knowledge gained in modern behavioural pharmacology labs to understand the etiology, development, and treatment of addiction. Most recently, the convergence of information from preclinical and clinical studies has led to a consensus that drug addiction is a progressive disorder: initial drug use is voluntary and controlled whereas the pathological state of drug addiction is characterized by compulsive and uninhibited drug intake. This depiction of drug addiction implies that the personal and environmental factors promoting initial drug intake are not the same as those controlling drug use in the chronically addicted state. Moreover, continued drug use itself may alter neural systems that underlie reward, learning, and self-control, thereby leading to further drug use. In this way, the cycle of drug addiction can be self-perpetuating and devastating.

The recognition that addiction represents a transition from controlled to uncontrolled drug use is manifested in the research community by the use of more complex behavioural paradigms to model different stages of this disorder. This book provides an up-to-date review of these paradigms and how each are used effectively to model the progression of drug addiction. The first half of the book describes the most common laboratory measures of addiction in animals, including intracranial self-stimulation (ICSS), drug self-administration, place conditioning and sensitization. The section concludes with recent and exciting developments in animal models of eating disorders, an area that is receiving increasing attention in health and research sectors as obesity is becoming a worldwide epidemic. The second half of the book describes how these paradigms are used to model the progression of drug addiction, providing insight into the clinical symptomatology of addiction from acquisition of drug use through compulsive drug taking to withdrawal and relapse. The book is aimed at a wide readership from students who are beginning to
explore this exciting field to established researchers who have already contributed to its success. Because it provides both methodological detail and a theoretical perspective, the book will appeal to readers who are, and are not, familiar with preclinical research on drug addiction. A major challenge in this field continues to be the translation of laboratory findings to therapeutic tools. My hope is that this book will provide a basis for future research that links the bench to the bedside in the treatment of drug addiction.

I am grateful to Tyson Baker, Katia Befort, Virginia Grant, Brigitte Kieffer, Bernard Le Foll and Thomas Tszechentke for constructive feedback on specific chapters in this book. I would also like to thank Richard Beninger, Hans Dringenberg, Eric Dumont and Janet Menard for ongoing discussions on related topics. Most importantly, I am indebted to a committed group of students whose enthusiasm and insight are a constant source of inspiration. My sincere appreciation to Scott Hayton, Matthew Lovett-Barron, Bonnie Lum, Sylvia Magrys, Megan Mahoney, Amanda Maracle, Apostolia Petropoulos and Ritu Sikka.

Kingston, ON, Canada

Mary C. Olmstead

Reference

Contents

Preface ................................................................. vii
Contributors ......................................................... xi

Part I Behavioral Paradigms

1 Intracranial Self-Stimulation ......................................... 3
   Styliani Vlachou and Athina Markou
2 Stimulant Self-Administration ........................................ 57
   Leigh V. Panlilio
3 Opiate Self-Administration .......................................... 83
   Francesco Leri
4 Nicotine Self-Administration ........................................ 101
   Robert E. Sorge and Paul B.S. Clarke
5 Alcohol Self-Administration ......................................... 133
   Friedbert Weiss
6 Place Conditioning ................................................ 167
   Christopher L. Cunningham, Peter A. Groblewski, and Charlene M. Voorhees
7 Sensitization ..................................................... 191
   Jessica A. Loweth and Paul Vezina
8 Animal Models of Eating Disorders .................................. 207
   Stephanie D. Hancock and Mary C. Olmstead

Part II Modeling Stages of Drug Addiction in Animals

9 Acquisition of Drug Self-Administration ................................ 237
   Marilyn E. Carroll and Richard A. Meisch
10 Escalation of Drug Use ............................................ 267
    Serge H. Ahmed
11 Environmental Modulation of Drug Taking ............................. 293
    Aldo Badiani, Daniele Caprioli, Arianna Testa, Maria Teresa De Luca, and Michele Celentano
12 Craving ........................................................ 311
    Jeffrey W. Grimm
13 Habit Formation and Compulsion .................................... 337
    David Belin, Daina Economidou, Yann Pelloux, and Barry J. Everitt
14 Impulsivity ...................................................... 379
    Andrea Bari, Trevor W. Robbins, and Jeffrey W. Dalley
15 Binge Drug Taking ................................................ 403
    Herbert E. Covington III and Klaus A. Miczek
16 Withdrawal .......................................................... 431
   Alasdair M. Barr, Heidi N. Boyda, and Ric M. Procyshyn

17 Relapse ........................................................... 461
   Suzanne Erb and Franca Placenza

Index ................................................................. 481
Contributors

Serge H. Ahmed • CNRS U MR5227 Laboratoire Mouvement Adaptation Cognition, Université Bordeaux 2; Université Bordeaux 1, Bordeaux, France
Aldo Badiani • Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy
Andrea Bari • Department of Experimental Psychology, Behavioural and Clinical Neuroscience Institute, University of Cambridge, Cambridge, UK
Alasdair M. Barr • Department of Anesthesiology, Pharmacology & Therapeutics, University of British Columbia, Vancouver, BC, CanadaBC Mental Health & Addiction Research Institute, Vancouver, BC, Canada
David Belin • Department of Experimental Psychology, University of Cambridge, Cambridge, UKAVENIR team Psychobiology of Compulsive Disorders, Pôle Biologie Santé CNRS UMR 6187 & Université de Poitiers, Poitiers, France
Heidi N. Boyda • Department of Anesthesiology, Pharmacology & Therapeutics, University of British Columbia, Vancouver, BC, CanadaBC Mental Health & Addiction Research Institute, Vancouver, BC, Canada
Daniele Caprioli • Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy
Marilyn E. Carroll • Department of Psychiatry, MMC 392, University of Minnesota, Minneapolis, MN, USA
Michele Celetano • Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy
Paul B.S. Clarke • Department of Pharmacology & Therapeutics, McGill University, Montreal, QC, Canada
Herbert E. Covington III • Fishberg Department of Neuroscience, Mount Sinai School of Medicine, New York, NY, USA
Christopher L. Cunningham • Department of Behavioral Neuroscience and Portland Alcohol Research Center, Oregon Health & Science University, Portland, OR, USA
Jeffrey W. Dalley • Department of Experimental Psychology, Behavioural and Clinical Neuroscience Institute, University of Cambridge, Cambridge, UK;
Department of Psychiatry, Addenbrooke’s Hospital, Cambridge, UK
Maria Teresa De Luca • Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy
Daina Economou • Department of Experimental Psychology, University of Cambridge, Cambridge, UK
Suzanne Erb • Department of Psychology, University of Toronto Scarborough, Toronto, ON, Canada
Barry J. Everitt • Department of Experimental Psychology, University of Cambridge, Cambridge, UK
JEFFREY W. GRIMM • Department of Psychology and Program in Behavioral Neuroscience, Western Washington University, Bellingham, WA, USA

PETER A. GROBLEWSKI • Department of Behavioral Neuroscience and Portland Alcohol Research Center, Oregon Health & Science University, Portland, OR, USA

STEPHANIE D. HANCOCK • Medicine Hat College, Medicine Hat, AL, Canada

FRANCESCO LERI • Department of Psychology, University of Guelph, Guelph, ON, Canada

JESSICA A. LOWETH • Department of Psychiatry and Behavioral Neuroscience, The University of Chicago, Chicago, IL, USA

ATHINA MARKOU • Department of Psychiatry, School of Medicine, University of California San Diego, La Jolla, CA, USA

RICHARD A. MEISCH • Department of Psychiatry and Behavioral Sciences, University of Texas Health Science Center at Houston, Houston, TX, USA

KLAAUS A. MICZEK • Departments of Psychology, Neuroscience, Psychiatry, Pharmacology, and Experimental Therapeutics, Tufts University, Boston/Medford, MA, USA

MARY C. OLMSTEAD • Department of Psychology, Centre for Neuroscience Studies, Queen’s University, Kingston, ON, Canada

LEIGH V. PANNILLO • Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, 251 Bayview Blvd Baltimore, MD, USA

YANN PELLOUX • Department of Experimental Psychology, University of Cambridge, Cambridge, UK

FRANCA PLEANZNA • Department of Psychology, University of Toronto Scarborough, Toronto, ON, Canada

RIC M. PROCYSHYN • BC Mental Health & Addiction Research Institute, Vancouver, BC, Canada; Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada

TREVOR W. ROBBINS • Department of Experimental Psychology, Behavioural and Clinical Neuroscience Institute, University of Cambridge, Cambridge, UK

ROBERT E. SORGE • Department of Pharmacology & Therapeutics, McGill University, Montreal, QC, Canada

ARIANNA TESTA • Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy

PAUL VEZINA • Department of Psychiatry and Behavioral Neuroscience, The University of Chicago, Chicago, IL, USA

STYLIANOS VLACHOU • Department of Psychiatry, School of Medicine, University of California San Diego, La Jolla, CA, USA

CHARLENE M. VOORHEES • Department of Behavioral Neuroscience and Portland Alcohol Research Center, Oregon Health & Science University, Portland, OR, USA

FRIEDBERT WEISS • Molecular and Integrative Neurosciences Department, The Scripps Research Institute, La Jolla, CA, USA
Part I

Behavioral Paradigms
Intracranial self-stimulation (ICSS) is an operant behavioral paradigm in which experimental animals learn to deliver brief electrical pulses into specific regions of their own brains that are considered to be part of the brain’s reward pathways mediating both natural and ICSS reward. Several brain sites support ICSS, with the lateral hypothalamus, medial forebrain (MFB) bundle, and ventral tegmental area (VTA) among the sites that produce the most vigorous ICSS responding. Various ICSS procedures have been designed and used during the last decades since the discovery of ICSS. Two of the most commonly used ICSS procedures, which have been experimentally validated and have shown to be reward-selective, are the rate-frequency curve-shift procedure and the discrete-trial current-intensity threshold procedure. In all ICSS procedures, lowering of ICSS thresholds indicates a facilitation of brain stimulation reward, whereas elevations in ICSS thresholds reflect the diminished reward value of the stimulation and thus an anhedonic state. Acute administration of most drugs of abuse, including cocaine, amphetamine, nicotine, morphine, and heroin, lower ICSS thresholds in experimental animals. By contrast, withdrawal from chronic administration of these compounds induces elevations in ICSS thresholds, indicating an anhedonic state that resembles the negative affective state of the drug withdrawal syndrome experienced by humans. However, certain drugs of abuse, such as ethanol and cannabinoids, have shown inconsistent effects in ICSS procedures, primarily because of the minimal effects induced by these drugs in the ICSS procedure. In summary, the ICSS procedure provides unique ways to investigate the anatomical basis of reward and motivation and is an important tool for the assessment of the reward-facilitating and anhedonic effects of various drugs of abuse with addictive properties.

**Key words:** Intracranial self-stimulation, Reward, Motivation, Drugs of abuse, Cocaine, Amphetamine, Nicotine, Heroin, Morphine, Δ9-Tetrahydrocannabinol, Ethanol, Benzodiazepines, Phencyclidine, Reward-facilitating effect, Anhedonia, Drug dependence, Depression

1. Introduction: History and Background of Brain Stimulation Reward

ICSS is an operant behavioral paradigm in which animals learn to deliver brief electrical pulses into specific parts of their own brain hypothesized to be part of the reward pathways that mediate both natural and ICSS reward (1–5). More than 50 years ago, Olds
and Milner (1953) discovered that rats returned to an area of a test chamber where they had previously received electrical stimulation. Although the brain site stimulated in the original rat in this study is not specifically known, some believe it was the septum (6). This serendipitous observation of conditioned place preference to the electrical stimulus indicated that the stimulation was rewarding and led Olds and Milner to further train animals to perform an operant response to self-stimulate discrete brain areas (6). This discovery spurred major excitement in the field of reward and motivation. The field was provided with an important tool to study the brain sites and neuromechanisms involved in reward processes. The search for brain reward pathways has been shown to be far more complex than simply tracing the brain sites that would support ICSS. Nevertheless, ICSS, also termed brain stimulation reward, has been demonstrated to be a highly useful procedure in the delineation of the neurobiology of reward and motivation. It is worth mentioning that the terms ICSS and brain stimulation reward are distinct. Specifically, ICSS refers to an operational definition of the procedure in which a subject performs an operant response to stimulate parts of its brain, while the term brain stimulation reward has the explicit connotation that the stimulation is rewarding. Nevertheless, the two terms are often used interchangeably in the literature.

2. Basic Features and Parameters of the ICSS Procedure

Electrical stimulation usually consists of a 100–500 ms train of repeated pulses of sinusoidal or rectangular waveforms usually with a duration of 0.1 ms. The original studies of brain stimulation reward used a sinusoidal waveform, whereas the rectangular waveform is preferred more recently because it is hypothesized to stimulate neuronal fibers in a physiologically relevant manner (7, 8). The electrical stimulation can be anodal, cathodal, or of alternating polarity. Cathodal stimulation is more effective than anodal stimulation in exciting neurons (7). Furthermore, anodal stimulation stimulates neurons in a more complex way, so it is less preferred than cathodal stimulation (7).

The electrodes used in ICSS are either monopolar or bipolar. Bipolar electrodes deliver biphasic stimulation, meaning that the polarity around each electrode tip switches from pulse to pulse between anodal and cathodal stimulation, whereas monopolar electrodes deliver monophasic stimulation. Some monopolar electrodes have been designed to be moveable such that several brain sites may be explored in the same subjects along the dorsal–ventral axis (9). Moveable electrodes allow for changes in the location of the electrode tip and are mainly used in anatomical studies aimed at
characterizing the sites supporting ICSS behavior (9). The effectiveness of a stimulating electrode depends on the size of the electrode tip, location of the electrode relative to the “reward” neurons and brain sites, current intensity, frequency, and train duration of the stimulation.

The most significant parameters of electrical brain stimulation are: (1) the current intensity (the maximal height of each pulse measured in μA); (2) the pulse width or duration of the stimulation pulses measured in milliseconds; (3) the frequency of the stimulation pulses that are a function of the width of each pulse and interpulse interval measured in Hz (i.e., number of pulses presented in 1 s), and (4) the train duration, measured in milliseconds, defined as the time period when pulses are delivered. Most recent studies use a pulse duration of 0.1 ms because it is considered the most physiologically relevant pulse duration that theoretically leads to one action potential (7). Any of the other three stimulation parameters – frequency (10), current-intensity (11), or train duration (12, 13) – can be varied to provide a reward threshold, whereas the other two parameters are kept constant. Such a threshold measure provides a quantitative assessment of stimulation efficacy and thus assesses brain reward pathway function. Different procedures are used to obtain and define reward thresholds (see below).

In most recent studies, either frequency or current intensity was chosen as the varied stimulation parameter. Changes in stimulation frequency are hypothesized to modify the firing frequency of the stimulated fibers, whereas changes in current-intensity modify the number of fibers that are activated around the stimulation tip (7, 14).

Lowering of ICSS threshold indicates an increase in the reward value of the stimulation because less electrical stimulation is required for the subject to perceive the stimulation as rewarding. Conversely, elevations in thresholds indicate a decrease in the reward value of the self-stimulation because higher frequencies or current-intensities are required before the subject perceives the stimulation as rewarding.

A number of different procedures have been developed and used in ICSS studies. Some of these procedures are the following: (1) rate-frequency (or rate-intensity or rate-duration) curve-shift procedure (12, 15–18), (2) discrete-trial current intensity procedure (11, 19, 20), (3) autotitration-of-threshold or set-reset procedure (21–24), (4) extinction or stimulus-control procedure (25–28), (5) post-reinforcement pause procedure (29, 30), (6) single-lever matching or response-strength procedure based on Herrnstein’s Matching Law (31, 32), and (7) one- and two-lever self-regulation of duration or On-Off procedure (33–35). However, not all of these procedures have been extensively used or experimentally validated. Furthermore, some of these procedures have limitations, the most important of which is that they are not
reward-selective (36, 37). In some of these procedures, the simple response rate generated by a single arbitrary set of stimulation parameters is used, thus not providing a reliable measure of brain reward function (36, 37). The two procedures that have been the most extensively validated and used in most ICSS studies conducted

The rate-frequency curve-shift threshold procedure

Fig. 1. Rate-frequency curve-shift method of intracranial self-stimulation. (a) Schematic of representative stimulation parameters used in the rate-frequency curve-shift ICSS procedure. Typically, a trial begins with three stimulation primes (50 pulses/0.4 s/prime), with each prime consisting of a 0.4 s period of non-contingent stimulation administered at a constant current and frequency followed by a timeout (TO) period. The primes (P1, P2, P3) are typically administered for 3–5 s. When the animal presses the bar or nosepokes (the two most commonly used manipulanda with this procedure), stimulation (Stim) is delivered at the appropriate current and frequency for 0.4 s. Each cathodal pulse of current has a duration of 0.1 ms. The frequency determines how many pulses are delivered during this period, which varies from trial to trial. After each 50–60 s test period, a 5–30 s TO period occurs during which the animal can press the lever, but no additional stimulation is available. (b) Schematic depicting the theoretical functions that relate response rates (presses) to stimulation frequency (Log Hz). The rewarding efficacy of stimulation is measured by nonlinear regression in which a theoretical line (dashed line) is drawn through the curve at 60%, 50%, 40%, 30%, and 20% of the maximum rate of responding. Either the stimulation frequency that maintains half-maximal responding (Maximum 50, \( M_{50} \); dashed line) or reflects the theoretical point at which the stimulation becomes rewarding (\( T_0 \); where dashed line 2 crosses the \( x \)-axis) can be used as the brain reward threshold. In this scenario, brain reward thresholds are either lowered (curve 1) (e.g., by increasing current or following acute administration of a drug of abuse) or elevated (curve 3) (e.g., by decreasing current or during withdrawal from a drug of abuse) compared with the control condition (curve 2). (c) Manipulations that affect response rates (e.g., decreasing (curve 1) or increasing (curve 3) the force required to depress the lever) can artificially affect \( M_{50} \) brain reward thresholds. The \( M_{50} \) (dashed lines) is a direct function of maximal response rates. By contrast, the \( T_0 \) (point of rise from \( x \)-axis) threshold measure is not affected by such motoric effects of manipulations because it is not a function of maximal response rates (Adapted from (39). With permission).
to date are the rate-frequency curve-shift procedure (Fig. 1) and the discrete-trial current intensity procedure (Fig. 2). These two procedures appear to have the reward selectivity that is required in psychopharmacological research (14). Brief descriptions of each of these procedures are provided below, as well as a summary.

**The discrete-trial current-intensity threshold procedure**

**Fig. 2. Discrete-trial current-intensity threshold intracranial self-stimulation procedure.**

Panels (a), (b), and (c) illustrate the timing of events during three hypothetical discrete trials. S1 refers to the non-contingent electrical stimulus that initiates the trial. S2 refers to the contingent electrical stimulus delivered after the completion of the operant response (see text for details). (a) A trial during which the animal did not respond (negative response). (b) A trial during which the animal responded within the 7.5 s response window (i.e., limited hold) after the delivery of the non-contingent stimulus (positive response). (c) A trial during which the animal responded during the inter-trial interval that resulted in the postponement of the initiation of the next trial by 12.5 s. The inter-response interval varies between 7.5 and 12.5 s. (d) A hypothetical session demonstrating how thresholds are defined for the four individual series of ascending and descending current intensities. The threshold of the session is the mean of the four series’ thresholds (Adapted from (11). With permission).
of the validation work conducted that has firmly established these two procedures in the ICSS field.

2.1. Rate-Frequency Curve-Shift Procedure

In the rate-frequency curve-shift procedure, the frequency of the stimulation is varied systematically, whereas the other parameters are held constant, so that the population of neurons that is stimulated is held constant. Animals typically press a lever manipulandum, nosepoke in a hole, turn a wheel manipulandum, or walk a runway to receive a train of stimulation. The completion of the operant response (e.g., lever-press) triggers a constant current generator to deliver a train of rectangular cathodal pulses of constant duration and intensity and variable frequency. The pulse frequency (i.e., number of pulses within a train) is progressively increased up to 40–50 pulses per stimulation train until the animal shows vigorous self-stimulation. As the frequency of the stimulation increases, the response rates rise accordingly until a maximal point of stimulation is delivered over which no further increases in response rates are produced and response rates reach an asymptote (15, 16).

During the acquisition phase, the animals are trained to self-stimulate for at least 3 consecutive days, using stimulation parameters that maintain near maximal rates of responding. After the self-stimulation behavior has been acquired and stabilized for a given pulse frequency that supports maximal response rates, animals are trained to self-stimulate using four alternating series of ascending and descending pulse frequencies. The pulse frequency is varied by steps of approximately 0.1 log units. Each frequency is tested within trials of 50–60 s duration, followed by a timeout period of 5–30 s. These parameters can be varied between studies. At the beginning of each trial, the animals receive three to five trains of priming stimulation at the frequency of the stimulation that is available for that trial. The mean number of the four alternating ascending and descending pulse frequencies in each step leads to the generation of a sigmoidal frequency-response function, very similar to the dose-response function seen in pharmacological studies. A frequency-response function is established daily until the self-stimulation indices (i.e., threshold and asymptote measures) stabilize. Each of these daily sessions has a duration of approximately 40–50 min. Threshold and response rate asymptote measures are estimated based on the response function. Threshold is defined as the frequency, intensity, pulse width, or train duration that supports an arbitrary level of performance (see below for methods of estimation). The asymptote is the maximal response level exhibited by the subject. Thresholds reflect the rewarding efficacy of the stimulation, and response rate asymptotes are an estimate of an animal’s ability to perform the task and reflect motor or performance effects. Different quantitative measures are used in the rate-frequency curve-shift procedure to obtain threshold and asymptote (performance capability) estimates. The most
Intracranial Self-Stimulation

reliable and commonly used measures are the $M_{50}$ (half-maximum) (15, 18), the Gompertz sigmoid growth model (which resembles the $M_{50}$) (38), and the $\Theta_0$ (39) (Fig. 1). The $M_{50}$ measure is analogous to the Effective Dose 50 ($ED_{50}$) used in pharmacology and refers to the stimulation frequency (or intensity or duration) that sustains responding at 50% of the maximal rate. The $\Theta_0$ measure refers to the theoretical point at which the stimulation becomes rewarding (i.e., response rates become higher than 0) (39). The $M_{50}$ measure can show artificial shifts attributable to increases or decreases in maximal response rates, whereas the $\Theta_0$ measure has been shown to be less affected by performance manipulations (8, 17, 18, 39). The Gompertz sigmoid growth model is a psychophysical method applied to the description of ICSS behavior:

$$f(X) = \alpha e^{-b(x-a)}$$

In this equation, $\alpha$ represents the maximal rate (asymptote), and $X_i$ (X at inflection) represents the threshold frequency. The latter is the pulse number producing 36.7% of the asymptotic rate (i.e., the rate lying on the fastest-accelerating region of the curve). Parameter $b$ represents an index of the slope, and $\varepsilon$ is the base of natural logarithms. The advantage of this model compared with other estimates is that it accounts for every data point in the dose-response curve to measure the threshold and asymptote values (38).

Thus, this method enables distinguishing between reward and performance and quantifying the drug effect (8, 11, 18, 37, 39–41). The quantitative scaling of changes in reward is important for two reasons. First, the size of the reward-facilitating or reward-attenuating effect is useful when comparing the effects of different manipulations. Second, even small changes in brain stimulation reward or performance capability can be detected because a quantitative measure is less likely to have ceiling or floor effects (42). The same procedure may be used while varying the current-intensity (43) or train duration (12), generating rate-intensity or rate-duration curve functions, respectively, while keeping the other two parameters constant.

2.2. Discrete-Trial Current-Intensity Threshold Procedure

The discrete-trial current-intensity threshold procedure is a modification of a task initially developed by Kornetsky and Esposito (19). This procedure consists of discrete trials. Each trial begins with the delivery of a non-contingent electrical stimulus followed by a 7.5 s response window within which the subject can make a response to receive a second contingent stimulus identical to the initial non-contingent stimulus. Similar to all other ICSS procedures, this response may be a lever press, nose poke, or turning a wheel manipulandum, with the latter being the most commonly used response with this procedure. A response during this time window is a positive response, and the lack of a response is a
negative response. Additional responses during a 2 s period immediately after a positive response have no consequence and are counted as extra responses. The inter-trial interval that follows either a positive response or the end of the response window (in the case of a negative response) has an average duration of 10 s (ranging from 7.5 to 12.5 s). Responses that occur during the inter-trial interval are timeout responses and result in a further 12.5 s delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the inter-trial interval and delay periods induced by timeout responses are gradually increased until animals perform consistently for a fixed stimulation intensity at standard test parameters. The animals are subsequently tested on the current-intensity threshold procedure in which stimulation intensities are varied according to the classical psychophysical method of limits (44). A test session consists of four alternating series of descending and ascending current intensities starting with a descending series (Fig. 2). Blocks of three to five trials are presented to the subject at a given stimulation intensity, and the current intensity is changed by steps of 5–10 μA between blocks of trials. The initial stimulus intensity is set approximately 40 μA above the baseline current-intensity threshold for each animal.

Each test session typically lasts 30–40 min and provides four dependent variables for behavioral assessment: threshold, response latency, extra responses, and timeout responses. The current-threshold for each descending series is defined as the stimulus intensity between the successful completion of a set of trials (i.e., positive responses during two or more of the three trials) and the stimulus intensity for the first set of trials, of two consecutive sets, during which the animal fails to respond positively on two or more of the three trials (in the case when three trials are presented at each current intensity). The current-threshold for each ascending series is defined as the stimulus intensity between a current intensity for which the animal fails to respond positively on two or more of the three trials and the first set of trials, of two consecutive sets, during which the animal responds positively on two or more of the three trials. The mean of the four series’ thresholds is defined as the threshold for the session. The time interval between the beginning of the non-contingent stimulus and a positive response is recorded as the response latency. The response latency for each session is defined as the mean response latency on all trials during which a positive response occurs. The extra responses and timeout responses for each test session are defined as the total number of extra responses and timeout responses that occur in the session, respectively. The response latency measure detects performance effects (11), extra responses primarily reflect the force with which a subject turns the wheel, and timeout responses reflect response inhibition (45). The main advantage of this
procedure is that it allows for a rate-independent measurement of reward thresholds. Thresholds are defined independently of rate of responding which can be low because timeout responses result in postponement of the next trial (11). The discrete-trial current-intensity procedure has been used primarily with variations in current intensity, although other stimulation parameters can also be varied while keeping the current-intensity constant (8, 11).

In both of the above procedures (rate-frequency curve-shift and discrete-trial current-intensity threshold), data obtained for the threshold and response asymptote or response latency are usually expressed as a percentage of baseline to avoid the complexity of individual subjects having different baseline values for each of the measures (39).

The ICSS procedure has several strengths and characteristics that distinguish it from other procedures used to study motivation, reward, or reinforcement, such as the self-administration and conditioned place preference paradigms. The ICSS procedure is considered a very powerful tool to assess whether a brain structure is involved in reward mechanisms because the electrode is implanted directly into the structure tested for brain stimulation reward (46). It is also a very flexible behavioral procedure that allows the implementation of different measurements. Furthermore, the ICSS paradigm offers one of the most direct measurements of drug effects on brain reward substrates (14, 46) (Table 1).

One of the most obvious strengths of brain stimulation reward is that it is a rapidly acquired response because of the powerful rewarding effects of ICSS and the fact that no delay occurs between the animal’s operant response and the delivery of the stimulation. Animals that respond for ICSS will choose to press a lever or turn a wheel to self-administer electrical stimulation over natural rewards, such as food and water, or in a subfreezing environment for hours or even days to the point of exhaustion (47). Moreover, ICSS directly activates the brain reward systems that drive behavior, bypassing most of the input side of these neuronal circuits (e.g., bypassing taste buds and their signal to the brain). No other behavior related to reward consumption can interfere with the operant response or complicate data interpretation.

Another advantage of ICSS procedures is that they provide quantitative measures of brain reward function and the effects of manipulations. Notably, however, because of the wide variety of ICSS procedures and stimulation parameters, direct comparisons of the magnitude of reward-enhancing or reward-attenuating effects are sometimes difficult to be determined between studies.
Table 1
Representative/selected articles on the effects of various drugs of abuse on ICSS thresholds

<table>
<thead>
<tr>
<th>Drug</th>
<th>ICSS method</th>
<th>Species</th>
<th>Threshold lowering during acute/chronic drug administration</th>
<th>Threshold elevation during acute/chronic drug administration</th>
<th>Threshold elevation during drug withdrawal</th>
<th>No effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychostimulants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>Continuous reinforcement schedule</td>
<td>Hooded rats</td>
<td>↑ Response rates, 5 mg/kg, IP (125)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Different stimulus parameter combinations</td>
<td>Wistar rats</td>
<td>↑ Response rates, 0.6–10 mg/kg, SC (101)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Discrete-trial method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Discrete-trial method</td>
<td>Albino male CDF rats</td>
<td>2.5–20 mg/kg, IP (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Train-duration curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>10–30 mg/kg, IP (103)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variant current-intensity method</td>
<td>Wistar rats</td>
<td>5 mg/kg, SC; 10, 30, 100 mg/kg, IP (104)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Current-intensity method</td>
<td>Wistar rats</td>
<td>5–20 mg/kg, IP (105)</td>
<td>30, 40 mg/kg/twice daily for 18 days, IP (105)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Train-duration method</td>
<td>Sprague-Dawley rats</td>
<td>↑ Response rates, 15 mg/kg, IP (106)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Current-intensity method</td>
<td>Sprague-Dawley rats</td>
<td>↑ Response rates, 5–30 mg/kg, IP (108)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>2.5–20 mg/kg, IP (109)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rate-intensity curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>1–16 mg/kg, IP (110)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>5–30 mg/kg, IP (111)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Therapy Method</td>
<td>Animal Model</td>
<td>Dosage Details</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Sprague-Dawley rats</td>
<td>1.8–18 mg/kg, IP (130)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>2.5–10 mg/kg, IP (112); 5 mg/kg, IP (114)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Swiss-Webster mice</td>
<td>2.5–20 mg/kg, IP (115)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Long-Evans rats</td>
<td>4 mg/kg, IP (89)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>10 and 20 self-injections of 0.25 mg/kg (116); 10 mg/kg, IP, 20 consecutive days (129); 10 mg/kg, IP (119, 217), 30 mg/kg/twice daily for 18 days, IP (105)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Long-Evans rats</td>
<td>10 mg/kg, IP (118)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>1.25–10 mg/kg, IP (120)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>1.25–10 mg/kg, IP (123)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>5 mg/kg, IP (124)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current-intensity method</td>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Train-duration method</td>
<td>Sprague-Dawley rats</td>
<td>10–15 mg/kg for 18 days, IP (103); ↑ response rates, 15 mg/kg, IP (126)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>“Binge” cocaine, 15 mg/kg/injection, IP, for 7 days (133)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Response rates, 25 mg/kg for 18 days or 30 mg/kg for 3 days, IP (126)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 1 (continued)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>ICSS method</th>
<th>Species</th>
<th>Threshold lowering during acute/chronic drug administration</th>
<th>Threshold elevation during acute/chronic drug administration</th>
<th>Threshold elevation during drug withdrawal</th>
<th>No effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
<td>8 Injections of 15 mg/kg over 9 h, IP (134)</td>
<td>No effect</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
<td>0.5 mg/kg “binge” intravenous self-administration, 3 h session for up to 4 days, 12 h session before final testing (53, 109, 131)</td>
<td>No effect</td>
</tr>
<tr>
<td>D-Amphetamine</td>
<td>Rate-intensity method</td>
<td>BALB/c, DBA/2 and C57BL/6 mice</td>
<td>$\uparrow$ Response rates, 0.25–8 mg/kg, IP (157)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-intensity method</td>
<td>Sprague-Dawley rats</td>
<td>$\uparrow$ Response rates, 0.25–2 mg/kg, IP (158)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-dependent various intensities method</td>
<td>Long-Evans rats</td>
<td>$\uparrow$ Response rates, 0.5–2 mg/kg, IP (156)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous reinforcement schedule</td>
<td>Long-Evans hooded rats, albino Walter Reed rats</td>
<td>$\uparrow$ Response rates, 0.5–5 mg/kg, IP (154)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-intensity method</td>
<td>Sprague-Dawley rats</td>
<td>$\uparrow$ Response rates, 0.1 mg/kg, SC (155)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-intensity method</td>
<td>Wistar rats</td>
<td>$\uparrow$ Response rates, 1.5 mg/kg for 9 days, IP (152)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Intracranial Self-Stimulation

<table>
<thead>
<tr>
<th>Method</th>
<th>Species</th>
<th>Dose Range (mg/kg or µg)</th>
<th>Route of Administration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photobeam modified hole-board task</td>
<td>Swiss mice</td>
<td>↑ Response rates, 0.5, 1, and 2 mg/kg, IP (51)</td>
<td>↓ Response rates, 3 and 5 mg/kg, IP (51)</td>
<td>51</td>
</tr>
<tr>
<td>Rate-intensity method</td>
<td>Charles River albino rats</td>
<td>2 and 4 mg/kg, IP (96)</td>
<td></td>
<td>96</td>
</tr>
<tr>
<td>Autotitration and discrete-trial threshold detection method</td>
<td>Sprague-Dawley rats</td>
<td>0.03–1 mg/kg, IP (138)</td>
<td></td>
<td>138</td>
</tr>
<tr>
<td>Variant train duration method</td>
<td>Sprague-Dawley rats</td>
<td>0.33, 0.66, and 1 mg/kg, IP for 3 days, IP (126)</td>
<td></td>
<td>126</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>1 µg/side into the nucleus accumbens shell (141)</td>
<td></td>
<td>141</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Dopamine D&lt;sub&gt;2&lt;/sub&gt; receptor, wildtype heterozygous, and knock-out mice</td>
<td>1–4 mg/kg, IP (139)</td>
<td></td>
<td>139</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Wistar rats</td>
<td>1–6 mg/kg for 2 days, IP (143)</td>
<td></td>
<td>143</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>5 mg/kg for 7 days, IP (160)</td>
<td></td>
<td>160</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>5 and 10 mg/kg/day for 7 days, SC (163)</td>
<td></td>
<td>163</td>
</tr>
<tr>
<td>Continuous reinforcement schedule</td>
<td>Hooded rats</td>
<td>2.5–10 mg/kg for 5 days, IP (171)</td>
<td></td>
<td>171</td>
</tr>
<tr>
<td>Decreased current-intensity method</td>
<td>Sprague-Dawley rats</td>
<td>5 mg/kg for 7 days followed by 10 mg/kg for 7 days, IP (160)</td>
<td></td>
<td>160</td>
</tr>
<tr>
<td>Response rates in 10 min sessions</td>
<td>Swiss mice</td>
<td>7.5 mg/kg twice daily for 10 days, IP (151)</td>
<td></td>
<td>151</td>
</tr>
<tr>
<td>Drug</td>
<td>ICSS method</td>
<td>Species</td>
<td>Threshold lowering during acute/chronic drug administration</td>
<td>Threshold elevation during acute/chronic drug administration</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------</td>
<td>-------------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Photobeam current-intensity ICSS task</td>
<td>Lister hooded rats</td>
<td>0.4 mg/kg, SC (177)</td>
<td>↓ Response rates, 0.03–1 mg/kg, SC (52)</td>
</tr>
<tr>
<td></td>
<td>Continuous reinforcement schedule and autotitration method</td>
<td>Sprague-Dawley rats</td>
<td>0.03–1 mg/kg, SC (52)</td>
<td>↓ Response rates, 0.03–1 mg/kg, SC (52)</td>
</tr>
<tr>
<td></td>
<td>Fixed-ratio 15 schedule</td>
<td>Sprague-Dawley rats</td>
<td>↑ Response rates, 0.1 mg/kg, SC (52)</td>
<td>↓ Response rates, 1 mg/kg, SC (52)</td>
</tr>
<tr>
<td></td>
<td>Rate-intensity discrimination method</td>
<td>Wistar rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rate-frequency curve-shift method</td>
<td>Long-Evans rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photobeam task</td>
<td>Male albino rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of ICSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-intensity</td>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>discrimination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>curve-shift</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrete-trial</td>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>current-intensity method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrete-trial</td>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>current-intensity method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 (continued)
<table>
<thead>
<tr>
<th>Method</th>
<th>Species</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different-schedules methods</td>
<td>Sprague-Dawley rats</td>
<td>↑ Response rates, 0.01–0.3 mg/kg, SC (188)</td>
</tr>
<tr>
<td>Autotitration method</td>
<td>Wistar rats</td>
<td>1 mg/kg, SC (180)</td>
</tr>
<tr>
<td>Progressive-ratio method</td>
<td>Wistar rats</td>
<td>1 mg/kg, SC (180)</td>
</tr>
<tr>
<td>Progressive-ratio method</td>
<td>Wistar rats</td>
<td>1 mg/kg, SC (180)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>0.125–0.5 mg/kg, SC (50)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>0.03 mg/kg/infusion for 1 h or 12 h daily (48)</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Sprague-Dawley rats</td>
<td>0.5 mg/kg, SC (114)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>0.25 mg/kg, SC, or 0.03 mg/kg/infusion (116, 182)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>3.16 mg/kg/day for 7 days, SC (55)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>3.16 mg/kg/day for 7–14 days, SC (50, 164, 194)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>Precipitated withdrawal, 9 mg/kg/day salt for 14 days, SC (195)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>Precipitated withdrawal, 3.16 mg/kg/day for 14 days, SC (209)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>Precipitated withdrawal, 3.16 mg/kg/day for 14 days, SC (209)</td>
</tr>
<tr>
<td>Drug</td>
<td>ICSS method</td>
<td>Species</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
</tr>
<tr>
<td></td>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
</tr>
<tr>
<td></td>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
</tr>
<tr>
<td></td>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
</tr>
<tr>
<td></td>
<td>Discrete-trial current-intensity method</td>
<td>C57BL6 mice</td>
</tr>
<tr>
<td>Method</td>
<td>Species</td>
<td>Dose/Measurements</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>---------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>3.16 mg/kg/day for 7 and 28 days, SC (183)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>Precipitated withdrawal, 3.16 mg/kg/day for 28 days, SC (208)</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>C57BL/6J mice</td>
<td>40 mg/kg/day for 28 days, SC (198)</td>
</tr>
</tbody>
</table>

**Opiates**

<table>
<thead>
<tr>
<th>Method</th>
<th>Species</th>
<th>Dose/Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrete-trial method</td>
<td>CDF rats</td>
<td>4–12 mg/kg twice daily for up to 25 days, IP (226)</td>
</tr>
<tr>
<td>Fixed-interval method</td>
<td>Sprague-Dawley rats</td>
<td>↓ Response rates, 3 and 5.6 mg/kg, IP (238)</td>
</tr>
<tr>
<td>Discrete-trial method</td>
<td>Fischer-344 rats</td>
<td>0.015–0.5 mg/kg, SC (230)</td>
</tr>
<tr>
<td>Autotitration and progressive ratio method</td>
<td>Wistar rats</td>
<td>1–10 mg/kg, SC (180)</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td>Long-Evans rats</td>
<td>Morphine-associated cues, 5 mg/kg, IP (118)</td>
</tr>
<tr>
<td>Autotitration method</td>
<td>Sprague-Dawley rats</td>
<td>3 and 10 mg/kg, SC, once or 15 mg/kg for 10 days, SC (243, 245)</td>
</tr>
<tr>
<td>Drug</td>
<td>ICSS method</td>
<td>Species</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td></td>
<td>Progressive-ratio schedule of ICSS</td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td></td>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
</tr>
<tr>
<td></td>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
</tr>
<tr>
<td>Heroin</td>
<td>Rate current-intensity method</td>
<td>Albino, Wistar-derived rats</td>
</tr>
<tr>
<td></td>
<td>Fixed-ratio 1 schedule</td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td>Method</td>
<td>Species</td>
<td>Dose</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>Non-dependent rats, 30 µg/kg, SC</td>
</tr>
<tr>
<td>Discrete-trial method</td>
<td>Fischer-344 rats</td>
<td>0.06 and 0.5 mg/kg, SC</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>Current intensity</td>
<td>Lewis rats</td>
</tr>
<tr>
<td>autotitration method</td>
<td></td>
<td>Lewis and Sprague-Dawley</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td></td>
<td>Fischer-344 rats</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current intensity method</td>
<td></td>
<td>Long-Evans rats</td>
</tr>
<tr>
<td>Levonantradol</td>
<td>Discrete-trial current-intensity method</td>
<td>Albino CDF rats</td>
</tr>
<tr>
<td>Rate-frequency curve-shift method</td>
<td></td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td>PCP</td>
<td>Fixed interval schedule</td>
<td>Sprague-Dawley rats</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Drug</th>
<th>ICSS method</th>
<th>Species</th>
<th>Threshold lowering during acute/chronic drug administration</th>
<th>Threshold elevation during acute/chronic drug administration</th>
<th>Threshold elevation during drug withdrawal</th>
<th>No effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Current intensity autotitration or progressive-ratio schedule</td>
<td>Wistar rats</td>
<td></td>
<td></td>
<td></td>
<td>0.3–5.6 mg/kg (180)</td>
</tr>
<tr>
<td></td>
<td>Rate-frequency curve-shift method</td>
<td>Long-Evans rats</td>
<td>2.5 and 5 mg/kg, IP (289); 0.5 μl, intra-nucleus accumbens (290)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td>Rate-independent discrete-trial method</td>
<td>F-344 rats</td>
<td>2 mg/kg, SC (295)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variable three-intensity method</td>
<td>Sprague-Dawley rats</td>
<td></td>
<td>↑ rates of pressing, 2 mg/kg, SC 296</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>Current intensity autotitration method</td>
<td>Sprague-Dawley rats</td>
<td>↓ Lever presses, 5% ethanol-containing liquid diet (275)</td>
<td></td>
<td></td>
<td>1.7 g/kg, IP or IG (274)</td>
</tr>
<tr>
<td></td>
<td>Rate-intensity ascending threshold schedule</td>
<td>Holtzman albino rats</td>
<td>0.9 and 1.2 g/kg, IP (272)</td>
<td></td>
<td></td>
<td>0.6 g/kg, IP (272)</td>
</tr>
<tr>
<td>Current intensity autotitration method</td>
<td>Wistar rats</td>
<td>0.6–2.4 g/kg, PO (180)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>------------</td>
<td>------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discrete-trial current-intensity method</td>
<td>Wistar rats</td>
<td>(\uparrow) Threshold, 22 mg/liter ethanol vapor for 17–20 days (54)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate-frequency method</td>
<td>High and low alcohol drinking N/NIH outbred rats</td>
<td>(\uparrow) Threshold and (\downarrow) lever presses in the low alcohol drinking group, 4 g/kg, IG, once (276)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Increase; ‡ decrease
Another advantage of the ICSS procedure is that all parameters of brain stimulation reward can be controlled and measured more precisely than those of natural rewards. Furthermore, the effects of various manipulations can be assessed using ICSS of different areas of the brain, potentially providing insights into the neuro-substrates of the effects of specific manipulations.

Another strength of the ICSS paradigm is that in almost all currently used ICSS procedures one can readily distinguish between the reward-enhancing (threshold) and motor/performance effects (response latency or maximal response rate) of manipulations. The ICSS procedure can be used to test either the acute or chronic effects of a drug, or even both (Table 1). Repeated testing, even several times daily, is feasible because no satiation occurs to the rewarding effects of the stimulation, thus allowing for detailed time-course assessments. Provided that the surgeries are conducted carefully to precisely position the electrode in the intended brain site and the head mount is well-secured to the skull, subjects can be tested routinely for periods of months or up to 1 year. During that time, unless manipulations are implemented, reward thresholds are extremely stable. Finally, the ICSS procedure does not require deprivation of any kind (e.g., food or water deprivation), which is the case for other reinforcers, for the animals to learn to perform reliably in these procedures.

In drug abuse research, the ICSS procedure provides a means of quantifying the reward-enhancing or reward-facilitating effects of drugs of abuse (48–50). ICSS procedures also allow the assessment of the reward deficits characterizing withdrawal from chronic exposure to drugs of abuse, reflected in elevated brain reward thresholds (51–57). In summary, the use of the ICSS procedure provides a unique way of studying the neurobiology of reward and motivational processes and provides quantitative measures of brain reward function and changes in brain reward function.

Nevertheless, the ICSS procedure also has some limitations. One of its limitations is the seemingly artificial nature of the reward. The procedure involves direct activation of brain reward circuits, bypassing the input side of the system. Although this is also an advantage of the procedure, researchers outside of the field may not readily know what the behavior is and what exactly is measured, thus sometimes making the communication of findings difficult. Another disadvantage of the procedure is that it requires surgery which can be labor-intensive. Nevertheless, this disadvantage is offset by the fact that the procedure can be used to provide multiple data points, including detailed time-course analyses. Finally, another disadvantage is that an electrode has to be implanted and secured to the skull, thus rendering the implementation of other brain manipulations, such as intracerebral injections, more difficult. Both the electrode and access to another...
Intracranial Self-Stimulation

brain device, such as an intracerebral cannula, need to be provided on the same small skull surface.

The brain pathways activated by ICSS are hypothesized to be the same pathways that are activated by natural rewards and by drugs of abuse that exert a powerful influence on pathways involved in incentive-motivation (1–4). Olds (58) and subsequent workers in the field (59–61) hypothesized that the posterior MFB region of the lateral hypothalamus was a major component of the brain reward system. At that time, when most researchers believed that the brain had only one unitary reward system, Stein proposed the noradrenergic hypothesis of brain stimulation reward, based on neuroanatomical and neurochemical evidence indicating a selective role of norepinephrine in the locus coeruleus (62, 63). However, subsequent neurochemical, mapping, lesion, and autoradiographic studies (64) showed that the brain reward system comprises several interconnected subsystems responsible for the rewarding effects of natural reinforcers and the reward-enhancing effects of addictive drugs (19, 65). Several brain sites will support brain stimulation reward, including the MFB region of the hypothalamus and especially the lateral and posterior part of the hypothalamus and regions of the mesolimbic dopamine system, the substantia nigra pars compacta, VTA (66), central nucleus of the amygdala (67), septum (6), bed nucleus of the stria terminalis, and nucleus accumbens (NAcc). The raphe nuclei (68) and different sites of the frontal and prefrontal cortex (PFC) (for review, see (69)) are also part of the brain reward system and support ICSS behavior. Many studies demonstrated that the medial PFC is a brain region highly implicated in ICSS behavior (70–73). Additional structures that support ICSS behavior include the hippocampus (74), amygdala, locus coeruleus (75, 76), caudate nucleus, olfactory bulb, tubercle (77), and cerebellum (78–80).

Notably, however, each of these structures that support ICSS cannot be considered a self-contained reward system that can generate rewarding effects as part of a unique reward system. The MFB, for example, has not just one unitary and exclusive reward system located in the MFB; multiple, parallel, and at times interdependent and interconnected reward systems may be located in different brain regions to mediate reward processes (65, 81). Although all of these structures were initially hypothesized to be part of the brain reward system and to be activated by ICSS to produce rewarding effects, extensive studies demonstrated that reward is not a unitary process, but rather a complex process.
Psychomotor stimulants, such as amphetamine, cocaine, and methamphetamine, are monoamine reuptake inhibitors or releasers, which exert their effects by activating mainly the dopaminergic, noradrenergic, serotonergic, and glutamatergic neurotransmitter systems of the brain (85–89). These brain systems are localized, among others, in brain regions highly implicated in brain reward functions, such as the NAcc, VTA, and PFC (90, 91).

Amphetamines and cocaine potentiate brain stimulation reward when they are administered either intracranially or systemically (3, 19, 20, 22, 35, 51, 53, 92–100). Specifically, a plethora of studies have shown that acute cocaine administration potentiates ICSS behavior reflected in increased response rates and lowering of ICSS thresholds in rats and mice (Fig. 3), whereas during cocaine withdrawal, decreased response rates and elevated ICSS thresholds were observed (19, 20, 89, 93, 101–128) (Fig. 4). Using different ICSS methods and stimulus parameters, acute administration of a variety of cocaine doses, ranging from 0.6 to 20 mg/kg, dose-dependently increased response rates (101) or lowered ICSS thresholds (19, 20, 93). Extending these findings, Markou and colleagues reported that repeated cocaine administration (10 mg/kg for 20 consecutive days) lowered ICSS thresholds, with no tolerance or sensitization to this effect (116). This latter procedure led to classical conditioning of the reward-facilitating effects of cocaine, such that after cessation of cocaine administration, a conditioned lowering of reward thresholds was observed upon re-exposure to the same testing conditions and following a saline injection (116). Kelley and Hodge shed more light on the reward-facilitating effects of cocaine by showing that these effects are mediated through 5-HT₃ receptor activity (89). Other research groups demonstrated that other systems in the brain, such as hypocretin, γ-aminobutyric acid, and cannabinoid, may also affect
Fig. 3. Drugs of abuse lower brain stimulation reward thresholds. Administration of a wide variety of psychoactive drugs leads to potentiation of activation of brain reward
the reward-facilitating effects of cocaine (119, 121, 123, 124). Interestingly, using the discrete-trial current-intensity method, Markou and colleagues found that self-administration of low doses of cocaine (10 and 20 injections of 0.25 mg/kg) lowered reward thresholds, an effect that only lasted for a short time (less than 2 h). Higher doses and testing in the ICSS procedure at later time points revealed no reward-enhancing effect of cocaine (129). These effects of self-administered cocaine were similar to those seen after intraperitoneal administration of 10 mg/kg cocaine (see above) and did not differ between male and female rats (130).

Other studies using the rate-intensity or rate-frequency curve-shift methods also showed that cocaine leads to facilitation of brain stimulation reward, indicated by lowering of ICSS thresholds (110, 111, 115). Frank and colleagues (103) and van Wolswinkel and colleagues (104) used even higher doses of cocaine (5–30 mg/kg, IP or SC) and tested the animals in a varied train duration or current-intensity ICSS procedure, respectively, with the ICSS electrode implanted at the level of the VTA. Cocaine significantly lowered train duration thresholds, again demonstrating a reward-facilitating effect of cocaine regardless of the ICSS method used (103, 104). Similar effects were also seen after acute or subchronic administration of cocaine when the electrode was located in the ventral pallidum (112), PFC (106), or medial PFC (107) or when the operant response varied (i.e., nosepoking or lever-pressing) in a rate-dependent ICSS procedure (108). Specifically, cocaine (1.5–10 mg/kg, IP) dose-dependently lowered ICSS thresholds of the ventral pallidum or lateral hypothalamus in the rate-frequency curve-shift procedure (112, 114), and this effect was not as pronounced when the animals were treated with methylphenidate during early developmental stages (120).

Using the rate-frequency curve-shift ICSS procedure in mice, acute cocaine administration (2.5–20 mg/kg, IP) dose-dependently lowered brain stimulation reward thresholds (115), whereas repeated administration of high-dose cocaine (40 mg/kg) decreased response rates and elevated thresholds (105). Consistent with one of the advantages of ICSS is the finding that these effects of cocaine are independent of the psychomotor stimulant effects of cocaine. Doses that induce enhancement of brain stimulation reward do not lead to significant effects on the performance capability of the animals (111, 112).

Fig. 4. (continued) By contrast, drugs that are not rewarding to humans, such as fluoxetine and clozapine (g) and (h), did not alter reward thresholds after termination of chronic administration, thereby demonstrating the discriminant validity of the paradigm. Notice that direct comparisons of effect magnitudes cannot be made among all figures because different stimulation parameters and procedures were used to conduct the cocaine, morphine, and ethanol studies (a–c: From (53, 164); d, f: From (54, 246); e: From (56); g, h: From (173, 296). With permission).
Withdrawal from psychoactive drugs is associated with elevations in brain reward thresholds. Withdrawal from a wide range of psychoactive drugs induces robust deficits in brain reward function. These affective sequelae can be quantified with the ICSS procedure. When responding for ICSS from electrodes, anhedonia is operationally defined as elevations in ICSS thresholds (either current-intensity, frequency, or train-duration thresholds). Withdrawal from drugs that are rewarding to humans, such as psychostimulants (cocaine (a), amphetamine (b), and nicotine (c)), depressants (ethanol (d) and morphine (f)), and an NMDA receptor antagonist (phencyclidine (e)), all elevated reward thresholds.

Fig. 4. Withdrawal from psychoactive drugs is associated with elevations in brain reward thresholds. Withdrawal from a wide range of psychoactive drugs induces robust deficits in brain reward function. These affective sequelae can be quantified with the ICSS procedure. When responding for ICSS from electrodes, anhedonia is operationally defined as elevations in ICSS thresholds (either current-intensity, frequency, or train-duration thresholds). Withdrawal from drugs that are rewarding to humans, such as psychostimulants (cocaine (a), amphetamine (b), and nicotine (c)), depressants (ethanol (d) and morphine (f)), and an NMDA receptor antagonist (phencyclidine (e)), all elevated reward thresholds.
During cocaine withdrawal, the impact of rewarding brain stimulation is attenuated, quantified by elevations in ICSS thresholds, indicating a depression-like state (53, 105, 109, 131–135) (Fig. 4). In a rate-intensity ICSS procedure, rats received repeated administration of a high cocaine dose (40 mg/kg, IP) immediately after each daily ICSS session for 7 days and were tested 24 h later (105). This repeated cocaine administration led to decreased response rates and elevated thresholds in the ICSS testing of each subsequent day (24 h post-injection). Repeated cocaine administration (30 mg/kg, twice daily) for 18 days led to lowering of thresholds and decreases in response rates when animals were tested for ICSS 5 days after discontinuation of cocaine administration (105). Importantly, in studies by Markou and Koob, in which the discrete-trial current-threshold ICSS procedure was used and cocaine (0.5 mg/kg/injection) was intravenously self-administered by rats under a fixed-ratio 5 schedule of reinforcement for 3–72 h continuously, thresholds were elevated for up to 72 h post-cocaine self-administration for the longest cocaine exposure (53, 109, 131). This anhedonic effect of cocaine withdrawal has been shown to be associated with decreased dopaminergic (109, 136), decreased noradrenergic (131), and increased adenosine (134, 135) neurotransmission. Furthermore, Markou and colleagues showed that repeated administration of the tricyclic antidepressant desmethylimipramine (DMI) (10 mg/kg, IP, twice daily for 5 days) significantly downregulated β-adrenergic receptors and shortened the duration of post-cocaine anhedonia reflected in reversal of the threshold elevations 1, 3, and 6 h after the termination of a 12 h cocaine self-administration session (0.5 mg/kg/injection) in the discrete-trial current-threshold ICSS procedure (131). In this study, the level of β-adrenergic receptor downregulation correlated significantly with the degree of effectiveness of DMI treatment in reversing post-cocaine anhedonia (131). Baldo and colleagues showed that repeated administration of cocaine (15 mg/kg, IP, eight injections over 9 h) produced elevations in thresholds 4, 8, and 12 h after cocaine administration, and this effect was reversed by administration of the adenosine-2 (A2) receptor-selective antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) (3 and 10 mg/kg) administered before both the 8 and 12 h post-cocaine self-stimulation testing sessions (134). Goussakov and colleagues recently showed using the rate-frequency curve-shift ICSS procedure that cocaine withdrawal attenuated the impact of rewarding brain stimulation, reflected in elevated ICSS thresholds, an effect that was evident after 3 and 5 days of a 7-day regimen of “binge” cocaine treatment (15 mg/kg/injection, IP, 3 daily injections at 1 h intervals) (133).

Similar to cocaine, D-amphetamine administration induces reward-enhancing effects in the ICSS procedure in rats, with
intracranial self-stimulation electrodes located in the lateral hypothalamus, dorsal noradrenergic bundle, PFC, substantia nigra, and VTA (22, 96, 137–159) (Fig. 3). Withdrawal from D-amphetamine results in elevations in ICSS reward thresholds (51, 160–172) (Fig. 4).

Cazala assessed the effects of D- and L-amphetamine in three different mouse strains with electrodes implanted in dorsal and ventral hypothalamic areas (0.25–8 mg/kg, IP) and found that both isomers increased self-stimulation response rates in all three strains, although with different sensitivity for each mouse strain (157). Using a modified hole-board task for self-stimulation in mice, Kokkinidis and Zacharko found that low D-amphetamine doses increased, and high doses decreased, ICSS response rates (51). A recent study by Elmer and colleagues, using the rate-frequency ICSS procedure in dopamine D₂ receptor deficient mice, showed that amphetamine (1–4 mg/kg, IP), in contrast to morphine, potentiated brain stimulation reward across the three genotypes used in the study (D₂ wildtype, heterozygous, and knockout mice), indicated by equal leftward shifts in the rate-frequency functions (139).

D-amphetamine appears to elicit differential anatomical sensitivity, measured by selective increases in response rates for ICSS on a fixed-ratio 1 schedule of reinforcement in rats prepared with electrodes in various brain sites (154). Specifically, rats prepared with electrodes in the septal area, the anterior and posterior hypothalamus (0.62, 2.5, and 5 mg/kg), or the ventromedial tegmentum and locus coeruleus (0.5–4 mg/kg) exhibited increased responding after D-amphetamine administration, with the highest responding when electrodes were implanted in the ventromedial tegmentum and posterior lateral hypothalamus (154). Another study showed that a low amphetamine dose (0.1 mg/kg) induced higher facilitation of brain stimulation reward when the electrode was placed more dorsally or medially in the substantia nigra than when it was placed close to the dorsal border of the substantia nigra (155). Additionally, self-stimulation of the PFC was facilitated by chronic D-amphetamine administration (1.5 mg/kg for 9 days), indicated by increases in response rates, whereas self-stimulation of the NAcc and supracallosal bundle remained unchanged or was suppressed, respectively (152).

In a study that used a rate-intensity ICSS procedure, 2 and 4 mg/kg of amphetamine shifted the curve to the left, indicating enhanced rewarding efficacy of brain stimulation (96). Consistent findings were observed in the autotitration procedure, in which D-amphetamine (0.03–1 mg/kg, IP) dose-dependently lowered thresholds (138). In a variant train duration procedure, amphetamine, administered at three different doses (0.33, 0.66, and 1 mg/kg, IP) counterbalanced over 3 consecutive days, dose-dependently lowered ICSS thresholds, indicating a reward-enhancing effect (145).
Using the discrete-trial current-intensity procedure, Markou and colleagues showed that exposure of rats to chronic mild stress for a period of 19 days potentiated the enhancement of lateral hypothalamic brain stimulation reward induced by acute administration of amphetamine (1–6 mg/kg, IP, for 2 days) compared with nonstressed animals (143). In a recent study using the rate-frequency curve-shift procedure, acute administration of D-amphetamine into the NAcc shell (1 µg/side) significantly lowered thresholds measured by decreases in $M_{50}$ values and did not affect maximal rates of responding (141).

Withdrawal from D-amphetamine decreases brain stimulation reward. Amphetamine administered three times per day for 5 days had an anhedonic effect, indicated by decreases in response rates of electrical brain stimulation when rats were tested 24 h following discontinuation of amphetamine administration and for at least 5 days (171). In studies by Markou and colleagues using the discrete-trial current-intensity procedure, continuous amphetamine exposure using 6-day subcutaneous osmotic minipumps (5, 10, and 15 mg/kg/day) dose-dependently lowered brain reward thresholds and decreased response latencies during two consecutive amphetamine exposures (163). By contrast, cessation of administration of the same amphetamine doses delivered via minipumps for 6 days dose-dependently elevated thresholds, an effect that lasted for 5 days for the highest amphetamine dose (163). Termination of intraperitoneal administration of a variety of amphetamine doses (1–5 mg/kg, three times daily for 1, 2, 4, or 6 days) led to ICSS threshold elevations (173). In a study by Markou and colleagues (164), amphetamine was administered intraperitoneally three times per day for 4 days in a rising-dose regimen starting from 1 mg/kg and stabilizing at 5 mg/kg. Thresholds and response latencies were determined for up to 156 h after the last administration in the discrete-trial current-intensity threshold ICSS procedure. Amphetamine withdrawal resulted in decreased reward, reflected in elevated brain reward thresholds, and these effects were alleviated by co-administration of the selective serotonin reuptake inhibitors fluoxetine or paroxetine and the serotonin 5-HT$_{1A}$ receptor antagonist p-MPPI, indicating that decreased serotonergic function may mediate the effects of amphetamine withdrawal (164, 167). Using a rate-intensity ICSS procedure and stabilizing amphetamine doses at 10 mg/kg (1–10 mg/kg, IP, three times per day for 4 days), Barr and colleagues found that rats exhibited decreased levels of ICSS responding for up to 60 h after the last amphetamine administration, an effect that was counteracted by repeated administration of electroconvulsive shock (172). Previous studies have also reported similar effects of amphetamine withdrawal after cessation of repeated intraperitoneal administration of amphetamine (162, 168, 170, 173).
As described above, the magnitude and duration of the effects of cocaine and amphetamine withdrawal on brain stimulation reward depend on the amount of drug that has been previously administered, as well as the duration of exposure to the psychostimulant (53, 173). However, the effects are similar, independent of whether the psychostimulant is self-administered or experimenter-administered.

Although less studied than cocaine and amphetamine, acute or chronic administration of other psychostimulant compounds, such as methamphetamine (142, 174, 175) and β-phenylethylamine (a structural analog of amphetamine (156)), induce effects similar to those observed after cocaine or amphetamine administration (see above).

In summary, acute administration of either cocaine or amphetamine potentiates brain stimulation reward, reflected in lowering of ICSS thresholds. Chronic administration of each of these drugs leads to prolonged lowering of ICSS thresholds that does not show either tolerance or sensitization (116, 163). Finally, withdrawal from either cocaine or amphetamine leads to elevations in brain reward thresholds, indicating an anhedonic state. Importantly, all of the reward-facilitating or reward-attenuating effects of cocaine and amphetamine are dose-dependent, highly consistent among studies and laboratories, and independent of the ICSS procedures or strain of animals or species used.

5.2. Nicotine

Nicotine is considered a mild psychomotor stimulant and shares several properties with classic psychostimulant compounds, such as amphetamine. Nicotine is one of the main psychoactive ingredients in tobacco smoke that contributes to addiction to tobacco smoking (176). Similar to the classic psychostimulants, acute nicotine administration (experimenter-delivered or self-administered) facilitates reward in a variety of ICSS procedures (48, 50, 114, 177–185) (Fig. 3), an effect that is dose-dependent (114, 178, 181, 186, 187). The time-, dose-, and procedure-dependency of the effects of nicotine were demonstrated in studies by Clarke and Kumar (177) and Schaefer and Michael (178). Nicotine administered at a dose of 0.4 mg/kg subcutaneously decreased the amount of time spent receiving self-stimulation and impaired locomotor performance, assessed by decreased responses (i.e., movements between photobeams) in rats permitted to turn on and off electrical stimulation by running along opposite walls of a shuttle box (177). However, this effect was only observed in the first few minutes after the nicotine injection. This initial aversive and motor-disruptive effect was later followed by a reward-facilitating effect and increased responding (i.e., increased movements between photobeams) even when the stimulation was not available (177). Consistent with these findings, studies by Schaefer and Michael found different effects of acute nicotine on
brain stimulation reward depending on the ICSS procedure used (178, 188). Nicotine (0.03–1 mg/kg, SC) had no effect or showed a decrease in response rates on the continuous reinforcement schedule and the autotitration procedure and did not affect the threshold for self-stimulation. By contrast, nicotine administered subcutaneously induced a robust biphasic effect when a fixed-ratio 15, fixed-ratio 30, fixed-interval 15, or fixed-interval 30 schedule of reinforcement was used, with the 0.1 mg/kg dose increasing and the higher doses (0.17, 0.3, or 1 mg/kg) decreasing response rates (178, 188). In contrast to these early experiments, subsequent studies using the discrete-trial and autotitration current-intensity procedures have generated consistent findings on the effects of nicotine on brain stimulation reward. Specifically, Markou and colleagues and Bespalov and colleagues demonstrated lower current-intensity thresholds after 0.25 mg/kg (50) or 1 mg/kg (180) nicotine administration, with no effect on response latencies (50) or a small reward-facilitating effect (164). The same dose of nicotine (1 mg/kg) had no effect in a progressive-ratio ICSS procedure (180). Importantly, using the same ICSS procedure, nicotine (0.03 mg/kg/infusion) self-administered for 1 or 12 h daily led to a long-lasting reward-enhancing effect, reflected by lowered ICSS thresholds during the nicotine self-administration days (before and after the daily nicotine self-administration session) that persisted for more than 1 month after self-administration ceased (48). These reward-enhancing effects of nicotine appear to share the same brain substrates with the reward-facilitating effect induced by other drugs of abuse (50, 181, 187). Specifically, these effects are partly attributable to the activation of nicotinic acetylcholine receptors (nAChRs) in the VTA (114), N-methyl-D-aspartate (NMDA) receptors potentially in the central nucleus of the amygdala and VTA (182), and orexin receptors in the insula (189). Similar to cocaine- (116) and amphetamine- (163) induced lowering of thresholds, no tolerance or sensitization to this effect of nicotine was observed (48).

The nicotine withdrawal syndrome in humans and animals includes both somatic and affective symptomatology (50, 55, 190–202). The ICSS procedure has provided one of the first affective measures of nicotine withdrawal in laboratory animals. A large number of ICSS studies have shown that withdrawal from experimenter-administered nicotine, regardless of whether it was spontaneous or precipitated by a nAChR antagonist in rats or mice chronically treated with nicotine, is associated with robust ICSS threshold elevations (50, 55, 164, 194–199, 203–208), indicating diminished interest in the rewarding stimulus and an anhedonic state (Fig. 4). Importantly, nicotine withdrawal does not affect performance in the ICSS procedure (i.e., no effect on response latencies in the discrete-trial current-intensity procedure), indicating that no motor disruption is
induced by cessation of nicotine administration (55). Interestingly, temporal associations between nicotine withdrawal and discrete environmental stimuli led to these stimuli acquiring conditioned properties. Subsequent presentation of these conditioned stimuli led to elevations in ICSS thresholds, reflecting conditioned nicotine withdrawal (209). In adolescent rats, the negative effects of nicotine withdrawal precipitated by the nAChR antagonist mecamylamine in subjects chronically treated with nicotine were smaller compared with adult rats (204). Interestingly, as mentioned above, withdrawal from nicotine self-administration leads to reward enhancement (i.e., lowering of ICSS thresholds) rather than reward deficits (i.e., elevations in ICSS thresholds), although the mechanism of action of this effect is not yet known (48).

Changes in cholinergic, dopaminergic, and glutamatergic neurotransmission, among other systems (193, 207, 210, 211), have been implicated in somatic and affective nicotine withdrawal signs in animals (212). Systemic administration of the nAChR antagonist dihydro-β-erythroidine (DHβE) or mecamylamine precipitated withdrawal-like elevations of ICSS thresholds in nicotine-dependent rats treated with 3.16 mg/kg/day nicotine base for 7–14 days (194, 213). Infusion of DHβE into the VTA, but not NAcc shell or bed nucleus of the stria terminalis, also precipitated elevations in ICSS thresholds in nicotine-dependent rats (195). Additionally, Hildebrand and colleagues showed that extracellular dopamine levels are reduced in the NAcc but not in the medial PFC in rats after systemic or intrategmental, but not intra-NAcc, administration of mecamylamine (193, 214, 215). The nAChR antagonist manipulations that decreased dopamine levels also precipitated the nicotine withdrawal syndrome when administered to subjects treated for 7 or 14 days with subcutaneous nicotine via osmotic minipumps (193, 214). Importantly, acute administration of bupropion (10–40 mg/kg), a US Food and Drug Administration-approved smoking cessation aid that inhibits dopamine and norepinephrine reuptake, dose-dependently reversed ICSS threshold elevations and somatic signs of nicotine withdrawal (216). Chronic administration of bupropion for 14 days also prevented the threshold elevations and increases in somatic signs during nicotine withdrawal and reversed the decrease in responsivity to potassium stimulation of the dopaminergic system in the NAcc shell in rats (211). Similarly, chronic, but not acute, administration with the tricyclic antidepressant desipramine, which primarily inhibits norepinephrine reuptake, prevented both the threshold elevations and the somatic signs of nicotine withdrawal in rats (207).

The metabotropic glutamate receptor II (mGluR2/3) antagonist LY314582, similar to nAChR antagonists, also precipitated withdrawal-like elevations in ICSS thresholds in nicotine-dependent
rats (217), suggesting that increased function or number of Group II mGluRs characterizes nicotine withdrawal. Accordingly, administration of the mGluR2/3 antagonist LY341495 attenuated threshold elevations in rats undergoing spontaneous nicotine withdrawal (217). Altogether, these findings suggest that chronic exposure to nicotine decreases cholinergic, dopaminergic, and glutamatergic transmission to mediate the affective aspects of nicotine withdrawal once nicotine administration is discontinued.

Recent studies have also implicated hyperactivity in the corticotropin-releasing factor (CRF) system in the anhedonic aspects of nicotine withdrawal. Mecamylamine-precipitated elevations in ICSS thresholds in nicotine-dependent rats were prevented by administration of the CRF receptor antagonist D-Phe CRF$_{12,41}$ in the lateral ventricle (intracerebroventricular injection), central nucleus of the amygdala, or NAcc shell, but not the lateral bed nucleus of the stria terminalis (218, 219). D-Phe CRF$_{12,41}$ had no effect on spontaneous nicotine withdrawal (218). More recently, two ICSS studies have provided evidence of decreased brain reward function during nicotine withdrawal in mice (196, 198). Both studies used the discrete-trial current-intensity ICSS procedure. In the first study, spontaneous withdrawal from experimenter-administered nicotine (2 mg/kg/injection, salt) for 7 consecutive days and withdrawal precipitated by the nAChR antagonist mecamylamine (2 mg/kg) in nicotine-dependent C57BL6 mice were associated with elevated ICSS thresholds. Removal of a subcutaneous osmotic minipump that delivered 24 mg/kg/day nicotine base for 8–10 days resulted in threshold elevations that persisted for 72 h after minipump removal (196). In the second study, C57BL/6J mice were used (198). Spontaneous nicotine withdrawal after 14-day exposure to 10–40 mg/kg/day nicotine administered continuously via subcutaneous osmotic minipump induced no changes in thresholds in C57BL/6J mice. However, termination of prolonged exposure (28 days) to a relatively high nicotine dose (40 mg/kg/day, base) administered through subcutaneous osmotic minipumps resulted in elevated ICSS thresholds in C57BL/6J mice. Similar threshold elevations were seen after administration of the nAChR antagonists mecamylamine (3 and 6 mg/kg) or DHβE (3 mg/kg) in C57BL/6J mice chronically treated with the same nicotine dose (40 mg/kg, base) (198). Both of these studies used the discrete-trial current-intensity ICSS procedure and demonstrated that the ICSS procedure can assess reward deficits during nicotine withdrawal in mice.

In summary, numerous studies have demonstrated that, similar to classic psychomotor stimulant drugs, acute experimenter-administered or self-administered nicotine leads to reward facilitation. With self-administered nicotine, this reward-enhancing effect persisted for an entire month of testing, demonstrating that important differences may exist in the effects of self-administered
nicotine and cocaine. However, withdrawal from chronic exposure to subcutaneous experimenter-administered nicotine results in robust ICSS threshold elevations, reflecting an anhedonic state. Thus, the anhedonic effects of nicotine withdrawal observed in the ICSS procedure are a reliable animal model of the anhedonic effects seen in humans during tobacco abstinence. The prolonged reward enhancement after chronic nicotine self-administration may reflect the prolonged reward-enhancing properties of nicotine that significantly contribute to the perpetuation of the tobacco smoking habit (48, 49, 220).

5.3. Opiates

Most opiate compounds, such as morphine and heroin, lower ICSS thresholds or increase response rates after acute or chronic administration, demonstrated by different ICSS procedures (57, 221–230) (Fig. 3). Nevertheless, studies have also shown a depressant or even biphasic effect (depression followed by facilitation of brain stimulation reward) of different opiates on ICSS response rates (231–235). This biphasic or depressant effect greatly depends on the time of opiate administration relative to ICSS testing. In the first studies on the reward-facilitating effects of opiates, morphine was hypothesized to act more as a negative than positive reinforcer that maintained behavior because it terminated the aversive withdrawal state (236, 237). Schaefer and Michael showed that morphine and naloxone dose-dependently decreased response rates in a fixed-interval ICSS procedure. The combination of the two drugs blocked the dose-dependent decrease induced by morphine (238). By contrast, heroin was found to increase rates of lever pressing for ICSS after five consecutive injections of 5 mg/kg or after heroin self-administration, an effect that was attenuated by pretreatment with naloxone (239, 240). With the extensive use of ICSS threshold procedures, particularly the discrete-trial current-intensity procedure, this early depressant effect on ICSS response rates was shown to reflect a motoric depressant effect. With chronic opiate administration, tolerance to this motor-suppressant effect of opiates occurred, which allowed the reward-facilitating effect to be revealed (241). Indeed, when the discrete-trial procedure was used, in which the determination of the ICSS threshold is independent of response rates, immediate reward facilitation was observed (226). In this study, rats received various doses of morphine (4–12 mg/kg) twice per day for up to 25 days (226). Using the current intensity and progressive-ratio ICSS procedures, morphine lowered the current-intensity threshold and increased the maximal ratio of reinforced and non-reinforced responses, respectively (180). In both of these studies (226), morphine-induced lowering of thresholds was observed that showed no tolerance or sensitization, similar to cocaine (116), amphetamine (163), and nicotine (48).
Heroin is more potent than morphine and 6-acetylmorphine in lowering ICSS reward thresholds (230). Using the discrete-trial current-intensity procedure, Kenny and colleagues found that intravenously self-administered heroin (20 μg/infusion) activated reward systems in nondependent rats, reflected in lowering of ICSS thresholds, and decreased reward sensitivity in dependent rats, reflected in elevated ICSS thresholds (57). Consistent with previous findings, naloxone administration (30 μg/kg) reversed the heroin-induced (20 μg/infusion) lowering of ICSS thresholds in nondependent rats. Interestingly, cues previously associated with morphine exposure (5 mg/kg, IP) lowered thresholds in the absence of morphine after 5 days of paired drug-cue training sessions in a rate-frequency procedure (118).

In contrast to the acute effects of opiates, opiate withdrawal leads to elevations in ICSS thresholds that appear to be more prominent during opiate antagonist-precipitated withdrawal in opiate-dependent subjects than spontaneous withdrawal (52). Antagonist-precipitated withdrawal can be induced by administration of opioid receptor antagonists, such as naloxone and naltrexone (57, 224, 235, 242–245). Opioid receptor antagonists induce withdrawal-like effects, such as elevated ICSS thresholds (Fig. 4) and decreased response rates for food (246), even after only a single dose of morphine, levorphanol, fentanyl, or methadone (247–249), as well as in heroin-dependent rats chronically self-administering heroin intravenously (57). Naltrexone suppressed responding in the autotitration or progressive-ratio ICSS procedure in rats chronically treated with morphine and in rats that had received only one dose of morphine (243–245). In opiate-dependent rats, very low doses of naloxone dose-dependently elevated ICSS thresholds (246). Importantly, administration of antagonists with high affinity for the μ opioid receptor, rather than the κ or δ receptor, resulted in a large response-rate-decreasing effect in rats that were pretreated with 10 mg/kg of morphine and were sensitized to the response-rate-decreasing effect of the antagonists in a frequency autotitration ICSS procedure (245). Thus, μ opioid receptors are mainly implicated in the reward deficits characterizing opiate withdrawal (245). Similar to cues associated with nicotine withdrawal (209), presentation of cues previously paired with naloxone-precipitated withdrawal elevated ICSS thresholds in heroin- or morphine-dependent rats in the absence of naloxone administration (57, 209).

In addition to heroin and morphine, numerous other opioid agonists, some of which have been used for the treatment of chronic pain (e.g., fentanyl) or are still under evaluation in clinical trials for pain, have been assessed in different ICSS procedures. For example, using the discrete-trial procedure, naloxone decreased brain reward function in rats chronically treated with the opioid receptor agonist fentanyl. The partial opioid receptor agonist...
buprenorphine prevented the affective and somatic symptoms associated with fentanyl withdrawal, indicating a possible treatment for the anhedonic state associated with withdrawal from fentanyl and possibly other opioids (250).

In summary, consistent with the effects of psychomotor stimulants, acute administration of opioid agonists leads to reward facilitation. Opiate withdrawal, including withdrawal from a single high dose of an opiate, is associated with reward deficits. Similar to nicotine, both spontaneous and antagonist-precipitated withdrawal in opiate-dependent subjects is characterized by ICSS threshold elevations that tend to be larger and more pronounced in antagonist-precipitated withdrawal compared with spontaneous withdrawal.

5.4. Cannabinoids

Cannabinoids have not been as extensively tested in the ICSS paradigm as the previously discussed drugs of abuse. Unlike other drugs of abuse that induce similar effects in different strains of rats in the ICSS procedure, different effects have been observed in various rat strains after the administration of the cannabinoid agonist Δ⁹-THC and other natural or synthetic cannabinoid 1 (CB₁) receptor agonists and analogs. Gardner and colleagues showed that low doses of Δ⁹-THC lowered ICSS thresholds (251–254). Specifically, using the autotitration ICSS procedure, 1.5 mg/kg Δ⁹-THC lowered thresholds in Lewis rats (251, 254).

In the rate-frequency curve-shift procedure, the most pronounced action of Δ⁹-THC was found after administration of 1 mg/kg in Lewis rats compared with Sprague-Dawley or Fisher rats. These differences in effects in various rat stains may be attributable to Lewis rats being more sensitive and vulnerable to the effects of addictive drugs than other strains (252), suggesting an important genetic component in the actions of cannabinoids. Notably, the reward-facilitating effects of Δ⁹-THC in the aforementioned studies are much less pronounced than those of other drugs of abuse, such as morphine, cocaine, amphetamine, or nicotine. These latter drugs can reliably shift the rate-frequency function to the left between 0.2 and 0.5 log units when the rate-frequency curve-shift procedure is used. However, in the study by Lepore and colleagues (252), Δ⁹-THC only shifted the rate-frequency function to the left by approximately 0.05 log units.

In contrast to the above studies, Stark and Dews (255), Kucharski and colleagues (256), and Vlachou and colleagues (257) have seen either no effect or an elevation in ICSS thresholds after administration of Δ⁹-THC or other cannabinoid analogs structurally related to Δ⁹-THC (Fig. 3). In the study by Stark and Dews, a 10 mg/kg dose of Δ⁹-THC administered orally induced a 50% decrease in response rates. The cannabinoid analogs nabilone (1 mg/kg, PO) and canbisol (0.32 mg/kg, PO) significantly reduced response rates (258). Later studies using the discrete-trial
current-intensity procedure \cite{256} or the rate-frequency curve-shift method \cite{257} showed significant elevations of reward thresholds after administration of 0.2 and 0.3 mg/kg levonantradol, a $\Delta^9$-THC cannabinoid analog, or 1 and 2 mg/kg of $\Delta^9$-THC, respectively.

The effects of various synthetic cannabinoid receptor agonists and antagonists on brain stimulation reward have also been examined. In a series of studies mainly by Vlachou and colleagues, the CB$_1$ receptor agonists WIN55,212-2, CP55,940, HU-210, and AMG-3 either did not affect the reinforcing properties of MFB self-stimulation or elevated ICSS thresholds depending on the dose used \cite{259–262}. Consistent with the above findings, the indirect cannabinoid receptor agonists PMSF, AM-404, OMDM-2, and URB-597 either did not affect or elevated ICSS thresholds, depending on the dose used \cite{263}. Low doses of the CB$_1$ receptor antagonists SR141716A and AM-251 did not affect brain reward thresholds, although some studies showed that high and likely nonselective doses of the CB$_1$ receptor antagonist SR141716A elevated thresholds \cite{259, 264}. Interestingly, clinical studies indicate that SR141716A (rimonabant) may induce anhedonic states and depressed mood \cite{265}.

In summary, most of the studies using the ICSS paradigm did not show reward-facilitating effects of $\Delta^9$-THC or other cannabinoids, or most often demonstrated anhedonic actions of these compounds after acute administration, consistent with observations in humans \cite{266–268}. These seemingly contrasting results may be attributable to differences in the pharmacological properties and dose ranges of the tested compounds, as well as the different levels of sensitivity to cannabinoids in different strains of animals \cite{252}.

**5.5. Ethanol**

Similar to cannabinoids, studies on the effects of ethanol on ICSS behavior have not produced very consistent findings. The difference in effectiveness has been proposed to depend on numerous experimenter parameters, such as the location of the stimulating electrode, ethanol dose administered, route of administration, and ICSS procedure \cite{269–271}. In one of the first ICSS studies with ethanol, 0.6 g/kg ethanol increased the rate of lever-pressing but did not affect the threshold measure. Higher doses of 0.9 and 1.2 g/kg elevated thresholds without affecting response rates \cite{272}. In another study \cite{273}, animals would not initiate brain stimulation behavior after a 2.1 g/kg dose of ethanol administered intraperitoneally, unless the experimenter first applied the stimulation, in which case animals exhibited approximately the same response rates as in their drug-free state, despite the fact that the animals were debilitated after the ethanol administration. The lack of effect of ethanol on response rates for ICSS was considered to be attributable to either the strong resistance of the brain reward system to the depressive effects of ethanol or the fact that ethanol
Intracranial Self-Stimulation renders the brain reward system more responsive to natural rewards and emotional stimuli, such as food, sex, or even social and aggressive stimuli (273). In Wistar rats, when ethanol was administered intraperitoneally or orally in a drinking solution at low doses up to 0.8 g/kg, the ICSS response rate and total time spent self-stimulating increased. Higher ethanol doses induced a dramatic and long-lasting decrease of both response rates and time self-stimulating (270). In the autotitration ICSS procedure, Sprague-Dawley rats showed no changes in reward thresholds, except at the highest ethanol dose (1.7 g/kg) when behavior was disrupted (274, 275). The route of ethanol administration (intraperitoneal or intragastric) appeared to affect the effects of ethanol on brain stimulation reward, demonstrated by a reduction in the response rate after intragastric administration (274). Additionally, chronic ethanol administration in the form of an ethanol liquid diet led to reduced responding in the 5% alcohol-containing diet group compared with the control diet groups (275).

In a recent study using the current-intensity autotitration and progressive-ratio ICSS procedures, ethanol lowered ICSS thresholds, although not dose-dependently (180) (Fig. 3). The intermediate dose of 1.2 g/kg ethanol lowered thresholds. Consistent with previous findings, the highest ethanol doses tested (1.8 and 2.4 g/kg, PO) did not have any reward-enhancing effects (180), possibly because of motor-induced incapacitation (273–275).

By contrast, withdrawal from chronic ethanol exposure (17–20 days) resulted in time-dependent ICSS threshold elevations in the discrete-trial current-intensity procedure, with peak elevations 6–8 h after cessation of ethanol exposure (54) (Fig. 4). ICSS threshold elevations and decreased response rates were also observed during alcohol withdrawal in rats selectively bred for low alcohol drinking, which exhibit alcohol avoidance, indicating a possible genetically exacerbated dysphoric-like experience during the withdrawal phase in these rats (276).

In addition to the most commonly used drugs of abuse, many other compounds and recreational drugs have been tested in the ICSS procedure, although not as extensively as psychostimulants, nicotine, and opiates. These drugs include benzodiazepines, barbiturates, phencyclidine, and club drugs such as 3,4-methylenedioxy-N-methylamphetamine (MDMA). Among these drugs, benzodiazepines and barbiturates, such as diazepam, chlordiazepoxide, meprobamate, bromazepam, and pentobarbital, are anxiolytics and sedative drugs that have high addiction potential and induce dependence. Benzodiazepines and barbiturates administered at low to moderate doses facilitated lever pressing and lowered ICSS thresholds (277–281). At high doses, they decreased response rates (159, 282, 283), possibly because of their muscle relaxant or sedative effects (284, 285). Importantly, in most of
these studies, the use of simplified ICSS procedures that do not dissociate between reward and performance effects made the reward-enhancing effects of benzodiazepines and barbiturates less readily detectable (285). Thus, these reward-facilitating effects show considerable variability in different studies, depending on the ICSS procedures used and the doses administered (281, 286–288). In the studies by Gomita and colleagues (286, 287), ICSS was combined with footshock punishment to test for the anxiolytic effects of benzodiazepines. In these studies, both diazepam (5–20 mg/kg, PO) and bromazepam (2–20 mg/kg, PO) dose-dependently increased response rates during the punished period, indicating an anxiolytic-like effect of the drugs. The same group of researchers showed, using the autotitration ICSS procedure, that chlordiazepoxide (5 and 20 mg/kg, PO) and meprobamate (100 mg/kg, PO) facilitated reward, reflected in lowering of the current-intensity at which the current was reset in this ICSS procedure (i.e., reset threshold) (288). Recently, Sagara and colleagues showed that diazepam (0.5 and 1 mg/kg, IP) produced a delayed extinction effect of running behavior in the ICSS runway paradigm with ICSS priming stimulation, indicating enhanced motivation induced by diazepam (281).

Phencyclidine (PCP), a dissociative anesthetic compound with hallucinogenic and neurotoxic effects, lowers brain stimulation thresholds, although to a lesser degree than cocaine or morphine (19, 20) (Fig. 3). PCP, administered either systemically (2.5 or 5 mg/kg) or centrally into the NAcc shell (0.5 μL), potentiated the rewarding impact of lateral hypothalamic self-stimulation in Long-Evans rats in the rate-frequency curve-shift procedure (289–291). Intraperitoneal administration of 3 mg/kg PCP increased response rates, and 5.6 mg/kg PCP induced behavioral disruptions reflected primarily in circling behavior using a fixed-interval schedule of reinforcement for ICSS in Sprague-Dawley rats (238). However, Bespalov and colleagues (180) found no significant reward-facilitating effects of PCP in Wistar rats using the current-intensity autotitration or progressive-ratio ICSS procedures, although they used doses similar to those used by Schaefer and Michael (0.3–5.6 mg/kg, IP) (238). Interestingly, chronic treatment with PCP (10, 15, and 20 mg/kg, SC) for 14 days resulted in a sustained and pronounced dose-dependent potentiation of brain stimulation reward (56). Similar to other drugs of abuse, acute withdrawal from a high PCP dose (5 and 10 mg/kg) or from chronic exposure to 10, 15, or 20 mg/kg/day for 14 days decreased brain reward function, reflected by large and prolonged elevations in thresholds that lasted for the entire period of observation (30 days) (56) (Fig. 4).

MDMA is a psychoactive phenylisopropylamine that is structurally similar to both amphetamine-related sympathomimetics and the hallucinogen mescaline (i.e., they both belong to the
Intracranial Self-Stimulation

phenethylamine class of compounds) (292, 293). Similar to all other drugs of abuse, MDMA produces pleasurable effects, including euphoria (294), in humans. Using a rate-independent discrete-trial procedure, Kornetsky and colleagues found that MDMA administration dose-dependently lowered reward threshold in the F-344 rat strain, an effect that was more pronounced at the 2 mg/kg dose (295). Reid and colleagues tested Sprague-Dawley rats in a variable three-intensity procedure in which they showed that after 5 days of injections (2 mg/kg, SC), MDMA significantly increased rates of lever-pressing at all three intensities tested (296).

In summary, benzodiazepines and club drugs appear to dose-dependently affect brain stimulation reward, with moderate doses having reward-facilitating effects and higher doses inducing disruption in behavior reflected in significant changes in the performance capability of the animals. Notably, the effects observed with these drugs in the ICSS procedures are not as clear as the effects demonstrated with psychomotor stimulants or opiates.

6. Summary

The serendipitous finding of Olds and Milner in the previous century that rats will work to self-stimulate parts of their own brain (6) led to the gradual development of different ICSS procedures that have been used for more than 50 years in behavioral neuropsychopharmacology research. The ICSS paradigm is considered a powerful tool for examining the anatomical basis of reward and motivation, as well as the reward-facilitating and anhedonic state induced by acute administration and withdrawal, respectively, of different compounds and drugs of abuse (8, 14, 39). Neuroanatomical and neurochemical studies have shown that the neurosubstrates of ICSS involve multiple brain sites comprising the brain’s reward pathways, the most important of which are the MFB and areas of the lateral hypothalamus and VTA. Various ICSS procedures have been used throughout the years. However, not all of these procedures have been experimentally validated to be reward-selective, and several of them are no longer in use. Two of the most commonly used ICSS procedures, because of their validation for the assessment of both reward and performance effects, are the rate-frequency curve-shift and the discrete-trial current-intensity threshold procedures. In all ICSS procedures, lowering of ICSS thresholds indicates a facilitation of brain stimulation reward, and elevations in ICSS thresholds reflect an anhedonic depressive-like state. Generally, most drugs of abuse, including cocaine, amphetamine, nicotine, morphine, and heroin,
lower ICSS thresholds after acute administration in experimental animals. Withdrawal from chronic exposure to these compounds leads to elevations in ICSS thresholds, indicating an anhedonic state that resembles the negative affective state of the drug withdrawal syndrome experienced by humans. However, other drugs of abuse, such as cannabinoids and ethanol, have shown inconsistent effects in the ICSS procedure. Factors such as the different strains of animals or different doses used may contribute to these inconsistencies, as well as the small effect size that is not always detectable. Overall, the ICSS paradigm is a powerful behavioral tool that allows the quantitative assessment of the effects of various manipulations and compounds on brain reward function.

Acknowledgments

This work was supported by National Institutes of Health research grants U01 MH69062 from the National Institute of Mental Health and R01 DA11946 and R01 DA232090 from the National Institute on Drug Abuse to AM. Dr. Styliani Vlachou was supported by individual Postdoctoral Research Fellowship 18FT-0048 from the Tobacco-Related Disease Research Program from the State of California. The authors wish to thank Janet Hightower for outstanding graphics.

References


100. Liebman JM, Segal DS (1976) Lack of tolerance or sensitization to the effects of chronic d-amphetamine on substantia nigra self-stimulation. Behav Biol 16:211–220


potentiation of brain stimulation reward in rats. Biol Psychiatry 57:120–125


144. Sweet KL, Neill DB (1999) Amphetamine injections into the nucleus accumbens
enhance the reward of stimulation of the subiculum. Ann NY Acad Sci 877:828–830


173. Lin D, Koob GF, Markou A (1999) Differential effects of withdrawal from chronic amphetamine or fluoxetine administration on brain stimulation reward in the rat: interactions between the two drugs. Psychopharmacology (Berl) 145:283–294


211. Hildebrand BE, Svensson TH (2000) Intraaccumbal mecamylamine infusion does not affect dopamine output in the nucleus...


Chapter 2

Stimulant Self-Administration

Leigh V. Panlilio

Abstract

Stimulants such as cocaine and the amphetamines are widely abused due to their rewarding effects. Much of what we know about drug abuse and drug reward comes from research involving stimulants, and much of this research involves using drug self-administration as an animal model of drug abuse. In this chapter, the example of stimulant self-administration is used to illustrate: (1) the basic methodology of drug self-administration procedures and (2) the behavioral principles that apply to addiction and animal models of addiction. Many variations of the self-administration procedure have been developed to model specific aspects of drug abuse, to assess the rewarding effects of drugs, and to assess the effects of treatments. The chapter describes how these variations are devised by stipulating the behavioral requirements for receiving the drug (i.e., the schedule of reinforcement) and incorporating drug-related environmental cues analogous to those that occur in the human drug-abuse environment.

Key words: Animal models, Drug abuse, Methodology, Stimulus, Reinforcement, Behavior, Learning

1. Introduction

Drug abuse is a behavioral phenomenon that has much in common with other behaviors, but also has unique aspects that set it apart. Addiction research is largely concerned with uncovering and explaining these unique aspects. Similarly, addictive drugs share certain properties with each other, but they also have differences that allow them to be divided into general classes. Drugs that give the user a feeling of alertness and energy are referred to as stimulants. This loose classification includes a range of drugs that vary with respect to their pharmacological actions and potential for abuse. Cocaine, amphetamine, and methamphetamine are the most widely abused drugs in this class, and also the most intensively studied. Along with opioids, such as heroin, these stimulants represent the de facto standard against which other addictive drugs are compared.
Drug self-administration is the primary animal model used to study drug abuse. In this chapter, the example of stimulant self-administration will be used to illustrate: (1) the basic methodology of drug self-administration procedures, and (2) the behavioral principles that apply to addiction and animal models of addiction. Examples will be given to illustrate certain points, but the chapter is not intended as a review of the literature. It is intended to provide a brief overview of the basic techniques used in this active area of research, to introduce the central concepts behind these techniques, and to provide a background for the other chapters in the book.

1.1. The Problem of Stimulant Abuse

In the United States alone, over two million people meet the criteria for dependence or abuse of cocaine or other stimulants (1). This abuse has high economic and social costs related to health care, crime, and loss of productivity (2). In the individual, stimulants can produce a variety of adverse effects, including insomnia, anorexia, tremors, teeth grinding, dizziness, repetitive movements (stereotypy), schizophrenia-like (psychotomimetic) symptoms, hyperthermia, a variety of cardiovascular effects, muscle rigidity, intracerebral hemorrhage, convulsions, respiratory depression, and sudden death (3, 4). Amphetamines and amphetamine derivatives such as methylenedioxymethamphetamine (“ecstasy”) can also have lasting neurotoxic effects (5, 6). The fact that addicted individuals continue to seek and consume stimulants despite these adverse consequences attests to the power of the drugs’ rewarding effects.

1.2. Drug Reward

The scientific consensus is that stimulants and other addictive drugs are abused (at least initially) because they increase neurotransmission in the mesolimbic dopamine pathway, a part of the reward system of the brain (7–9). Cocaine elevates dopamine levels by blocking the reuptake of dopamine after it has been released into the synaptic cleft (10). Amphetamine and methamphetamine elevate dopamine levels by several mechanisms, including stimulating dopamine synthesis and inverting the direction of dopamine uptake, causing a massive release of dopamine into the synaptic cleft (11, 12). While non-stimulant drugs of abuse also increase mesolimbic dopamine transmission, they do so less directly, generally by altering the activity of neurons “upstream” of the neurons that release dopamine.

The normal function of the reward system is to increase contact with biologically meaningful substances, stimuli, and events in the natural environment, such as food, water, and sex. This normal function is “hijacked” when addictive drugs pharmacologically activate the system. The effects of the drug reinforce drug-taking behavior, making it more likely to be repeated in the future. As drug use continues, the people, places, and things associated with
the rewarding effects of the drug come to function as cues that have rewarding effects of their own. These cues motivate the individual to seek the drug, and they guide the complex sequences of actions involved in obtaining, preparing, and administering the drug. Thus, the behavior of abusing drugs is shaped and maintained by the effects of both the drug itself and drug-related features of the environment.

To understand the etiology of addiction and to develop therapeutic treatments, it is necessary to study how drugs and drug-related cues affect behavior and the brain. Drug self-administration provides an animal model of drug abuse that is ideal for studying the rewarding effects of drugs and drug-related cues. These procedures typically involve implanting an intravenous catheter that allows a rat or monkey to self-inject a drug by performing a simple response (e.g., pressing a lever) that activates a motor-driven syringe pump. Laboratory animals will self-administer most of the same drugs that are abused by humans (13), and stimulants are readily self-administered by rodents and nonhuman primates. The basic drug self-administration procedure is highly flexible, and many variations have been developed to focus on specific aspects of drug reward, abuse, and addiction. Due to its flexibility, face validity, and close correspondence to human drug-taking behavior, drug self-administration is the “gold standard” among animal models of drug abuse.

Drug self-administration procedures do have some disadvantages compared to other animal models of drug abuse, such as drug-discrimination and place-conditioning procedures (see Chap. 6). For example, self-administration procedures generally require many training sessions per subject, and these sessions are relatively long. This limits the number of subjects that can be studied, reducing the statistical power of these studies. However, in most cases, small group size can be compensated for by using a within-subject experimental design that increases power by studying each subject under multiple conditions. Self-administration procedures can also be technically difficult to implement, requiring specialized equipment and skills. For example, the experimenter must monitor each animal’s progress closely and sometimes adjust the training parameters for specific subjects to obtain consistency of performance across subjects (see (14)). The experimenter must be skilled in shaping animal behavior, programming the experimental events, and implanting catheters. Catheter failure represents one of the most significant obstacles to this research, often causing subjects to be dropped from a study before it is finished. In rats, catheters typically last about 3–6 months. In monkeys, a catheter usually lasts a year or longer, and once it fails a new catheter can be implanted in the same vein or a different vein. So, individual monkeys can be studied for many years and are well
suited for long-term studies. Rats are better suited than monkeys for short-term studies, such as those focusing on the initial acquisition of drug use or involving irreversible treatments.

The self-administration of drugs by animals is presumed to be directly analogous to drug use by humans. Although similar drug self-administration procedures can be used in a laboratory setting with either human or animal subjects, there can be distinct advantages to using animals. The scientific method requires that researchers have the ability to control and manipulate the conditions that produce addiction. But, it would be unethical to induce addiction in humans for research purposes, and drug-experienced human volunteers have varied and complex histories that can interact with the experimentally imposed conditions being studied. In contrast, the environment and life experiences of laboratory animals can be carefully monitored and controlled. In addition, many techniques for studying the brain mechanisms involved in drug self-administration are not feasible or appropriate for use in humans and can only be implemented in animals.

The main drawback to studying drug self-administration in animals rather than humans is that species sometimes differ with respect to the reward-related effects of drugs. For example, rodents seem to differ from humans and other primates with respect to the rewarding effects of THC, the main psychoactive component of marijuana (15). But, even though the details can differ between species, the basic principles and phenomena do generalize across species, and the overall similarities between animals and humans support the validity of using animal models. In fact, a productive and efficient use of resources for medication development is to use rodents to test hypotheses generated from in vitro findings, then use primates to verify the most promising findings from testing in rodents, and finally to use human volunteers to evaluate the treatments that were found to be effective in primates.

2. Schedules of Reinforcement

Historically, drug self-administration procedures were directly adapted from the techniques used by experimental psychologists to study behavior maintained by natural rewards, such as food. Focusing on the rewarding effects of drugs in this manner places the study of addiction within a comprehensive approach to the study of behavior and how it is shaped by its interactions with the environment. Using this approach, researchers have created many variations of the self-administration procedure that focus on specific addiction-related aspects of the behavior, such as how much the drug is valued as a reward (see Sect. 3), how drug intake is
Variations of the self-administration procedure can be created by modifying the **schedule of reinforcement**, the set of contingencies that define the relationship between behavior and the delivery of reinforcement (16). The schedule specifies requirements such as what response produces the reinforcer, the number of times the response must occur, the amount of time that must pass before another reinforcer can be obtained, and whether any cues are provided to signal the availability of the reinforcer. With drug and nondrug reinforcers, in the laboratory and in the natural environment, the schedule of reinforcement is a powerful determinant of the pattern of responding. The sections below briefly describe the most commonly used schedules of drug self-administration and the specialized purposes they serve.

### 2.1. Continuous Reinforcement

Continuous reinforcement is the simplest schedule of reinforcement: only one response is required for each injection of the drug. However, it should be noted that in practice, not every response will produce an injection; since the injection takes some time to deliver, there is typically a *timeout* period of at least a few seconds following each injection under continuous reinforcement as well as most other schedules of reinforcement. Any responses that occur during the timeout period do not produce additional drug. In many studies, longer timeouts (typically 30–60 s) are instituted to prevent the animal from self-administering another injection before the previous injection has been delivered and adequately distributed to the brain.

One way that continuous reinforcement is used is to study the acquisition of drug self-administration (see Chap. 9). Even when an experiment will involve a more complex schedule later in training, a continuous-reinforcement schedule is used during initial training because consistently and repeatedly associating the response with the reinforcing effects of the drug facilitates acquisition of the response. Once responding has been established, more complex response requirements (such as those described in the sections below) can be gradually introduced.

The simple, direct relationship between responding and receiving the drug under continuous reinforcement makes this schedule well suited for studying rates and patterns of drug intake. Under this schedule, self-administration tends to occur in a specific pattern. Early in the session there is a period known as the *loading phase*, in which several injections are taken in relatively rapid succession. The remainder of the session is known as the *maintenance phase*, in which there is a pause of fairly constant duration following each injection. If the dose is changed, the duration of pausing changes, with higher doses producing longer pauses.
Over the course of the session, the level of drug in the animal’s body also follows a predictable pattern. During the loading phase, when injections occur rapidly, the drug level steadily increases. During the maintenance phase, when injections are evenly spaced in time, the drug level rises quickly after each injection, then gradually decreases as the drug is eliminated from the body during the post-injection pause. Most interestingly, the drug level where the next response occurs tends to be about the same with each successive injection. Thus, the animal appears to regulate its drug intake in such a way that the level of effect is not allowed to fall below a certain minimum (17–21).

This phenomenon of regulated drug intake is a quintessential aspect of drug self-administration, occurring reliably across many different laboratories, species, and drug classes. Consequently, understanding why it occurs might provide insight into the unique nature of drug reward. There are four basic mechanisms that might contribute to the highly regular post-injection pausing that underlies regulated drug intake (21–23). First, the drug may produce behavioral “side effects” (i.e., effects not related to reinforcement) that cause responding to slow down or cease temporarily. Second, the drug may have aversive, punishing effects when a high drug level is reached (see Sect. 2.8). Third, achieving a certain level of drug effect may produce satiation, such that the animal is no longer motivated to obtain reward. Fourth, even though the animal is still motivated, taking more drug may have no effect when the reward system becomes saturated by a high level of drug, so the animal learns to detect when this happens and pause until it detects that the drug level has dropped (24).

Understanding the mechanisms underlying regulated drug intake may also provide insight into the dysregulation of drug intake that is often associated with severe addiction. It has long been known that animals given access to stimulants 24 h per day develop excessive levels of intake (25–27). In recent years, it has been shown that when rats are provided with extended access to the drug (e.g., sessions lasting 6 h or more), they develop many of the hallmarks of addiction, such as escalated intake ((18); see Chap. 10) and increased susceptibility to relapse (28). Thus, extended-access conditions can be used to provide an animal model of drug abuse that more closely approximates addiction, as opposed to casual, controlled use.

### 2.2. Ratio Schedules

Ratio schedules specify the number of responses that are required for each injection. Under a fixed-ratio schedule, the same number of responses is required for each injection. This schedule is usually designated by the abbreviation “FR” followed by the response requirement. For example, under FR 10 an injection is delivered for every tenth response. Continuous reinforcement is sometimes referred to as FR 1. With reinforcing drugs and with nondrug
reinforcers such as food, FR schedules that require a substantial number of responses for each reinforcer typically produce a specific pattern of responding known as “break and run”; there is an initial period of nonresponding (i.e., a break) after delivery of the reinforcer, followed by a period of rapid responding (i.e., a run) that continues until the required number has been reached.

In many cases, FR schedules of drug self-administration are used as baselines in studies that involve the testing of potential treatments for addiction (see Sect. 4). In other cases, fixed-ratio schedules are used to assess whether a novel drug has reinforcing effects. For example, when a medication is developed for a purpose such as treating pain or obesity, it is important to determine whether it also has rewarding effects that make it liable to be abused. One way to evaluate the rewarding effects of the new drug is to train animals to self-administer a drug with known abuse liability, such as cocaine, then see if the self-administration response is maintained when the syringe is filled with the new drug instead of cocaine. Ratio schedules are advantageous for these purposes because they engender substantial rates of responding if the new drug is an effective reinforcer (see Sect. 3). In contrast, the rate of responding maintained by a reinforcing drug under a continuous-reinforcement schedule can be quite low, especially if the drug has long-lasting effects, and can sometimes be difficult to distinguish from the rate that occurs when the drug is replaced by a placebo such as saline solution.

Continuous reinforcement is used to determine when the animal will take the drug if it is freely available. In contrast, a progressive-ratio schedule is used to determine how much the animal will “work for the drug.” Under a progressive-ratio schedule, the required number of responses is increased with each successive injection. The increases usually occur in steps according to an exponential progression. For example, a commonly used progression is 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, and 603. This kind of schedule is designed to determine the point at which the response requirement becomes so high that the drug’s reinforcing effects no longer maintain responding. Typically, the pattern of responding will become less consistent and long pauses will begin to appear as the requirement increases. Once a designated criterion is reached, for example, 1 h without a response, the final response requirement that was successfully completed is taken as the “breaking point.” The breaking point is a relatively direct measure of the strength of the drug as a reinforcer at the tested dose (see Sect. 3.3). The criterion chosen for determining the breaking point should be chosen to be substantially longer than the post-injection pause that would occur under continuous reinforcement or a low-requirement fixed-ratio schedule. The progression listed above was designed to start with a ratio of 1 and escalate quickly
enough that the animal will cease responding within a 5 h session (29). If the criterion is not met within a session, researchers sometimes simply use the highest ratio completed within the allotted session time as the datum. Although this may give orderly results, it is not clear whether these results would be comparable to “true” breaking points, defined by a nonresponse criterion. An alternative procedure when the criterion is not met within a single session is to start the next day’s session with a response requirement that is the same as (or one or two steps lower than) the highest step already reached, rather than starting again at the first step of the full progression.

2.3. Second-Order Schedules

Second-order schedules are one kind of procedure that incorporates the effects of drug-related environmental cues into the drug self-administration procedure (30). These schedules represent a combination of two simpler schedules, such as a fixed-ratio schedule and a fixed-interval schedule (the latter of which specifies the amount of time that must pass before the reinforcer becomes available). Under such a schedule, completing the requirements of the fixed-ratio schedule produces a brief stimulus presentation (e.g., a colored light for 2 s). Once the amount of time specified by the fixed-interval schedule elapses, the drug is given along with the next brief stimulus presentation. For example, Katz and Goldberg (31) used a second-order schedule of cocaine self-administration with squirrel monkeys. The brief stimulus was presented on a fixed-ratio 10 schedule (i.e., every tenth response produced the stimulus), and the first stimulus presented after 5 min was accompanied by an injection of cocaine. For comparison, a simple fixed-interval schedule was also studied, in which cocaine was delivered for the first response that occurred after 5 min, and the stimulus was only presented during the injection. As is typical of this kind of schedule, the second-order schedule generated much higher rates of responding than the simple fixed-interval schedule.

Responding occurs at higher rates under second-order schedules because the brief stimulus comes to have reinforcing effects of its own. These reinforcing effects result from classical conditioning (32), in which the stimulus associated with the effects of cocaine becomes capable of eliciting responses similar to those elicited by cocaine itself. The conditioned-reinforcing effects of the drug-associated stimulus can be seen most clearly when delivery of the drug is discontinued; even though the response produces only the stimulus, responding can be maintained at high levels for many sessions. These conditioned-reinforcing effects of the brief stimulus model the effects of cues that influence human behavior in the drug-abuse environment. For example, in order to experience the effects of cocaine, a person must come into contact with a series of specific cues: money, the drug supplier, the
2.4. Conditioned Reinforcement

packaging and physical properties of the drug, the place where the drug will be used, the injection paraphernalia, the sensation of the needle puncturing the skin. Each of these cues acts as a conditioned reinforcer that maintains part of the sequence of behavior.

Second-order schedules are valuable because they incorporate the effects of drug-related cues into an elegant animal model that begins to approach the complexity of drug abuse in the natural environment. However, it can also be valuable to have a simpler model of conditioned reinforcement. This can be accomplished with procedures in which a stimulus is first associated with the effects of a drug, then used to reinforce a novel response. During the conditioning phase, the drug can either be delivered automatically by the experimenter or self-administered by the animal (33). For example, Di Ciano and Everitt (34) trained rats to self-administer cocaine by poking their nose into a small hole in the wall of the training apparatus. Each cocaine injection was paired with a 20-s presentation of a cue light. Then, during a test session, the rat was presented for the first time with two levers, one of which produced the cue light for 1 s for every third response, and one of which had no effect. The rats responded more on the lever that produced the light, indicating that this drug-paired stimulus had become a conditioned reinforcer capable of establishing a new response.

Even when conditioned reinforcement or environmental cues are not the focus of a drug self-administration study, it is common practice to present a visual or auditory stimulus each time the drug is delivered. And, even when such stimuli are not intentionally provided by the experimenter, it is still likely that there are injection-related cues, such as the sound of the motorized syringe pump and the feeling of room-temperature fluid being injected. Whether incidental or intentionally programmed by the experimenter, these injection-related cues can have an important influence on drug self-administration. Such cues provide immediate feedback that the drug is being delivered, bridging the delay between the response and the onset of the drug’s effects, and thereby making the drug more effective at reinforcing the response. This situation is comparable to the standard procedure for studying food reinforcement, in which a stimulus such as a feeder click is immediately provided when the response occurs, but there is a delay between the pressing of the lever and the food pellet being taken into the mouth. Providing a drug-paired stimulus also makes the laboratory model more comparable to the natural drug-abuse environment, where ingestion of a drug is almost always accompanied by some kind of cue. Interestingly, one effect that stimulant drugs can have is to enhance the effectiveness of conditioned reinforcers. For example, amphetamine
and other stimulants can increase the conditioned-reinforcing effects of stimuli associated with food or water (35).

The conditioned-reinforcing effects of stimuli associated with drug delivery can induce relapse to drug use in humans, and this phenomenon can be modeled in laboratory animals with cue-induced reinstatement procedures (e.g., see (36); see Sect. 2.7, and Chap. 17). It should also be noted that conditioned reinforcement provides the basis for place-conditioning procedures (see Chap. 10), an animal model of drug reward in which the drug’s effects are associated with the features of an environmental context.

2.5. Multiple Schedules

As described above, drug-related cues act as conditioned reinforcers to establish and maintain the sequences of behavior that ultimately lead to experiencing the effects of the drug. An equally important role of environmental cues is to guide behavior by indicating what response is required at each step of the sequence. Cues that provide this guiding effect – by signaling when a specific response can produce a specific reinforcer or conditioned reinforcer – are known as discriminative stimuli. Discriminative stimuli can be incorporated into drug self-administration procedures by using a multiple schedule in which the experimenter presents tones or lights to signal when the drug is available. Under these conditions, the animal’s response will readily come under control of the discriminative stimulus, increasing in frequency when the stimulus is present and decreasing when it is absent (14, 37). In both the laboratory and the human drug-abuse environment, these cues exert considerable power over drug-related behavior, determining when and where specific responses will occur. For example, like conditioned reinforcers (which are produced by the animal’s response), discriminative stimuli (which are presented automatically by the experimenter) can reinstate drug seeking in an animal model of cue-induced relapse (e.g., (38)).

Although drug-related cues are often studied in the laboratory by focusing on the effects of a single cue, the natural environment is composed of a vast number of cues that can be encountered in various configurations. Multiple schedules can be used to study how the individual cues that make up these configurations interact to influence drug self-administration. For example, a tone and a light can be presented separately to signal the availability of cocaine during training, then these cues can be presented together for the first time during a test session. The combined power of these cues can cause rats to double their intake of cocaine (37). In contrast, if a tone signals when cocaine is available and a light signals when cocaine is not available during training, the light acquires inhibitory properties that can substantially decrease the amount of responding controlled by the tone during a subsequent test in which the cues are combined (39). Thus, individual
Stimulant Self-Administration

Cues can increase or decrease drug seeking depending on their relationship to the drug, and when multiple cues are encountered they interact to influence behavior. The effects of combined cues depend on both the discriminative control exerted by each cue (i.e., whether it occasions a habitual increase or decrease in responding) and the conditioned-incentive effects of the cue (i.e., whether it is associated with an increase or decrease in the rate of reinforcement; see (40)).

Another way that multiple schedules are used is to evaluate whether a potentially therapeutic treatment can selectively decrease drug self-administration. For this purpose, a multiple schedule is used in which responding produces food pellets in the presence of one stimulus and drug injections in the presence of another stimulus (e.g., (41)). The goal is to discover treatments that decrease drug-reinforced responding while leaving food-reinforced responding intact. If a treatment decreases both food and drug responding, it might be producing general sedative or motor-depressant effects rather than altering the reinforcing effects of the drug. Or, it might be blocking the reinforcing effects of both the drug and natural reinforcers, an effect that could make it less desirable as a treatment.

2.6. Chained Schedules

Chained schedules are related to second-order and multiple schedules in that (1) they are complex schedules composed of simpler components and (2) they incorporate the effects of environmental cues. The difference is that in chained schedules, the responding in one component of the schedule has the effect of advancing the schedule to the next component, with the drug only received in the final component. For example, Olmstead et al. (42) used a chained schedule of cocaine self-administration in which rats’ “drug-seeking” and “drug-taking” responses occurred on separate, retractable levers. At the beginning of a reinforcement cycle, the seeking lever was inserted and a light was presented to indicate that responses on that lever could provide access to the taking lever. Once the rat had responded on the seeking lever for about 30 s, the next response on the seeking lever caused the taking lever to be inserted. The first response on the taking lever produced an injection of cocaine, and the lights were turned off to signal a timeout period. In this schedule, the retractable levers not only provided a means of responding, but served as cues. That is, insertion of either lever functioned as a discriminative stimulus to respond on that lever, and insertion of the taking lever functioned as a conditioned reinforcer for responding on the seeking lever.

The main advantage of this seeking–taking chained schedule is that it isolates drug seeking from drug taking. The ability to distinguish between drug-seeking and drug-taking responses is important because different mechanisms may underlie these
behaviors (e.g., see (43)). This kind of distinction can also be achieved with second-order schedules by either focusing on the behavior that occurs prior to the first injection of the session or by using a procedure in which the drug is only injected at the very end of the session (44).

2.7. Extinction

Extinction refers to discontinuing reinforcement. When a behavior such as lever pressing is no longer reinforced, its frequency will eventually drop to a very low level. However, when extinction is first instituted, the animal’s response rate will often increase for a short time before it decreases; this temporary increase in responding is known as an “extinction burst.” Furthermore, once the response has decreased to a low, stable level, it may increase again (i.e., show spontaneous recovery) if the animal is reexposed to the training apparatus after a hiatus. Thus, even though a response no longer produces the drug and does not occur for extended periods of time, it does not disappear completely.

Extinction is most widely used in addiction research as a phase of training in the reinstatement model of relapse ((45); see Chap. 17). In this procedure, extinction is used to parallel abstinence from drug use. For example, in a typical reinstatement procedure, rats are trained to self-administer cocaine, then an extinction phase is instituted in which drug delivery is discontinued until responding drops to a low level. Finally, a reinstatement test is performed in which the animal is given a treatment and allowed to respond, but the response still does not produce the drug. If the treatment increases the frequency of responding (i.e., responding is reinstated), this is considered to be analogous to a relapse of drug-seeking behavior. Three general kinds of treatment are effective in producing reinstatement in the laboratory: exposure to drugs (either the training drug or a different drug), exposure to stress (usually a series of mild footshocks), or exposure to drug-related cues (discriminative stimuli or response-produced conditioned reinforcers). These treatments correspond to the triggers that are known to induce relapse to drug use in humans.

Extinction can also be used as one part of a complex schedule of reinforcement, such as a multiple schedule that includes signaled periods in which the drug is not available. As mentioned above (see Sect. 2.5), stimuli that signal a period of extinction can produce inhibitory effects when combined with other drug-related stimuli. Extinction can also be used to assess the persistence of drug seeking. For example, there is evidence that addiction-like states in animals may cause resistance to extinction in heroin-trained rats (46) but not cocaine-trained rats (28, 47, 48). It has long been known that a history of intermittent reinforcement (i.e., training with schedules other than continuous reinforcement) makes behavior resistant to extinction, and that certain cues and contexts can maintain responding during extinction.
Understanding the factors that contribute to the persistence of behavior in the face of changes such as extinction could provide new avenues for the prevention and treatment of addiction (49).

2.8. Punishment

Continuation of drug use despite adverse consequences is a primary symptom of drug addiction. Such consequences can be modeled in the laboratory by using punishment procedures. Punishment occurs when a response produces something aversive that decreases the likelihood of the response occurring in the future. For example, if a brief footshock is delivered whenever the rat presses the lever in a drug self-administration procedure, the rate of self-administration will usually decrease. However, resistance to punishment, like resistance to extinction and resistance to conditioned suppression (see Sect. 2.9), may reveal increased compulsivity (see Chap. 13) or addiction-like increases in the motivation to receive the drug (48, 50).

Punishment can occur in the human drug-abuse environment in several different ways. Often, it is imposed by other people (e.g., employers, law enforcement) to decrease drug use. But, there can also be an inherently aversive, punishing component to the effects of the abused drug itself. For example, self-administered cocaine can produce both reinforcing and punishing effects in the same animal (51). Unfortunately, both the punitive measures meted out by society and the inherently aversive effects of abused drugs tend to be delayed relative to the rewarding effects, and this delay reduces their ability to decrease drug use. Nevertheless, adverse consequences can be one factor that limits drug use or promotes abstinence in addicted individuals.

It is worth noting that a number of abused drugs (e.g., benzodiazepines, barbiturates, ethanol) have rewarding effects but can also reverse the effects of punishment. The anti-punishment and anti-anxiety effects of these drugs might promote their coadministration with stimulants (52). For example, drugs such as diazepam and buspirone may counteract the inherent aversive effects of self-administered cocaine (53). Furthermore, anti-punishment drugs might directly induce relapse to drug use when abstinence has been achieved through punishment (54).

2.9. Conditioned Suppression

Conditioned suppression is a phenomenon in which ongoing behavior is disrupted by a conditioned stimulus. It is used in two ways in addiction research. First, like punishment, it can be used to assess the persistence of self-administration behavior; the main difference is that the aversive event is produced by the response in punishment procedures, but the aversive event occurs regardless of the animal’s behavior in conditioned suppression. For example, rats that are self-administering cocaine can be periodically presented with a visual stimulus signaling that an unavoidable footshock will be delivered. Responding will normally decrease
during presentation of the conditioned stimulus, but resistance to this suppression may be an indicator of addiction (55).

Second, conditioned-suppression procedures can be used as a model of conditioned drug effects (see (56)). For example, rats that are responding on a schedule of food reinforcement can be presented with a visual stimulus signaling that an intravenous injection of cocaine will be automatically delivered. The cocaine-associated stimulus will disrupt responding (57). This suppression induced by cocaine-associated stimuli in rats may be analogous to the cue-induced effects that humans describe as drug craving. Since conditioned suppression can be produced by either hedonically negative events (e.g., footshock) or hedonically positive events (e.g., delivery of food; (58)), the suppression induced by cocaine-paired cues could be due to either reward-related or aversive effects.

Reinforcement is said to occur when a response has an effect on the environment that makes the response more likely to be repeated in the future. The reinforcement discussed in the chapter thus far is positive reinforcement, which occurs when a response has the effect of producing something, like a drug injection or food pellet. In contrast, negative reinforcement occurs when the response becomes more likely because it eliminates something aversive. Some drugs can produce negative reinforcement by providing relief from pain, stress, or anxiety. Since withdrawal from chronically administered drugs of abuse is usually unpleasant, avoiding or escaping from this state can be negatively reinforcing. For example, morphine-dependent monkeys will press a lever that prevents or terminates injections of opioid antagonists that precipitate withdrawal symptoms (59). Although the symptoms produced by withdrawal from cocaine and other stimulants are not as severe as those produced by opioid withdrawal, stimulant withdrawal can produce depression-like effects ((60, 61); see Chap. 1). Avoiding or escaping from these unpleasant states might contribute to the persistence of drug use ((62); see Chap. 16).

However, there are several reasons to believe that addiction stems primarily from the positive reinforcement produced by drugs, rather than the negative reinforcement produced by avoidance of withdrawal symptoms or other unpleasant states (63). Neither precipitated withdrawal from heroin nor presentation of stimuli associated with withdrawal appear to motivate heroin seeking under a seeking–taking chained schedule in a way that would be consistent with negative reinforcement (64). Medicines that provide relief from unpleasant conditions such as pain but do not activate the reward system are not addictive, and many drugs that produce unpleasant effects during withdrawal (e.g., antidepressants, antihistamines; (65)) are not addictive. Perhaps most
importantly, escape from withdrawal symptoms cannot account for relapse to drug use in individuals who have been abstinent for a long period of time. This suggests that treatments that alleviate withdrawal symptoms might assist in achieving abstinence, but by themselves are unlikely to prevent relapse.

3. Assessing Reinforcing Effects

The assumption that the reinforcing effects of drugs underlie their potential for abuse and addiction is central to the drug self-administration model of human drug abuse. It is worth noting that these reinforcing effects are not an immutable property of the drug, but depend on a number of factors such as the animal’s reinforcement history, drug history, current state, access to alternative sources of reinforcement, and genetic makeup (see Chap. 11). As a result, being able to assess the reinforcing effects of a drug under various conditions is critical to conducting drug self-administration research. The process of assessing reinforcing effects essentially involves making comparisons. Does any dose of drug X have a reinforcing effect compared to a saline solution? How do various doses of drug X compare to each other? How do the reinforcing effects of drug X over a range of doses compare to those of drug Y? Does a potentially therapeutic treatment change these effects? Does inactivating a certain brain area change these effects?

3.1. Control Procedures

The most basic of these questions is whether a certain dose of a drug is having a reinforcing effect. For example, a rat may repeatedly press a lever that produces intravenous injections of a drug, but this in itself does not demonstrate that the drug’s effects are reinforcing. By definition, reinforcement is evident when the response that produces the drug becomes more likely to be repeated. This increase in likelihood must be measured relative to some control condition. A commonly used control procedure is to provide the rat with a second lever (an “inactive” lever) that does not produce the drug. If the rat presses the active lever more than the inactive lever, this indicates that the drug is having a reinforcing effect. This two-lever control procedure is an efficient and valid way to verify that the injections are reinforcing.

However, it should be noted that responding on the inactive lever in this two-lever control procedure cannot be considered a truly neutral, nonreinforced behavior. There can be generalization (66) of responding from the reinforced lever to the inactive lever due to their physical similarity. Furthermore, sometimes by chance, the drug may be delivered when the rat responds on the active lever soon after responding on the inactive lever, which can lead to “superstitious” responding on the inactive lever (67).
As a result, responding on the inactive lever should not be considered a measure of general locomotor activity independent of the reinforced responding that occurs on the active lever.

An informative but less commonly used control procedure is to have additional, independent groups of rats that have access to a lever but never receive the drug, or that only receive the drug passively, regardless of whether they press the lever. Even if the experimenter never programs anything to occur when the lever is pressed, rats will occasionally press it incidentally as they explore their surroundings. If the experimenter simply programs the lever to turn stimuli such as lights on and off, these stimuli changes can have an inherently reinforcing effect that maintains a certain level of responding. Importantly, passively received drugs can sometimes enhance the inherently reinforcing effects of these stimulus changes, causing substantial rates of responding ([68]; see Chap. 4). Thus, depending on how they are implemented, independent-group control procedures can provide a more interpretable test for reinforcement than a two-lever procedure, and they can also provide unique insight into how drugs affect behavior.

The most important control procedure for confirming that a drug is having a reinforcing effect is to discontinue drug delivery and use the drug’s vehicle as a placebo. If the response is maintained by the reinforcing effects of the drug, responding should cease or at least decrease due to extinction when all conditions are kept the same except that the drug is no longer delivered. This demonstration of reinforcement is even clearer if the drug and extinction conditions are then repeated and the behavior increases and decreases accordingly. It is important to point out that, typically, responding only decreases to low levels after a number of extinction sessions. However, if animals are repeatedly tested with drug and extinction conditions, the extinction-induced decreases in responding will occur more rapidly. This kind of training can be valuable when the animals will be used to test the effects of treatments expected to block the reinforcing effects of the drug; animals that have learned to abruptly stop responding when the injections are not having a reinforcing effect may provide a more sensitive and valid test for blockade.

3.2. Dose–Effect Curves

The procedure of comparing responding under drug and placebo conditions can (and usually should) be extended to comparing several different doses of the drug within each animal. Dose-dependence is an expected characteristic of any pharmacological effect. In the case of drug self-administration, demonstrating dose-dependence helps confirm that the drug is having a reinforcing effect and allows an accurate description of how a treatment changes the drug’s effects on behavior (see Sect. 4.1). When comparing doses, ideally each dose should be studied for several sessions, until the rate of responding stabilizes. The order in
which the doses are tested should be counterbalanced across animals. When each dose has been tested, the data are used to construct a *dose–effect curve* for each animal, showing the response rate (or some other measure) as a function of dose. These individual curves can be averaged together into a group curve to facilitate presentation of the results. But, it only makes sense to do this if the individual curves are generally consistent with each other.

Dose–effect curves describing response rates or injection rates under drug self-administration schedules typically exhibit an inverted-U shape. The peak of the curve occurs at a dose that is high enough to be reinforcing, but not so high that it produces long post-injection pauses. Along the *descending limb* of the curve (i.e., at doses higher than the peak), the rate of responding decreases as the dose increases, because higher doses produce longer post-injection pauses. Along the *ascending limb* of the curve (i.e., at doses lower than the peak), less responding is maintained than at the peak dose, probably because doses on the ascending limb are only weakly reinforcing. Response rates on the ascending limb often represent the averaging of alternating periods of rapid responding and periods of nonresponding, rather than a steady pattern of moderate responding. In many studies an ascending limb is not obtained, and responding at doses lower than the peak dose is comparable to responding under placebo conditions. Although the determinants of whether an ascending limb is obtained have not been studied extensively, likely factors include the schedule of reinforcement, the pharmacokinetic properties of the self-administered drug (i.e., how quickly and for how long it acts), and the animal’s training history.

**3.3. Reinforcing Efficacy**

Beyond the question of whether a drug is functioning as a reinforcer, there is the question of *reinforcing efficacy*, or how effective the drug is at maintaining the response. Reinforcing efficacy is measured by comparing the effects of different doses, different drugs, or even drug and nondrug reinforcers. However, these comparisons cannot be performed by simply measuring the response rate maintained by a reinforcer; response rates are influenced by too many factors. So, three specialized procedures have been developed for comparing reinforcing effects: progressive-ratio schedules, choice schedules, and behavioral economics analyses.

As described earlier (see *Progressive ratio*, above), breaking points under progressive-ratio schedules provide a direct assessment of how effectively a reinforcer maintains a response. Typically, the higher the dose, the higher the breaking point. However, in some studies there is a peak in the dose–effect curve above which the breaking point starts to decrease, possibly indicating that aversive side effects are beginning to appear, or perhaps that the
nonresponse criterion used to determine the breaking point is too short. An advantage of progressive-ratio schedules is that the breaking-point measure is relatively independent of the animal’s response rate. This independence is important because most drugs of abuse have behavioral side effects that can alter ongoing responding even when the drug is passively received. Depending on the drug, dose, schedule of reinforcement, and the animal’s history, such effects might involve either an increase or a decrease in response rate, and the size and direction of this effect may have nothing to do with the drug’s effectiveness as a reinforcer.

Choice procedures provide another means of comparing the efficacy of various reinforcers. Like progressive-ratio procedures, the measure of reinforcing efficacy obtained with choice procedures is independent of response rate. Choice procedures typically involve providing the animal with two levers: one lever produces a drug, and – depending on the study – the other lever produces the same drug at a different dose, produces a different drug, or produces a nondrug reinforcer such as food. If one lever is consistently chosen over the other, the reinforcer associated with that lever presumably has a higher reinforcing efficacy. However, it should be noted that the availability of two different reinforcers in the same session can sometimes alter their efficacies, such that the results obtained with the choice procedure do not agree with those obtained with a progressive-ratio or behavioral-economics procedure in which the reinforcers are studied separately. As with other self-administration procedures, it is important to assess choice over a range of doses. In addition, control procedures should be used to ensure that an apparent preference for a specific dose is not just due to a side bias (e.g., the animal habitually choosing the left lever); this usually entails switching the outcomes associated with the two levers and verifying that the behavior shifts appropriately.

A behavioral-economics approach to studying drug self-administration combines principles of psychology and microeconomics (69). Drugs are viewed as commodities, and the animal’s response output is viewed as the allocation of a resource. Typically, this kind of research involves using fixed-ratio schedules of drug self-administration and comparing various combinations of response requirements and doses. The response requirement and dose are converted to a unit price. For example, under a FR 10 schedule in which each injection contains 1 mg of cocaine, the unit price of cocaine would be 10 responses per mg; the same unit price (10 responses per mg) could be studied by giving 0.1 mg of cocaine in each injection under a FR 1 schedule. A demand curve is generated, depicting the amount of drug consumed as a function of unit price. These curves usually descend from left to right, with consumption decreasing as the price increases. The steepness of this curve indicates the elasticity of demand, how sensitive
consumption is to changes in price. If consumption drops off quickly as price is increased, the demand is elastic. If consumption remains stable even when the price is increased, the demand is inelastic, which indicates that the drug is highly valued and treated as a necessity.

As a general approach to studying the allocation of behavior, a behavioral-economics analysis can be applied to many situations, including progressive-ratio and choice procedures. The decreased consumption seen under high costs in a demand-curve analysis is presumably related to the cessation of responding that defines the breaking point under a progressive-ratio schedule. With regard to choice schedules, the behavioral-economics approach provides an established theoretical framework for analyzing interactions between different commodities. Such interactions are important to consider, since the availability of one commodity may decrease or increase the demand for another. For example, food and water have a complementary relationship: consuming food makes water more reinforcing. Certain commodities can also “substitute” for each other, suggesting that they satisfy the same demand. For example, when both cocaine and the short-acting opioid remifentanil were made available under a choice schedule, and the price of one drug was manipulated while holding the price of the other drug constant, monkeys increased their consumption of the fixed-price drug when the cost of the variable-price drug went up, indicating that these drugs substitute for each other as commodities (70). In contrast, in an experiment where monkeys were allowed to choose between responses that delivered ethanol or water to drinking spouts, when the price of both fluids was increased the monkeys maintained their ethanol intake by increasing their responding but did not maintain their water intake; this indicates that the demand for ethanol was less elastic than the demand for water (71).

4. Assessing Treatment Effects

4.1. Interpreting Shifts in the Dose–Effect Curve

Studying a range of doses is essential for evaluating whether a drug is having reinforcing effects. It is equally essential when evaluating whether an experimental treatment is altering the drug’s effects. For example, measuring the effects of a treatment on cocaine self-administration under a single dose of cocaine would not provide a complete picture. If the cocaine dose is near the peak of the dose–effect curve, rates of self-administration might be decreased equally by treatments that increase the effects of cocaine (i.e., make it function like a dose on the ascending limb) or decrease the effects of cocaine (making it function like a dose on the descending limb). Therefore, the effects of treatments that
The shifts in the dose–effect curve alter self-administration responding should be analyzed as *shifts in the dose–effect curve*.

If a treatment shifts the dose–effect curve to the left or right but the shape is maintained, this indicates that sensitivity to the drug has changed. If a treatment causes a rightward shift, it decreases sensitivity to the self-administered drug; this kind of effect is typically obtained when a treatment blocks the reinforcing effect of the drug, but the blockade can be overcome by increasing drug intake. A leftward shift indicates that the treatment increases sensitivity to the drug, potentiating its effects. If the curve shifts over time even when no treatments are given, leftward and rightward shifts indicate the development of sensitization or tolerance, respectively, to the self-administered drug.

Upward and downward shifts of the dose–effect curve generally indicate that a treatment alters the efficacy of the drug at producing the effect being measured. A treatment that shifts the self-administration curve upward increases the maximum rate of responding that can be maintained by the self-administered drug. A downward shift or flattening of the curve often occurs when the treatment blocks the reinforcing effects of the drug in a way that cannot be overcome by increasing drug intake. A downward shift may also occur if the treatment does not alter the reinforcing effects of the drug, but somehow interferes with performance of the self-administration response; to evaluate this possibility, separate experiments can be conducted to measure the drug’s effects on spontaneous locomotor activity or food-reinforced responding. It should be noted that for most schedules other than progressive ratio, the drug’s efficacy in maintaining high rates of responding does not necessarily correspond to its reinforcing efficacy. For example, an upward shift of the dose–effect curve might result from tolerance to response-suppressant side effects of the drug.

Detecting shifts in a dose–effect curve usually requires many sessions of testing. For example, the following would be a thorough approach to testing the effects of a treatment on drug self-administration. The first step is to study several doses of the self-administered drug to establish a preliminary dose–effect curve. Each dose is made available for several sessions until a stable performance develops. This preliminary phase serves several purposes. It provides the animals with experience self-administering the drug under a range of doses, and it allows the experimenter to verify that the range of doses is sufficient to observe the typical inverted-U dose–effect curve. Once this preliminary curve has been established, two more curves are determined by studying each dose of the drug with and without the treatment in each subject. These dose-by-treatment combinations are studied in a mixed order, counterbalanced across subjects. The treatment and no-treatment curves are determined during the same phase of
training – rather than simply comparing a treatment curve to the preliminary curve obtained earlier – to prevent extraneous variables such as sensitization and tolerance from confounding the results. As during determination of the preliminary curve, each condition is studied for several sessions until behavior becomes stable; this allows an assessment of whether a treatment effect is consistent from day to day, takes time to fully develop, or disappears after repeated exposures.

The thorough approach described above is designed to produce the most valid and reliable results. But, it is quite time consuming, and it becomes even more time consuming when expanded to study more than one level of the treatment or to study combinations of treatments. Consequently, some alternative procedures have been developed to streamline the process. One way to quickly obtain dose–effect curves is by using a multi-dose schedule, in which the session is divided into several periods, with a different dose of the drug made available during each period (e.g., (72)). For example, a multiple schedule can be used in which there are five 30-min periods in each session, with a different stimulus presented and a different dose of cocaine made available during each period (e.g., see (73)). Once the animals have been trained with this schedule, a dose–effect curve can be obtained within each session.

Within-session dose–effect curves can also be produced with a variable-dose schedule (74). This procedure takes advantage of the fact that the duration of post-injection pausing is dose-dependent (see Sect. 2.1). In a variable-dose schedule, the dose is varied unpredictably throughout the session, with no signal to indicate which dose will be delivered by the next response. When the post-injection pauses from the session are plotted as a function of dose, the pauses are seen to be longer at higher doses. Like continuous reinforcement, variable-dose schedules are useful for studying changes in drug intake but are not appropriate for measuring reinforcing efficacy. The dose–effect curves obtained with continuous-reinforcement (when the dose is fixed within sessions but varied across sessions) and variable-dose schedules are typically congruous, except at low, nonreinforcing doses. At these doses, responding is infrequent and pauses are extremely long under continuous reinforcement. In contrast, a nonreinforcing dose will produce very short post-injection pauses under a variable-dose schedule because the animal will respond quickly until a higher dose is received.

Cumulative dosing is a within-session strategy that involves studying several doses of a treatment drug instead of several dose of the self-administered drug (e.g., see (75)). In this procedure, the session is divided into periods, and some amount of the treatment drug is injected at the beginning of each period. The cumulative amount of treatment drug affecting the animal
during each period is calculated based on the rate at which it is known to be eliminated from the body. Thus, a low dose is in effect early in the session, and the cumulative dose increases with each successive period. Since cumulative dosing usually entails removing the animal from the chamber and giving it an intraperitoneal injection before each period during test sessions, the animals must first be acclimated to being handled and receiving vehicle injections during training sessions. A disadvantage of cumulative dosing procedures is that low doses are tested early in the session and higher doses later in the session, a potentially problematic confound.

With all of these streamlined procedures, the researcher must weigh the advantages of rapid testing against disadvantages such as the lack of complete counterbalancing and the small amount of exposure to each condition. But, it should be noted that saving time is not the only potential advantage of rapid testing. Streamlined techniques may be less open to interference from extraneous variables such as gradual drifts in the baseline rate of self-administration. Or, the focus of the study may be on changes in the effectiveness of a treatment over time, making it advantageous to obtain a “snapshot” of the dose–effect curve during each daily session.

Animal models of drug abuse are easily integrated with most of the procedures currently used in neuroscience. This multidisciplinary approach has significantly advanced our understanding of the brain mechanisms that underlie reward and addiction. Unfortunately, the very ease and success of this integration can lead to the impression that drug self-administration and other behavioral methods are merely tools that serve other areas of research. But, behavior is also the clinical endpoint, and the value of any treatment for addiction will depend on how it alters what people do. Environmental determinants such as schedules of reinforcement and exposure to drug-related cues are critical to the etiology and progression of addiction, and manipulating these environmental variables can have robust therapeutic effects. For example, one of the most effective clinical means of maintaining abstinence in addicted individuals is through contingency management, a treatment strategy in which nondrug reinforcers are used to reinforce behavior incompatible with drug use (76). Thus, in developing clinical interventions, conducting laboratory research with animal models, and generally working toward a comprehensive theoretical account of addiction, it is important to appreciate that addiction is a behavioral phenomenon that involves a complex interaction between the environment, drugs, and the brain.
Acknowledgements

This chapter was written with support from the Intramural Research Program of the NIH, National Institute on Drug Abuse. Thanks to Steven R. Goldberg, Charles W. Schindler, and Gianluigi Tanda for constructive comments on the manuscript.

References

a function of dosage per injection in the rhesus monkey. Psychopharmacologia 22:271–281


Chapter 3

Opiate Self-Administration

Francesco Leri

Abstract

This chapter discusses some of the motivational drives behind opiate addiction discovered over approximately 80 years of studies of opiate self-administration in animals. The focus of the discussion is on regulation of opiate intake by physiological dependence, pain, and learned habits. Drug intake by animals is fairly well regulated by satiety levels. Under appropriate experimental conditions, a satiety level can be altered by physiological dependence or pain. Furthermore, during self-administration, there are several sources of learning that will modulate drug intake. It is concluded that these drives and learning dynamically interact with each other to influence the direction and the strength of opiate habits.

Keywords: Opiate, Opioid, Morphine, Heroin, Dependence, Withdrawal, Satiety, Pain, Conditioning, Relapse

1. Introduction

Last November, I took a walk in Downtown Eastside Vancouver, an area where many disadvantaged individuals converge attracted by low-cost temporary housing and tolerance for open drug use. I was interested in getting a sense of the local heroin problem because I study the motivational properties of opiates using laboratory rats. A in behavioral pharmacology, however, did not prepare me for what I was about to see. While counting used syringe caps and empty vials of saline tossed over the sidewalks, I tried not to stare at people injecting or smoking drugs in abandoned storefronts. Most of them were lying or sitting on the floor, covered by bags of all colors to shield themselves from the incessant rain and the eyes of society. One of these people particularly struck me; she must have been 20–30 years old, but her face displayed the scars of a much longer life. She was on her knees, searching for something lost under a pile of wet clothes, holding a syringe between her teeth. The syringe was half-full of a brownish–reddish fluid,
most probably heroin mixed with blood. Her searching behavior was intense, but sluggish and confused. And her friend could not even help; he was trying to stand against the gravitational force of acute intoxication. His face was scarred as well, but by large red wounds and sores. When I got back to the hotel, besides experiencing the emotional burden of witnessing human suffering, I asked myself a series of questions: why are these people living in such terrible conditions? Is it possible to answer this question by giving heroin to rats? What have we learned about opiate addiction from studying the effects of opiates (i.e., natural alkaloids, such as morphine, that are derived from the opium plant) and other opioid drugs (opiate-like synthetic and semisynthetic compounds, such as heroin and methadone) in animals?

It is obvious that rats, mice, and nonhuman primates exposed to opiate or opioid drugs that preferentially activate mu-opioid receptors in the central nervous system, will never “be like” these people. After all, besides significant biological differences, there are social, economic, and medical factors at the core of human opiate addiction that cannot be modeled in other species. This, however, does not trivialize the value of our studies in animals and the importance of our research findings. On the contrary, it suggests that one strategy to explore the complexities of human opiate addiction is the systematic investigation of its specific behavioral and pharmacological features using simpler biological systems. Furthermore, as suggested by Charles Schuster: “The investigator using infra-human organisms is less likely to involve untestable mentalistic constructs as the factors generating the self-administration of drugs” (1).

The goal of this chapter is to discuss what we have learned about the motivational forces behind opiate addiction in approximately 80 years of studies of opiate self-administration in animals. The theoretical framework adopted for this discussion is based on Hull’s theory of goal-directed behavior (2), which suggests that the strength of a habit (i.e., expression of a learned response) results from an interaction between drives (i.e., sources of “energy” that activate behavior) and previously acquired habits. This chapter will first review evidence that opiate self-administration in experimental animals is goal-directed, and will then present the results of studies suggesting that drug intake is sensitive to manipulation of drives and acquired habits. The drives of interest are physiological dependence and pain. The role of learned habits will be exemplified by an analysis of relapse. Because this chapter provides only few details about methodologies employed in the studies reviewed, the interested reader is encouraged to refer to the original reports for additional information.
2. Some Methodological Considerations

It has been clear for some time that a variety of animal species will voluntarily self-administer opioids and mu-opioid agonists via various routes (1). When the delivery of a drug is the consequence of a particular behavior, and the behavior changes according to the schedule of drug delivery, then the drug is considered to serve as a reinforcing stimulus (3). That opioids could reinforce arbitrarily learned responses was presaged by Shirley Spragg, who showed that physically dependent chimpanzees would gradually learn to select a box concealing a syringe filled with morphine (4). Perhaps, the first demonstration of operant self-administration was by Headlee, Coppock, and Nichols, who reported that morphine/cocaine-pretreated rats would learn to make head movements in order to receive IP injections of morphine and codeine (5).

An initial issue of contention was whether animals required some type of preexposure to opioids in order to subsequently acquire self-administration behavior. It soon became clear, however, that this was a methodological rather than a pharmacological issue (see below), and a distinction between “inducing” versus “non-inducing” procedures was proposed (3). Inducing procedures are employed in situations where drug-naive animals have to surmount some aversive effect of the drug such as, for example, the bitter taste of morphine in oral self-administration studies. Thus, naive rats do not normally drink solutions of morphine, but they will learn to prefer such solutions over water if they are repeatedly “forced” to drink them in order to relieve thirst (6), or if they are chronically pretreated with morphine prior to oral self-administration training (7). In contrast, non-inducing procedures simply make the drug available; acquisition of self-administration is accomplished with no further behavioral or pharmacological manipulation (3). These procedures are typically employed when opioids are available intravenously to freely moving animals, a method pioneered in the 1960s by Schuster and Thompson and by Daneau and Yanagita in monkeys, and by Weeks in rats (8). Pre-training animals to respond for food, or other nondrug reinforcers, is generally not necessary for the acquisition of intravenous self-administration of opioids. In addition, although this type of induction procedure is likely to accelerate the rate of learning, and can be useful in identifying “nonresponders,” food pre-training involves food restriction, which is a powerful stressor that can alter subsequent responses to opioids (9, 10). Food pre-training is also not appropriate for studies designed to determine whether animals acquire self-administration of new compounds with untested reinforcing efficacy.
3. Regulation of Opiate Intake

The most convincing evidence suggesting that opiate self-administration is goal-directed is the observation that animals regulate drug intake to achieve and maintain some level of satiety. James Weeks (11) may have been the first to report that intravenous opiate self-administration in rats is characterized by prolonged periods of not responding alternating with brief periods of high rates of responding terminated by an injection. From this, he concluded that morphine was a reinforcer that produced almost immediate satiation. It is now generally accepted that animals dynamically regulate drug intake by adjusting operant responding to the available unit dose. That is, at low ratio schedules, the rate of opiate-reinforced behavior is inversely related to drug dosage (12), and the number of infusions increases or decreases with decreases or increases in the unit dose, respectively (13). This is achieved by altering post-infusion pauses in responding: inter-dose intervals increase following increases in doses, and decrease following decreases in doses (14–16). In addition, mu-antagonist pretreatment usually increases opiate intake, while agonist pretreatment decreases it (1). It should be noted, however, that the relationship between dose and responding is also modulated by the schedule of reinforcement. For example, Roberts et al. (17) explored the progressive ratio (PR) schedule, which restricts drug intake by requiring progressively more operant behavior for each successive infusion. At heroin doses ranging between 12.5 and 100 \( \mu g/kg/infusion \), an inverted U-curve was observed. Although the exact shape of the curve is regulated by the rate of ratio progression escalation (18) and by the pharmacological properties of the opiate used in self-administration (19), it is clear that unit dose and intensity of responding for the drug are not always linearly related. Therefore, studies using PR schedules have indicated that it may be misleading to estimate the level of motivation to self-administer opiates from an analysis of self-administration behavior at low ratio schedules.

Additional evidence for regulation of opiate intake by satiety levels comes from studies whereby animals are given long periods of access to drugs. In fact, when given unlimited (i.e., 24 h a day) access to opiates, self-administration typically shows an escalation followed by a stabilization. For example, Gracin et al. (20) found that morphine intake in rats increased over 14 days of unlimited access and then stabilized for the rest of the self-administration period (over 30 days) at a relatively constant amount of daily morphine intake (about 240 mg/kg/day). Bozarth and Wise (21) reported that rats given unlimited access to heroin self-administration (0.1 mg/kg/infusion) showed a gradual increase in 24 h drug intake during the first 2 weeks of testing, but after this time, daily
intake remained constant. Interestingly, Ahmed et al. (22) demonstrated that escalation induced by extending the duration of access to opiates can largely be attributed to increases in opiate intake during the initial period of each daily session, and a recent study by Chen et al. (23) suggested that these changes are associated with the development of dependence. More specifically, rats given unlimited (23 h a day) access to heroin for 35 days stabilized their intake after approximately 14 days of self-administration, and escalation was marked by an increase of heroin intake in the first hour of each session. Importantly, somatic signs of naloxone (1 mg/kg)-precipitated withdrawal were maximal after 14 days of self-administration, suggesting that stabilization of heroin intake occurred when a new “dependence level” of satiety was achieved.

It should be noted, however, that dependence does not necessarily require escalation of intake. In fact, when heroin access per hour is limited and animals still have access to the drug 24 h a day (i.e., discrete-trials procedure), escalation is not observed, but dependence still develops as indicated by loss of body-weight within 24 h following the termination of self-administration (24). Clearly, escalation and dependence are not all-or-none phenomena, and their relationship may be more or less clear depending on the experimental conditions employed.

One possible explanation for why animals self-impose limits on drug intake within- and between-sessions of self-administration may be impaired motor functions rather than attainment of satiety. After all, behavior on the ascending limb of the opiate dose–effect curve is thought to be modulated by reinforcing value, whereas the descending limb is thought to be controlled both by the reinforcing and response-suppressing effects of the self-administered drug (see (25) for review). However, the results of intracranial self-administration studies argue against this possibility. In fact, drug-naïve and untrained rats will readily learn to self-administer morphine and other mu-agonists directly into the ventral tegmental area (26, 27). Importantly, as observed in intravenous studies, rates of operant responding during the first hour of intra-VTA self-administration are significantly higher than that of each subsequent hour of testing. Given that activation of mu-receptors in the VTA cause increases in motor activity (28), not decreases, it is quite likely that changes in within-session response rates are indeed related to the differential requirements for establishing and maintaining satiating drug concentrations in the brain (26).

4. Role of Physiological Dependence and Withdrawal

During the development of inducing and non-inducing protocols, the role of physiological dependence and withdrawal in opiate
self-administration became a subject of critical interest. In turn, the analysis of when and how animals self-administer opiates has been instrumental to the understanding of motivational drives for opiate-seeking and opiate-taking behaviors. The central question of this research has been: is learning to seek opiates dependent on drive reduction (i.e., reduction of a need state) or on some pleasurable euphoric effect? (29). The studies of Spragg in chimpanzees strongly supported the drive-reduction hypothesis (4). In fact, he showed that chimpanzees gradually learned to voluntarily accept morphine injections applied to different parts of their body. Over the period of regular drug injections (twice per day, about 160 injections), animals developed tolerance as well as physiological dependence characterized by a variety of emotional and physiological signs of withdrawal. In rats, chronic injections of morphine (100 mg/kg/day × 48 days) lead to the development of dependence, which manifests itself as the occurrence of signs of abstinence within 6 h from the last injection. These signs, which include decreases in eating, drinking, body weight, locomotion, and increases in defecation, are dose-dependently alleviated by administration of additional morphine (6), and can be precipitated by administration of opioid antagonists such as naloxone or naltrexone (30).

Interestingly, the chimpanzees of Spragg also displayed intense behavioral manifestations of “desire” for morphine when experiencing withdrawal. These included vigorous tugging of the experimenter toward the compound where injections were usually administered, and preference for the hypodermic syringe over food when both hungry and in withdrawal from morphine. However, when hungry and recently injected with morphine, dependent chimpanzees chose food. Furthermore, no interest for the syringe or injection room was noted 2 weeks following abrupt withdrawal from morphine. From this, Spragg concluded that alleviation of withdrawal was the critical drive for opiate-seeking behavior.

In support of the drive-reduction hypothesis, Beach and Hebb (29) reported that rats would learn a preference for a compartment associated with morphine injections. Although induction of morphine dependence prior to training did not seem to alter the development or expression of this preference, after 3 weeks of withdrawal, only animals trained when dependent still showed a preference. Thus, only rats that had presumably experienced drive reduction during training showed a persistent morphine-seeking habit.

The drive-reduction hypothesis of opiate self-administration was further developed and expanded in scope by Abraham Wikler (31–33), who argued that opiates reduce a variety of drives even in opiate-naïve organisms. These drives include pain, hunger, anxiety, and sex. When the drug effect wears off, these drives rebound
Opiate Self-Administration

with increased vigor as a result of homeostatic counter-adaptations. Their return is experienced as tensions, which eventually increase to the point of distress. From this analysis, it was concluded that opiates have greater behavior-enhancing effects if taken when the subject is dependent, since they would relieve the punishment of impeding withdrawal reactions (8, 34). Hence, “escape training” was considered the primary drive of opioid seeking and taking, and this form of learning was considered to be critically dependent on the development of a withdrawal state induced by homeostatic counter-adaptations to the direct effects of the drug.

Working within this framework, John Nichols and colleagues demonstrated sustained opiate-directed behavior in rats that drank morphine solutions while experiencing morphine withdrawal (7). Opiate dependence was considered essential for self-injection learning even in the absence of overt signs of abstinence. James Weeks (11, 35), for example, reported that rats whose physical dependence was significantly reduced by decreasing the dosage of morphine pre-exposure from 40 to 20 mg/kg/h, self-administered less morphine on a continuous schedule of reinforcement. Further, progressive decreases in infusion dose led to an increase in number of infusions taken. But compensation was found to be incomplete; that is, total daily morphine intake ended up decreasing. This was taken as additional evidence supporting the escape-learning hypothesis because self-administration of lower doses may have produced a loss of dependence and thus reduction of morphine “need.” Within similar lines, Wikler (36) reported that rats in acute withdrawal from morphine chose a solution containing 5–10 mg/mL of etonitazene (a potent mu-opioid agonist that is orally consumed by rats without severe water deprivation or pre-training) over water, and drank enough of it to the point of showing no physiological signs of withdrawal. This was in contrast to morphine-tolerant, but not abstinent, rats that drank a solution of etonitazene at volumes not significantly different from water.

Since these early studies, additional evidence accumulated suggesting that, indeed, physiological dependence modulates opiate self-administration. For example, opioid dependence facilitates self-administration of otherwise weak reinforcing drugs such as mixed opioid agonist–antagonists (see (37) for review), and reliably increases intravenous self-administration of fast acting mu-agonists such as remifentanil (38). But, the nature of dependence-induced enhancement of opiate reinforcement has been reconsidered. More specifically, the view that drug taking in dependent animals is maintained by reduction of withdrawal has been challenged by the incentive-motivational view initially proposed by Dalbir Bindra (39), which posits that the withdrawal state enhances the incentive value of the drug and of drug-predictive stimuli. In support of this interpretation, using an experimental
design whereby responding on a “seeking” lever gave rats access
to a “taking” lever, Hutcheson et al. (40) found that rats responded
more vigorously on the seeking lever only after having self-
administered heroin in a state of withdrawal, and only when tested
in a state of withdrawal.

But, the evidence reviewed above does not imply that acquisi-
tion of opiate self-administration will occur only in the presence
of dependence and withdrawal. In fact, Kumar (41) demonstrated
that it was possible to eliminate morphine pretreatment and still
convert an initial rejection of morphine into a marked preference,
as measured by the proportion of morphine solution drunk by
rats when both morphine and water were made available. In addi-
tion, pretreatment and withdrawal were found to have no effect
on the development and expression of the preference (42, 43),
and rats made passively dependent did not begin self-administra-
tion of morphine unless previously trained to drink morphine
solutions when thirsty (44). From this, Kumar concluded that a
morphine-seeking habit can be induced in animals to satisfy a
drive different from relief of withdrawal.

A similar conclusion was drawn from a study of Weeks (45)
who demonstrated that, within a certain dose range, morphine is
self-administered in the absence of dependence, as revealed by
sensitivity to precipitation of withdrawal by opioid antagonists.
This observation was replicated by Dai et al. (46) studying intra-
venous heroin self-administration, and by Amit et al. (47) and
De Vry et al. (48) studying intra-cerebroventricular (ICV) morphine
and heroin self-administration, respectively. Amit et al. demon-
strated that rats, which were neither shaped, food-deprived, nor
pre-dependent on morphine, learned to perform an operant
response for ICV infusions of morphine. At the termination of
self-administration, some rats were injected with naloxone (1 mg/
kg, IP). During the following 30 min, rats did not display signs of
withdrawal (wet-dog shakes, tremors, or hyperactivity). In the
study of De Vry et al., rats were tested for sensitivity to pain (hot-
plate) and withdrawal precipitated by naloxone (10 mg/kg),
immediately after self-administration of different heroin doses.
No differences were found in hotplate latencies between dose
groups, and signs of withdrawal were small in all groups. The
authors concluded that the effects of heroin on self-administra-
tion could be dissociated from its analgesic and physical-depen-
dence-inducing actions.

Hence, opiates and other mu-opioid agonists can serve as
reinforcing stimuli in drug-naïve animals because, at appropriate
doses and administration routes, they will promote acquisition
and maintenance of responses leading to their delivery. But, is this
effect attributable to the induction of an positive affective state
(i.e., reward) or to a reduction of unobservable drives? It may be
too simplistic to attribute acquisition of self-administration solely
to opiate-induced pleasure/reward as not all effects of opiates are pleasurable in humans (49), and rodents can develop both preferences and aversions to stimuli predictive of opiate effects (50, 51). Furthermore, the “onset” of dependence may be quite difficult to identify. That is, even when physical signs of withdrawal are not manifested, animals may be in a state of affective anhedonia (52) characterized by reduced sensitivity of brain reward systems (as measured by increases in intracranial self-stimulation (ICSS) thresholds (53)). In support of this possibility, Kenny et al. (54) demonstrated that increases in heroin self-administration occur in parallel to increases in ICSS thresholds in the absence of somatic signs (tremor, teeth chattering, wet-dog shakes) of withdrawal. Coupled with the observation that physiological tolerance can be observed even after a single administration of opiates (33), it may very well be that opiate self-administration is motivated by reduction of withdrawal, immediately after the first self-administered dose.

Beside dependence and withdrawal, what other drive could influence opiate self-administration? One obvious answer is pain, not only because most animal species seek to avoid it and will learn behaviors to escape it (55), but also because mu-opioid agonists are effective pain-relieving agents (49).

There is evidence that pain can influence opiate self-administration in animals, but whether this drive facilitates or impedes it remains unclear. From a drive-reduction point of view, pain should enhance opiate self-administration because it creates one additional drive to be reduced by the drug. The results of Beck and O’Brien (56) are consistent with this prediction. In fact, drug-naïve and previously untrained rats were allowed to press a lever to receive intravenous infusions of morphine (1.0–2.4 mg/kg, IV) 24 h a day, until morphine intake stabilized (i.e., 100 mg/kg a day for 3 consecutive days). Then, a period of classical conditioning was initiated whereby animals received electrical shocks to one foreleg (conditioned stimulus) just prior to the delivery of the drug. But, conditioning was unsuccessful because most animals developed an abnormal pattern of morphine intake characterized by clusters of several infusions followed by inactivity and eventually death by overdose (56). This result suggests that the acute pain caused by the shock may have elevated the dose required to achieve satiety beyond levels that caused respiratory depression and death. Similarly, Dib et al. (57) reported that nociceptive stimulation (electrical stimulation of the tail) enhanced ICV self-administration of morphine and produced overdose in
some animals. Finally, Francis Colpaert (58, 59) reported that relative oral intake of fentanyl was higher in rats injected with Freund’s adjuvant to induce rheumatoid arthritis.

Others, however, have suggested that pain decreases opiate self-administration. For example, Lyness et al. (60) reported that arthritic rats self-administered less morphine (5 mg/kg, 24 h a day, FR1–FR15) over 35 self-administration sessions than controls. The authors argued that the nature of the reinforcement sought by the two groups was different and that these effects (analgesia vs reward) showed tolerance at different rates. But, there was no attempt to explain why morphine analgesia would substitute, rather than add to, morphine reward. Furthermore, if these drive sources are indeed additive rather than substitutive, then post-infusion drug effects should be larger in animals in pain, and this should decrease overall morphine intake. That is, at low response requirements, decreases in drug intake could reflect increases, not decreases, in drug potency. A similar interpretation can be applied to the findings of Martin et al. (61) who induced allodynia in rats by ligature of spinal nerves L5 and L6. These animals were found to self-administer (FR10) less heroin (9–15 µg/kg/inf), methadone (38–75 µg/kg/inf), morphine (180 µg/kg/inf), fentanyl (0.8–5 µg/kg/inf), and hydromorphone (5–20 µg/kg/inf). Arguably, the effect of pain states on the self-administration of opiates should be reexamined using more demanding schedules of reinforcement such as the PR schedule.

6. Role of Learned Habits

The question of what drives opiate self-administration becomes particularly interesting and clinically relevant when considering the problem of relapse. That is, what can motivate an individual to seek and use a drug after the body has had enough time to adapt to its absence? Wikler (36) argued for a complex interaction between pharmacological factors and learned responses. According to him, vulnerability to relapse in previously dependent individuals results from: (1) long-term persistence of low-grade physiological deviations from normal; (2) classical conditioning of abstinence phenomena to environmental situations frequently associated with acute withdrawal distress; and (3) operant conditioning of opioid-seeking behavior through reduction of acute withdrawal distress by self-administration of such drugs.

One of the first animal models of relapse was described by Thompson and Ostlund (62). Animals were forced to drink a morphine solution (acquisition), then water (extinction), and then morphine again. The interest was in studying factors that
would modulate drug intake when animals had renewed access to the drug following extinction: a period they called “reacquisition.” A very similar approach was adopted by Garcin et al. (20) who used the term “primary dependence” to refer to dependence induced in drug-naïve animals by acquisition of opiate self-administration. This was followed by abstinence, which was characterized by three phases. During the early phase (4–5 days after termination of self-administration) there was a decrease in body weight as well as food and water intake. During the intermediary phase (5 days to 4 weeks), there was a gradual recovery of weight and food/water intake. In the late (or protracted) phase, all parameters were normal. Other researchers described similar phases of morphine abstinence and withdrawal in rats (although sometimes they used a biphasic model; see (20) for review). After all phases of abstinence, animals were allowed to reacquire morphine self-administration, and the term “secondary dependence” was used to refer to “relapse.”

During tests of secondary dependence to morphine in ex-addicted animals, Garcin et al. (20) found that morphine intake was significantly higher than that observed during primary dependence, and that the level of intake was only slightly modulated by the duration of the period of forced withdrawal. These results were then replicated by other investigators who found that ex-addicted rats self-administered higher amounts of opiates either intravenously or orally (46). Increases in opioid intake observed following protracted abstinence were taken to represent a long-lasting persistence of a “need” state. More specifically, long-lasting metabolic alterations (63) were considered to induce a hyper-sensitivity to stressful stimuli and therefore higher intake of substances that would reduce stress such as opiates (64).

Wikler agreed that a potent factor in predisposing to relapse was long-term persistence of homeostatic disturbances. But, he also argued that these disturbances provide a source of reinforcement for operant conditioning of opioid-seeking behavior during relapse (36). More specifically, his group proposed a learning-based hypothesis that explains why post-dependent rats self-administered more drug than naïve rats (65). According to this hypothesis, a subject dependent on opiates regularly experiences periods of mild opiate withdrawal, which are alleviated by further drug intake. Such a training schedule provides the basis for interoceptive conditioning of suppression of opiate abstinence to the independent, but concomitant, internal sensorial effects of the drug. Therefore, internal sensorial effects of opiates acquire the properties of a secondary or conditioned reinforcer, and become effective in reinforcing drug-taking behavior when the subject is re-exposed to the drug after long periods of abstinence (i.e., relapse).
To test this hypothesis, two groups of rats were pre-exposed to morphine either by daily injections, or by continuous intravenous infusions of the same dose (65), and only the former group was expected to undergo interoceptive conditioning. It was found that animals treated by repeated acute injections of 200 mg/kg morphine became “irritable,” and the administration of the drug induced a short period of catalepsy, followed by stereotyped chewing and forepaw movements. However, rats that received daily morphine infusions (200 mg/kg/day) were more active than saline-infused rats, and displayed no irritability. Body weights decreased during morphine exposure, and even more so during withdrawal, but no differences were found between regimens of morphine pre-exposure. Normal patterns of water intake were altered by morphine, and intake decreased during withdrawal equally in the morphine groups. Importantly, when these rats were given relapse tests following withdrawal, the injection group consumed significantly more of an opiate solution (etontizene) than the infusion group (66). Therefore, these results supported the interoceptive conditioning hypothesis, suggesting that interoceptive stimuli associated with the drug injections during dependence acquire conditioned reinforcing properties and served to reinforce self-administration behavior after the dissipation of withdrawal.

Other experimental findings emphasized the role of exteroceptive (environment, lights, tones, taste) conditioned reinforcers in sustaining opiate seeking. Thompson and Ostlund (62) showed that environmental stimuli present during morphine self-administration and extinction directly controlled opiate intake during reacquisition of morphine self-administration. Schuster and Woods (67) trained dependent rhesus monkeys to self-administer morphine in conjunction with the activation of a red light. On alternate days, lever presses produced either no programmed consequences or saline injections accompanied by the light. It was found that response rates were considerably higher on days when saline and the red light were present.

The effects of learning on the expression of opiate habits can be long lasting. Mumford et al. (43) tested choices between morphine and water in morphine-dependent rats. In these animals, naloxone-precipitated withdrawal was conditioned to a particular taste. After 24 days of “voluntary” abstinence induced by lacing the morphine solution with the taste associated with naloxone-precipitated withdrawal, rats were given additional morphine–water tests. It was found that rats preferred the morphine solution as much as before abstinence. Kumar (68) studied endurance of relapse tendencies in morphine-dependent rats who had learned to prefer a morphine solution (which is bitter) over water. After developing a reliable preference for morphine, they were given access to water only for 110 days (forced abstinence). During
Opiate Self-Administration

tests, morphine/water choice was compared in previously trained rats and drug-naïve rats. It was found that choice for morphine was higher in ex-addicted rats than in naïve animals even after almost 3 months of abstinence. An additional interesting finding of this study was that abstinent rats also drank more bitter water (i.e., same taste of morphine), suggesting that the taste of the solution was critical for the expression of the preference. In a follow-up experiment, during the withdrawal period, rats were allowed to drink the bitter solution, and then they were tested for morphine/water choice. It was found that the period of extinction reduced choice for morphine. On the basis of this observation, it was concluded that “extinguishing secondary reinforcement may therefore make an appreciable contribution to the treatment of drug dependence” (6).

Although it is fairly well accepted that conditioning plays an important role in the reemergence of opiate habits, its modulation by dependence and the nature of the conditioned responses are topics that have been the subject of debate. In fact, in contrast to the predictions made by dependence-based models of opioid-seeking behavior, there is substantial experimental evidence suggesting that robust conditioning can be observed even in the absence of physiological dependence. For example, Smith and Davis (69) reported that an audio-visual stimulus paired with the delivery of intravenous infusions of morphine acquired the ability to control operant responding even in morphine-naïve rats. Zhang et al. (70) found a positive relationship between dose of heroin available during self-administration and intensity of operant responding maintained by a context and by a cue associated with heroin infusions. The various dose groups, however, did not develop physical dependence because the number of infusions per day was limited to 25. Similar effects have been reported in self-administration experiments whereby conditioning parameters (71) or schedule of reinforcement (72) were manipulated without inducing overt physical dependence. Finally, using the reinstatement model, it has been found that acute withdrawal does not precipitate heroin seeking, but exposure to heroin itself or to heroin associated cues does (73, 74).

The other controversial topic is the nature of opiate-conditioned responses. In fact, it has been suggested that increases in opiate intake during relapse tests reflect decreases in drug potency caused by conditioned “compensatory” responses (75), rather than enhanced motivation to self-administer the drug. For example, in a number of elegant experiments, Shepard Siegel demonstrated that stimuli predictive of drug availability elicit conditioned responses that counter the unconditioned effects of a drug (76). These stimuli can be exteroceptive or interoceptive, or both. Interoceptive stimuli include the initial sensations associated with the onset of a drug effect (intra-administration cues), as described
by Wikler, as well as interoceptive response-initiating (or response produced) sensations that occur before (or after) the delivery of a self-administered drug infusion. Weise-Kelly and Siegel (77) demonstrated that the presence of these stimuli decrease the direct effect of heroin on ataxia, and reported more pronounced morphine withdrawal in animals that actively self-administered morphine in comparison to yoked animals. Similarly, Hinson et al. (78) reported that rats drank more morphine in an environment paired with morphine injections, but these rats also displayed conditioned tolerance to the analgesic effects of morphine, suggesting that the conditioned environment may have enhanced self-administration by reducing the depressant effect of morphine responding.

In conclusion, the studies reviewed above indicate that there are many sources of learning during opiate self-administration. Stimuli predictive of drug availability, drug delivery, or interoceptive sensations of drug action, can all become powerful drives of opioid seeking either because of drug-like (arousal of incentive motivation) or drug-opposite (compensatory reactions) conditioned responses (79). In addition, the withdrawal state can also be conditioned to exteroceptive and interoceptive cues (80–83). Therefore, similarly to changes in intrinsic motivation to self-administer opiates, repeated drug exposure and development of physiological dependence are likely to modulate the acquisition of learned responses and thus influence the direction and the strength of opiate habits.

7. Implications

There are several implications of these findings in animals to the understanding and treatment of heroin-dependent individuals populating the streets of a modern metropolis such as Vancouver. First, regardless of why they initiated use, a significant portion of their daily behavior is driven by the pharmacological action of the drug (84). From this, it follows that mu-agonists with different pharmacological profiles can alter drug-motivated behaviors in ways that may be beneficial to the health of these individuals. This partially explains why drugs with slow onset and long duration of action such as methadone and buprenorphine are highly effective in reducing harm associated with opiate addiction (85). Second, alternative treatment options should be made available to those who do not benefit from traditional maintenance-based options, and these include prescribed heroin. The observation that animals given unlimited access to heroin self-administration regulate intake and do not overdose leads to the prediction that humans too should be able to regulate intake of medical heroin. So far,
the results of the few clinical trials of prescribed heroin (86–89) have been consistent with this prediction. Finally, it is clear that the treatment of opiate addiction requires intense cognitive interventions aimed at recognizing and eliminating sources of learned responses conducive to drug use, as well as acquisition of alternative harm-reductive habits (90).

References

motivational markers of opiate dependence. Neropsychopharmacology 31:2692–2707
Chapter 4

Nicotine Self-Administration

Robert E. Sorge and Paul B.S. Clarke

Abstract

Intravenous self-administration (IVSA) studies have shown that nicotine can serve as a reinforcer in animals and humans. Brain mechanisms underlying nicotine IVSA, as well as the effects of pharmacological interventions, have also been widely investigated and are summarized. However, the conditions under which nicotine self-administration has been observed do not closely model the way in which nicotine is consumed by cigarette smokers, in two important respects: conventional IVSA procedures typically employ ultra-rapid infusions and high unit doses of nicotine that approximate the content of one or two cigarettes. In contrast, recent evidence shows that rats will also self-administer nicotine in doses equivalent to about two cigarette puffs, delivered into the circulation at a much slower rate that more closely approximates the delivery rate in cigarette smokers. Possibly, adoption of these refinements will help to provide an animal model with greater predictive clinical validity in order to advance the search for more effective smoking cessation pharmacotherapies.

Key words: Nicotine, Tobacco, Intravenous self-administration, Self-administration, Reinforcement, Review, Nicotinic receptors, Animal models

1. Scope

It is widely believed that cigarette smoking stems largely from nicotine addiction. Reinforcing effects of nicotine have been extensively investigated using the nicotine intravenous self-administration (IVSA) procedure in adult rats. After describing methodological aspects of the technique, we highlight its main applications in this species, particularly related to pharmacotherapy development. Finally, we discuss further refinements of the procedure that may provide a closer model of human cigarette smoking. (Please note that we will use the terms “dependence” and “addiction” interchangeably in this chapter.) Important research has also been performed in other species, notably squirrel monkeys (1, 2), rhesus monkeys (3, 4), and in wild type and...
genetically modified mice (5–7). These species fall outside the scope of this review and the reader is referred to recent reviews by others (8, 9).

2. The Problem of Tobacco Addiction

Tobacco dependence is a major preventable cause of death. In developed countries, tobacco smoking is estimated to cause about 30% of all deaths from cancer, and an even greater number of deaths from other diseases (10). Globally, smoking accounts for 4–5 millions deaths each year (10). The primary problem facing smokers who attempt to quit is the high rate of relapse and the poor effectiveness of current pharmacological and non-pharmacological therapies (11). A better understanding of tobacco dependence appears necessary in order to provide more effective pharmacotherapies.

3. Tobacco Dependence versus Nicotine Addiction

Cigarette smoke contains about 4,000 identified chemicals, the great majority of which have never been characterized pharmacologically or behaviorally. Nevertheless, the 1988 US Surgeon General’s Report concluded that “nicotine is the drug in tobacco that causes addiction” (12). This widely held view has provided the rationale for well over 100 published reports of nicotine self-administration. Indeed, nicotine IVSA is currently the dominant animal model related to cigarette smoking. Recently, nicotine’s central role in tobacco dependence has been vigorously questioned (13, 14), and a more nuanced view appears to be emerging (15, 16).

4. Oral Nicotine Self-Administration in the Rat

Most nicotine self-administration studies employ intravenous delivery, but there are also several published reports of oral self-administration. The latter approach offers several advantages: catheterization surgery is avoided, extensive training is not required, 24-h access is convenient, and long-term testing is straightforward. One obvious drawback is that nicotine absorption is slow and potentially variable. Moreover, since nicotine solutions are bitter, rats need to be encouraged to consume nicotine solutions, either by schedule-induced polydipsia (17), sweetening with sucrose (18), or by gradually increasing the
nicotine concentration (19). Results to date are somewhat equivocal. For example, in one study, rats lever-pressed more for oral solutions of nicotine + sucrose than of sucrose alone, yet drank less when consumption was unrestricted. In a further experiment, rats could choose to respond for either aqueous nicotine solutions or water; weaker nicotine solutions were mildly preferred, whereas stronger solutions were avoided (18).

Cigarette smoking is a complex behavior modulated by pharmacological, psychological, social, and economic variables. Which aspects of this behavior, then, does nicotine self-administration model? In general terms, the self-administration procedure has been used to model the acquisition, maintenance, extinction, and reinstatement of nicotine-taking behavior. Self-administration behavior, tested in limited-access sessions, is presumably controlled by the acute positive consequences of drug administration. With more extended daily exposure, withdrawal signs can emerge (20, 21); here, negative reinforcement may play an important role. Importantly, nicotine self-administration behavior tends to be weak unless the drug is paired with sensory cues, and it has been proposed that animals self-administer intravenous nicotine primarily because the drug makes these ancillary cues more reinforcing (see below).

Although compulsion is a hallmark of drug addiction, nicotine self-administration studies to date have provided little if any evidence of compulsive drug seeking (but see (20, 21)). However, most studies of nicotine self-administration are quite possibly not long enough for compulsive behavior to manifest itself. It is worth noting that even with cocaine, compulsive drug-seeking behavior can require several months of repeated exposure and/or testing before motivated drug seeking increases sufficiently to be regarded as compulsive (22, 23).

Self-administration studies have been instrumental in identifying molecular and neuronal mechanisms that are critical to the acute reinforcing effects of nicotine. Nicotinic receptors are widely expressed in both the periphery and CNS, but it is the central action of nicotine that appears important to self-administration (24). Several brain mechanisms have been identified that are critical to nicotine IVSA, as described below. In the search for more effective pharmacotherapies for cigarette smoking, numerous drugs have been tested in animals self-administering intravenous nicotine. However, more testing will be necessary to determine whether the standard nicotine self-administration procedure can usefully predict clinical efficacy (25, 26).
The Surgeon General’s 1988 conclusions relied quite heavily on two published reports of intravenous nicotine self-administration in human subjects (27, 28). However, these studies have been strongly criticized on a number of grounds (13). First, few subjects were tested in any condition; several had a history of drug abuse. Second, few subjects appeared to self-administer more nicotine than saline. Third, several reported observations about nicotine self-administration were not sufficiently supported by data. Fourth, subjects were told that they might receive nicotine, but nevertheless identified the drug as cocaine. This last observation might be related to the rapidity of drug delivery (9 s), or the use of unit doses (approx. 10–40 µg/kg) that far exceed the nicotine yield from single cigarette puffs (approx. 1–2 µg/kg) (29).

Two recent reports provide stronger evidence of IVSA in human smokers (30, 31). In one of these studies (31) subjects were willing to respond 1,600 times for each nicotine infusion and responded very little for saline infusions. However, several subjects were past or present drug users, and it is not clear how far these results would generalize to the average cigarette smoker (31). A further complication in human IVSA studies is that nicotine produces a recognizable cue, and many subjects believe that nicotine is addictive. Therefore, nicotine self-administration, where observed, could potentially be generated by expectation, unless this possibility is explicitly ruled out (13, 32).

Human subjects have also been tested for self-administration of nicotine in the form of nasal spray and polacrilex gum. In several acute studies, Perkins et al. have compared self-administration of nicotine versus placebo nasal spray in a forced choice task; overall, fewer than half of the smokers chose nicotine consistently over saline, even when abstinent from cigarettes (33, 34). However, a longer-term study suggested that reinforcing effects of nicotine nasal spray may take several days to emerge (35). Similarly, most subjects prefer placebo chewing gum to nicotine gum in acute tests (36, 37), whereas nicotine gum is preferred with prolonged access (35).

In summary, a clear demonstration of nicotine self-administration in humans remains elusive (32), although animals will clearly self-administer the drug. Animal models of drug self-administration offer some significant benefits; for example, more rigorous controls are possible, and the problems of past drug history and biases based on expectations are avoided. The following sections deal with self-administration of nicotine in animals and will be focused primarily on one animal – the adult rat.
The IVSA procedure represents the gold standard for studying the motivational aspects of a drug in animals. This procedure possesses significant face validity (38), and it results in patterns of intake that closely match human drug consumption (39–42). In addition, the progressive ratio schedule of reinforcement provides a measure of how motivated the animal is to obtain drug infusions (43). In this procedure, the amount of work required to receive each successive infusion of a drug increases exponentially. At some point, the response requirement becomes too high and the animal ceases to respond for the drug, and thus, the amount of responding required to obtain the last infusion is termed the “breakpoint.” Other drugs can be tested within this procedure to measure any change in the motivation to self-administer a particular drug (see (44) for a review).

The standard nicotine IVSA procedure in the rat is essentially the same as that for other drugs of abuse. Under general anesthesia, the animal is catheterized in the right jugular vein and the catheter is passed subcutaneously to a cannula that is either fixed to the skull or positioned to exit between the scapulae. Catheterization surgery is described in detail elsewhere (45). In order to obtain nicotine infusions, the animal makes an operant response. This response may be a lever press, a nose-poke entry into a receptacle, or movement of a wheel manipulandum. During the self-administration session, a houselight may be illuminated. The infusion is almost invariably coupled with a sensory cue, usually a light placed above the lever or an auditory signal (i.e., a tone). Such sensory cues play a critical role in nicotine self-administration, as discussed below. Following the infusion, a timeout period is typically imposed, lasting from seconds to minutes, during which no further infusion is available; this period serves to minimize aversive drug effects and prevent overdose. Usually, a second, “inactive” lever is provided; responses on this lever do not result in drug delivery and instead serve as a control for general increases in activity caused by the drug or by some other experimental manipulation.

The first demonstrations of intravenous nicotine self-administration in rats employed a variety of methods. For example, Singer et al. obtained high levels of nicotine self-administration by using a procedure normally associated with schedule-induced polydipsia (46). Accordingly, rats were food-deprived and placed on a fixed interval 60 s (FI 60) schedule of food delivery. Cox and coworkers provided an early demonstration of IVSA using a more conventional approach (47). These authors showed that rats would press a lever to obtain intravenous nicotine at doses of 10
and 30 µg/kg (but not 3 µg/kg), and would redirect their responding when nicotine was reassigned to a previously inactive lever. Nicotine self-administration did not require schedule induction, pre-exposure or food restriction. However, only a subset of the rats responded preferentially for nicotine in a consistent manner.

Nicotine self-administration was further refined and characterized by DeNoble and Mele, in a paper whose publication was involuntarily delayed by 20 years (48). Meanwhile, in 1989, Corrigall and Coen described a nicotine IVSA procedure that has served as a template for many subsequent studies (49). Their seminal paper described robust self-administration of nicotine across a range of doses and across various schedules of reinforcement (see Fig. 1).

The refinements associated with the Corrigall and Coen (1989) procedure are as follows:

1. Rats acquire nicotine self-administration at greater rates when initially trained to lever press for food and/or are food-restricted during IVSA training (46, 50). Depending on the study, food restriction has been found essential to nicotine IVSA (50) or not (47).

2. Nicotine infusions are typically given by rapid (e.g., 1–3 s) infusion (20, 51–66). Such rapidity is commonly believed to be essential to nicotine IVSA, but recent evidence suggests otherwise (see below).

3. Nicotine solutions are pH-neutralized. This is presumably important, because nicotine is commonly used as a bitartrate salt, which forms highly acidic aqueous solutions.

4. A timeout period (e.g., 1 min) is thought likely to reduce aversive effects of nicotine (45).

5. Limited access sessions (e.g., 1 h/day) elicit higher rates of responding than near-continuous drug access (20, 67, 68).

6. On fixed-ratio schedules, peak rates of responding and number of infusions are typically obtained at doses of 10–30 µg/kg/infusion (20, 51–53, 55, 57, 60, 66, 69–71). However, compared to other drugs such as cocaine and opiates, fixed-ratio responding appears less sensitive to infusion dose (72). In contrast, on a progressive ratio schedule, responding and nicotine intake increase monotonically with infusion dose (73, 74).

7. Diurnal cycle – most IVSA takes place during the active period (21, 47, 75).
Several intrinsic factors affect rates of nicotine IVSA in rodents, including genetic strain (57, 60, 65, 76) and sex (63, 70, 77). Developmental stage also plays a role, and several groups have reported that adolescent rats self-administer intravenous nicotine more than adult rats (58, 76, 78). However, when response demands are increased, adolescent rats may actually self-administer less than adult subjects (79).

The widely used Corrigall–Coen procedure permits limited, 1-h daily access to nicotine. However, some researchers provide more extended access. For example, Sharp and associates permitted rats to lever-press for infusions of nicotine over a 23-h period (51). This procedure has better face validity, and provides nicotine plasma levels closer to those found in human smokers (80). Interestingly, total daily nicotine intake in the unlimited access procedure is not significantly different than in the limited access procedure. Extended-access nicotine IVSA has been studied with respect to strain (57), age (81), extinction behavior (55) and pregnancy (70). Somewhat disheartening, from a treatment standpoint, is the finding that reduction in access to nicotine results in a compensatory increase in daily intake that rebounds above baseline levels upon resumption of normal access (68).

10. Drug Treatments that Reduce Intravenous Nicotine Self-Administration in Rodents

Many drugs have been tested in rodent nicotine IVSA procedures, providing some insight into the neurobiological mechanisms underpinning this behavior. Typically, drug challenges are presented acutely to rats that have already acquired nicotine IVSA in limited-access sessions on a fixed-ratio schedule of reinforcement. This behavioral assay is also widely used to screen for novel drugs that might inhibit cigarette smoking, although the predictive validity of this procedure has not been established (26, 38). Here, we highlight some of the main findings in the drug-testing arena; more detailed reviews are available elsewhere (82–84). Unless otherwise stated, studies refer to acute drug effects on established nicotine IVSA.

10.1. Nicotinic Treatments

All first-line smoking cessation drugs (nicotine, varenicline, bupropion) act on nicotinic acetylcholine receptors (nAChRs). The effects of nicotine replacement therapy have been modeled in rats receiving chronic passive subcutaneous or intravenous infusion of nicotine (80, 85). Nicotine IVSA was markedly reduced, but it is not clear whether the passive nicotine regimen was effective: (1) by substituting for the reinforcing effect of self-administered nicotine, (2) by inhibiting the reinforcing effect of self-administer nicotine (e.g., by inducing nAChR desensitization), or (3) whether it allowed an aversive threshold to be reached more readily.

Nicotinic receptor antagonists have been extensively evaluated in the IVSA paradigm. The broad-spectrum nAChR blocker
mecamylamine reduces nicotine self-administration reliably when administered acutely to rats (73, 86–89) and mice (5, 90); mecamylamine has even been used as a positive control substance for drug screening purposes (91–93). With several self-administered drugs, operant responding transiently increases when saline is substituted. In rats trained to self-administer intravenous nicotine, only a small “extinction-like” burst of responding has been observed upon saline substitution (55), and none after acute mecamylamine challenge (49, 55, 65) (see Fig. 2).

A number of other nAChR antagonists have also been shown to reduce intravenous nicotine self-administration. These include: dihydro-beta-erythroidine (DHβE), considered to be a β2*-selective antagonist (92, 93); methyllycaconitine (MLA), a poorly selective α7 nAChR antagonist (94); the α3β4 antagonist 18-methoxy-coronaridine (18-MC) (87, 95); N, N’-dodecy-l-bis-picolinium dibromide (bPiDDB) (96); and bupropion (see below). Chlorisondamine, which blocks central nAChRs for many weeks after a single administration (97), produced a successive decline in nicotine self-administration in the days following antagonist administration (24). Intravenous nicotine self-administration is also inhibited by negative allosteric modulators of nAChR (98),

Fig. 2. Effects of saline substitution and mecamylamine (nicotinic antagonist) challenge on intravenous nicotine self-administration. Adult rats were first trained to lever-press for infusions of 0.06 mg/kg on a progressive ratio (PR) schedule of reinforcement, in daily 1-h sessions. After a pre-extinction baseline period, each rat was tested under two conditions for five consecutive days, in a counterbalanced order: after saline-for-nicotine substitution, and after acute challenge with mecamylamine with nicotine still available. Both conditions led to an extinction-like decrement in responding. Data are mean ± SEM (Taken from (73). With kind permission).
the nAChR agonists SSR591813 (99) and isorecolone (100), and by nAChR partial agonists including varenicline (91, 101). It is perhaps surprising that nicotine and nicotinic antagonists both reduce nicotine IVSA; conceivably, acute challenge with nicotine produces significant desensitization at nAChRs that are critical to IVSA (102).

The atypical antidepressant bupropion (Welbutrin™, Zyban™) is a moderately effective smoking cessation aid (103). However, in rodent studies of nicotine self-administration, bupropion has met with mixed results: acute and chronic treatments have been shown to reduce (87, 104–106), have no effect on (107), or increase responding for nicotine (105, 108). Response inhibition reliably occurs after acute high doses of bupropion (38, 105), but this is behaviorally nonspecific, insofar as high doses also attenuate responding for sucrose (104, 105) and food (108). Bupropion possesses some antagonistic activity at nAChRs (109), although behaviorally effective doses do not appear sufficient to inhibit nAChRs in rats (110); the drug can also inhibit plasmalemmal monoamine transporters (110). However, the mechanisms underlying bupropion’s therapeutic action remain obscure, and pharmacological analysis of bupropion’s behavioral effects is complicated by the formation of long-lasting active metabolites and also by species differences in metabolism (111, 112).

Lastly, active immunization against nicotine reduced drug intake substantially in rats (113), and nicotine vaccines are currently in clinical trials.

10.2. Dopaminergic Treatments

Dopamine (DA) has received a great deal of attention as a putative “reward” transmitter. Extracellular DA levels are increased during nicotine self-administration in limited-access sessions (114), but the effects of DAergic drugs on nicotine IVSA have been little studied. Corrigall and Coen demonstrated that the DA D2 receptor antagonists haloperidol and spiperone, as well as the D1 receptor antagonist SCH 23390, reduced nicotine intake; however, these drugs also reduced locomotor activity. Furthermore, SCH 23390 also reduced food self-administration (115), suggesting a generalized behavioral suppression. More recently, a DA D3 receptor antagonist SB277011A was shown to reduce nicotine IVSA, while sparing food-reinforced responding (116); a high dose was required, suggesting that D3 receptors may not have been responsible. Nicotine IVSA is also affected by acute challenge with the DA agonists apomorphine (105) and methylphenidate (117).

10.3. Glutamatergic Treatments

Glutamate (Glu) is the primary excitatory neurotransmitter in the brain and it provides some promising targets for addiction treatments. For example, chronic treatment with N-acetylcysteine, a nutritional supplement that increases cystine–glutamate exchange,
Nicotine Self-Administration was recently shown to reduce cigarette smoking (118). Initial studies in rats showed that the NMDA receptor antagonist dextromethorphan reduced nicotine self-administration (87, 119), but also self-administration of water (119). Antagonists targeting the mGluR5 glutamate receptor subtype have received considerable attention, particularly 2-methyl-6-(phenylethynyl) pyridine (i.e., MPEP). MPEP and its more selective analog MTEP consistently reduced nicotine self-administration in rats with little or no effect on food-reinforced responding (120–124). Agonists and antagonists of mGluR2/3 receptors have also been reported to reduce nicotine self-administration (see Fig. 3), when given either alone (LY379268) (125) or in combination (LY341495) with MPEP (122).

**10.4. Gamma Aminobutyric Acid (GABA) Treatments**

The GABA<sub>a</sub> receptor agonist baclofen exerts acute biphasic effects on intravenous nicotine self-administration, with low doses increasing responding and high doses decreasing responding for nicotine (126). Both GABA<sub>a</sub> agonists and newer GABA<sub>a</sub> positive allosteric modulators reduce nicotine self-administration, with the latter showing useful selectivity for nicotine- versus food-reinforced responding (127–129).

**10.5. Noradrenergic and Serotonergic Treatments**

There is little published information about noradrenergic and serotonergic drugs, and results are not promising as regards smoking cessation. Reboxetine, which potently inhibits certain
nAChRs as well as the norepinephrine transporter (NET), reduced self-administration of nicotine, but also inhibited sucrose self-administration (86). Similarly, the NET blocker desipramine inhibited both nicotine and food self-administration (130). Serotonergic (5-HT) antagonists have not been systematically studied, although in one report 5-HT₃ receptor blockers were ineffective in reducing nicotine intake (131). In squirrel monkeys, the 5-HT transporter blocker sertraline failed to affect established nicotine IVSA (132).

10.6. Opioid Treatments

There is evidence both for and against opioid receptor involvement in nicotine’s rewarding effects (133). For example, nicotine conditioned place preference was found to be absent in mu-opioid knockout mice (134) and in wild type animals, the expression of place preference was abolished by systemic administration of the opioid antagonist naloxone (135). In contrast, the opioid antagonists naltrexone and naloxone had no effect on intravenous nicotine self-administration, despite reducing cocaine self-administration (48, 136). A recent, more detailed analysis suggests that naltrexone reduces the incentive properties of nicotine-associated sensory cues without reducing the primary reinforcing effects of the drug (133). However, human studies have revealed mixed effects of naltrexone on cue-induced craving and smoking behavior (137).

10.7. Cannabinoid Treatments

In animal studies, CB₁ receptor antagonists have been shown to reduce self-administration of several drugs of abuse, including nicotine. However, the effect of CB₁ receptor antagonists may be partially nonspecific, in that SR141716 (i.e., rimonabant) reduced inactive lever pressing (138) and AM251 inhibited responding for food reward (139). As discussed elsewhere, CB₁ antagonists appear to inhibit IVSA at least partly by attenuating the impact of nicotine-associated cues (140, 141).

10.8. Brain Lesions

Several types of anatomical lesions have been applied to nicotine IVSA in rats. In particular, 6-hydroxydopamine lesions of the nucleus accumbens (NAcc) markedly attenuated this behavior (24), consistent with a role for mesolimbic dopamine in mediating nicotine reward or some related motivational process (Fig. 4). Excitotoxic lesions of pedunculopontine tegmental nucleus (PPTg) also reduced established nicotine self-administration behavior (142), while lesions of the posterior (but not anterior) PPTg (143), and neonatal frontal cortex (144) reduced acquisition of nicotine self-administration.

10.9. Central Drug Administration

Intracerebral administration of agonists or antagonists in rats has provided information about potential mechanisms underlying nicotine IVSA behavior. Three brain areas have been targeted: the
Nicotine Self-Administration

NAc, ventral tegmental area (VTA), and PPTg. Intra-NAc administration of the mGluR2/3 agonist LY379268 inhibited nicotine self-administration (125), whereas focal infusion of the nicotinic antagonist DHβE did not (145). Intra-VTA administration of several drugs – notably DHβE (145), LY379268 (125), muscimol, baclofen, and the mu opioid receptor agonist DAMGO (146) inhibited nicotine IVSA. Finally, DHβE (142) and GABA<sub>B</sub> agonists also reduced nicotine self-administration when they were given into the PPTg (147).

Nicotinic receptor (nAChR) subtypes are defined primarily by subunit composition, and are highly heterogeneous. Knockout and transgenic mice have implicated a subset of nAChR subunits in nicotine IVSA, as reviewed previously (8, 102). Nicotinic AChR deletion can produce behaviorally selective effects, shown by the finding that β2 mutant mice self-administered cocaine but not nicotine (148, 149). Recent studies have combined two experimental strategies: genetic knockout, followed by intracerebral injection of a viral vector in order to express the deleted nAChR subunit within a brain structure of interest. This approach has identified α4β2- and α6β2-containing nAChRs in the VTA as critical to nicotine IVSA in the mouse (150).
11. Drug Treatments that Reduce Relapse to Nicotine Seeking

Relapse is a recurring problem in the treatment of smoking. Indeed, of smokers who quit without pharmacological or psychological support, only 3–5% remain abstinent after 6–12 months (151). In abstinent subjects, drug seeking can be triggered by a variety of stimuli including drug-associated cues, exposure to the drug itself, or stress. Different relapse scenarios are modeled in the rodent reinstatement procedure of drug self-administration, pioneered by de Wit and Stewart (152). In this procedure, rats are first trained to self-administer a drug. Self-administration behavior is then extinguished over multiple sessions, during which drug-associated cues may or may not be available. Animals are then acutely exposed to three types of stimuli: the drug itself, stressors, or drug-associated cues provided the latter have not been already extinguished. The resultant increase in (unreinforced) responding provides a measure of drug seeking. Brain mechanisms underlying reinstatement are highly dependent on the nature of the triggering stimulus (153). This behavioral procedure is now widely used to screen drugs for therapeutic potential.

Reinstatement related to nicotine IVSA has recently been reviewed in detail (9). Reinstatement has been induced by intravenous and systemic injections of nicotine (154, 155), footshock stress (156) and re-exposure to nicotine-associated stimuli (157). Nicotine-induced reinstatement was inhibited by antagonists of dopaminergic (SB277011A), glutamatergic (MPEP, EMQMCM) (158, 159), and cannabinoid (AM251) (139) receptors; the mGluR1 antagonist EMQMCM also reduced reinstatement of food seeking (159). In contrast, stress-induced reinstatement was attenuated by both the corticotropin-releasing factor (CRF) receptor 1/2 antagonist D-Phe CRF (12–41) (administered icv) and the α2 adrenergic receptor agonist clonidine (administered systemically) (160).

Studies of cue reactivity in cigarette smokers have largely focused on craving and physiological measures rather than drug seeking behavior itself (161). Indeed, there is surprisingly little direct evidence that smoking cues actually promote the occurrence of smoking behavior (161–163). Nevertheless, various drugs have been screened in animals for their ability to block reinstatement of nicotine seeking triggered by nicotine-associated cues. Drugs that inhibit reinstatement include the nAChR antagonist mecamylamine (164, 165), and modulators of GABA (CGP44532) (127), glutamate (MPEP, EMQMCM) (159, 166) and cannabinoid receptors (SR141716, AM251) (139, 167). In contrast, bupropion inhibited nicotine self-administration prior to extinction, but acutely increased reinstatement triggered by
nicotine-associated cues (157). Speculatively, the latter result may be related to the limited efficacy of bupropion in smoking cessation (but see above).

Through conditioning, stimuli associated with smoking can elicit effects in their own right (168). For example, initially neutral cue complexes (visual, auditory and olfactory components) can elicit high levels of cigarette craving after they have been repeatedly paired with smoking (169). With the average smoker taking thousands of puffs a year, the extensive range of stimuli that can become associated with smoking are significant hurdles for cessation treatment. In human smokers, individuals who relapse tend to react more strongly to smoking-related cues than those who remain abstinent, and reactivity to these cues has some predictive validity in terms of successful quitting (170).

Caggiula and colleagues have recently shed new light on behavioral processes maintaining nicotine IVSA in the Corrigall–Coen model (171). Specifically, these investigators have systematically shown that intravenous nicotine exerts only weak primary reinforcing effects, and instead maintains self-administration behavior mainly by a process which they term “reinforcement enhancement.” The main evidence for reinforcement enhancement is as follows. Nicotine IVSA procedures incorporate a visual (or auditory) stimulus that is paired with each nicotine infusion, and nicotine IVSA is dependent on the presence of this stimulus (88, 92, 172–179). However, such stimuli tend to be reinforcing in their own right. For example, rats will typically press a lever in order to briefly illuminate a light stimulus, even when this is never paired with nicotine (171, 180, 181). Critically, responding for a visual stimulus can be enhanced by non-contingent administration of nicotine (see Fig. 5), delivered either via yoked passive infusions (173, 174, 179) or by continuous infusion (173). This reinforcement-enhancing effect of nicotine has been seen on most schedules of reinforcement (but not on FR1 (182)) and across a wide dose range (174).

Interactions between nicotine and environmental stimuli suggest one mechanism by which the drug could help to maintain cigarette smoking. Another widely discussed possibility is that the reinforcing effects of nicotine in smokers are potentiated by other smoke constituents, particularly monoamine oxidase (MAO) inhibitors. The basis for this notion is as follows. Cigarette smokers possess lower levels of brain and peripheral MAO compared to non-smokers and ex-smokers, with reductions in both MAO-A

12. Miscellaneous Issues and Recent Developments

12.1. Reinforcement Enhancement by Nicotine

Interactions between nicotine and environmental stimuli suggest one mechanism by which the drug could help to maintain cigarette smoking. Another widely discussed possibility is that the reinforcing effects of nicotine in smokers are potentiated by other smoke constituents, particularly monoamine oxidase (MAO) inhibitors. The basis for this notion is as follows. Cigarette smokers possess lower levels of brain and peripheral MAO compared to non-smokers and ex-smokers, with reductions in both MAO-A
and MAO-B isoforms (183, 184). Cigarette smoke contains several identified MAO inhibitors (185). In rats, MAO inhibition results in increased extracellular DA in the NAc; whereas, local destruction of DA terminals is associated with a profound reduction in nicotine IVSA (24). Hence, tobacco-associated MAO inhibitors may potentially augment DA-dependent reinforcing effects of nicotine (or perhaps promote cigarette smoking by some other mechanism).

Several MAO inhibitors have been tested for their effects on nicotine IVSA in rats. Published work in this area has originated from two groups. Stinus and associates administered several MAO inhibitors (tranylcypromine, phenelzine, clorgyline and norharman) on a daily basis during acquisition of fixed-ratio schedule responding and after transfer to a partial reinforcement schedule (186, 187). MAO inhibitor treatment selectively increased responding on the PR schedule (Fig. 6). These findings suggest that chronic MAOII treatment did not enhance learning, and may instead have increased the rats’ motivation to self-administer nicotine (an aspect thought...
Nicotine Self-Administration

It is also possible, however, that the effects of these MAO inhibitors took time to emerge or required chronic administration in order to develop. In contrast, Leslie’s group found that daily MAO inhibitor pretreatment (tranylcypromine) did enhance acquisition of nicotine IVSA (188–190), an effect that was blocked via the α1-adrenergic receptor antagonist prazosin (189).

These findings are certainly of interest but require careful interpretation. First, all published animal studies employed high
doses of MAO inhibitors, resulting in a near-total inhibition of enzyme activity. In contrast, MAO-A and -B isoforms are only inhibited by about 30–40% in human brain (183, 184). Second, animal studies have largely relied on frequent (i.e., daily) administration of irreversible MAO inhibitors, even though MAO activity returns only slowly after irreversible inhibition, with a recovery half-life exceeding one week in rats (191). Use of repeated high doses is of particular concern since MAO inhibitors exert numerous off-target actions. Third, in some studies (188–190) nicotine IVSA itself occurred only in the presence of MAO inhibition. The latter studies examined only the first 5 days of IVSA training, and it would be interesting to know whether the enhancement would persist with further testing.

Aside from MAO inhibitors, only two other tobacco smoke constituents have been examined in the nicotine IVSA paradigm: acetaldehyde and nornicotine. Acetaldehyde, at a dose that was not itself self-administered, increased nicotine self-administration in adolescent rats; however, this facilitatory effect was not observed in adults (192). Furthermore, the locomotor stimulatory effect of nicotine was also increased by pretreatment with acetaldehyde in both adolescents and adult rats (193), suggesting that generalized activity may be partly responsible for the increase in IVSA. These findings clearly merit further study.

Nornicotine is a nicotinic agonist that is present in tobacco smoke and also formed from nicotine metabolism. It has a longer elimination half-life than nicotine, and may reach pharmacologically significant concentrations in cigarette smokers (194). Rats have been found to self-administer a range of nornicotine doses (peak responding at approximately 300 μg/kg/infusion), showing normal extinction and reinstatement upon removal and replacement of the drug (195). It should be noted, however, that nornicotine is less prevalent than nicotine in cigarette smoke, and much of it is formed in vivo from nicotine. Hence, the pharmacological impact of nornicotine may be slowed by the need for nicotine metabolism.

Collectively, the findings reviewed in this section suggest that some non-nicotine tobacco constituents may enhance the reinforcing effect of nicotine. Tobacco smoke may also contain chemical reinforcers other than nicotine. Although only one such candidate (nornicotine) has come to light so far, it is worth bearing in mind that tobacco addiction, from a pharmacological point of view, may constitute more than just an addiction to nicotine.

Several studies have described the effects of chronic nicotine self-administration on stress-related processes in adult rats. For example, rats given prolonged (e.g., 40 days) access to nicotine not only displayed higher levels of social anxiety (196) but also
mounted exaggerated adrenocorticotropic hormone (ACTH) and corticosterone (CORT) responses to a mild stressor (197). Chronic nicotine self-administration, similar to chronic stress (198), also increased CRF mRNA in the paraventricular nucleus (PVN) of the hypothalamus (199). Furthermore, chronic nicotine increased NMDA and glutamate (Glu) receptor prevalence in the medial prefrontal cortex (mPFC) and VTA respectively (200), as well as Glu release in these areas (201) – another effect common to chronic stress (202). Somewhat surprisingly, however, norepinephrine (NE) release in the amygdala was enhanced only during acquisition of nicotine self-administration, but normalized over time (203), suggesting tolerance.

Numerous neuropharmacological and behavioral changes have been reported in rats self-administering nicotine, and the following overview is not intended to be exhaustive. Some aspects are reviewed in more detail elsewhere (82, 204, 205). Although the focus has been on changes occurring in the CNS, chronic nicotine exposure and/or IVSA can also lead to various peripheral changes, such as in T-cell responsiveness (206).

The immediate early gene product Fos is commonly used as a marker of neuronal activation. Intravenous nicotine increases Fos expression in a variety of brain areas, both after passive infusions (207, 208) and self-administration (207, 209) in rats. In one IVSA study, Fos expression was increased in 43 of 77 brain areas examined, including the mPFC, NAc shell (NAcSh), and cingulate cortex (209). However, it is not clear whether this Fos induction was related to the reinforcing effects of nicotine, since the experimental design did not include a control group receiving passively administered nicotine. A passive nicotine control was included in a subsequent study (207), but this group was not matched for prior nicotine exposure. Acute passive administration of intravenous nicotine also increased glucose utilization, another marker of neuronal activity, but unlike Fos induction, this effect was apparently restricted to NAcSh (210).

In a limited sample of monkeys, it was reported that baseline levels of nAChR (α4β2) significantly (p<0.05) predicted levels of nicotine IVSA in a puzzling inverse relationship (211). This is interesting because studies have shown that passive nicotine administration can increase the density of brain nAChRs (212–214), which might suggest a lowered motivation to self-administer nicotine over time. Nicotine IVSA for an extended period also led to widespread receptor upregulation, and an analysis of cell-surface expression revealed preferential increases in α6 and β2 subunits (215). This finding is potentially significant since α6β2-containing nAChRs expressed in VTA DAergic neurons appear important for nicotine self-administration, as demonstrated recently in mice (150). Furthermore, established nicotine IVSA in rats was associated with
raised extracellular DA levels in the NAc, with a larger effect in the NAc shell than core (114).

Certain consequences of nicotine IVSA are extremely persistent. For example, chronic nicotine IVSA has been observed to reduce intracranial self-stimulation (ICSS) thresholds for 36 days post-withdrawal, with no sign of recovery (67). Another example of persistence is that chronic nicotine IVSA can also promote cell death and inhibit neurogenesis in the hippocampus (216).

Virtually all nicotine IVSA studies published in the past 15 years have employed the Corrigall–Coen procedure or some variant of it. Although this procedure has generated a rich literature, several features are of questionable utility: food restriction and/or prior food training, limited daily access, rapid infusions, and high unit doses. These aspects are now discussed in turn.

Food restriction. Food-restricted animals appear more motivated in many behavioral test procedures (217). Although Corrigall and Coen (1989) tested their animals while hungry, they did not determine whether acute food deprivation enhanced nicotine IVSA. Subsequently, Donny and associates demonstrated that this is indeed the case; rats lever-pressed more vigorously for intravenous nicotine when tested before rather than after a daily meal (182). It is possible therefore that hungry rats self-administer nicotine at least partly to obtain its appetite suppressive properties (218, 219).

Prior food training. In many nicotine IVSA studies, animals are initially trained to respond for food reward. Once this operant behavior is acquired, intravenous nicotine is made available instead. In principle, this procedure makes it difficult to distinguish the extent to which rats are responding for nicotine versus still attempting to self-administer food (especially when hungry). However, when this issue was investigated systematically, it was found to have little practical importance (164). Thus, when nicotine infusions were substituted for food pellet reinforcers, the animals expressed only a transient response bias towards the lever previously associated with food.

Limited daily access. Short test sessions (e.g., 1 h/day) are convenient and permit a high throughput of experimental animals. Corrigall and Coen (1991) first suggested that limited access might also promote self-administration of nicotine. This has recently been confirmed in studies (20, 67, 68), in which short (1–2 h) sessions generated greater hourly nicotine intake than sessions lasting 6, 12, or 23 h. One disadvantage of short daily nicotine IVSA sessions is that rats typically self-administer 10–20 cigarettes worth of nicotine (on a mg/kg basis) within a single 1-h session, resulting in plasma nicotine levels that are at least twice the daytime levels measured in cigarette smokers (85, 220).

A second, obvious drawback of short IVSA sessions is that, while total daily nicotine intake may be similar to that of human
Accordingly, some researchers have adopted an “unlimited access” schedule of nicotine self-administration that features 23-h/day access to nicotine with ad-lib food and water (51). This schedule results in a circadian pattern of intake (21) and provides plasma nicotine levels comparable to cigarette smokers (85). In these respects, the 23-h/day access IVSA procedure appears to model human smoking behavior better than the limited access procedure.

**Infusion speed.** A major assumption in nicotine IVSA research is that nicotine is only reinforcing when delivered via rapid infusions (e.g., of 1–3 s duration). However, the relevant published evidence is either anecdotal (51) or based on sub-optimal study design (4). That said, rapid intravenous infusions of nicotine are considerably more effective in promoting locomotor sensitization and in triggering immediate early gene induction in mesolimbic structures (208). Interestingly, in human smokers with previous drug exposure, rapid intravenous infusions of nicotine are typically identified as psychostimulant-like (28, 221–223), suggesting that nicotine may have cocaine-like subjective effects if delivered rapidly enough and in a sufficient dose.

The use of rapid nicotine infusions was probably inspired by the long-held belief that each cigarette puff delivers a discrete bolus of nicotine rapidly to the brain. However, Rose and colleagues demonstrated several years ago that the peak of arterial nicotine occurs much later (i.e., 20–30 s post-puff, Fig. 7) and is

![Fig. 7. Arterial nicotine concentrations following individual cigarette puffs in a single smoker. Nicotine concentrations, sampled every 5 s from a radial artery, rose gradually and with an appreciable delay after each puff. These data suggest that the brain does not receive a short-latency spike of nicotine (bolus) (Taken from (224). With kind permission).](image)
much lower (ten as opposed to 100 ng/ml) than once believed (224). Accordingly, we recently investigated whether slower intravenous infusions of nicotine could support IVSA in rats. Contrary to expectations, rats not only self-administered slower infusions of nicotine (e.g., 30 s), but even preferred them to fast (3 s) infusions when subjects were offered a simultaneous choice (225). Furthermore, preferential responding on the active lever was observed not only for fast (3 s) infusions, but also for infusions lasting 30, 60, or 120 s. Importantly, acute challenge with a DA antagonist produced opposite effects on IVSA, depending on the speed of infusion (225). This result highlights the importance of the choice of animal model when investigating brain mechanisms.

Unit dose of nicotine. Infusion doses of 15–30 μg/kg have become widely adopted because they generate the highest rates of lever pressing (47, 49, 53, 59, 65, 73, 75, 81, 93). However, this infusion dose corresponds, on a mg/kg basis, to 1–2 cigarettes worth of nicotine (29) – typically delivered in ~1 s!

12.6. “Slow/low” Nicotine: A New IVSA Procedure

Isolated reports, largely ignored, have suggested that rats may occasionally self-administer much lower doses of nicotine (3–7.5 μg/kg/infusion) (51, 75). Recently, however, we have demonstrated that, when nicotine is available in slow (30 s) infusions, even doses as low as 3 μg/kg will support IVSA (225). Hence, in our new procedure, IVSA can be maintained by a unit dose corresponding to ~2 cigarette puffs (29), delivered at a rate which is closer to that experienced by smokers (224). Recently, we have compared the effects of drug challenges in two nicotine IVSA procedures: our standard “slow/low” procedure (3 μg/kg delivered in 30 s) versus a conventional procedure (30 μg/kg delivered in 3 s). Importantly, the effects of DA antagonists differ greatly between these two procedures (225).

13. Conclusions

Intravenous self-administration studies have shown that nicotine can serve as a reinforcer in animals and humans. However, virtually all demonstrations to date have made use of high infusion doses and/or rapid infusions (or both), which are of questionable relevance to human smoking behavior. In the standard limited-access procedure, plasma nicotine levels exceed those found in most human smokers (220). We therefore suggest that a better model of human smoking would incorporate slow infusions and extended access. Brain mechanisms underlying nicotine IVSA have been widely investigated, but only time will tell how much
this burgeoning literature really tells us about cigarette smoking. Animal models related to nicotine dependence are currently under scrutiny, with concerns about their predictive clinical validity (25). Possibly, adoption of lower doses and slower infusions will help to fill this need.

Acknowledgments

Our own research findings were supported by Canadian Institutes of Health Research (CIHR), the Canadian Tobacco Control Research Initiative (CTCRI), and the Natural Sciences and Engineering Research Council of Canada.

References


102. Picciotto MR, Addy NA, Mineur YS et al (2008) It is not “either/or”: activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. Prog Neurobiol 84:329–342


reinforcement in the maintenance of cigarette smoking. Br J Addict 86:605–609


Chapter 5

Alcohol Self-Administration*

Friedbert Weiss

Abstract

This chapter provides an exhaustive overview of the current repertoire of animal models for alcoholism research. The chapter covers behavioral procedures modeling different stages of the alcohol addiction cycle, including strategies for investigating ethanol reinforcement, ethanol dependence, binge drinking, ethanol craving, and susceptibility to relapse. Moreover, the description and evaluation of the utility of these models is presented within a historical context, as well as an assessment of specific needs for future model development. The chapter emphasizes that the primary objective of contemporary research on alcohol abuse and addiction is to explain the processes that compel some but not other individuals to drink excessively, the identification of brain mechanisms that support the acute reinforcing actions of alcohol in nondependent subjects, and abnormalities in these mechanisms that are responsible for the development of dependence and the compulsive character of ethanol seeking and use in alcohol-addicted individuals. With respect to the development and successful implementation of valid animal models of self-administration and addiction, a number of issues must be considered in the case of alcohol due to the fact that (a) voluntary alcohol consumption in animals is generally low, except in animals that have been genetically selected for high spontaneous ethanol preference, and (b) that the conditions that make excessive alcohol consumption a reinforcing event in some subjects and not others are complex because they involve interactions among genetic, psychosocial-environmental, and neurobiological factors. Despite these challenges, a wide array of animal models is available that permits investigation of behaviors directed at obtaining access to and consuming alcohol as well as the identification of neurobiological, genetic, environmental, and motivational factors regulating these behaviors in both the nondependent and dependent states. These models also are instrumental for identifying pharmacological treatment targets for intervention at different stages of the addiction cycle and as preclinical tools for evaluating the efficacy of potential medications for the treatment of excessive alcohol use and the prevention of alcohol craving and relapse.

Key words: Alcohol deprivation effect, Alcohol acclimation procedures, Binge drinking, Conditioned place preference, Dependence, Reinforcement, Reinstatement, Self-administration, Withdrawal

*Modeling Stages of Alcohol Addiction: From Reinforcement to Dependence and Long-Lasting Susceptibility to Craving and Relapse.
1. Introduction

Ethanol reinforcement and ethanol-seeking behavior are the primary behavioral measures of interest in contemporary research on alcohol abuse and addiction. The objective of this research – to explain the processes that compel some but not other individuals to drink excessively – involves the identification of brain mechanisms that support the acute reinforcing actions of alcohol in nondependent subjects as well as abnormalities in these mechanisms that are responsible for the development of the pathological, compulsive character of ethanol reinforcement and use in dependent subjects. The acute reinforcing effects of alcohol are defined as those that increase the probability of its consumption in the future by producing hedonically positive consequences. However, alcohol addiction, by definition, is associated with the experience of ethanol withdrawal such that drug-related learning associated with ethanol consumption during withdrawal modifies an individual’s ethanol reinforcement history by introducing the novel dimension of learning about negative reinforcement (i.e., alleviation or prevention of withdrawal-associated manifestations of negative affect including tension, anxiety and dysphoria) as a significant aspect of ethanol’s actions that drive ethanol abuse in a self-medication sense. Thus, ethanol, when available and ingested during withdrawal, may acquire increased incentive value and potency as a reinforcer that may contribute to the lasting and compulsive pattern of ethanol abuse in addicted persons. In addition, there is likely to be a concomitant increase in the conditioned incentive value of ethanol-associated environmental stimuli or events that augment their impact as triggers of ethanol craving and may thereby increase susceptibility to relapse.

Animal models have and continue to be developed that permit the investigation of behaviors directed at obtaining access to and consuming alcohol as well as the identification of neurobiological, genetic, environmental, and motivational factors regulating these behaviors in both the nondependent and dependent state. These animal models also are instrumental for identifying potential neurochemical and molecular treatment targets for intervention at different stages of the addictive cycle, and as preclinical tools for evaluating the efficacy of potential medications for the treatment of excessive alcohol use and the prevention of craving relapse in abstinent individuals (1, 2).

As in the case of animal models designed to investigate the addictive effects of other drugs of abuse, animal models of ethanol seeking and reinforcement represent an experimental strategy for studying biological, environmental, and genetic determinants of ethanol preference and reinforcement as well as the neurobiological and behavioral consequences of chronic drug use and
abuse. However, in the case of ethanol, special considerations apply to the development and successful implementation of animal models of drug seeking, self-administration, and addiction. This is so because spontaneous voluntary alcohol consumption in animals is generally low and not compellingly pharmacologically motivated, except in animals that have been genetically selected and outbred for high spontaneous ethanol preference. Therefore, the demonstration and investigation of ethanol reinforcement requires special initiation or acclimation procedures to overcome the innate avoidance by many species of higher ethanol concentrations and, therefore, the difficulty in establishing ethanol intake at levels that produce pharmacologically meaningful blood alcohol concentrations (BACs) sufficient to draw valid conclusions about pharmacologically motivated (i.e., addiction relevant) reinforcing actions of ethanol. A further challenge for the development of valid models of ethanol self-administration and addiction is that the conditions that make excessive alcohol consumption a reinforcing event in some subjects and not others are complex because they involve interactions among genetic, psychosocial-environmental, and neurobiological factors.

In spite of these complexities, research with animal models has provided critical information about neural systems with which alcohol interacts and that participate in maintaining the desire and motivation to drink. Moreover, substantial advances have been made with the development of animal models that permit exploration of how the diverse behavioral effects of alcohol, which range from its reinforcing effects to motor coordination, aggression, and violence, are coupled to specific ethanol-induced actions or long-term ethanol-induced neuroadaptive changes in the brain. Finally, animal models of ethanol seeking and reinforcement have provided valuable information that ultimately led to the identification and clinical use of treatment drugs for alcohol addiction (e.g., (3)).

This chapter will provide an overview of the large repertoire of animal model tools available to date. In doing so, the description of these procedures will emphasize the development of contemporary models of alcohol addiction within a historical context because, with few exceptions, even the most effective contemporary models have their roots in and evolved from early attempts to scientifically approach the study of ethanol reinforcement, addiction, and proclivity to relapse.

2. Ethanol Self-Administration and Reinforcement

To demonstrate that a model taps into behavior motivated by ethanol reward, two criteria must be fulfilled. First, it is essential to demonstrate that ethanol intake is maintained by
pharmacological motivation rather than factors related to nutritional need, thirst, or palatability. Second, ethanol must be shown to act as a reinforcer by changing and maintaining behavior such as, for example, the learning of an alcohol-reinforced operant response, in addition to maintaining mere ethanol drinking (4–7).

Once these criteria are met, an important strength of contemporary animal models of alcohol self-administration lies in their power to provide detailed information on the degree to which ethanol serves as a reinforcer under specific conditions (e.g., varying ethanol concentrations, reinforcement contingencies, and the degree of an alcohol deprivation status). This includes their utility to reveal factors that drive and modify the motivation to consume or to expend “work” to obtain alcohol. In other words, these models are effective tools for establishing the reinforcing strength or magnitude of alcohol under different experimental conditions, for identifying factors that determine the onset, maintenance, and termination of drinking episodes (technically termed “bouts”), for analyzing drinking patterns such as the distribution of drinking bouts over given periods of access to ethanol, and revealing factors that influence variations in the motivation to seek and consume ethanol. These models have also provided valuable information about how environmental factors such as stress, the availability of alternative reinforcers, or the “cost” (i.e., the schedule or “work requirement” imposed to obtain alcohol) influence ethanol self-administration, and how such environmental manipulations interact with genetic propensity for heightened ethanol intake, the history of prior ethanol exposure, or the ethanol deprivation status in terms of the development and maintenance of excessive alcohol drinking.

It is important to point out that the development of experimental models of ethanol reinforcement is faced with unique challenges because this method development must take into consideration and overcome several problems that historically have hampered studies of ethanol reward. First, most animals exhibit innate taste preference–avoidance behavior depending on the concentration of ethanol. Most animal species accept and consume low concentration (1–5%) ethanol solutions, but avoid higher concentrations. As a result, voluntary ethanol consumption in animals is generally not sufficient to produce pharmacologically meaningful (i.e., reinforcing) blood alcohol levels (BALs) and, therefore, not sufficient to permit investigation of the reinforcing effects of ethanol as a pharmacological agent. Second, given the pharmacokinetics of small orally administered ethanol doses (as in most self-administration models), the delay between alcohol ingestion and the onset of its pharmacological (reinforcing) effects will be substantial, impeding the development of a reinforcing relationship between pharmacological stimulation induced by the reinforcer and the behavioral response that produced it (for review: (7–10)).
In order to establish a reinforcing relationship between ethanol consumption and a given behavioral response that makes available the ethanol reinforcer, it is necessary that animals receive an effective (self-administered) dose of ethanol over a time period that is sufficiently short to produce pharmacological effects. As described in the next section, to develop voluntary ethanol drinking at pharmacologically meaningful levels, the field has relied on a variety of special procedures to initiate ethanol self-administration under contingencies that meet these essential conditions. Procedural details for the major methods currently in use are shown in Boxes 1–6.

**Box 1. Sweet Solution Fading Procedures**

Sweet solution fading procedures are variants of the “taste adulteration” procedure with the difference that the tastant, typically a sweetener, is not abruptly but gradually removed (faded) from the ethanol solution. The procedure was originally developed by Samson and colleagues in the mid-1980s using sucrose as a tastant, but variants using noncaloric sweeteners such as saccharin, or more recently, “SuperSac” – a 3.0%/0.125% (w/v) glucose/saccharin solution that generates higher rates of ethanol intake than other sweet solution fading procedures have begun to be employed based on early work by Valenstain (30) and further developed by Walker and colleagues (31). Sweet solution fading is likely the most widely used procedure to establish ethanol-reinforced operant responding. Reliable ethanol intake is maintained following removal of the sweetener and is sufficient to generate significant BALs ranging between 25 and 75 mg% in a typical 30-min session. Technically, the procedure is designed to establish response-contingent oral self-administration of ethanol or water in a two-lever, free-choice operant task. To accomplish this, animals are initially trained in daily short-access session (e.g., 30 min) to respond at either of the two levers for small amounts of a sweet solution (e.g., 0.1 mL) reinforcer until stable responding is obtained. Animals are then introduced to a free-choice task in which responses at one lever produce ethanol solution while responses at the other lever result in delivery of water. Ethanol self-administration then is initiated by adding a low concentration of ethanol (e.g., 5% w/v) to the sweet solution. During subsequent training, ethanol concentrations are gradually raised to 10% (w/v) or more. At the same time, the concentration of sweetener is slowly decreased and eventually eliminated completely from the drinking solution.
This technique takes advantage of the adjunctive drinking behavior typically associated with food-reinforced responding on some schedules of reinforcement. Intermittent availability of food pellets in food-deprived animals that have concurrent access to a liquid produces excessive drinking of water or ethanol (i.e., schedule-induced polydipsia). When the food reinforcement contingency is terminated, water intake reverts to normal levels, but ethanol intake can persist at significant levels. When ethanol then is made available response contingently, typical schedule controlled behavior can be obtained and maintained by ethanol presentation on fixed-interval or fixed-ratio response contingencies. Success with ethanol initiation by schedule-induced polydipsia, however, does not seem entirely clear-cut, particularly in rats, because this procedure often depends on the continued presence of the original inducing condition (i.e., food deprivation with intermittent food pellet presentation) to maintain excessive drinking. On the other hand, the procedure continues to be employed with great success in monkeys where it has been demonstrated that the drinking typographies emerging with initial ethanol intakes of only 1.5 g/kg are highly predictive of daily ethanol consumption over the course of 1 year post induction. Specifically, the frequency with which monkeys ingest 1.5 g/kg ethanol during induction strongly predicts subsequent drinking with a near-perfect correlation with later ethanol intake and BALs ranging from 100 to 160 mg% when the animals drink their 1.5 g/kg dose in a single bout. Thus, this model of ethanol self-administration is suitable for identifying early alcohol drinking typographies particularly in nonhuman primates (with consumption of the equivalent of six drinks) that evolve into chronic heavy alcohol consumption, corresponding to the equivalent of 16–20 drinks per day.
One of the original and most widely employed methods to study the effects of drug and ethanol cues on the recovery of extinguished drug seeking behavior. In this procedure, animals are trained to respond at a lever, with completion of each response requirement resulting in delivery of an oral ethanol dose (typically 0.1 mL PF 10% w/v ethanol). Each response producing the drug reinforcer is contiguously paired with a brief presentation of an environmental stimulus (e.g., a tone or cue light) that by virtue of these repeated pairings comes to serve as a conditioned stimulus (CS). Both drug administration and presentation of the CS are contingent upon a response by the animal. Once reliable ethanol self-administration is acquired, drug-reinforced instrumental responding is “extinguished” by ceasing to reinforce responses by ethanol delivery and withholding presentation of the CS. Extinction sessions are conducted until responding is substantially lower than that maintained by delivery of the drug and deemed incidental rather than motivated by expectation of obtaining the drug. Subsequently, reinstatement tests are conducted in which the degree of recovery of responding at the previously ethanol-paired lever, now maintained by response-contingent presentation of the CS only (i.e., without further ethanol availability), is operationally defined as a measure of craving or relapse.

**Box 5. Conditioned Reinstatement: Discrete Cues**

In the area of alcohol addiction research, seeking-taking schedules are often employed that are variants of “chained schedules of reinforcement” that dissociate ethanol-reinforced consummatory behavior (i.e., ethanol drinking) from appetitive ethanol-seeking responses (i.e., behavior induced and maintained by the incentive-motivational effects of ethanol-associated contextual cues present in the self-administration environment). In this procedure, rats must complete a set of responses at a lever operandum during which time ethanol is not available (appetitive phase). Completion of a response requirement within a specified time period results in retraction of the lever and presentation of a sipper tube containing ethanol solution from which rats are then allowed to freely drink for a given amount of time (consummatory phase). Thus, responding during the appetitive phase in this model provides a measure of the daily strength of the animal’s motivation to initiate and engage in ethanol-seeking behavior when exposed to an ethanol-predictive stimulus environment (i.e., the operant conditioning chamber).

**Box 4. Seeking-Taking Chained Schedules**
To study the effects of environmental context on the recovery of ethanol-seeking, drug availability is conditioned to stimuli (i.e., olfactory, auditory, tactile or visual cues) present in the self-administration environment, and these stimuli are not paired contiguously with drug infusions nor are contingent upon a response. Owing to their predictive nature for drug availability, these stimuli “set the occasion” for engaging in reward seeking (i.e., lead to the initiation of responding). Except for using context or noncontingent presentation of discriminative stimuli as a cue manipulation, these models are identical to the discrete cue (CS) model in terms of the training and experimental sequence, with conditioning followed by extinction in the absence of the drug and its associated context and, subsequent, initiation of reinstatement tests in the presence of the drug-associated context. Multiple contextual reinstatement procedures exist.

The basic model utilizes differential reinforcement of behavior in the presence of discriminative stimuli. In this procedure, during self-administration learning, responses at the operandum are reinforced by ethanol only in the presence of this stimulus. In the absence of the stimulus (or, in presence of a distinctly different cue) responses remain non-reinforced.

A second frequently employed contextual conditioning model utilizes distinct environments that provide compound contextual cues (i.e., the concurrent presence of olfactory, auditory, tactile, and visual cues). Here, responding is reinforced by an ethanol reinforcer in one context. Drug-reinforced responding then is extinguished in a second distinctly different environmental context. Subjects subsequently tested in the second (i.e., nondrug-paired) context show low ethanol-seeking because the behavior has been extinguished in this context. In contrast, animals tested in the first (drug-paired) context show reactivation or renewal of responding at the previously ethanol-associated active operandum.

A variant of the contextual reinstatement procedure above is based on learning of a conditioned place preference (CPP) for an environment paired with the experience of ethanol. Following extinction of CPP, accomplished by pairing vehicle rather than ethanol with the environment, reestablishment (technically termed renewal or reactivation of CPP), is produced by an ethanol injection. This model can also be employed to study reactivation of an acquired CPP following an imposed period of abstinence, and therefore can be adapted for studies of relapse (i.e., a condition typically preceded by a period of abstinence).
This section will begin with a historical account of the development and evolution of animal models of alcohol self-administration and reinforcement, beginning with the original two-bottle, water-ethanol, free-choice drinking model and proceeding to contemporary models that sustain remarkably high levels of voluntary ethanol intake even in animals without a genetically determined high ethanol preference. In all models, ethanol is said to serve as a drug reinforcer if it is consumed in greater quantities than a concurrently offered nondrug fluid such as water or sweet solution, and if the intake of ethanol is sufficient to produce pharmacologically meaningful BALs, that is, BALs known to exert minimum effects indicative of positive reinforcement such as facilitation of brain stimulation reward, stimulation of locomotor activity, or mild anxiolytic actions (11, 12). Although spontaneous alcohol consumption in animals is generally low except in subjects with a genetic drinking preference, efforts over the past several decades have been successful in establishing that, with appropriate “acclimation” to the aversive taste or smell, ethanol readily becomes a reinforcing substance that is self-administered by several species including monkeys, rats, and mice (e.g., (13, 14)). This review will focus on model development and studies in rats, as these represent the most ubiquitously used species for studies of ethanol reinforcement. Similarly, because of constraints in scope and space, this review will focus largely on insights gained from studies with standard laboratory rats rather than lines genetically selected for ethanol preference.

Although not an “initiation” model per se, the free-choice water/ alcohol drinking procedure, originally introduced by Richter and Campbell (15), was the first to be widely implemented for studies of voluntary ethanol intake. In this procedure, rats are allowed to drink freely from bottles containing water or ethanol solution (at one or several different concentrations). Ethanol intake and preference (ethanol/total fluid intake) are then determined during 24-h unlimited access, or under limited access conditions of varying duration, depending on the experimental objective. Rats genetically selected for ethanol preference consume ethanol under these conditions at pharmacologically significant levels without the need for special initiation procedures (e.g., (16)). However, in standard laboratory rats, the total amount of ethanol consumed in the free-drinking model is typically below the metabolic capacity of rats and not sufficient to provide confidence that the ingestion of ethanol is pharmacologically motivated.

To overcome the limitations of the free-drinking model and to enhance voluntary ethanol intake, several procedures have been developed that involve gradual acclimation to ethanol as well as habituation to its aversive taste. Early attempts sought to
accomplish this by mixing alcohol solutions with flavors or tastants (so-called taste adulteration procedures), or by enhancing animal’s motivation to drink by placing them on food deprivation schedules. However, increases in drinking induced by these means generally are not sustained when the tastant, fluid, or food restrictions are removed. Moreover, these manipulations do not provide compelling evidence that ethanol intake is maintained by pharmacological motivation rather than factors related to nutritional need, thirst, or taste (4, 17). Another common means to acclimate rats to ethanol utilized access to gradually increasing ethanol concentrations up to 6% (18, 19); however, intake of these low ethanol concentrations generally does not result in pharmacologically meaningful BALs. This said, it is important to keep in mind, as pointed out astutely by McClearn (20), that a model with utility for one aspect of alcoholism may be entirely irrelevant or even misleading with respect to another, and the value of a particular model must be evaluated and validated with respect to the particular facet of alcohol-related physiological or behavioral changes the model is designed to address.

A significant step toward demonstrating a reinforcing action of ethanol was the implementation of operant conditioning procedures where presentation of small volumes of alcohol was contingent upon the performance of a distinct behavioral response such as pressing a lever. This strategy is frequently employed in the context of the models described below.

This acclimation procedure makes use of the increase in water intake that typically follows feeding (i.e., postprandial drinking). Food-deprived animals are given access to increasing concentrations of ethanol along with their daily food ration during short daily sessions (21). Once postprandial ethanol intake stabilizes, animals continue to drink ethanol in the absence of food and without further food restriction, albeit at reduced levels. Prandial procedures have been developed to initiate operant responding for ethanol, but have also been adapted for increasing two-bottle choice ethanol intake (22–24).

Indeed, only mixed success has been achieved with the procedures described above. Following removal of tastants, ethanol intake generally declines toward pre-tastant exposure levels within a few weeks (9, 21). Similarly, after prandial procedures, ethanol intake decreases below pharmacologically meaningful levels once food restriction is removed (23, 25, 26).

In what follows, models will be described that have been adequate and often highly successful in generating voluntary levels of ethanol intake at pharmacologically relevant levels. These models represent the most common ethanol initiation procedures in contemporary ethanol research. Some of these methods have been developed decades ago but are still widely in use, albeit often in
modified form. Others have been developed more recently and are finding increasingly more widespread application.

These are variants of the taste adulteration procedure with the difference that the tastant, typically a sweetener, is not abruptly but gradually removed (faded) from the ethanol solution. The procedure was originally developed by Samson and colleagues in the mid-1980s using sucrose as a tastant (10, 27), but variants using noncaloric sweeteners such as saccharin (e.g., (28, 29)), or more recently, “SuperSac” – a 3.0%/0.125% (w/v) glucose/saccharin solution that generates higher rates of ethanol intake than other sweet solution fading procedures – have begun to be employed based on early work by Valenstain (30) and further developed by Walker and colleagues (31). Sweet solution fading is likely the most widely used procedure to establish ethanol-reinforced operant responding. Reliable ethanol intake is maintained following removal of the sweetener and sufficient to generate significant BALs ranging between 25 and 75 mg% in a typical 30-min session (e.g., (28, 29)). Technically, the procedure is designed to establish response-contingent oral self-administration of ethanol or water in a two-lever, free-choice operant task. To accomplish this, animals are initially trained in daily short-access sessions (e.g., 30 min) to respond at either of the two levers for small amounts of a sweet solution (e.g., 0.1 mL) reinforcer until stable responding is obtained. Animals then are introduced to a free-choice task in which responses at one lever produce ethanol solution while responses at the other lever result in delivery of water. Technical details of this procedure are described in Box 1. The significance and power of this procedure is that following completion of this initiation procedure, rats will continue to self-administer ethanol without any addition of sweetener and intake is typically maintained at levels that satisfy the criteria of pharmacological motivations, or, in other words, the maintenance of behavior by the reinforcing actions of ethanol per se.

Some investigators have attempted to bypass use of sweet solutions to initiate ethanol intake by providing 10% ethanol as the only available fluid for up to 3 days before operant training commences. However, even here, addition of sucrose or saccharin to increase the reinforcing strength of the ethanol solution, or water deprivation regimens need to be imposed to motivate consumption of ethanol solutions (32). Addition of sucrose, in particular, adds as a complicating factor changes in ethanol absorption by either decreasing gastric emptying or altering ethanol metabolism such that blood or brain alcohol levels equivalent to other procedures are not achieved (e.g., (33)). This is a major factor having led many investigators to employ saccharin (28) or a saccharin–polycose mixture (34) rather than sucrose for the sweet solution fading procedure discussed above.
This procedure takes advantage of the observation that following brief periods of forced abstinence, in which ethanol and water are switched daily as the sole source of fluid over a period of time, voluntary ethanol intake gradually increases (35–38) possibly akin to the alcohol deprivation effect discussed later in this chapter (see Box 2 for technical details). This methodology, therefore, may evolve into a valuable future tool for research on the neurobiological basis of ethanol reinforcement and addiction. What remains unclear at present, however, is whether significant amounts of ethanol intake are maintained once rats are exposed to ethanol self-administration tasks utilizing operant conditioning procedures that require lever responses (see, e.g., (28)).

Very recently, a highly effective novel procedure for the initiation of voluntary ethanol consumption in rats has been developed. This procedure, utilizes “jello shots” consisting of 10% ethanol, 10% polycose, and 0.25% gelatin. With this procedure, rats orally were reported to consume nearly 9 g/kg of ethanol in a 24-h period (approaching levels of ethanol intake in lines of rats genetically selected for ethanol preference), and between 1.5 and 3 mg/kg in 1- and 3-h access periods, respectively (39). Moreover, as measured by microdialysis, brain ethanol concentrations were closely correlated with “jello shot” intake and reached approximately 7 mM following ethanol intake of up to 44 mL after 24 h of access to an ethanol–polycose mixture (40). While these procedures are still under development, the results to date are encouraging, leading to ethanol intakes similar to and exceeding those obtained with many other initiation procedures. More importantly, these procedures eliminate conceptual issues having to do with the impact of “history of reinforcement” associated with the need for water deprivation necessary with many other models to establish initial operant reinforcement maintained by a fluid such as water. On the other hand, the impact of the caloric and sweetener content of polycose mixtures on ethanol reinforcement remains to be determined.

An important consideration for the analysis of alcohol seeking and reinforcement is the distinction between alcohol preference in two-bottle free-drinking tests and behavior maintained by the reinforcing actions of ethanol when the reinforcer is contingent upon a discrete behavioral response.

As outlined above, preference is typically established by determining which of two solutions freely available from drinking bottles – one containing ethanol, the other a nondrug reinforcer – is ingested preferentially. This task requires little learning and work. Response-contingent availability of ethanol, on the other hand, requires completion of learned sequences of behavior under
restrictive conditions in order to gain access to alcohol. In this situation, the amount of ethanol that is consumed depends on the amount of work an animal is willing to expend. Thus, rather than “preference,” these tests probe an animal’s “motivation” or degree of persistence in the effort to obtain alcohol at a particular time and a particular set of circumstances. The importance of this distinction has been illustrated in an early series of studies that examined the extent to which alcohol serves as a reinforcer across lines of rats genetically selected for high vs. low alcohol consumption in two-bottle preference tests. Surprisingly, in a line of rats selected for alcohol avoidance in preference tests (Indiana alcohol nonpreferring NP rats), ethanol actually served as a potent reinforcer when offered response contingently (41–43). Moreover, when the number of responses required for alcohol delivery was progressively increased until rats ceased responding (i.e., reached the break point in a progressive ratio schedule), alcohol nonpreferring NP rats were willing to work harder than rats of a genetically selected high alcohol drinking line (HAD rats) that shows high spontaneous alcohol intake in two-bottle preference tests (41). Similarly, a reduced “willingness” to work for alcohol under reinforcement contingencies with higher response requirements has been observed in rats of another genetically high alcohol preferring line, the Finnish AA rats (44). Only in one alcohol preferring line (Indiana P rats) was response-contingent alcohol intake in operant self-administration tests consistent with high alcohol consumption in two-bottle preference tests typically shown by this line of rats.

These findings demonstrate that the factors that determine whether alcohol will come to serve as a preferred substance in preference tests differ from those that mediate the incentive value of alcohol as measured by the amount of work that an animal will expend to gain access to the drug. Results such as these illustrate the importance of studying the motivating strength of alcohol as a factor in the proclivity to develop compulsive alcohol-seeking and abuse patterns, and suggest that this distinction may be an important one to consider in the development of both pharmacological and non-pharmacological treatment strategies for alcohol abuse.

An ethanol self-administration model that has found increasing application (45–47) dissociates ethanol-reinforced consummatory behavior (i.e., free 10% w/v ethanol drinking) from appetitive ethanol seeking (i.e., behavioral responses directed at obtaining ethanol induced and maintained by the incentive-motivational effects of ethanol-associated contextual cues). This procedure is essentially a modification of chained schedules of reinforcement that are widely employed for studies of psychostimulant-maintained conditioned and primary reinforcement. In this
“seeking-taking” procedure, rats are subjected to an “appetitive” phase during which they must complete a set of responses at a lever in an ethanol-associated context (i.e., operant self-administration chamber) during which time lever responses do not make ethanol available. Completion of a given number of responses within a specified time results in retraction of the lever and initiation of a “consummatory” phase by presentation of a sipper tube containing ethanol solution from which rats are allowed to freely drink. Thus, responding during the appetitive phase provides a measure of the day-to-day strength of animals’ motivation to initiate and engage in ethanol-seeking behavior when exposed to the ethanol-predictive stimulus environment, and can also serve as a measure of the desire to drink (48, 49). Behavior during the consummatory phase on the other hand, provides a measure of actual ethanol consumption as an index of the acute reinforcing strength of ethanol. In this model, seeking and consumption are not necessarily correlated. More importantly, this model allows for the investigation of neural mechanisms that control seeking or approach responses (i.e., ethanol “craving”) vs. mechanisms that control the reinforcing effects of ethanol. From a treatment drug development perspective, this model provides an effective tool to evaluate the relative efficacy of potential drug treatments for preferential therapeutic actions on ethanol craving vs. actual ethanol intake (e.g., (50, 51)).

An alternative approach to studying the reinforcing effects of ethanol are conditioned preference tasks (for review, see, e.g., (52, 53) and Chap. 6 in this volume). Here, the reinforcing value of ethanol is measured by the degree to which animals seek and spend time in an environment (place preference) or prefer a flavored solution (taste preference) that has previously been paired with systemic administration of alcohol. Thus, these procedures rely on the conditioning of an environmental (or orosensory) context with the hedonic effects of alcohol, and the extent to which the animal prefers the ethanol-associated environment or tastant while not under the influence of ethanol provides a measure of the reinforcing strength of alcohol. The basic principles of conditioned preference procedures are described in Chap. 6 of this volume. However, certain unique considerations apply to the implementation of these procedures in the case of ethanol. With a single pairing, alcohol often induces conditioned aversion rather than preference in rodents, particularly in standard laboratory rats (54–57). Repeated exposure to alcohol, however, attenuates these aversive actions (58, 59) and can result in conditioned place preference (60, 61), suggesting that tolerance to the aversive effects of alcohol is an important factor leading to the acceptance of alcohol and possibly its abuse. Indeed, more rapid development of tolerance or inherited lower sensitivity to the postingestional
aversive effects of alcohol may contribute to genetically determined alcohol preference. Alcohol produces taste aversion in rats of the genetically selected alcohol nonpreferring NP line but not in the alcohol preferring P line. While high doses of ethanol also induce taste aversion in P rats, this aversion is stronger and more persistent in the NP line (62). On the other hand, both P and NP rats show alcohol-induced place aversion without apparent line differences (63). Thus, genetic factors appear to account for reduced sensitivity to some, but not other aspects of ethanol’s aversive properties in the P rats. In contrast to rats, most mice develop a clear preference for environments associated with the effects of alcohol (64). This effect is greatest when animals are exposed to the alcohol-associated environment only for a short period of time after ethanol administration (65), suggesting that ethanol exerts reinforcing actions, particularly, during the early rising phase of BAC.

To study the conditions and degree to which alcohol serves as a reinforcer, powerful behavioral analyses derived from the field of behavioral economics have been employed. For example, rats given concurrent access to a sucrose solution and a solution containing ethanol plus sucrose, show and maintain preference for alcohol over sucrose even when the work output required to gain access to alcohol-containing sucrose solution was selectively increased (66, 67). However, when both solutions contained only sucrose, an increase in the response requirement for one of the solutions shifted preference toward the solution that required only low work output. In the terminology of economics, the degree to which changes in price affect changes in demand depends on the availability of substitutable commodities. For example, demand for a particular commodity that is widely available is elastic, while demand for a rare, difficult-to-obtain commodity such as a life-saving drug or organ is inelastic. Thus, these findings illustrate that ethanol represents an inelastic commodity because sucrose was a poor substitute for ethanol and, by inference, that ethanol’s reinforcing effects depend on its pharmacological actions (66). One can also conclude from this outcome that the reinforcing effects of sucrose and ethanol differ in nature and that the reinforcing effects of sucrose and ethanol are regulated by different neural mechanisms.

Important also is the role of environmental factors that either induce alcohol-seeking and consumption, or control its maintenance once drinking has begun. With respect to the latter, several variables are known to alter behavior maintained by alcohol, presumably by influencing its “valence” as a reinforcer. These include changes in the palatability of the alcohol solution (10), the concurrent availability of another palatable reinforcer (68), the concentration of alcohol (69, 70), and whether access to
ethanol is unlimited or restricted in time (71). One variable, the price of alcohol, appears particularly important. For example, using a “closed economy” (another economic concept for the analysis of choice behavior) in which all daily nutritional and fluid supplies as well as alcohol were available only on the basis of work (i.e., performance of an operant response), researchers have compared the effect of varying the “cost” of alcohol on intake in rats genetically selected for alcohol preference (P rats) to genetically heterogeneous rats (72). When the concentration of an ethanol solution or the number of lever presses rats had to emit to obtain alcohol was varied, both increases in ethanol concentration and reductions in work requirements enhanced alcohol intake. Consumption was greatest, however, when the “cost” per reinforcer was lowest, that is, under conditions that simultaneously held the response requirements low and ethanol concentrations high. This relationship was similar in alcohol preferring (P) and genetically heterogeneous rats. Overall, however, P rats worked harder for the alcohol reinforcer and consumed greater volumes of ethanol than rats without a genetic ethanol preference. Thus, whereas genetic predisposition toward high alcohol intake is associated with greater motivation to work for alcohol, making alcohol a qualitatively more potent reinforcer (i.e., increasing alcohol concentrations), alcohol intake is essentially controlled by the same environmental variable (i.e., “cost”) in strains with different innate ethanol preference. Increasing the concentration of alcohol not only enhances alcohol intake but also makes alcohol self-administration more persistent (69). Moreover, lengthening the time interval between responding and delivery of alcohol—a procedure that normally reduces responding for a reinforcer—concomitant increases in alcohol concentrations made alcohol-seeking actually more resistant to such time-dependent disruption. Models such as these are valuable because they shed light on motivating factors that regulate alcohol-seeking and consumption. In particular, based on these findings one would predict that alcohol abuse involving more potent forms of this drug (e.g., spirits vs. beer) is less amenable to therapeutic intervention. A possible beneficial clinical strategy suggested by these findings is to substitute beverages of lesser potency in order to create a more sensitive window for therapeutic intervention (69).

Studies concerned with the influence of environmental factors that contribute to the initiation of or enhance early alcohol drinking have focused on the role of stress associated with social interactions or rearing conditions. Earlier studies have revealed that emotional stressors such as intermittent social separation during adolescence increase alcohol consumption of rhesus monkeys in adulthood (73). Similarly, social stress associated with early social
Alcohol Self-Administration

separation (74) or subordinate social status in rats can induce alcohol drinking (75). Maternal separation in rhesus monkeys for the first 6 months of life also increases the likelihood to engage in alcohol consumption later in life compared to monkeys reared by their mothers (76). More recent research has confirmed and extended these findings by showing that after the establishment of social hierarchies, fight-stressed submissive mice exhibit increased alcohol consumption (77). Moreover, a stressor consisting of a signal predicting an aversive loud noise increased alcohol self-administration in mice (78). In a systematic investigation of the relationship between the quality of stress and the motivation to drink alcohol in rats, chronic isolation housing but not chronic intermittent immobilization stress increased alcohol consumption, suggesting that social isolation stress, in particular, elicits alcohol-seeking behavior (79–81).

3. Modeling Ethanol Abuse and Addiction States

3.1. Animal Models of Ethanol Self-Administration in Dependent Subjects

A major procedural and conceptual issue for experimental studies of alcohol addiction is the need for alcohol dependence induction procedures, because with the exception of several genetically selected rodent lines (e.g., (82–87)), rats do not voluntarily consume sufficient amounts of ethanol to produce marked intoxication, much less dependence. The most frequently employed dependence induction methods all involve some form of involuntary or forced ethanol administration, including forced high-dose aggressive (88) or more physiologically relevant (89) intragastric ethanol intubation, forced ethanol vapor inhalation using continuous (90) or intermittent procedures resembling the timing of intake patterns in alcoholics (91), and presentation of an ethanol liquid-diet dissolved in a nutritional vehicle as the animal’s sole source of fluid and nutrition (91, 92). Moreover, using such involuntary dependence induction procedures, it has traditionally been difficult to demonstrate that ethanol dependence or withdrawal increases ethanol consumption in animals (for review, see (93–95)). More recently, however, positive results have been obtained in rats given the opportunity to associate ethanol intake with the alleviation of withdrawal symptoms over multiple episodes of forced abstinence (96). Indeed, it is now well established that with appropriate ethanol-initiation procedures, rats will self-administer significant amounts of ethanol during acute ethanol withdrawal and transiently maintain elevated levels of ethanol intake often long after detoxification and withdrawal (97, 98). These procedures now permit thorough investigation of neuroplasticity associated with chronic high-dose ethanol in mediating the transition from casual ethanol use to dependence the maintenance of an addicted state.
For example, early studies have revealed that dependent rats withdrawn from an ethanol liquid diet regulate their ethanol consumption in a manner that restores and maintains withdrawal-induced deficiencies in extracellular dopamine efflux at pre-withdrawal levels (99). Similarly, an intragastric ethanol challenge dose was shown to restore deficient dopamine release in the ventral striatum and to reverse ethanol withdrawal symptoms (100).

The emerging problem of binge drinking with significant adverse health consequences presents potentially severe problems leading to substantial cognitive and other health detriments in adulthood. Binge-like ethanol self-administration is defined by the National Institute on Alcoholism and Alcohol Abuse (NIAAA) as an excessive pattern of alcohol drinking that produces BACs greater than 0.8 mg% within a 2-h period.

In order to conduct meaningful pharmacological studies within model of binge drinking in rats, it is imperative to confirm that animals are reaching the criterion BAC level of 0.8 mg% within a 2-h period that poses a significant challenge in the context of limited-access ethanol self-administration procedures. Adolescents that consume alcohol do so during periods of neural plasticity and maturation, which likely affects their mental health and performance in adulthood. The development of valid binge drinking models is still in its infancy, but important strides have been made toward establishing appropriate animal analogs of this drinking phenomenon (e.g., (101)).

Recently, advances have been made toward establishing binge-like operant self-administration in male rats that facilitates multiple bouts of voluntary high alcohol intake per day during adolescent development (34). In this procedure, juvenile animals (28–42 days of age) undergo multiple short periods of access to sweetened alcohol solution (or sweetened water in controls) in the presence of ad libitum food and water during overnight operant self-administration sessions. These rats voluntarily self-administer on average approximately 4.0 g/kg ethanol across six 30-min “mini-sessions” permitted during 12-h cycles over 14 consecutive days. Rats subsequently tested in adulthood for alcohol self-administration before and after dependence induction by chronic intermittent alcohol vapor exposure showed little change in baseline drinking, but exhibited excessive dependence-induced drinking with BALs substantially higher in rats with a history of alcohol binge drinking during adolescence. Moreover, these rats also showed a tendency toward higher anxiety-like behavior several weeks after removal from ethanol vapor inhalation, an effect not evident in dependent rats with a history of drinking a control solution only during adolescence. Thus, as suggested by this animal model, adolescent binge drinking may have long-lasting effects on mental health.
effects on vulnerability to alcohol effects, and possibly stress-related disorders in adulthood (34). Other ethanol binge-drinking models exist that utilize, for example, rats selectively bred for high alcohol preference, and the binge-drinking induction procedure in these animals differ in important respects from those described above (102) and are discussed in detail below.

Other compelling evidence for adolescent vulnerability comes from a modification of the CPP model (103). Adolescence is a transitional period during development that is associated with a greater likelihood of addiction to drugs than any other age. One possibility for this observation is that learned associations between the rewarding experience of drugs and drug-related cues may produce greater motivational salience, and thus are more difficult to extinguish. Using an unbiased place-conditioning paradigm with two doses of cocaine (10 or 20 mg/kg), the authors show that adolescent rats require in excess of 75% more extinction trials than adults to extinguish cocaine place preferences. Furthermore, once extinguished, adolescents display a greater preference for a previously cocaine-paired environment upon drug-primed reinstatement of cocaine seeking compared with adults. These results suggest that adolescent vulnerability to addiction involves robust memories for drug-associated cues that are difficult to extinguish. Therefore, drug-addicted adolescents may have a higher risk of relapse than adults, leading to greater prevalence of addiction in this population.

A well-described phenomenon in the alcohol literature is a marked increase in ethanol consumption that follows both short (≥24 h) and long (≤1 week) periods of alcohol deprivation ((35, 104, 105) for review). The alcohol deprivation effect (ADE) is considered a measure of the motivation to seek and consume alcohol (35, 106, 107), loss of control (108), or relapse (85, 109). Similarities exist between the alcohol deprivation effect in animals and human alcohol abuse such as enhanced ethanol consumption after abstinence in social drinkers (110), and the “loss of control” phenomenon surrounding the first drink after abstinence in alcoholics (111–113). With repeated cycles of deprivation and increased deprivation periods, this phenomenon becomes resistant to manipulations of ethanol concentration, taste, and environmental factors ((114); for review, see (2)). The increase in ethanol intake produced by repeated deprivation outlasts long abstinence phases (114) and may become irreversible (108). Extending these observations, access to multiple concentrations of ethanol and exposure to multiple deprivation cycles can partially overcome the genetic predisposition of NP, LAD-1, and LAD-2 rats for low alcohol consumption. These findings support the unexpected conclusion that genetic control of low alcohol consumption in rats is not associated with the inability to display an ADE (102). Overall, these observations support the view that the ADE provides an effective
Ample evidence exists to show that alcohol-associated stimuli or events can evoke drug desire that may lead to the resumption of drinking in abstinent alcoholics (115–121). Therefore, a number of conditioning models have been established to mimic and reproduce this effect in animals to study the neurobiological basis of this phenomenon.

In the context of the drug addiction literature, conditioned reinstatement refers to the resumption of extinguished instrumental responding induced by noncontingent exposure to a drug-related cue (for review see (122–124)). The most widely employed animal model of craving and relapse use is the extinction/reinstatement model. This model has perhaps been most extensively applied for investigations of the significance of environmental stimuli conditioned to the reinforcing actions of drugs of abuse or alcohol in the relapse process, but is also widely employed to study the effects of stress and drug priming on the resumption of drug seeking. It should be noted that the validity of this model has not gone unchallenged, and the literature is replete with both critical (e.g., (125)) and supportive (126) appraisals of the procedure. Taking into account both the limitations and advantages of this model, it is perhaps safe to say that the reinstatement model is likely the most valuable procedure available to date both for investigating the neural basis of craving and relapse and for evaluating the potential of treatment drugs for the prevention of craving and relapse.

Both response-contingent and nonresponse-contingent exposure to ethanol-associated contextual stimuli (or an ethanol-paired environmental context) very reliably elicits recovery of extinguished responding at a previously ethanol-paired lever without further alcohol availability (127–133). The conditioned effects of these stimuli are remarkably resistant to extinction in that recovery of ethanol-seeking does not diminish when these cues are presented repeatedly under non-reinforced conditions (134), and can even increase in magnitude over time, a phenomenon that has been referred to as “incubation of craving” (124, 135). Consistent with clinical findings, reinstatement induced by alcohol cues is sensitive to reversal by opioid antagonist administration (127, 130–132, 136). This is so, because in alcoholics, naltrexone attenuates cue-induced craving (137, 138) and reduces relapse rates (139, 140). Moreover, excellent correspondence exists
between neural mapping data in animals (141, 142) and functional brain imaging studies in drinkers (e.g., (143–149)) with respect to the neurocircuitry activated by alcohol cue manipulations that, in humans, is closely linked with self-reports of craving. Conditioned reinstatement of ethanol-seeking in animals, therefore, has good predictive validity as a model of craving and relapse linked to alcohol cue exposure.

It is well established that small doses of drugs of abuse including ethanol, rather than reducing drug desire, elicit further drug craving (e.g., (112, 150)). Moreover, in alcoholics, the first drink after abstinence if often associated with “loss of control” leading to severe intoxication and return to continued alcohol abuse (112). This priming effect can readily be demonstrated in animals and provides an effective means to experimentally study the neural and molecular basis of the “loss of control” phenomenon that frequently is the heart of the relapse process in alcoholics or people at risk for alcohol abuse (e.g., (2)).

Stress has an established role in alcohol abuse in humans and is a major determinant of relapse (151–157). The significance of stress in drug seeking behavior is well documented also in the animal literature. Physical, social, and emotional stress can facilitate acquisition or increase self-administration of ethanol in rodents and nonhuman primates (75, 76, 78, 158).

Stress consistently elicits reinstatement of ethanol seeking in drug-free animals, with footshock being the predominant experimental model of stress-induced relapse ((124) for review). Interactive effects of stress and drug-related cues on drug seeking were modeled in an original series of studies by testing for the concurrent effects of footshock stress and an ethanol-associated conditioned stimulus (CS) on reinstatement (80, 81). Rats were trained to self-administer ethanol and reinforced responses were paired with brief presentation of a simple (i.e., noncompound) CS. After withdrawal, ethanol-reinforced responding was extinguished, and the reinstatement of alcohol-seeking was studied under three conditions: (a) during response-contingent presentation of the CS alone, (b) after exposure to 10 min of intermittent footshock stress alone, and (c) during response-contingent presentation of the CS following exposure to footshock stress. Under these conditions the ethanol CS and footshock, when presented alone, produced only threshold effects on alcohol-seeking behavior. However, the ethanol CS elicited strong responding in animals that had been subjected to footshock stress before the session. Thus, interactive effects between stress and drug-related cues exacerbating drug seeking can readily be demonstrated in animal models. Indeed, the existence of such interactive effects has recently been confirmed with another drug of abuse and in the context of a pharmacological
stress manipulation, using yohimbine. This $\alpha_2/\alpha_1$ noradrenergic receptor antagonist with anxiogenic action – that has found increasing application as an alternative stressor in animal models of drug (159, 160) and reward (161–163) seeking.

In humans, the severity of alcohol withdrawal increases with the number of previous withdrawal episodes (164–166). Moreover, the severity of craving induced by alcohol cues is positively correlated with the history and degree of ethanol dependence (119, 167, 168). Considering the significance of a history of ethanol dependence in relapse risk (169) it stands to reason that a dependence history will have an impact on other “triggers” for ethanol-seeking. To examine this, a group of animals was made dependent via an ethanol vapor inhalation procedure following acquisition of operant ethanol self-administration. During the last 2 days of dependence induction, the rats were removed from the vapor chambers for 12 h/day, but allowed to self-administer ethanol paired with response-contingent presentation of an ethanol-paired CS. Rats then were withdrawn from ethanol, and subjected to reinstatement tests 3 weeks after termination of ethanol vapor exposure. In these previously dependent rats, response-contingent presentation of the ethanol CS after footshock produces synergistic effects on ethanol-seeking by more than doubling the interactive effects of the stress and ethanol-paired stimuli in a synergistic manner compared to their effects in nondependent rats (81).

The significance of a dependence history with respect to its role in the effects of alcohol cues and stress is illustrated further by the finding that previously ethanol-dependent rats not only show enhanced reinstatement induced by footshock stress, but also by a stimulus (CS) conditioned to footshock stress. Again, both significant individual and interactive effects of the stimulus conditioned to ethanol reward and the conditioned stressor were observed in rats with a history of ethanol dependence, but not in rats without such a history (81). An important point to be gleaned from these findings is that risk factors for relapse have traditionally been studied in isolation and it has been difficult to establish an unequivocal predictive causal relationship for either stress, ethanol cues, or dependence history (e.g., (125) as necessary and sufficient determinants for relapse to actually occur). However, abstinent individuals are likely to be frequently exposed to multiple environmental risk factors, while at the same time suffering various degrees of anxiety or mood-dysregulation, caused by enduring neuroadaptive changes as a result of chronic drug abuse. Consistent with this scenario, the interactive findings described above illustrate that the probability of relapse is likely to vary as a function of the number of risk factors operative at a given time and under particular circumstances, with relapse
occurring when the sum total of these pressures reaches a critical mass or threshold.

Place conditioning procedures permit examination of neuropharmacological substrates involved in the acquisition and expression of the conditioned reinforcing effects of ethanol. It is important to note that in this model, manipulations that interfere specifically with the expression of conditioned place preference (CPP), once acquired, provide information on the neural and motivating forces of the conditioned rewarding effects of ethanol leading to ethanol-seeking or “craving.” In contrast, interference with the acquisition of a CPP is relevant for the understanding of neural mechanisms mediating the acute reinforcing effects of ethanol as inferred by the establishment of Pavlovian associations between the ethanol reinforcer and the place-conditioning environment and, therefore, of lesser importance for the understanding of factors that drive the desire to obtain ethanol (craving) and relapse-like behavioral responses. Like the ethanol “seeking-taking” model, studies of the expression of ethanol CPP do not generally impose a period of abstinence and, thus, have perhaps some limitations with respect to providing a valid measure of craving and relapse. Nonetheless, the degree of preference for a previously ethanol-paired environment provides an index of the strength of ethanol seeking associated with the incentive-motivational effects of the previously alcohol-associated stimulus context.

It is important to note that the CPP model generally employs forced administration of ethanol. The reinforcing actions of ethanol under these conditions may differ from those associated with voluntary oral self-administration. As a result, the strength or nature of associations that are formed between ethanol and environmental stimuli may differ in CPP vs. self-administration procedures. Importantly, as well, the number of learning trials in models of ethanol-seeking that involve the conditioning of the effects of self-administered ethanol with environmental stimuli typically is considerably greater than in the CPP procedure. Associations that are produced between specific environmental stimuli and ethanol are therefore likely to be weaker in the CPP model. Due to these differences, the expression of conditioned ethanol-seeking responses may be differentially sensitive to pharmacological manipulation in CPP vs. self-administration models. Lastly, it cannot be ruled out that different neural substrates are involved in contextual conditioning in the case of CPP as opposed to stimuli associated with active self-administration.

The increase in voluntary alcohol consumption after forced deprivation (alcohol deprivation effect (ADE)) discussed above is also widely implemented as a tool to study the neurobiology of alcohol craving and relapse (170). Similarities exist between the
alcohol deprivation effect in animals and certain characteristic of human alcohol abuse, including enhanced ethanol consumption after abstinence in social drinkers (110), binge drinking in which alcohol is followed by a period of abstinence (171, 172), and aspects of the “loss of control” phenomenon surrounding the first drink after abstinence in alcoholics (111–113). In view of these similarities, this procedure appears to have appropriate face validity as a model for certain aspects of the relapse process. Indeed, many consider the ADE a “true” model of relapse compared to reinstatement models that perhaps more accurately model craving processes. Moreover, findings that pharmacological agents that suppress ethanol intake and reduce the likelihood of relapse in humans effectively attenuate the alcohol deprivation effect in animals (see below) have added support for the predictive validity of this procedure as a model of relapse. The alcohol deprivation effect can be demonstrated under both limited and unlimited-access conditions and with both home-cage free drinking and operant self-administration models (e.g., (170)), although it appears most robust in the two-bottle free-choice procedure using genetically alcohol preferring lines, or after extensive repeated cycles of intoxication and deprivation. Nonetheless, given the stability and robustness of the alcohol deprivation effect under many experimental conditions, this phenomenon may have great utility for the exploration of diverse variables contributing to the relapse process.

Early experiments have revealed that rats show marked increases in voluntary ethanol consumption after periods of forced abstinence (35, 114, 173, 174). This so-called alcohol deprivation effect has since been confirmed in mice (175), rats (108, 176), monkeys (114, 177), and human social drinkers (109), and is well established as a robust and reliable phenomenon in animal models of alcohol drinking.

The alcohol deprivation effect can be readily demonstrated in nondependent animals and may provide a potentially valuable model for understanding of changes in the reinforcing efficacy of ethanol that occur with abstinence (110, 178). More importantly, however, under appropriate conditions, this phenomenon appears to become resistant to manipulations of ethanol concentration, taste, and environmental factors (179) and, therefore, may prove useful as a model for compulsive alcohol-seeking behavior and loss of control that characterize substance dependence on alcohol. Studies that have characterized the alcohol deprivation effect in rats given long-term (8–24 months) continuous free access to different concentrations of ethanol and water, interspersed with deprivation periods of varying lengths, indicate that ethanol consumption increases significantly over baseline as a result of deprivation (108, 114) reaching levels of intake similar to those in rats selectively bred for alcohol
preference (114). The increase in ethanol intake associated with alcohol deprivation was characterized not only by enhanced preference for ethanol over water but preference for higher ethanol concentrations (>10% v/v) and resistance to change by altering the palatability of the ethanol solution by either quinine or sucrose, or manipulation of environmental and social conditions such as isolation or changing dominance hierarchies (108). Moreover, ethanol deprivation under these exposure conditions revealed a behavioral withdrawal syndrome, as measured by lowered thresholds of footshock reactivity, which reached maximum on the second day of abstinence and persisted for up to 5 days post-ethanol (180). Finally, the alcohol deprivation effect appears to outlast long abstinence phases (114). Indeed, it has been suggested that this effect is irreversible since it remained unaltered after 9 months of abstinence (114, 181). In particular, the loss of reversibility of this effect and the reduced adaptability of ethanol-seeking behavior in response to environmental or taste manipulations suggests that these procedures may provide an effective model to study mechanisms underlying specific aspects of ethanol-maintained addictive behavior and loss of control.

The search for neural substrates, genetic, epigenetic, environmental, and social determinants of craving and relapse has generated an ever-expanding literature that will be beyond the scope of the present review. In particular, genetic factors have received much attention in studies with rodent lines genetically selected for high ethanol drinking and preference. Outstanding reviews on these topics have been written by Rodd and colleagues (84) who focus on research with several lines of ethanol-preferring lines and on “spontaneous recovery” as an alternative to the more commonly employed conditioned reinstatement procedure, by Heilig and Egli (182) as well as Koob et al. (183) who take up the important issue of novel pharmacological treatments for alcohol dependence, by Weiss and Porrino (1) who discuss the neurobiological basis responsible for persistent alcohol seeking, and by Spanagel (2) who provides an excellent exhaustive account on the status current knowledge of alcoholism as an addictive behavior. These reviews all are highly recommended as supplementary reading, and will complement in the information provided within the context of the present brief review.

Acknowledgments

The author was supported by grants AA10531, AA014351, and AA018010 from the National Institutes of Health/National Institute on Alcohol Abuse and Alcoholism.
References

15. Richter CP, Campbell KH (1940) Alcohol taste thresholds and concentrations of solution preferred by rats. Science 91:507–508
23. Linseman MA (1988) Consumption of alcohol compared to another bitter solution in a limited access drinking paradigm. Alcohol 5:301–303
38. Wise RA (1973) Voluntary ethanol intake in rats following exposure to ethanol on various schedules. Psychopharmacology 29:203–210
63. Scheckter MD (1992) Locomotor activity but not conditioned place preference is differentially altered by moderate doses of ethanol administered to P and NP rats. Alcohol 9:185–188
64. Cunningham CL, Nichus JS, Noble D (1993) Species difference in sensitivity to ethanol’s hedonic effects. Alcohol 10:97–102
on ethanol. Brain Res Mol Brain Res 50: 221–229


stimuli in rats. Neuropsychopharmacology 27:391–399


of stress, drug abuse, and development, Bethesda, MD, 2000


Chapter 6

Place Conditioning

Christopher L. Cunningham, Peter A. Groblewski, and Charlene M. Voorhees

Abstract

Place conditioning is a form of stimulus–outcome learning that is commonly used to draw inferences about the rewarding and aversive effects of psychoactive drugs. This chapter focuses primarily on methodological issues that arise in the implementation and interpretation of place-conditioning studies. A description of the basic procedure is followed by a discussion of several key methodological issues, including compartment configuration, apparatus bias, stimulus selection, temporal parameters (interstimulus interval, trial duration, intertrial interval), experimental design and controls, dependent variables, and locomotor activity. Consideration is then given to methodological and interpretative issues that arise when using the place-conditioning procedure to study acquisition versus expression, extinction, and reinstatement of place conditioning. The chapter concludes with a brief discussion of the potential relevance of the place-conditioning procedure for understanding drug seeking and addiction in humans.

Key words: Conditioned place preference (CPP), Conditioned place aversion (CPA), Reinstatement, Relapse, Extinction, Locomotion, Stimulus selection, Configural cues, Conditioning, Drug reward, Drug aversion, Conditioned reinforcement, Drug seeking behavior, Unbiased procedure, Preference test

1. Introduction

Place conditioning is a form of stimulus–outcome learning that is commonly used to draw inferences about the motivational effects of psychoactive drugs. It is based on the observation that animals will learn to approach or avoid spatially distinct environmental cues that have previously been associated with rewarding or aversive drug effects, respectively. In its simplest form, place conditioning is implemented as a two-phase procedure. During the first phase, drug administration is paired with exposure to a unique set of environmental cues on one or more occasions. Exposure to a different set of environmental cues is paired with
vehicle administration. During the second (test) phase, animals are given the opportunity to choose between the two sets of environmental cues by moving between adjacent spatial locations that contain those cues, typically in the absence of the conditioning drug. Greater approach and contact with the drug-paired cue are interpreted as evidence for a rewarding drug effect, whereas withdrawal and avoidance of the drug-paired cue are seen as evidence for an aversive drug effect. Because animals display a preference or aversion for drug-related cues, the place-conditioning procedure has relevance for understanding the general phenomenon of “drug seeking.” Moreover, examination of the learning and motivational processes that underlie place conditioning might improve our understanding of how those processes are involved in the development, maintenance, and elimination of human drug addiction.

There are two major theoretical explanations for place conditioning. Early investigators (1) adopted a two-process theory framework, viewing drug-paired stimuli as conditioned reinforcers (when using rewarding drugs) or conditioned punishers (when using aversive drugs). It was assumed that drug-paired environmental cues acquired conditioned motivational value through a Pavlovian process during the first phase and that choice of a spatial location during the test phase was secondarily reinforced (or punished) by response-contingent exposure to the previously drug-paired cue during the test phase (2). More recently, some investigators have suggested that behavior during a place-conditioning choice test can also be viewed as an instance of “sign-tracking” behavior (3–5). That is, the drug-paired cue might directly elicit a Pavlovian conditioned approach (or conditioned withdrawal) response that does not depend on an instrumental (i.e., action-outcome) contingency. It is very difficult to distinguish between these alternative interpretations experimentally, and it is reasonable to suppose that both of these types of learning might affect performance during place-conditioning tests (6).

This chapter will focus primarily on methodological issues that should be considered in the implementation and interpretation of place-conditioning studies. Detailed discussion of many of these issues as well as summaries of empirical findings can be found in several previously published descriptions and reviews of the place-conditioning technique (7–18).

2. Basic Procedure

2.1. Habituation or Pretest

Many place-conditioning protocols begin either with a habituation session or with a pretest session. The general purpose of a habituation session is to familiarize animals with nonspecific, possibly
stressful aspects of the procedure such as handling, transport to the test room and (vehicle) injection in order to reduce the likelihood that such events will interfere with later learning about the cue–drug relationship. Habituation might also involve exposure to the test apparatus. In some cases, investigators will begin with one or more pretest sessions in which animals have access to the entire apparatus with the stimulus alternatives configured as in the final choice test. The general purpose of such pretests is two-fold. First, a pretest familiarizes animals with the test configuration of the apparatus, reducing the possibility that novelty will interfere with the later expression of a conditioned preference or aversion. Second, it provides a basis for assessing each animal’s initial “bias” for the to-be-conditioned stimuli (CSs). Although such pretesting can sometimes be useful, there are reasons for carefully considering how pretests might impact results and their interpretation. One concern is that preexposure to CSs can interfere with later conditioning, a phenomenon known as latent inhibition (9). Another concern is that an animal’s bias for a particular CS might not be apparent on an initial test, or it may change over time (8), raising questions about the value of using pretest scores when assigning the cue to be paired with drug. These problems and the need for pretesting can be avoided by constructing an apparatus that can be shown to be “unbiased” for the target animal population (see later Sect. 3.2).

2.2. Conditioning Trials

During the conditioning phase, animals are given one or more exposures to each of the stimulus alternatives, preferably in a counterbalanced order. One of the stimulus alternatives (labeled the CS+) is consistently paired with drug whereas the other stimulus is consistently paired with vehicle (labeled the CS−). Because relative novelty can influence approach toward a stimulus during choice tests (12), animals should receive an equal number of exposures to each CS during the acquisition phase of the experiment. Consistent with the principles of Pavlovian conditioning, the strength of place conditioning is positively related to the total number of CS+ conditioning trials (19). When conditioning parameters are optimized, learning can be quite rapid. Four to six conditioning trials of each type are usually sufficient to produce reliable place conditioning, but significant conditioning has also been reported with as few as one (20) or two (19) conditioning trials. Temporal and other parameters that influence the strength of place conditioning are discussed in more detail in Sect. 3.

As an alternative to the standard approach for conducting place-conditioning studies, some investigators have recommended the “reference-dose” procedure (21, 22). Whereas the standard approach typically involves a comparison between drug- and vehicle- paired cues, the reference-dose procedure involves a comparison between cues that have both been paired with drug. For example,
the two cues might be paired with different doses of the same
drug in order to test whether animals can distinguish between
their rewarding effects. It has been suggested that this approach
might be especially useful for determining dose–effect curves and
for revealing dose effects that are difficult to detect using the
standard procedure (22, 23). The reference-dose procedure has
also been used successfully to test effects of a pharmacological
treatment on the acquisition of conditioned place preference
(CPP) (24). A recent empirical and theoretical analysis of the
reference-dose procedure can be found elsewhere (23).

In most place-conditioning studies, the preference test is con-
ducted 24 h or later after the last conditioning trial by offering
the animal a choice between compartments that contain the drug-
paired and vehicle-paired CSs. The exact configuration of these
compartments varies across studies (see Sect. 3.1) and the transi-
tion between compartments might involve traversing a “neutral”
compartment. Preference tests are most often conducted under
drug-free conditions (or after a vehicle injection) in order to avoid
interpretative complications created by sensory–motor drug
effects. In some cases, however, the purpose of the study might
be to examine effects of a specific drug pretreatment manipula-
tion (e.g., injection of a drug antagonist or agonist). Test session
durations typically range between 10 and 30 min, though longer
durations are sometimes used. Long test durations are typically
less useful when working with rats because they tend to become
relatively inactive after 20–30 min. Mice, however, remain active
over longer periods of time and long-duration test sessions (i.e.,
60 min) have been found to be informative in some cases (2, 25).

Place-conditioning chambers have been constructed in many
different configurations using a wide variety of cues. Most often,
place conditioning has been successfully reported using apparatu-
ses consisting of one, two, or three compartments. Although
these apparatuses differ somewhat in their configuration during
training and testing, they all offer a choice between two stimulus
environments (i.e., CS+ vs. CS−) during the test. One-compartment
apparatuses typically have a set of interchangeable tactile cues
(flooring) such as the apparatus described in detail by Cunningham
et al. (13). During conditioning, animals are free to explore the
entire apparatus with either the drug- or vehicle-paired cues
present throughout. During testing, however, each half of the
compartment contains a different cue (counterbalanced). Two-
compartment apparatuses consist of two equal sized chambers
Place Conditioning

separated by either a wall or guillotine door that is removed or opened during the preference tests. These apparatuses typically contain a combination of visual (e.g., white, black, gray, or striped walls) and tactile (e.g., metal bars, sandpaper, or wood shavings) cues that differentiate the two compartments, but olfactory cues have sometimes been used as well (e.g., almond, lemon). During conditioning, the animal is confined to one of the two chambers, but is allowed to freely explore both chambers during preference testing. Three-compartment apparatuses typically include two conditioning chambers similar to those in a two-compartment apparatus but are separated by a third, usually smaller, “neutral” chamber. This center compartment is not used during the conditioning phase of the experiment, but serves as a starting chamber during the preference test when all three compartments are available.

The advantages and disadvantages of each configuration continue to be the subject of much debate. For instance, one advantage of a one-compartment apparatus is that it reduces the potential confounding effects of novelty on preference expression because the animal has experienced the entire apparatus during both drug and saline conditioning sessions (26). However, although a one-compartment apparatus works well with tactile CSs, it has not been effective for conditioning visual cues that are readily conditioned in a two-compartment apparatus (3). A three-compartment apparatus has the advantage of possibly reducing unintentional bias related to the animal’s initial placement in the apparatus (10), but also introduces the complication of interpreting potential treatment effects on the amount of time spent in the neutral chamber during choice tests. A broader disadvantage of all two-choice apparatuses is that they do not permit the experimenter to differentiate between an animal’s approach to (or preference for) the drug-paired chamber versus withdrawal from (or aversion to) the saline-paired chamber. One solution to this problem is to use a three-choice apparatus in which three distinct compartments are connected via a neutral transition compartment (27). This approach allows the experimenter to include a third, novel chamber during the choice test in addition to drug-paired and vehicle-paired chambers. In an elegant series of experiments using a three-choice apparatus, Parker (27) showed that rats preferred a morphine-, amphetamine-, or apomorphine-paired chamber over a saline-paired or novel chamber, suggesting that the ability of a drug to induce place preference is not due simply to its ability to reduce habituation to (i.e., maintain novelty of) the drug-paired chamber. Rather, choice of the drug-paired compartment appears to reflect the animal’s preference for the conditioned rewarding effects of drug-paired cues. Given these findings, use of a simpler two-choice apparatus is reasonable and sufficient in most cases.
3.2. Apparatus Bias

A place-conditioning apparatus is considered to be “unbiased” when a group of untrained animals, on average, shows no preference for one stimulus alternative compared to another. In contrast, a place-conditioning apparatus is labeled as “biased” when the average response of a group of untrained animals shows a consistent preference (or aversion) for one of the stimulus alternatives. For example, rodents generally tend to prefer a darkened compartment with black walls over a brightly illuminated compartment with white walls (28). Although it is convenient to apply these labels to the apparatus itself, they are more properly described as characteristics of the subject population. That is, the same apparatus might be “unbiased” for one particular mouse strain, but be “biased” for a different mouse strain (29). Therefore, it is essential that apparatus bias be carefully determined for every subject population under study.

Determination of apparatus bias is important because it can impact the ability to detect effects of drug-induced preference or aversion. For example, if a rewarding drug is paired with an already preferred cue, it may be difficult to detect conditioning-related increments in preference due to a ceiling effect (30). Furthermore, use of a biased apparatus raises problems in the interpretation of place-conditioning studies. For instance, if conditioning involves pairing the non-preferred chamber with a putative rewarding drug, it will be unclear whether the response shown during a post-conditioning choice test is due to direct rewarding effects of the drug or is due instead to drug-induced alleviation of whatever motivational processes underlie the unlearned aversion for the non-preferred chamber (i.e., an antiaversive or anti-anxiety drug effect; see (12, 15, 18)). Using a biased black-versus-white apparatus, for example, drug pairing might increase preference for the less-preferred white compartment because the drug reduces the stress or anxiety normally elicited by exposure to a brightly illuminated place, but not because the drug has a direct rewarding effect. For these and other reasons, use of an unbiased apparatus is strongly recommended, as is use of an unbiased procedure for assigning the specific cue to be paired with drug (i.e., a random, counterbalanced assignment; see (30)).

The impact of apparatus and design bias on the outcome and interpretation of place-conditioning results has recently been discussed in detail by Cunningham et al. (30). These investigators reported that mice showed significant preference for an ethanol-paired tactile cue only when ethanol was paired with the initially non-preferred tactile cue in a biased apparatus. On the other hand, in an unbiased apparatus, mice showed significant conditioned preference, regardless of which tactile cue was paired with ethanol. Overall, these studies suggest that it is especially important to consider each animal’s initial bias when assigning the
drug-paired chamber in a biased apparatus (i.e., whether the preferred or non-preferred chamber is paired with drug), but that this issue may be less important when using an unbiased apparatus.

Interestingly, there are cases where use of a biased animal assignment procedure may be necessary for detecting conditioned place preference, even when an apparatus is relatively unbiased. For example, Le Foll and Goldberg (31) concluded, both from an extensive review of the literature as well as from empirical evidence, that a biased procedure in which drug was paired with the animal’s non-preferred chamber was more sensitive for detecting nicotine-induced place preference in rats. No significant preference was detected in rats that received nicotine paired with their initially preferred chamber. The authors noted that one possible interpretation of this finding is that nicotine was able to induce conditioned preference by alleviating stress produced by exposure to the non-preferred chamber.

3.3. Stimulus Selection

Although some apparatuses use CSs from only one sensory modality (e.g., tactile floor cues), most place-conditioning apparatuses use compound CSs composed of several elements from different sensory modalities. For example, visual (e.g., brightness, patterns), olfactory (e.g., scented bedding, wood chips), and tactile cues (e.g., floor textures, metal grids, sandpaper) are often used in various combinations. Some researchers have suggested that tactile floor cues presented in the dark may be more effective than visual or olfactory cues for eliciting approach responses because the animal must actually touch the drug-paired cue in order to elicit a conditioned motivational response (10, 26). In recent studies that compared the efficacy of tactile and visual CSs, we found that tactile cues were able to support development of ethanol-induced conditioned place preference in mice trained in the dark or in the light, using both one- and two-compartment procedures. However, visual cues (presented in the light) were effective only when the cues were presented in a consistent spatial location using a two-compartment procedure (3). Similar assessments of olfactory cues have not yet been reported. Although some investigators have suggested that place conditioning is stronger when multiple-element cues are used (12), there have been no rigorous empirical assessments of this hypothesis.

3.4. Temporal Parameters

Like other forms of Pavlovian learning, place conditioning is sensitive to several important temporal parameters, including: (1) the time delay between exposure to the CS and exposure to the drug (interstimulus interval or ISI), (2) the duration of CS exposure, and (3) the time delay between consecutive conditioning trials (intertrial interval or ITI). Unfortunately, there is no single optimum combination of temporal parameters. Rather, it appears
that the optimal parameters vary depending on the nature of the drug, the route of administration, the animal’s species/genotype and static features of the apparatus (e.g., cue modality/intensity, apparatus bias). Thus, investigators must determine optimal parameters empirically for their target population, drug, and apparatus.

3.4.1. Interstimulus Interval

Most place-conditioning procedures attempt to overlap onset of a drug’s central nervous system effects with cue exposure by injecting the drug immediately or several minutes before exposure to the CS+. Injection of drug at a long delay before or after exposure to the CS will not produce conditioning (32). Selection of an appropriate delay interval presumably depends on the specific drug’s pharmacokinetics and may be influenced by route of administration. For example, routes that require longer periods of time for absorption and distribution (oral, intragastric, subcutaneous) may require somewhat longer delays than routes that produce a relatively rapid onset of drug effects (intraperitoneal, intravenous). Also, drugs with relatively slow rates of elimination may be less sensitive to the detrimental effects on conditioning of longer delays between drug and cue exposure than drugs that are rapidly eliminated. The timing and order of exposure to a CS and drug can have profound effects on the direction of place conditioning. In one study, intragastric infusion of ethanol immediately before cue exposure produced conditioned place aversion (CPA) whereas infusion of the same dose 5 min before cue exposure produced conditioned place preference (33). In other studies involving nicotine, ethanol, or amphetamine, injection immediately before CS exposure produced conditioned place preference while injection immediately after CS exposure produced conditioned place aversion (32, 34–36).

3.4.2. CS Duration

Place conditioning has been demonstrated over a wide range of CS (trial) durations, but most often in the range between 10 and 30 min. Some data suggest that the optimal CS duration might depend on both the drug and the animal genotype. For example, in our studies of ethanol-induced place conditioning in DBA/2J mice, a 5-min trial duration produced stronger place preference than 15- or 30-min trials (37). However, when the same strain and apparatus were used with cocaine, place conditioning was more robust with a relatively long, 60-min trial duration than with 15- or 30-min trials. In contrast, a different mouse strain (C57BL/6J) showed similar cocaine-induced place preference at all three trial durations (38). Thus, one cannot assume that the optimal trial duration determined for a particular drug and apparatus will necessarily be optimal when using a different drug, genotype, or species.
In contrast to Pavlovian conditioning procedures that involve short-lived unconditioned stimuli like food pellets or electric shock, ITIs in drug conditioning studies are typically measured in hours or days rather than seconds or minutes. In most place-conditioning studies, CS+ (drug) and CS− (vehicle) trials are typically separated by intervals of 24 h or more, providing more than enough time for effects of most drugs to dissipate between trials. This strategy also allows both types of trial to be conducted at the same time of day, eliminating a possible influence of circadian variables. In some studies, however, investigators administer both types of trial on the same day, raising the possibility that the conditioning of one cue is influenced by its temporal proximity to the trial that precedes or follows it. For example, if the first trial of the day involves injection of a drug with a long half-life, residual drug effects may affect learning on the second trial of the day. Alternatively, if the first trial involves a drug with a relatively short half-life, learning on the second trial might be influenced by acute withdrawal ("hangover") from those drug effects, which could condition an aversion to the second trial cues. Some investigators have attempted to avoid such problems by always making the CS+ (drug) trial the second trial of the day. However, because trial order is not counterbalanced, this approach confounds drug effects with time-of-day (circadian) effects. Moreover, if the interval between the CS− and CS+ trials is very short, learning on the CS− trial might be affected by drug exposure on the following CS+ trial (38). The best way to avoid all of these potential problems is to separate the drug and vehicle trials by 24 h or more.

The expression of a conditioned place preference or aversion is assumed to depend on the formation of a learned association between contextual stimuli and the drug’s rewarding or aversive effects. Because there are many reasons why exposure to a drug might alter preference for a contextual stimulus, it is important to include proper controls in the experimental design to isolate effects that reflect associative cue–drug learning (39). One common approach is to compare an experimental group (i.e., a group that receives cue–drug pairings) to a vehicle-only control group that receives equivalent exposure to the CSs, but always receives vehicle injections instead of drug. Although a vehicle-only group is useful for detecting unlearned biases or shifts in biases that might occur due to repeated cue exposure or the passage of time, it does not control for any non-associative effects produced by drug exposure. For example, a drug that had no direct rewarding or aversive effects might nevertheless produce changes in cue preference due to chronic changes in sensory–motor function or motivational tone. Thus, a better control is provided by making comparisons between groups that are matched for overall exposure to both the CS and drug (39, 40).
One such strategy involves use of an “unpaired drug” control group. This group receives exposure to the CSs without drug (i.e., with vehicle), but also receives exposure to the drug unpaired with the CSs. For example, drug exposure might occur in the home cage several hours after each exposure to the cue that is normally paired with drug in experimental animals. Conditioning is indexed by the difference between the paired experimental group and the unpaired control group. One problem with an unpaired drug group, however, is that it does not control for possible conditioning of responses to features of the apparatus or test room that are common to both the drug- and vehicle-paired compartments. For example, in contrast to an experimental group that receives drug in the apparatus on half of its trials (i.e., on CS+ trials), an unpaired group is unlikely to acquire a conditioned activity responses to general cues of the apparatus (41), thus providing an alternative interpretation of test differences between paired and unpaired groups. An arguably better control strategy involves using a counterbalanced discrimination control procedure in which one cue is consistently paired with drug (CS+) whereas the other cue is paired with vehicle (CS−) in all animals. The specific CS paired with the drug is counterbalanced across groups, and successful conditioning is indexed by analyzing the difference between the counterbalanced conditioning subgroups. Because these groups are matched for exposure to the apparatus, CSs, and drug, but differ in the paired relation between each cue and drug, group differences are assumed to reflect the underlying cue–drug association (13, 30, 39).

3.6. Dependent Variables

Investigators have used several different dependent variables to index performance during place-conditioning test sessions. The most common measures are: (a) raw time scores (e.g., number of seconds spent in compartment A), (b) percentage time spent in the drug-paired compartment, (c) difference between time spent in the drug-paired compartment and time spent in the vehicle-paired compartment, or (d) the difference between time or percentage time spent in the drug-paired compartment during the post-conditioning test and time or percentage time in that compartment during a pretest. Two critical issues in the selection of a dependent variable are: (a) whether the apparatus and subject assignment procedure are biased or unbiased and (b) the nature of the experimental design and the specific comparisons that are used to draw conclusions about the development of a conditioned place preference or aversion. For example, experiments that use a biased subject assignment procedure will typically include a pretest as part of the procedure and then index place conditioning by calculating a post-conditioning minus pretest difference score. Although such changes might be informative, it is important to note that within-subject preferences might change between a pretest and posttest for reasons that are unrelated to drug-induced
conditioning. In cases where a pretest is not required (e.g., when using an unbiased apparatus and unbiased subject assignment procedure), however, the dependent variable can be based entirely on behavior measured during the post-conditioning test. As noted earlier, the most informative comparisons will be between groups that have been matched for their overall exposure to the choice stimuli and drug (e.g., between paired vs. unpaired groups or between the counterbalanced subgroups in a discrimination design, that is, A+, B− vs. A−, B+). When the apparatus is unbiased and an unbiased subject assignment procedure has been used, we recommend a comparison of raw time scores between the counterbalanced subgroups as the most direct way to provide evidence of successful place conditioning. Cunningham et al. (30) have discussed the rationales for all of these dependent variables as well as their advantages and disadvantages. They also provided an empirical comparison of all four measures in experiments involving either a biased or unbiased apparatus.

3.7. Locomotor Activity Processes unrelated to drug-induced conditioning can disrupt the expression of conditioned-place preference or aversion. The simplest way of disrupting or altering expression is by introducing a manipulation or experimental variable that alters locomotor activity during the preference test session. For example, the design of a place-conditioning study might include administration of a pharmacological agonist or antagonist before the choice test in order to assess whether the treatment drug alters the motivational processes underlying expression of conditioned-place preference or aversion. While such tests might be informative, their interpretation becomes complicated if the drug elicits a significant increase in activity, a behavior that could compete with the behavior of maintaining contact or proximity to the drug-paired cue. Subject variables that are linked to differences in basal activity (e.g., genotype, sex) might also confound interpretation of differences in expression of place conditioning. A recent study highlighted the importance of these concerns by showing an inverse relationship between the strength of conditioned-place preference and the level of test activity induced by a pretest injection of an activating dose of ethanol (42). This relationship was most apparent in the DBA/2J mouse strain, which is strongly activated by ethanol. In fact, the high levels of activity in these mice after ethanol completely interfered with the expression of place preference. The inverse relationship between preference and test activity was also observed in another mouse strain (NBZ/B1NJ) that is much less activated by ethanol. In that case, however, the ethanol-induced increase in activity was not sufficient to interfere with the expression of preference. Overall, these data underscore the importance of measuring locomotor activity during place conditioning tests and considering that information in the interpretation of results.
4. Using the Place-Conditioning Procedure

4.1. Acquisition Versus Expression

Procedural separation of the training (acquisition) and test (expression) phases during place-conditioning studies enables investigators to easily examine and compare experimental manipulations thought to alter drug effects or learning about those effects (acquisition phase) to manipulations that might affect retrieval of that learning or the performance of behaviors that reflect that learning (expression phase). Perhaps the most common use of the place-conditioning procedure has been to study the neurobiological mechanisms that underlie the putative rewarding or reinforcing effects of drugs abused by humans (12, 14, 16, 17). Investigators focused on the primary (direct) rewarding effects of drugs have typically administered treatments during the acquisition phase of a place-conditioning study. For example, before each CS+ trial, one might inject (systemically or intracranially) a pharmacological antagonist that blocks a neurotransmitter receptor hypothesized to mediate or modulate the rewarding effect of the conditioning drug. Alternatively, one might temporarily inactivate a particular brain nucleus (e.g., by intracranially infusing a local anesthetic drug) to map the neuroanatomical circuit that mediates drug reward. Similarly, one might temporarily manipulate a specific gene (e.g., by using a conditional gene knockout or by intracranially injecting a viral vector) to determine that gene’s influence on drug reward. In all cases, the prediction is that place conditioning in experimental animals will differ from that seen in animals that have received an appropriate control treatment.

Several important methodological considerations arise in the design and interpretation of such acquisition studies. One critical issue is whether the treatment has an effect during CS− conditioning trials. For example, in studies involving pharmacological antagonists, some investigators have administered the treatment drug before both CS+ and CS− trials. An interpretive danger in this approach is that treatment-related changes in test performance might be explained by the ability of the treatment drug to directly condition a preference or aversion for the CS− instead of or in addition to treatment drug effects on drug reward during CS+ trials. Thus, most investigators administer the treatment only on CS+ trials and apply a control treatment (e.g., vehicle injection) on CS− trials. It is important to note, however, that this approach does not eliminate the need to consider the possibility that a treatment might directly condition a preference or aversion for the CS+. For example, a treatment drug that is found to interfere with development of conditioned place preference might have this effect because it directly conditioned an aversion to the CS+, rather than because it reduced the paired drug’s rewarding effect (i.e., interference via behavioral antagonism rather than
pharmacological antagonism). Thus, whenever a treatment given before CS+ trials is found to alter place conditioning produced by an abused drug, one must also test the ability of the treatment alone to induce place conditioning (43).

Another interpretive issue that arises whenever an acquisition treatment is found to alter place conditioning is whether the treatment is selectively modifying the rewarding (or aversive) effect of the conditioning drug as opposed to having a more general, non-specific effect on sensory–motor function or learning/memory processes. In other words, instead of reducing the conditioning drug’s rewarding effect, a treatment might impair acquisition of place conditioning because it raises sensory thresholds for detecting the CSs or because it impairs learning or encoding of the CS–drug association. The best strategies for addressing such possibilities are to directly assess the treatment’s effect on sensory function using an appropriate behavioral or physiological assay and to assess the treatment’s effect on: (a) place conditioning induced by a different outcome (e.g., a different drug or a natural reinforcer like food) or (b) a different form of learning. One example of the latter approach was provided by a recent series of studies designed to examine various NMDA-receptor-system treatments on the acquisition of ethanol-induced conditioned place preference. After finding that a competitive NMDA-receptor antagonist (CGP-37849) interfered with the acquisition of ethanol-induced place preference but did not condition a preference or aversion on its own, the investigators tested effects of the antagonist on the acquisition of place aversions induced by pre-CS injection of lithium chloride or by post-CS injection of ethanol (43). Because the antagonist interfered with both types of aversive place conditioning, the authors concluded that the antagonist interfered with ethanol-induced place conditioning by impairing the animal’s ability to learn the CS–ethanol association rather than by altering ethanol’s rewarding effect.

In cases where a treatment given before acquisition has a long-term or permanent effect (e.g., chronic lesion, genetic mutation), consideration must also be given to the possibility that the treatment alters performance (expression) of the place-conditioning response rather than or in addition to modifying its acquisition. In the case of lesions, one solution to this problem might be to compare a group that receives the lesion before acquisition and testing with a group that receives the lesion between acquisition and testing (44). However, it is difficult to isolate chronic treatment effects on acquisition when a treatment given after acquisition is also found to have an effect on expression. In such situations, consideration should be given to using treatment manipulations during acquisition that have relatively short-lived effects (e.g., temporary inactivation of a brain area, intracranial microinfusion of a selective pharmacological antagonist).
As an alternative to testing manipulations during the acquisition of place conditioning, investigators can introduce treatments at the time of testing to examine their impact on the performance (expression) of conditioned place preference or aversion (45, 46). Because the conditioning drug is typically not given during such preference tests, any effects of the treatment manipulation cannot be attributed to alterations in the primary (direct) rewarding or aversive effects of the conditioning drug. Rather, treatment effects will presumably be due to alterations in the behavioral processes that underlie performance of the cue-directed approach or avoidance response used to index place conditioning. In most cases, investigators introduce treatments at the time of testing in order to examine their influence on the putative conditioned rewarding/reinforcing or conditioned aversive/punishing effects of a CS previously paired with drug (i.e., CS+). Arguably, treatments that impair performance of drug seeking during conditioned place preference tests might have significant translational relevance for identifying potential therapies for drug addiction.

As in the case of acquisition studies, however, consideration must be given to several different possible interpretations of treatment effects on the expression of place conditioning. For example, a treatment might produce its effect on performance by selectively altering the conditioned motivational state or motivational processes triggered by exposure to the CS+ (a motivational treatment effect). Alternatively, the treatment might produce its effect by altering retrieval of the CS–drug memory or by affecting other learning processes engaged during the test (a learning-memory treatment effect). Finally, a test treatment might produce its effect in a relatively nonspecific way by altering sensory thresholds or locomotor activity levels (a sensory–motor treatment effect). Differentiating among these alternative interpretations can be difficult. Although interpretations based on alterations in locomotor activity can be directly addressed by concurrent measurement of activity during the place-conditioning test (42), assessment of other interpretations will usually require additional experiments designed to test specific alternative hypotheses. For example, to test whether a treatment uniquely affects the learned motivational response induced by conditioning with a particular drug, investigators can test effects of the same treatment on expression of place conditioning induced by conditioning with several different drugs or with natural reinforcers like food. Finding a treatment that only affects expression of place conditioning induced by one drug or a small number of similar drugs will imply a relatively selective mechanism of action. Moreover, a lack of treatment effects on expression of place conditioning induced by dissimilar drugs or natural reinforcers will eliminate more general alternative interpretations based on alterations in learning and memory or sensory–motor function.
4.2. Extinction

Like other forms of Pavlovian conditioned behavior, an established conditioned place preference or aversion can be weakened by repeated exposure to the CS without the unconditioned stimulus (i.e., drug), a phenomenon known as extinction. Extinction of place conditioning is of potential relevance to human cue-exposure therapies that involve presenting stimuli previously associated with drug in the absence of drug with the goal of reducing cue-elicited craving and drug seeking (47). Two types of extinction procedures have been used to reduce the expression of conditioned preference or aversion: forced and choice. Forced extinction typically consists of exposing the animal to the previous drug- and vehicle-paired CSs in separate sessions in the absence of drug. These trials are often similar to conditioning trials except that the drug is omitted (or vehicle is given during exposures to both CSs). Researchers have referred to this type of extinction procedure as forced exposure (48) because the experimenter controls exposure to each CS throughout extinction. Animals presumably learn an inhibitory CS–no drug relationship during these sessions and will subsequently spend less time in the compartment previously paired with drug compared to pre-extinction choice tests. Although this procedure can be quite effective for reducing or eliminating the expression of conditioned place preference and aversion (49), the primary disadvantage of the forced extinction method is that the presumed trial-by-trial decrease in the strength of place conditioning cannot be monitored without periodically inserting probe choice test trials.

An alternative approach is simply to extinguish place conditioning through extended choice testing in the absence of drug (“choice extinction”). On the initial test, the animal expresses a place preference or aversion, but with prolonged or repeated exposure to the test cues, performance of this response decreases. The primary difference from forced extinction is that the animal controls the amount of exposure to each CS through its choice behavior. When tests are sufficiently long, choice extinction can be used to examine within-session extinction as well as extinction over multiple sessions. Responding decreases in both forced and choice extinction procedures due to a presumed decrement in the strength of conditioned reinforcement or other underlying motivational processes (25). However, the behavioral and neurobiological differences between these two extinction procedures have not yet been well studied.

Given the Pavlovian nature of place conditioning, it is reasonable to assume that the time course of extinction will be affected by the strength of initial conditioning as well as by such parameters as CS duration and ITI. Conditioned place preference can be very strong and resistant to extinction, often requiring much longer exposure to CSs in a drug-free state than the exposure required for initial conditioning (25). Intermittent extinction testing
(i.e., very long ITIs between successive CS exposures) may be especially effective for maintenance of place conditioning over long periods of time (50), an outcome that mirrors the persistence of drug seeking in human addicts. Despite the translational significance of extinction, however, systematic study of the variables that affect extinction of place conditioning has been relatively limited.

Of special interest to the problem of human addiction are behavioral and pharmacological manipulations that might facilitate extinction of drug seeking responses. Extinction of place conditioning using either a forced or choice procedure can be used to address this question. For example, administration of the opioid receptor antagonist naloxone before choice extinction trials was shown to facilitate extinction of ethanol-induced conditioned place preference (2, 25). This particular example has good external validity because opioid receptor antagonist treatment (with naltrexone) has also been found to be effective in reducing craving elicited by alcohol-associated cues in alcoholics (51). Naloxone appeared to facilitate extinction of conditioned place preference by disrupting opioid-mediated maintenance of ethanol’s conditioned rewarding effect during choice tests. Other facilitators of extinction such as d-cycloserine (DCS) may work through different mechanisms, for example, by facilitating new learning of the inhibitory CS–no drug association during extinction sessions (52, 53). Facilitation of extinction of place preference by DCS may be limited, however, to certain extinction procedures, drugs, or species (53, 54).

Neurobiological mechanisms underlying extinction of drug-induced conditioned place preference and aversion have also been studied with the same techniques used to study mechanisms underlying acquisition and expression of place conditioning (i.e., lesions, intracranial microinfusions). For example, post-conditioning excitotoxic lesions of the basolateral amygdala have been found to retard choice extinction of cocaine-induced conditioned place preference in rats, an outcome that was attributed to impairment in the ability to modify CS–drug associations (55). In another study, temporary inactivation of the medial prefrontal cortex (mPFC) or infusion of an NMDA-receptor antagonist (AP-5) into mPFC immediately before each forced extinction trial blocked extinction of amphetamine-induced conditioned place preference (56), suggesting that NMDA receptors within mPFC normally play a role in the extinction of this behavior. Much more research is needed to identify the unique neurobiological mechanisms underlying the acquisition, expression, and extinction of drug-induced conditioned place preference and aversion.

4.3. Relapse Models

Relapse to drug seeking and drug taking is a significant problem in human addict populations. The place-conditioning procedure
Place Conditioning

has been used to model relapse to drug seeking by examining manipulations that produce a recovery in conditioned place preference after extinction. Several methods have been used to restore conditioned preference after extinction: drug priming, CS exposure, stressor exposure, contextual renewal, and reconditioning. Each of these methods represents a different approach to re-establishing the conditioned behavior and all support the notion that extinction learning does not erase the original CS–drug association that underlies the expression of a conditioned place preference. Each method potentially serves a unique role in our efforts to understand the environmental and biological triggers that contribute to relapse behavior in human addicts.

An important consideration in the design and interpretation of relapse studies is the specific comparisons that are used to decide whether response recovery has occurred. Relapse studies generally involve an acquisition and extinction phase followed by a relapse manipulation that often (but not always) occurs just before the final preference test. The strongest evidence for relapse is provided when the magnitude of preference during that final test significantly exceeds preference measured during a post-extinction test (i.e., a within-subject comparison). Relapse can be considered complete when test performance matches that seen during a post-conditioning (pre-extinction) preference test (7). To isolate critical features of the relapse-inducing event, it might also be appropriate to include a between-subject control comparison. For example, when studying drug-priming reinstatement, a vehicle-treated control group would address the possibility of reinstatement induced simply by handling and injection. When interpreting the outcomes of relapse studies, consideration should be given to the possibility that the relapse-inducing treatment might produce its effects through a relatively nonspecific mechanism (e.g., by altering locomotor activity or general arousal).

Drug priming has been the most commonly used method for reinstating an extinguished conditioned place preference. In most cases, after extinction training is completed, an injection of the conditioning drug is administered immediately before a reinstatement preference test. This method has been used successfully in both rats and mice and with many drugs of abuse including cocaine, morphine, heroin, ethanol, meth- and d-amphetamine, and nicotine (for an extensive review see (7)). Several of these studies have shown that place preference can be reinstated by a broad range of doses of the conditioning drug. One such study showed that an extinguished place preference initially induced by a high dose of morphine (40 mg/kg) could be reinstated by priming with the same morphine dose or with much lower doses (5, 10, and 20 mg/kg) but not by very low doses (2 or 3 mg/kg; (57)). Other studies have shown cross-reinstatement, a phenomenon in
which an extinguished place preference is reinstated by priming injection of a different drug. For instance, Romieu et al. (58) reported that an extinguished preference originally induced by cocaine was significantly reinstated by cocaine as well as by phencyclidine, nicotine, morphine, and ethanol.

4.3.2. CS Reinstatement

Although cue-induced reinstatement is often used as a model of relapse in drug self-administration procedures, CSs previously paired with drug have not been used to reinstate conditioned place preference after extinction. Although a few studies have reported CS-induced reinstatement of place preference, these studies have used cues that were previously paired with other unconditioned stimuli, but not with the drug originally used for conditioning. For instance, it has been reported that the visual and tactile cues of a place-conditioning chamber can reinstate an extinguished morphine-induced conditioned place preference after the chamber has been repeatedly paired with morphine withdrawal (59). In a similarly designed study, Sanchez and Sorg (60) showed that auditory or olfactory conditioned fear stimuli were able to reinstate an extinguished cocaine-induced conditioned place preference. However, the fear-arousing CS also induced substantial freezing during the preference test, raising the possibility that the enhancement of preference was simply a byproduct of reduced activity during the test. Overall, these studies suggest that it is possible to reinstate an extinguished place preference using a Pavlovian CS, but this effect remains to be shown with a CS previously paired with the drug originally used to induce place preference.

4.3.3. Stressor-Induced Reinstatement

Stressors have been shown to play a major role in relapse to drug seeking and drug taking behaviors in drug addicts and alcoholics. Thus, it is no surprise that stress-eliciting agents, both physical and psychological, can trigger relapse in preclinical models of drug seeking behavior. A single session of intermittent, uncontrollable footshock is the most commonly reported method of stressor-induced reinstatement. For example, this stressor has been shown to reinstate extinguished cocaine- and morphine-induced conditioned place preference in mice and rats (61, 62). Other relapse-inducing stressors capable of reinstating extinguished conditioned place preference in animals include: restraint-immobilization, social defeat, and forced-swimming (61, 63, 64). Overall, these results show that a variety of physical and psychological stressors are capable of reinstating extinguished drug seeking behavior as indexed by conditioned place preference.

4.3.4. Contextual Renewal

Contextual renewal refers to the ability of a set of distinct contextual cues to reinstate a behavior that was extinguished in the presence of an alternative set of cues. Specifically, a conditioned
behavior that is initially learned in Context A and subsequently extinguished in a different context (Context B) can be restored when tested in the original non-extinguished context (Context A) or in a novel context (65). This phenomenon has received substantial attention in both the fear conditioning and self-administration literature, but there is currently only one published paper examining contextual renewal of conditioned place preference (48). In these studies, distinctive tactile floor cues served as CS+ and CS−, while context was manipulated by varying the color of the compartment walls and lid (black vs. white). Results showed that both cocaine- and morphine-induced conditioned place preferences were restored when animals were tested in Context A after training in Context A and extinction in Context B (i.e., ABA contextual renewal). Thus, as with other types of Pavlovian conditioning, environmental cues present during conditioning and extinction play an important role in modulating drug-induced conditioned place preference.

Reconditioning of the extinguished CS is another method that can be used as a model of relapse to drug seeking. Reconditioning, or reacquisition, most often consists of a single session (or small number of sessions) of CS–drug pairings identical to those performed during the initial conditioning trials. Reconditioning typically occurs more rapidly than does initial conditioning. Most published reports of reconditioning of conditioned place preference have involved heroin as the paired drug. For example, Leri and Rizos (66) reported that a single reconditioning session with heroin could reinstate an extinguished heroin-induced place preference in rats. This effect was not seen when the reconditioning heroin injection was given outside of the conditioning environment. Furthermore, the basolateral amygdala appears to be necessary for rapid reconditioning because pharmacological inactivation of this brain area 15 min after the reconditioning trial prevented reinstatement of the heroin-induced place preference (67). Rapid reacquisition of extinguished ethanol- and morphine-induced place preference have also been shown after a single reconditioning session (54, 68).

5. Conclusions

Although the place-conditioning procedure involves stimulus-contingent rather than response-contingent administration of a drug, we and others have suggested that this procedure is potentially relevant to understanding the phenomenon of drug seeking in human addicts because the target behavior is strongly controlled by environmental stimuli that have previously been paired
with abused drugs. In fact, the apparent lack of self-control that characterizes alcoholics and drug addicts might well be a by-product of an irresistible attraction conditioned to drug-paired stimuli through learning processes similar to those involved in place conditioning. Much like human drug seeking behavior, place conditioning can be rapidly acquired and shows substantial resistance to extinction. The fact that this behavior can be so rapidly established in drug-naïve organisms suggests the procedure might be especially useful for understanding processes involved in the initiation of drug seeking. The procedure has also been found to be useful for modeling the effects of variables thought to influence individual differences in sensitivity to the ability of abused drugs to induce seeking behavior (e.g., genotype, drug history). Moreover, the ability to reinstate conditioned place preference after extinction using a variety of manipulations (e.g., drug priming, context, stressors) makes this procedure a potentially valuable tool in the study of processes underlying relapse. Finally, the demonstrated utility of this procedure for studying neuropharmacological and other neurobiological mechanisms involved in the acquisition, expression, extinction, and reinstatement of place conditioning holds promise for the identification of more effective treatments for eliminating drug seeking behavior and preventing relapse.

Acknowledgments

Preparation of this chapter was supported in part by NIH-NIAAA grants AA007702, AA013479, AA018052, and AA007468. Thanks are extended to K. Matthew Lattal and Tamara Phillips for their helpful comments and suggestions.

References


Place Conditioning

Reinstatement of morphine-induced conditioned place preference in mice by priming injections. Neural Plast 10(4):279–290


Chapter 7

Sensitization

Jessica A. Loweth and Paul Vezina

Abstract

Exposure to psychostimulant drugs leads to sensitized locomotor responding, nucleus accumbens (NAcc) dopamine (DA) overflow, and drug self-administration upon reexposure to the drug weeks to months later. Calcium-dependent signaling pathways contribute importantly to both the induction and expression of sensitization by these drugs. In particular, calcium-calmodulin (CaM)-dependent protein kinase II (CaMKII), a serine/threonine kinase that is highly expressed in forebrain regions such as the NAcc, is known to contribute to the expression of several forms of plasticity including sensitization. Notably, pharmacologically inhibiting CaMKII in the NAcc prevents the expression of sensitized locomotion, NAcc DA overflow, and drug taking. Evidence indicates that CaMKII may act both pre- and postsynaptically in this site to influence the expression of these manifestations of sensitization. Presynaptically in DA neuron terminals, CaMKII regulates sensitized DA overflow. Postsynaptically in medium spiny neurons, it contributes to the upregulation of AMPA receptors, the activation of which is necessary for the expression of sensitization. It is conceivable that interactions between CaMKII-mediated neuroadaptations in both of these sites may underlie the long-lasting maintenance of sensitization by psychostimulant drugs.

Key words: CaMKII, Dopamine, Drug self-administration, Glutamate, Locomotion, Nucleus accumbens, Reinstatement, Sensitization

1. Stimulant Sensitization

Psychostimulant drugs like amphetamine and cocaine increase extracellular levels of dopamine (DA) in brain. They achieve this effect in the somatodendritic and terminal regions of DA neurons by blocking uptake of DA into the cell or causing reverse transport of DA through the DA transporter into the extracellular space (1). Several lines of evidence indicate that the locomotor-activating, reinforcing, and incentive effects of psychostimulants are mediated by transmission in the mesocorticolimbic DA pathways. These pathways consist of DA cell bodies located in the midbrain ventral tegmental area (VTA) and their ascending
projections primarily to the nucleus accumbens (NAcc) as well as other brain regions including the prefrontal cortex, hippocampus, and amygdala (2). It has been known for some time that repeated exposure to psychostimulants leads to sensitized locomotor responding to these drugs when rats are reexposed to the drug weeks to months later (3). This long-lasting effect is paralleled by equally long-lasting enhancements in NAcc DA neuron reactivity (4–6). More recently, it was shown that repeated exposure to psychostimulants also facilitates acquisition of drug self-administration (7, 8) and enhances the subsequent reinstatement of drug seeking (9) as well as the amount of work animals will emit to self-administer the drug (10). Evidence indicates that sensitization of stimulant-induced locomotion, drug self-administration, and NAcc DA overflow are produced by the same drug exposure regimens via actions in the same nuclei (5, 10, 11) and are prevented by the same pharmacological interventions (6, 12–14). Such findings provide strong support for the idea that the enhanced expression of complex motivated behaviors like drug seeking and drug taking represent, like enhanced locomotion, important manifestations of behavioral sensitization. Thus, sensitization of midbrain DA neuron reactivity has been proposed to underlie the transition from casual drug use to craving and abuse (15, 16). The growing number of findings showing that enhanced behavioral and neuronal responding to stimulants also occurs in humans (see (17, 18)) highlights the importance of understanding sensitization at the molecular, cellular, systems, and behavioral levels so as to promote the development of the therapeutic interventions necessary to reverse it.

Activity in VTA-NAcc DA neurons has long been associated, not only with drug-induced locomotion, but also with self-administration supported by amphetamine and other psychostimulant drugs. For example, both effects are prevented by DA receptor blockade or 6-OHDA lesions of DA neuron terminals in the NAcc (for discussion and references, see (16)). It would thus be expected that the progressive enhancement in the reactivity of these neurons should lead to the progressive enhancement of the manifestation of both of these behavioral outputs, as the above results indicate. In their seminal paper (15), Robinson and Berridge proposed that activity in VTA-NAcc DA neurons encodes the incentive valence of drug cues. Sensitization of the reactivity of these neurons would thus be expected to enhance the incentive valence of these cues, allowing them to more readily illicit approach and as a result to augment the pursuit of the drug, a result again indicated by the above findings.

Stimulant sensitization can be divided into two distinct components, each associated with a separate brain region and different sets of identified neuroadaptations. Induction takes place in midbrain nuclei such as the VTA and expression occurs in forebrain
sites like the NAcc (19, 20). The former encompasses the transient neuronal events that are produced by repeated, intermittent exposure to the drug. Because the VTA is richly innervated, a number of neurotransmitter systems have been implicated in the induction of sensitization with glutamate and somatodendritic derived DA proposed to play a major role in this effect (16, 21). Pharmacological activation and inhibition studies suggest an important role in this site for calcium-initiated signaling pathways including those involving calcium-calmodulin (CaM)-dependent protein kinase II (CaMKII) and mitogen-activated protein kinase, as well as protein kinase A and C (16, 22). As a site of expression, the NAcc is home to a number of long-lasting alterations in neuronal signaling that have been linked to enhanced neurochemical and behavioral responding to psychostimulant drugs. Of all neuroadaptations known to follow exposure to such drugs, the one most consistently associated with the expression of locomotor sensitization is the enhancement in the ability of psychostimulants to increase extracellular levels of DA in the NAcc (5, 6, 16). A number of other long-lasting adaptations have also been reported to occur in this site, including alterations in dendritic morphology (23), increased glutamate overflow (24, 25), functional upregulation of AMPA receptors (9, 25), changes in synaptic strength at cortico-accumbens glutamate synapses (26), as well as changes in AMPA receptor subunit expression and membrane insertion (27–29). As with induction in the VTA, CaMKII is again one of a number of proteins implicated in the expression of stimulant sensitization (30–33). Others include PKA, Darpp-32, cAMP response element binding protein, extracellular signal-regulated kinase, and immediate early gene Fos-related antigens like delta FosB (34–36). Of these, CaMKII has attracted considerable attention for its potential role in long-term memory storage due to its abundance in brain, its prominence in the postsynaptic density and its ability to remain phosphorylated after activation (37). CaMKII is also well positioned in post-receptor signaling pathways to bridge converging DA and glutamate neurotransmission in the NAcc (38). Considering that stimulant sensitization is long lasting and that both DA and glutamate contribute to its expression, CaMKII has been investigated particularly for its contribution to the expression of sensitization by psychostimulant drugs. The evidence for this contribution is reviewed below.

2. CaMKII Structure and Function

CaMKII is a serine/threonine kinase that belongs to the multifunctional CaMK family (CaMKI, II, and IV) and phosphorylates a wide array of downstream targets. CaMKII is highly expressed
in forebrain sites like the NAcc, hippocampus, amygdala, and prefrontal cortex (39, 40). Four different CaMKII subunits have been identified: α, β, γ, and δ. The α and β subunits are the principal isoforms found in brain, where they form dodecameric holoenzymes made up of one or both subunits (41, 42). In rat forebrain, the α subunit predominates at a ratio of approximately 3:1 over the β subunit (41, 43).

The unique activation properties of this kinase play an important role in its ability to significantly alter synaptic strength and neuronal activity, and thus, it has attracted attention as a major contributor to various types of neuronal plasticity including the sensitization produced by psychostimulant drugs. When CaM is not bound to the kinase, the autoinhibitory domain binds to and occludes the catalytic domain, where the substrate-binding site is located (Fig. 1). With rising calcium levels, CaM binds to the autoinhibitory domain and temporarily activates the kinase (44). This sequence of events also exposes a threonine residue (Thr286) on the autoinhibitory domain and permits its autophosphorylation, thereby preventing inactivation of CaMKII. In this constitutive active state, CaMKII can remain active in the absence of CaM and thus exhibit calcium-independent activity (42, 45).

![Fig. 1. Illustration of the principal functional domains of CaMKII.](image)

When CaMKII is inactive (a), the Thr286 (α-isof orm; Thr287 on the β-isof orm) and pseudosubstrate segments of the autoinhibitory domain bind to the T site and substrate-binding site (S site) of the catalytic domain, respectively, to prevent kinase activity. Binding of CaM to a region overlapping with the pseudosubstrate region removes it and the autoinhibitory domain from the S site and activates the kinase (b). Once the kinase is active and the autoinhibitory domain has been removed from the catalytic domain, the latter can now be autophosphorylated at Thr286 (c), thereby preventing binding of this segment to the T site and deactivation of the kinase. Binding of the T site to a region on the NMDA NR2B subunit produces the same effect. This constitutively active form of CaMKII no longer requires CaM to remain active, exhibits calcium-independent activity, and has been proposed to serve as a molecular switch capable of long-term memory storage (37).
This property of CaMKII has been proposed to contribute importantly to its impact on neuronal activity and especially to its role in the production of long-term potentiation, a primary model of the cellular and molecular mechanisms underlying learning and memory (37). It remains unknown whether this constitutive activity of CaMKII is also necessary either for the induction or the long-term maintenance of stimulant sensitization. Considering its wide array of substrates, it is equally feasible that CaMKII acts as a trigger to initiate downstream post-phosphorylation cascades that themselves mediate the long-lasting expression of sensitized responding to psychostimulant drugs. Whichever the case, a number of reports have indicated that CaMKII is in fact necessary for the expression of stimulant sensitization.

3. CaMKII and the Expression of Stimulant Sensitization: Behavioral Evidence

As indicated above, CaMKII has been shown to be necessary for the induction of long-term potentiation (37, 46) and locomotor sensitization by psychostimulant drugs (47). Although there have been relatively few investigations, the evidence obtained to date indicates that CaMKII is also necessary for the expression of behavioral sensitization by cocaine and amphetamine (Fig. 2).

Fig. 2. Inhibition of CaMKII in the NAcc shell decreases the enhanced locomotion and drug self-administration normally observed in rats previously exposed to psychostimulant drugs. In both cases, the effect of increasing concentrations of the CaMKII inhibitor KN-93 on the expression of sensitized behavior was assessed. Data are shown as (a) 2-h total locomotor activity counts (group means ± SEM) following a systemic injection of cocaine or (b) amphetamine infusions (group means ± SEM) obtained on a progressive ratio schedule of reinforcement (cumulative presses required are also shown). KN-93 was administered before the cocaine injection in (a) and before the amphetamine self-administration session in (b). Non-sensitized behaviors were not affected by KN-93 (not shown). Significantly different from: * the not-sensitized condition; † the sensitized condition (Adapted from (31, 33). With kind permission).
For example, microinjecting the CaMKII inhibitor KN-93 into the shell of the NAcc attenuates the expression of locomotor sensitization in rats previously exposed to cocaine. Similar findings were obtained following infusions into the NAcc of L-type calcium channel blockers (31, 48). More recently, infusion of KN-93 into the NAcc shell, but not into the NAcc core, was shown to decrease the enhanced work output and self-administration of amphetamine normally observed in rats previously exposed to the drug (33). These findings indicate that CaMKII is necessary for the enhanced expression of locomotor activity as well as complex motivated behaviors, both behavioral correlates of sensitized midbrain DA neurotransmission (16). Indeed, several reports indicate that CaMKII is necessary for the expression of the latter as well.

4. CaMKII: Presynaptic Effects

Amphetamine is known to acutely increase extracellular DA levels via a non-calcium-dependent mechanism. However, the enhanced portion of DA overflow observed in the NAcc of rats previously exposed to amphetamine does require calcium. For example, L-type (diltiazem) and N-type (conotoxin) calcium channel blockers inhibit the expression of sensitized NAcc DA overflow by amphetamine while sparing the ability of the drug to increase overflow acutely (30). These results obtained in vivo do not rule out the recruitment by calcium of postsynaptic signaling pathways in medium spiny neurons and their modulation of DA neuron firing via descending projections to the VTA. Nonetheless, it is likely that the entry of calcium into DA neuron terminals in the NAcc modulates, at least in part, the expression of amphetamine sensitization and that it does so by activating CaMKII. Inhibiting this kinase in the NAcc blocks the expression of sensitized DA overflow in this site (30). Reports showing that application of the CaMKII inhibitor KN-93 to striatal slices from amphetamine-exposed rats blocks sensitized DA release support a presynaptic site of action ((32), Fig. 3). These findings indicate that CaMKII can enhance DA release in the striatum, and by extension in the NAcc, through a presynaptic mechanism by interacting with substrates within the terminals of midbrain DA neurons. Although CaMKII is known to phosphorylate synapsin I at a site that frees synaptic vesicles for exocytosis (49), this kinase appears to mediate enhanced amphetamine-induced DA release via a non-exocytotic mechanism. Inhibition of CaMKII, for example, does not block enhanced K+-evoked DA release in rat striatal slices and maintains its ability to block enhanced amphetamine-induced DA release even after depletion of DA vesicles by reserpine (32). CaMKII may thus mediate enhanced amphetamine-induced NAcc DA
Sensitization

overflow by phosphorylating the dopamine transporter or proteins in contact with it at several potential consensus sites (50). For example, there is evidence that CaMKII promotes acute amphetamine-induced DA release by interacting with the C-terminus of the DA transporter (51) or by supporting association of the transporter with syntaxin 1A (52). Alterations in these or other interactions could conceivably lead to enhanced DA release. While the mechanisms whereby CaMKII promotes the expression of sensitized NAcc DA release remain elusive, taken together these findings suggest that calcium-mediated second messenger pathways are altered in sensitized midbrain DA neurons and are well positioned to mediate enhanced DA release. Given that drug self-administration is enhanced in animals sensitized to amphetamine and is accompanied by enhanced reactivity in midbrain DA neurons, these presynaptic calcium signaling pathways may be important for the expression of excessive drug taking.

Levels of calmodulin (which binds calcium to form CaM that activates CaMKII) are elevated following exposure to amphetamine. This has been demonstrated in striatal tissue (53–55) and striatal synaptosomes (56). As CaM activates CaMKII, changes in calmodulin protein levels following exposure to amphetamine would be expected to alter CaMKII activity. Indeed, CaMKII activity (but not protein levels) has been reported to be increased in striatal synaptosomes prepared from amphetamine-exposed rats (49), again supporting a presynaptic site of action for CaMKII in sensitized amphetamine-induced DA release. Interestingly, findings obtained in the NAcc have been less consistent. In this site, previous exposure to amphetamine has been reported to decrease calmodulin levels (57) and to leave levels of CaMKII and

Fig. 3. Inhibition of CaMKII prevents the enhanced amphetamine-induced DA release normally observed in striatal slices obtained from rats previously exposed to amphetamine (b). Slices prepared from rats previously exposed to saline are shown in (a). Slices were perfused with Kreb buffer and amphetamine (Amph), the CaMKII inhibitor KN-93, or a combination of the two was introduced for 2.5 min starting at the arrow. Samples were collected every 5 min. Data are shown as group mean (±SEM) pmol DA released per mg wet weight tissue per sample. Significantly different from: *, the KN-93 and Kreb-alone conditions; †, the amphetamine-alone condition (Adapted from (32). With kind permission).
its constitutively active form unchanged (58). Thus, it is possible that CaMKII contributes differentially to the expression of sensitization in the striatum and the NAcc. Alternatively, as both of these reports assayed these proteins in NAcc punches or hand-dissected tissues as opposed to synaptosomes, it is conceivable that the results obtained were influenced by different alterations in CaMKII signaling in pre- and postsynaptic sites (59). As with its presynaptic actions, CaM may also contribute to the initiation of neuroadaptations by activating CaMKII postsynaptically where it recognizes numerous substrates.

5. CaMKII: Postsynaptic Effects

CaMKII is ubiquitously expressed in brain and is abundant in both pre- and postsynaptic sites where it phosphorylates many functional proteins (60). The GluR1 subunit of AMPA receptors expressed by intrinsic NAcc neurons is known to express such targets. CaMKII directly phosphorylates the AMPA receptor GluR1 subunit at serine residue 831 to increase unitary conductance (61, 62) and response quantal size (63). In addition, GluR1-containing receptors are delivered to synapses in an activity-dependent manner that involves entry into the extrasynaptic site and lateral movement into the synapse (64). CaMKII activity is also required for insertion of GluR1 into the cell membrane although the specifically targeted substrate remains unknown (65). These CaMKII-mediated, activity-dependent changes in AMPA receptor function are known to play an important role in altering synaptic strength and producing long-term potentiation (66, 67). It is likely that they also underlie sensitized behavioral responding to psychostimulant drugs as changes in glutamatergic transmission are known to contribute importantly to the expression of stimulant sensitization (21). For example, AMPA receptor antagonists, administered either systemically or into the NAcc, block the expression of locomotor sensitization by AMPH or cocaine (68–71); cf (72). Conversely, microinjection of AMPA into the NAcc produces enhanced cocaine-induced locomotor activity (25, 73) and reinstatement of cocaine seeking (9) in stimulant exposed rats compared to nonexposed controls. Rats showing sensitized locomotor responding to cocaine also display enhanced levels of GluR1 surface expression (27, 28, 74) and increased AMPA/NMDA receptor ratios in the NAcc (26), both findings indicative of enhanced synaptic excitability in this site. Finally, increased membrane insertion of AMPA receptors lacking the GluR2 subunit have been shown to mediate enhanced cue-induced cocaine seeking (29). In these cross-linking experiments, AMPA receptors detected on the cell surface were found to be associated with the
postsynaptic protein PSD95 (27). Notably, experiments conducted in co-cultures of primary NAcc and prefrontal cortex neurons showed that CaMKII activity is required for the enhanced cell surface expression of AMPA receptors in NAcc neurons following repeated DA (75). Taken together, these findings are consistent with the possibility that CaMKII activity in intrinsic NAcc neurons regulates postsynaptic glutamate-dependent mechanisms underlying the expression of sensitization.

Evidence, more directly illustrating a role for CaMKII in NAcc neurons in the expression of behavioral sensitization by amphetamine, was recently obtained in experiments using viral-mediated gene transfer. In these experiments, herpes simplex viral (HSV) vectors were microinjected into the NAcc to overexpress CaMKII in neurons adjacent to the injection site (76). Using vectors expressing green florescent protein, whole brain immunohistochemical analyses revealed that infection was limited to these neurons in the NAcc and that neurons in nuclei sending projections to this site were spared (77). Remarkably, overexpressing CaMKII in the NAcc of drug naïve rats produced a sensitized locomotor response to amphetamine (78) and increased work output and amphetamine self-administration (79) relative to control rats that were not infected or infected with a reporter gene. As illustrated in Fig. 4, infected rats showed a sensitized locomotor response to a challenge injection of amphetamine even though they had not previously been exposed to the drug. These findings extend those described above and strongly support an important role for CaMKII signaling in NAcc neurons in the expression of stimulant sensitization. Thus, overexpressing CaMKII in the NAcc may mimic some of the changes produced by amphetamine exposure that lead to long-term maintenance of sensitization. Together with the findings reviewed earlier, these results also suggest that overexpressing CaMKII may enhance behavioral responding to amphetamine by functionally upregulating AMPA receptors in these neurons.

6. CaMKII and the Expression of Stimulant Sensitization

The results discussed in the previous sections show that CaMKII can act presynaptically in midbrain DA neuron terminals in the NAcc and striatum as well as postsynaptically in medium spiny neurons in these DA projection fields to contribute to the expression of behavioral sensitization by psychostimulant drugs. It is not yet clear how this enzyme might coordinate its effects in these and possibly other sites to influence the expression of behavioral sensitization. One possibility is that CaMKII exerts effects in both pre- and postsynaptic sites to produce a sensitizing feed-forward
loop, possibly initiated by enhanced NAcc DA overflow, leading to upregulation of medium spiny neuron AMPA receptors, increased excitability in these cells, and enhanced behavioral output via projections from the NAcc to pre-motor nuclei. Importantly, the increased excitability in medium spiny neurons could be self-enhancing because these cells also send descending feedback projections to the VTA where, via disinhibition (80–82), they can lead to increased activity in midbrain DA neurons and more DA release in the NAcc. Thus, the enhanced NAcc DA overflow resulting from exposure to psychostimulant drugs could lead to increased medium spiny neuron excitability, which would lead to further enhancements in NAcc DA release and further increases in medium spiny neuron excitability. Interestingly, different lines of evidence indicate that CaMKII in NAcc neurons can be recruited by a pathway initiated by the activation of D1 DA receptors. Stimulation of these receptors activates cyclic AMP and protein kinase A, which can then phosphorylate L-type cal-

Fig. 4. Overexpressing CaMKII in the NAcc of drug-naïve rats enhances locomotor responding to amphetamine. Herpes simplex virus vectors were constructed by inserting rat CaMKII cDNA into the vector pHSV-PrpUC, depicted in (a). The main elements of the vector necessary for packaging and transgene expression are the packaging site (HSV-1 “α”), the immediate early gene promoter (HSV-1 IE 4/5), the sequence of origin (OriS), and the simian virus 40 (SV-40) polyadenylation (poly A) signal. The transgene was subcloned into the HSV amplicon, transfected into 2–2 African green monkey kidney epithelium cells, and superinfected with replication-deficient helper virus. After several passages, the virus was purified, pelleted, and resuspended in 10% sucrose. (b) Preliminary data demonstrating that viral-mediated overexpression of CaMKII in the NAcc of drug-naïve rats leads to enhanced locomotor responding to amphetamine when compared to control rats that were not infected (10% sucrose infusions) or infected with a reporter gene (HSV-LacZ). Data are shown as locomotor activity counts (group means ± SEM) obtained before and after a systemic injection of amphetamine (arrow). Inset shows 2-h total locomotor activity counts obtained after amphetamine. *Significantly different from controls (78) (a: Adapted from (88). With kind permission).
Calcium channels to increase inward calcium conductance (83, 84) and lead to the activation of CaMKII (Fig. 5). A recent study showed that cocaine-induced reinstatement is dependent on this signaling pathway in the NAcc as well as the cell surface expression of the AMPA receptor GluR1 subunit in this site (38). Consistent with these findings, activation of D1 DA receptors has been shown to promote the cell surface expression of GluR1 in primary NAcc neuron cultures through a pathway dependent on protein kinase A (85–87). Together, these findings identify a signaling cascade through which stimulant-induced DA overflow in the NAcc could functionally upregulate AMPA receptors in these neurons. CaMKII may thus act as a bridge to integrate DA and glutamate neurotransmission converging onto medium spiny neurons in the NAcc (38). Repeated exposure to psychostimulant drugs may exploit this function to produce interacting multi-site neuroadaptations that underlie the long-lasting maintenance of sensitization by these drugs.

Acknowledgments

Supported by a grant from the Peter F. McManus Charitable Trust (PV) as well as grants R01-DA-09397 (PV) and F31-DA-022834 (JAL) from the National Institutes of Health.
References

24. Kim JH, Austin J, Tanabe L et al (2005) Activation of group II mGluR receptors
blocks the enhanced drug taking induced by previous exposure to amphetamine. Eur J Neurosci 21:295–300
27. Boudreau AC, Wolf ME (2005) Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. J Neurosci 25:9144–9151


Animal Models of Eating Disorders

Stephanie D. Hancock and Mary C. Olmstead

Abstract

Eating disorders and drug addiction share many common traits. This includes biological and environmental factors that predispose individuals to develop either disorder, an increased risk for anxiety and depression when the disorders are present, and heightened trait levels of impulsivity and compulsion. Animal models of eating disorders are not as well established as those that model drug addiction, but the research in this area is progressing rapidly. In this chapter, we discuss anorexia nervosa, bulimia nervosa, binge eating disorder, and obesity as these encompass the majority of maladaptive eating behaviors in humans. We begin by outlining the important features that characterize each disorder and that should thereby be present in an animal model. An overview of peptide control of feeding is provided to help the reader evaluate the animal models presented. These are based principally on genetic variation and stressful life events. In general, most animal models based on genetic alterations have limited applicability to humans, at least to date. Those based on stressful life events appear more promising in that they more accurately reproduce alterations in feeding and neuroendocrine function that are characteristic of each disorder. The next obvious step in eating disorder research is to combine the two approaches to determine how genetic alterations and stressful events interact to produce maladaptive eating and physiological changes.

Key words: Anorexia nervosa, Bulimia nervosa, Binge eating disorder, Obesity, Stress, Genetics, Neuroendocrine function, Feeding peptides, Hypothalamus

1. Introduction

Eating disorders and drug addiction share many common traits. This includes biological and environmental factors that predispose individuals to develop either disorder, an increased risk for anxiety and depression when the disorders are present, and heightened trait levels of impulsivity and compulsion. Like drug addiction, the chance of recovery from eating disorders is dismally low: more than 50% of patients in partial remission relapse within a year (1, 2). The overlap between eating disorders and drug addiction should not be surprising as drugs of abuse activate
neural systems that evolved to reinforce behaviors, such as feeding, that enhance species’ survival (3, 4).

Animal models of eating disorders are not as well established as those that model drug addiction, but the research in this area is progressing rapidly. As with drug addiction, no single paradigm can model the complexity of pathological eating, particularly as this is a large category that can be divided into several distinct disorders. In this chapter, we discuss anorexia nervosa, bulimia nervosa, binge eating disorder, and obesity as these encompass the majority of maladaptive eating behaviors in humans. Anorexia nervosa is characterized by self-imposed weight loss to a body weight less than 85% of that expected for the individual’s age and height. Anorexics exhibit increased activity levels and food obsessions (related to the handling and preparing of food), but no significant disturbances in appetite (5). The disorder may be separated into subtypes: restricting and binge eating/purging. Bulimia nervosa is sometimes considered a subtype of anorexia nervosa as both involve excessive preoccupation with food and body image, and bulimia is often preceded by a history of anorexia. The defining feature of bulimia is repeated episodes of overeating followed by compensatory behaviors such as vomiting, laxative use, or excessive exercise. Thus, the binge eating/purging subtype of anorexia nervosa overlaps considerably with bulimia nervosa, with the exception that anorexics are underweight whereas bulimics are typically of normal to low weight. Recurrent bouts of extreme overeating also occur in binge eating disorder, but with no subsequent compensatory behavior. Obesity is distinct from these other three disorders in that maladaptive eating (either restricted or uncontrolled) is not a criterion for this condition: obesity is defined solely as a state of excess body fat with a corresponding body mass index (BMI) greater than 30 (6). Indeed, obesity is not even listed as a psychiatric disorder in DSM-IV (7). Nonetheless, with very few exceptions, obese individuals display disrupted eating patterns and/or activity levels that jeopardize their health. By this criterion, obesity is clearly an eating disorder and is discussed in this chapter as such. Moreover, the increasing prevalence of obesity makes it a global health issue and one that requires the attention of the research community.

Given that a primary feature of all eating disorders is disrupted feeding, any animal model must reproduce the maladaptive eating that is characteristic of that disorder. In the case of anorexia nervosa, food restriction should be self-imposed such that animals forego food consumption even when it is readily available. Despite the reduced intake of calories, anorexics exhibit normal to high activity levels, which should also be manifested in an animal model. Bulimia nervosa cannot be modeled fully in animals as there is no evidence that rodents or nonhuman primates initiate compensatory behaviors following binge eating episodes.
By that criterion, gut emptying via gastric fistula does not model purging. In contrast, binge eating can be modeled effectively in animals if brief bouts of excessive food intake occur repeatedly over time, are enhanced by food palatability, but are independent of food deprivation and circadian rhythms. To conform to the human disorder, food consumption of bingeing animals must be exaggerated compared to control animals exposed to the same environmental conditions. Obesity can be reproduced in animals through a variety of means, but the relationship between this state and the human condition depends on whether these animals also exhibit alterations in food intake. We use this as a criterion to evaluate animal models of obesity because, with the exception of a very small percentage of people, obese humans show different patterns of food consumption than do individuals of normal weight.

In addition to these fundamental changes in feeding behavior, physiological and neuroendocrine changes accompany most eating disorders. A detailed analysis of these changes in anorexia nervosa suggests that some, but not all, are adaptations to reduced food intake and weight loss (8). Bulimics, binge eaters, and obese humans exhibit distinct profiles of neuroendocrine changes although, in the absence of large longitudinal studies, it is impossible to determine which predates the other. Regardless of the causal relationship, the same changes should be evident in animal models of a particular disorder. Finally, any animal model should attempt to explain the higher rates of all eating disorders in females and, with the exception of obesity, the increased propensity for adolescent onset. In the following sections, we evaluate animal models of eating disorders, based on how well they reproduce altered eating patterns and the accompanying physiological and neuroendocrine changes characteristic of each disorder.

2. Peptide Control of Feeding

In order to evaluate animal models of eating disorders, it is important to understand the complex network of central and peripheral signaling peptides that control feeding, simplified in Fig. 1. Food intake and body weight are regulated, primarily, through neuropeptide production in the hypothalamus. The arcuate nucleus in this structure contains two groups of interconnected, “first-order” neurons that send axon terminals into the hypothalamic median eminence. The blood brain barrier is incomplete in the median eminence, allowing these neurons to contact the bloodstream (9). One group of first-order neurons produces anorexigenic (appetite-suppressing) peptides such as cocaine- and amphetamine-regulated transcript (CART) and the prohormone pro-opiomelanocortin (POMC). Cleavage
products of POMC include the melanocortins, adrenocorticotropic hormone (ACTH), and alpha-melanocyte-stimulating hormone (α-MSH), the latter of which acts as an agonist at melanocortin-3 (MC3) and -4 (MC4) receptors to suppress food intake and weight gain (10). MC3 receptors are located primarily within the arcuate nucleus whereas MC4 receptors are found in many hypothalamic nuclei and widely distributed throughout the brain (11, 12). The second group of first-order neurons acts in opposition to the first, releasing orexigenic (appetite-stimulating) peptides that include neuropeptide Y (NPY) and agouti-gene-related protein (AgRP). AgRP acts as an endogenous antagonist at MC3 and MC4 receptors (10, 11), helping to maintain a balance between signals that increase and decrease food intake.

First-order neurons in the arcuate nucleus send signals to “second-order” neurons in the dorsomedial, ventromedial, lateral/perifornical, and paraventricular nuclei of the hypothalamus. Within second-order nuclei, orexigenic and anorexigenic peptides are co-localized and co-released, activating receptors in overlapping...
regions. For instance, the lateral/perifornical hypothalamus contains the orexigenic peptides melanin-concentrating hormone (MCH) and orexin, as well as the anorexigenic peptide, CART. The paraventricular nucleus contains the anorexins cholecystokinin (CCK) and corticotropin-releasing hormone (CRH), but also orexigenic endogenous opioids. Whether orexigenic or anorexigenic peptides are released is largely dependent upon peripheral signals relating to the body’s energy balance. One such signal comes from leptin, an anorexigenic peptide produced by adipocytes (fat cells) in direct relation to fat tissue mass. Leptin activates Ob receptors, the product of the diabetes (Db) gene (13), within the median eminence; it also crosses the blood brain barrier to activate Ob receptors located on orexigenic- and anorexigenic-producing neurons in the arcuate, dorsomedial, ventromedial, lateral/perifornical, and paraventricular nuclei. In a state of positive energy balance, this adiposity signal reaches the arcuate nucleus where POMC and CART neurons are stimulated and NPY and AgRP neurons are inhibited. In a negative energy balance, the opposite occurs. Ghrelin is another peripheral signaling peptide, produced by the stomach and, in smaller amounts, by the arcuate nucleus and pituitary (14, 15). In contrast to leptin, ghrelin exerts orexigenic effects by stimulating NPY and AgRP expression in the hypothalamus (16, 17) and by antagonizing the leptin pathway (18). It is through these mechanisms that peripheral signals play an important role in regulating hypothalamic feeding-peptide function.

### 3. Animal Models Based on Genetic Variation

Anorexia nervosa, bulimia nervosa, binge eating disorder, and obesity are all associated with genetic variations in the peptide feeding system described above. This may explain, at least partially, differences in the vulnerability to develop these disorders. Indeed, estimates for the heritability of anorexia range from 33% to 84%, bulimia from 28% to 83%, and binge eating disorder from 38% to 61% with the remaining variance dependent upon individual experiences (19, 20). Heritability of obesity is the most substantial of all eating disorders, ranging from 77% to 94%. It is not surprising, therefore, that the majority of animal models based on genetic variation relate to this disorder.

#### 3.1. Obesity

The human obesity gene map (2005 update) references 135 candidate genes and more than 600 loci where mutation or transgenic expression in mice alter body weight and adiposity (21). Heritability of obesity, therefore, is probably polygenetic and likely interacts with environmental factors such as over-responsiveness
to external stimuli (22) or the availability of calorically dense comfort foods (3). Despite the difficulty of establishing gene–obesity connections, animal models of spontaneous single-gene mutations have provided valuable insight into the neuroendocrine changes that accompany obesity. Currently, there are 10 spontaneous single-gene mutations in mice and rats that result in an obese phenotype (23); those that are relevant to human obesity (24) are discussed below.

The most common genetic alterations that produce obesity in animals involve dysfunction in leptin signaling. Early experiments indicated that the profoundly obese ob/ob mouse lacks a circulating factor (25), later identified as leptin (26, 27). The animals exhibit hyperphagia combined with reduced locomotor activity and metabolic rate (28, 29). Importantly, this phenotype is normalized by exogenous leptin administration (29, 30). Loss-of-function mutations in the leptin receptor gene result in a similar phenotype, as seen in the diabetic db/db mouse and the Zucker fatty fa/fa rat (31). Deletion of hypothalamic Ob receptors does not produce profound obesity characteristic of ob/ob mice, although the reduction in central leptin receptors correlates with the severity of obesity in these animals (32, 33). The leptin-induced control of food intake appears to be mediated through interactions with hypothalamic peptides: obese and leptin-receptor deficient rodents show decreased POMC and CART expression, increased AgRP, NPY, and melanin-concentrating hormone expression, and are less sensitive to CCK-induced reductions in food intake and meal termination (31, 34–40).

Like animals, humans may exhibit genetic deficiencies in leptin production and loss-of-function leptin-receptor mutations. These alterations produce hyperphagia, severe early-onset obesity, and rapid weight gain during development (41). In leptin-deficient individuals, leptin administration normalizes hyperphagia and dramatically reduces body weight and adiposity. Congenital leptin and leptin-receptor deficiencies are extremely rare and cannot explain the growing obesity epidemic. On the other hand, serum leptin and adipocyte Ob gene expression are strongly correlated with percent body fat in obese individuals. This suggests that, even in the absence of congenital leptin-signaling defects, obesity may represent a self-propelling state wherein high serum leptin levels lead to resistance and a loss of leptin-induced appetite suppression (42). The proposed mechanisms of leptin resistance include deficiencies in the leptin blood-brain-barrier transport system (43, 44), impaired rhythmicity of plasma leptin levels (45), adipocyte leptin-receptor blockade (46), and leptin signaling defects within the hypothalamus (47). Leptin resistance may be regulated, at least partially, by external factors as mice show increased resistance to leptin during consumption of a high-fat diet (48).
Hyperphagia, obesity, and increased linear growth rate also occur with genetic alteration of the melanocortin system, which includes POMC, α-MSH, AgRP, and MC4 receptors. POMC gene deletion in the mouse creates a loss of α-MSH signaling at MC4 receptors, resulting in hyperphagia and obesity that can be partly reversed by peripheral α-MSH treatment (49). Human POMC gene mutations have been linked, as in the animal model, to overeating and early-onset obesity (50). These mutations need not be homozygous: individuals exhibiting heterozygous point mutations that reduce hypothalamic MC4 receptor signaling have a high prevalence of obesity, suggesting that even partial defects in melanocortin function predispose individuals to obesity (51). Further, genetic deficiencies in the production of POMC cleavage enzymes, such as prohormone convertase 1, result in severe early-onset obesity (52). Like POMC gene deletion, targeted deletion of the MC4 receptor in mice produces hyperphagia and obesity (53, 54). MC4 receptor knockout mice are particularly susceptible to increased weight gain on a high-fat diet (55). These findings accord with evidence that the MC4 receptor selectively modulates dietary fat intake, as MC4 receptor knockout mice do not exhibit hyperphagia when placed on a low-fat diet, and MC4 receptor agonists selectively reduce fat, but not protein or carbohydrate, consumption (56). Like leptin-deficient animals, MC4 receptor knockout mice are largely insensitive to CCK-induced satiety (57).

Consistent with animal studies, MC4 receptor gene mutations in humans contribute to hyperphagia, increased fat mass, and increased growth rate in 0.5% to 1.0% of obese adults and 6% of individuals with childhood-onset obesity (24, 58–63). Although homozygous mutations produce a more severe phenotype, heterozygous mutations and the resultant loss of MC4 receptor function also lead to hyperphagia and obesity (59). In addition, DNA sequence variation in the regulatory region of the MC4 receptor gene is associated with increased waist circumference and fat mass (64, 65). One MC4 receptor variant, discovered in 25% of severely obese individuals, corresponds with increased intake of high-fat and high-protein foods (66). Overexpression of the AgRP gene decreases MC4 receptor activity and increases obesity in mice (67), and obese humans exhibit high levels of plasma AgRP (68). In rats, intracerebroventricular injections of AgRP selectively enhance intake of high-fat, but not low-fat, foods (69), reminiscent of the human MC4 receptor-variant phenotype (66). Genetic variation in the human AgRP gene (70) as well as an upstream promoter region (71) are associated with late-onset obesity. These animal models of genetic variation highlight the importance of heritability in susceptibility to obesity. They also demonstrate that neuropeptide feeding system function is modified by environmental factors, such as high-fat diet
consumption, as these may influence gene expression and other processes that maintain balance in the feeding peptide system.

Like obesity, anorexia nervosa is strongly familial (72), and is associated with genetic mutation or variation. Still, there is considerable debate regarding susceptible genes and this disorder (73). The most widely implicated candidate genes for anorexia are those encoding serotonin (5-HT) receptors, promoter regions, and transporter proteins (73–79). The anx/anx mouse supports this proposed connection between disruptions in 5-HT function and anorexia. This spontaneous mutation produces increased 5-HT neurotransmission (80, 81) and anorexic traits early in life, including dysregulated food intake, failure to grow, emaciation, and hyperactivity (82). The anx/anx mouse also exhibits reduced serum leptin levels and abnormalities in hypothalamic neuropeptide systems, including NPY, AgRP, POMC, and CART (83, 84), changes that are associated with anorexia in humans. These animals, however, also display traits uncharacteristic of the disorder, including body tremors, ataxia, and head weaving (82). Many of the animals die between 20 and 30 days of age, severely restricting the validity of this model.

Other proposed animal models of anorexia also lack validity to the human condition. This includes models based on inactivation of genes encoding melanin-concentrating hormone (85), CRH type 2 receptors (86), muscarinic M3 receptors (87), and tyrosine hydroxylase (88). Although all of these models replicate some feature of anorexia (e.g., hypophagia, emaciation, hypoleptinemia, altered metabolic rate, and/or neuropeptide system dysfunction), genetic studies in humans have failed to support their validity (79). Similarly, a number of neuropeptide systems are dysregulated in anorexia nervosa (8, 89), but there is little evidence that these are candidates for genetic predisposition to the disorder (73). The development of an animal model based on these irregularities, therefore, is unwarranted. Variations in the delta-1 opioid receptor gene are associated with anorexia in humans (74, 75, 90), but an animal model based on this variant has yet to be developed. Finally, binge/purge anorexia (91), bulimia (92, 93), and binge eating disorder (94) have all been linked to variants of ghrelin or preproghrelin genes, but these findings are not universal (95, 96) and ghrelin knockout mice show no alterations in 24-h food intake or body weight gain (97).

In contrast to these poorly validated models, there are a handful of animal models based on genetic variation that show promise for understanding eating disorders. First, variation in the DNA sequence encoding the AgRP gene is associated with the restricting subtype of anorexia nervosa (98) and central AgRP infusions increase food intake and prevent starvation in a rat model of anorexia (99). Second, restricting and binge/purge subtypes of anorexia have been associated with different alleles of the
central cannabinoid receptor 1 (CB1) gene (100), and CB1 receptor knockout mice eat less than wild-type mice following temporary food restriction (101). Third, both anorexia and bulimia nervosa are linked to variants of genes encoding estrogen receptors (102–104), which may account, in part, for the increased prevalence of these disorders in females (105). In rats, estrogen increases activity of the CCK signaling pathway and reduces food intake and body weight (106, 107). Fourth, hypothalamic MC4 receptor signaling modulates meal size and meal choice (108), and early-onset obesity in MC4 receptor knockout mice is the result of hyperphagia (109). Moreover, in patients undergoing laparoscopic gastric banding as a treatment for obesity, 6.3% carried MC4 receptor gene variants and all met the criteria for binge eating disorder (110). Similarly, in a sample of severely obese individuals, 5.1% exhibited MC4 receptor mutations with 100% of carriers reporting binge eating; in obese and normal-weight subjects without MC4 receptor mutation, rates of binge eating were 14.2 and 0%, respectively (111). MC4 receptor mutation is not, however, always associated with an increased risk for binge eating (112, 113), emphasizing that other factors play an important role in the etiology of this disorder.

Many animal models of psychiatric disorders, including some discussed above, produce genetic alterations using gene knockout technology. These studies must be interpreted with caution because the redundancy of orexigenic pathways allows ample opportunity for compensation of function during development. This may explain some apparent discrepancies, such as the finding that NPY modulates eating, but NPY gene deletion does not affect food intake or body weight (114). It should also be noted that our preceding discussion focused on genetic alterations related to the neuropeptide feeding system. In contrast, eating disorders are complex psychiatric conditions that undoubtedly involve disturbances in a variety of cognitive and behavioral processes. Both animals and humans that exhibit these changes may display genetic alterations in neural systems that mediate reward, emotion, learning, or inhibition, among others.

4. Animal Models Based on Stressful Events

Eating disorder patients exhibit heightened responses to stress, particularly as a trigger for binge eating (115). Obese individuals are stress-sensitive in that overeating in these individuals is more likely to be triggered by internal stress responses than by physiological signals of hunger. It is not surprising, therefore, that stressful life events often precipitate anorexic, binge eating, or bulimic episodes (8, 106, 116–118) which, over time, may lead to obesity (119).
The effect of stress on food intake is moderated by stressor intensity in both humans and animals: mild or acute stress increases eating whereas severe stress decreases eating (120–122). When foods high in fat and sugar are available, stress increases intake of these “comfort” foods (119, 123), even under conditions when reductions in eating are normally exhibited (124). Given the well-established links between stress and eating disorders (115), a number of animal models have been developed that show altered food consumption and body weight following stress. Prior to discussing these, we briefly outline interactions between stress hormones and feeding peptides, providing a framework for discussing animal models based on stress responses.

4.1. Stress Hormone-Feeding Peptide Interactions

Stress, defined as a physical or psychological event that causes a disruption in homeostasis (125), activates the hypothalamic–pituitary–adrenal (HPA) axis initiating a cascade-like secretion of hormones. The stress response is initiated in the paraventricular nucleus with the release of CRH. CRH stimulates high-affinity type 1 (CRH1) receptors located on anterior pituitary corticotrophs (126), resulting in ACTH and α-MSH secretion as well as increased POMC synthesis and gene transcription (127). Although ACTH and α-MSH suppress food intake, the anorexigenic effects of CRH are likely mediated directly through binding to low-affinity CRH type 2 (CRH2) receptors in the ventromedial (128) and paraventricular (129) nuclei of the hypothalamus, as neither hypophysectomy nor CRH1 receptor antagonists prevent CRH-induced anorexia in rats (130, 131). Stimulation of CRH2 receptors may be the mechanism through which leptin induces anorexic effects; leptin-stimulated release of CRH (44, 132), upregulation of CRH2 receptor mRNA in the ventromedial hypothalamus of rats (133, 134), and NPY inhibition (135, 136) all induce anorexic effects that can be prevented by CRH antagonists (137, 138).

CRH-stimulated release of ACTH from pituitary corticotrophs activates melanocortin receptors in the adrenal cortex, resulting in glucocorticoid (Cort) secretion, namely, cortisol in humans and corticosterone in rodents. Within the CNS, Cort binds to two types of receptors. The high-affinity mineralocorticoid receptor, located primarily in the hippocampus, is tonically activated and sensitive to the low Cort concentrations present under rest at the circadian trough (139). Under nonstressful conditions, mineralocorticoid receptor occupation maintains low ACTH levels, guarding Cort concentrations at a minimum to preserve metabolic homeostasis (140). The low-affinity glucocorticoid receptor, widely distributed throughout the brain, is phasically activated by higher Cort concentrations which are present during the circadian peak preceding daily activity and, particularly, during periods of stress (140–142). In response to stress, Cort binds to glucocorticoid receptors within the paraventricular nucleus,
pituitary corticotrophs, and most notably the hippocampus, activating a negative feedback loop to reduce HPA-axis activity and halt further Cort secretion. This mechanism protects the brain from the detrimental effects of prolonged Cort exposure (143). Higher hippocampal glucocorticoid receptor density translates into greater sensitivity to the negative-feedback effects of circulating Cort and a faster “turning off” of the stress response (144, 145). Glucocorticoid receptors are also found in high density in adipocytes, particularly intra-abdominal visceral fat, where their activation leads to lipid accumulation (146).

The HPA-axis response to stress is intensity-dependent with the magnitude of Cort release related to the perceived severity of the stressor. At low concentrations, Cort enhances body weight gain through facilitation of visceral fat deposition (146, 147), and promotes food intake via reductions in hypothalamic CRH and increases in hypothalamic NPY (148–154). NPY-mediated increases in food intake and weight gain depend upon circulating Cort; NPY infusions fail to increase food intake and body weight in adrenalectomized rats unless these animals are co-treated with synthetic Cort (155). These orexigenic effects are counteracted by a Cort-induced increase in Ob gene expression and plasma leptin levels (156–158). Leptin also acts at the adrenal level to dampen further Cort release (159, 160). In the absence of Cort, the anorexigenic effect of leptin is maximized: leptin-induced decreases in body weight are greater and longer lasting in adrenalectomized animals and are dose-dependently reduced by exogenously administered Cort (161). Adrenalectomy also alleviates obesity in leptin-deficient ob/ob and db/db mice (162, 163).

In contrast to mineralocorticoid receptor occupancy at low concentrations, high Cort concentrations increase glucocorticoid receptor occupancy to produce dramatically different effects on feeding behavior. High-dose Cort decreases body weight (164–166), feeding efficiency (i.e., body weight gain per kcal of food intake; 147), and food intake (165), likely through increases in leptin secretion and upregulation of ventromedial hypothalamic CRH2 receptors (165). The weight reduction associated with glucocorticoid receptor stimulation is more pronounced in lean, than in fat, body mass. This results in an increased proportion of fat to lean tissue, particularly around the abdomen (147, 164, 167), and may account for the higher proportion of visceral to subcutaneous fat mass in anorexics (168). In sum, the modulation of feeding peptide systems by acute or chronic stress provides a mechanism whereby stress may increase the propensity to develop an eating disorder.

4.2. Stress Manipulations

In animal models, mild stressors such as brief footshock (169), mild tail pinch (170), bursts of noise (171), temporary restraint...
or handling (172), short-term social stress (173), saline injection (174), and a small amount of wheel running (175) all increase food intake and/or body weight. These acute stressors produce transient perturbations in the glucocorticoid circadian rhythm and only slight increases in daily mean Cort levels (140). Chronic stressors of greater intensity have the opposite effect: prolonged footshock (169, 176), repeated tail pinch (170), sustained loud noise (177), extended immobilization (178, 179), 23 h/day social separation (180), long-term social stress (173), and shifting animals from individual to paired housing (181) all decrease food intake and/or body weight. These moderate stressors produce significant elevations in Cort so that mean daily levels are increased fivefold, and glucocorticoid receptor occupation is chronic (140). Given the differential effect on food intake and body weight, acute stressors are commonly used to model bulimia and binge eating, whereas chronic stressors are incorporated into animal models of anorexia.

4.3. Activity-Based Anorexia Model

The activity-stress (182) or activity-based anorexia paradigm (183, 184) is the best established animal model of anorexia. This protocol involves unlimited running-wheel access (~22–23 h daily), combined with limited food access (~1–2 h daily). In both humans and rodents, running increases CRH, ACTH, and Cort secretion (185–193), particularly when it is combined with food restriction (194, 195). Early life events that heighten HPA-axis reactivity to stress increase susceptibility to activity-based anorexia (196, 197) and are thought to precipitate the disorder in humans (198). In the activity-based anorexia model, animals exhibit suppressed food intake during the once-daily meal, lower than normal body weight, and hyperactivity. These symptoms become more severe across days (183, 199) and, in the absence of intervention, may lead to death (200).

The activity-based anorexia model replicates many of the core behavioral and physiological characteristics of anorexia. For example, weight loss, food intake suppression, and hyperactivity are more pronounced in young (201) and in female (197, 202) animals subjected to this protocol. Anorexics exhibit increased restlessness and excessive exercise that correlates with decreases in leptin levels (203). In females, this leads to hypothalamic-gonadal dysfunction and amenorrhea (204). In the activity-based anorexia model, estrous cyclicity is lost as activity levels increase (205) and leptin administration suppresses starvation-induced increases in wheel running (206). Wheel-running animals show increased hypothalamic 5-HT (207) which, through stimulation of hypothalamic CRH release (208), has been proposed to mediate stress-induced anorexia (130). The role of 5-HT in anorexia nervosa is well documented (8), providing further support for the validity of the activity-based anorexia model.
Finally, the activity-based anorexia model appears to mimic changes in cognitive function that develop with this disorder. Upon initial loss of 10–15% body weight, anorexia nervosa patients are cheerful, energetic, and mentally alert; with further weight loss, fatigue, irritability, and cognitive dysfunction ensue (209–212). In animals, wheel running (207) and moderate food restriction (213) improve cognitive performance, as measured in a spatial-learning task, whereas extreme food deprivation leads to deficits in performance (213). This suggests that the activity-based anorexia model may replicate some of the cognitive deficits displayed by anorexic patients. Assessment of other cognitive dysfunctions prevalent in anorexia nervosa, such as attentional set-shifting deficits and obsessive-compulsive traits, would add to the validity of this model. Given that these tests of animal cognition are already well-established (214, 215), this area of research could progress quickly.

Animal models of binge eating incorporate the phenomenon, well documented in humans, that stress-induced increases in feeding are magnified in the presence of calorie-laden snack foods (216). Consumption of high-fat, high-sugar “comfort” foods may occur because stress-induced opioid release suppresses HPA-axis activity (217). Intake of comfort foods sustains opioid release and increasingly inhibits HPA-axis activity (218, 219), creating a circle of events whereby humans and other animals may use comfort foods to “self-medicate” against stress (123). High dietary restraint (216), which in women is correlated with hypercortisol (220–223), and Cort hyperreactivity (224, 225) render individuals particularly susceptible to stress-induced bingeing. Eating comfort foods reduces Cort response to stress (226) and provides short-term alleviation of negative emotions (227). In rats, voluntary and periodic intake of food with high fat and high sugar content blunts HPA-axis reactivity by lowering hypothalamic CRH mRNA expression and reducing ACTH and Cort responses to stress (228–232). In contrast, animals that only have access to a high-fat diet exhibit increases, rather than decreases, in HPA-axis activity (233–235). The high-fat-only diet appears to act as a nutritional stressor, highlighting the importance of intermittent and voluntary intake of high-fat food in stress reduction.

Corwin and colleagues developed an animal model of binge eating disorder based on clinical findings that human binge eating occurs in the absence of hunger (236) and is directed toward high-fat foods that are deemed “forbidden” and therefore self-restricted (237). In addition to unlimited chow, rats are given limited access (e.g., 2 h) to high-fat vegetable shortening, multiple times per week for several weeks. Bingeing develops over time in that rats increase their intake of fat when it is available and restrict their intake of chow when fat is unavailable (238, 239).
This behavior mimics the binge/compensation pattern typical of binge eating disorder and bulimia. Although bingeing on highly palatable food may alleviate anxiety and reduce HPA-axis activity in the short term, anxiety behaviors are increased in bingeing animals over the long term (240, 241). This also characterizes bulimic and binge eating patients who experience increased anxiety following binges once the disorder is established.

A separate model of binge eating uses a restriction-refeeding/stress protocol to model dietary restriction and stress as precipitating factors in this disorder (242, 243). Rats that experience repeated food restriction and refeeding, analogous to human “yo-yo dieting,” binge on high-fat, high-sugar food in the absence of hunger, long after restriction has ended (244). When food restriction-refeeding cycles are followed by a mild footshock, bingeing on highly palatable food is initiated sooner and is more dramatic (245). Bingeing is also more profound when cycles are introduced during the “adolescent” period in rats that experienced low levels of early-life maternal care (246), known to heighten HPA-axis reactivity to stress (247). Repeated fasting, stress, and bingeing on foods rich in sugar and/or fat decrease POMC and CRH2 receptor expression in the arcuate and ventromedial hypothalamus, respectively, increase NPY and Cort activity, and alter functioning of 5-HT, dopamine, and opioid systems in rats (165, 248–256). Disruptions in these systems are characteristic of both binge eating disorder and bulimia (8, 89, 257), providing further face validity to the restriction-refeeding/stress model.

4.5. Obesity Models

As noted previously, most animal models of obesity involve genetic variations that alter food intake and/or body weight. Chronic, mild stress can also produce obesity in animals and, like humans, this effect is associated with HPA dysregulation culminating in heightened CRH activity in the paraventricular nucleus, ineffective glucocorticoid feedback, and sustained HPA-axis stimulation (141, 165, 255, 258–262). Obese humans show elevated Cort secretion and altered Cort clearance that result in prolonged adipocyte glucocorticoid receptor activation, excess deposition of intra-abdominal fat, and leptin resistance which, together, increase obesity (146, 161, 263–266). Women with increased central fat distribution, as measured by waist-to-hip ratio, exhibit HPA-axis hyperactivity, secrete more cortisol during a laboratory stressor, and report more chronic stress compared to women with low waist-to-hip ratios (267–270). In genetically obese mice and rats, the HPA axis is hyperactive; expression of CRH2 receptor mRNA is reduced in the ventromedial hypothalamus (271, 272) whereas CRH1 receptor and hypophysiotropic CRH neuron activity is increased (134). Cort levels are elevated (273) and correspond with increased NPY mRNA content in the arcuate and paraventricular nuclei (274). These animal models therefore add credence
to the idea that, although genetic makeup influences susceptibility to obesity, environmental (i.e., stress) factors play a large role in regulating expression of these genes.

Finally, some animal models of obesity incorporate developmental processes by examining how early life experiences may render animals prone to later-life obesity (275). During gestation and early preweaning, maternal consumption of a high-fat diet increases the body fat of pups at weaning (276, 277), particularly in rat lines selectively bred for diet-induced obesity (278). This is paralleled in humans as obesity during pregnancy more than doubles the likelihood of childhood obesity (279). Perhaps counter-intuitively, maternal malnutrition, specifically during development of hypothalamic feeding centers in early to mid-pregnancy, increases the risk of adult obesity (280). In rats, gestational malnutrition may increase metabolic efficiency as developing animals adapt to an insufficient nutrient supply (281); with food abundance in adulthood, these adaptations may lead to increased body weight gain (282–285). Postnatally, rat pups raised in small litters exhibit adult obesity due to increased milk consumption preweaning and/or increased metabolic efficiency (286–289). Increased energy intake in human infants is associated with increased body weight in childhood and the propensity for obesity (290–292). These studies highlight the fact that environmental factors have a significant impact on body weight and adiposity and must be considered in conjunction with genetic predispositions and environmental stressors in an animal model of obesity.

5. Conclusions

Our discussion of animal models of eating disorders focused on models based on genetic alteration and those based on stressful events. The first analysis pointed to important roles for leptin and the melanocortin system in obesity, AgRP, 5-HT, estrogen, and CB1 receptor signaling in anorexia, and MC4 receptor signaling in binge eating. In general, however, most animal models based on genetic alterations have limited applicability to humans, at least to date. Those based on stressful events appear more promising in that they more accurately reproduce alterations in feeding and neuroendocrine function that are characteristic of each disorder. The next obvious step is to combine the two approaches to determine how genetic alterations and stressful events interact to produce maladaptive eating and physiological changes. Both processes clearly impact eating disorders in humans, a fact which should be reflected in animal models.

In addition to feeding and physiological changes, cognitive dysfunction is a primary characteristic of most eating disorders.
This includes exaggerated food obsessions, distortions in body image, compulsive food restriction, and/or the inability to control food intake. Indeed, approximately 15% of anorexic patients and 21% of bulimic patients meet criteria for obsessive compulsive disorder (293). The restricting subtype of anorexia nervosa is typically associated with emotional, cognitive, and social inhibitions, including perfectionist and obsessive traits; in contrast binge eaters tend to be extroverted but emotionally labile to the point of being impulsive (294). In the absence of large, prospective studies of eating disorders, it is difficult to determine whether these cognitive changes precede or follow the development of an eating disorder. It is also not clear how cognitive factors would be manifested in an animal model of obesity as these have not been studied in humans. Nonetheless, the fact that disrupted feeding patterns and cognitive changes co-occur in most eating disorders suggests that animal models should include assessments of both functions.

Eating disorders are also associated with altered affective states including heightened anxiety, restlessness, and agitation in anorexia nervosa (209, 211), and a high comorbidity with mood disorders in bulimia nervosa (295). Again, the face validity of an animal model would be strengthened substantially if it reproduced one or more of these affective changes. Many of these affective and cognitive changes also typify drug addicts, adding further credence to the proposed connection between eating disorders and drug addiction (3). Indeed, evidence is accumulating that alterations in neural systems controlling reward, inhibition, motivation, and emotion contribute to maladaptive behaviors in both drug addiction and obesity (296). Whether these can be extended to other eating disorders remains an open question, one that animal research is primed to answer.

References


Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. Diabetes 47:294–297


in anorexia and bulimia nervosa. Curr Drug Targets CNS Neurol Disord 2:53–59


different obesity phenotypes. Int J Obes 24:S47–S49


174. Booth DA, Campbell CS (1975) Relation of fatty acids to feeding behaviour: Effects of palmitic acid infusions, lighting variation and pent-4-enoate, insulin or propranolol injection. Physiol Behav 15:523–535


Part II

Modeling Stages of Drug Addiction in Animals
Acquisition of Drug Self-Administration

Marilyn E. Carroll and Richard A. Meisch

Abstract

This review provides an overview of animal models of acquisition of drug reinforcement by discussing research findings from studies that used drugs such as cocaine, methamphetamine (METH), phencyclidine, and nicotine, as well as several routes of drug self-administration (SA). Theoretical perspectives are given for the acquisition models indicating that the animal models are valid predictors of human behavior. Common organismic factors that contribute to the acquisition of drug abuse are also discussed, such as sex, hormonal influences, innate preference for sweet substances, impulsivity of choice, impaired inhibition, and avidity for physical activity. Differential rates of acquisition of drug SA in rats selectively bred for high and low sweet intake, ethanol intake, or avoidance responding are also discussed. Environmental factors such as enriched versus impoverished conditions, and the effect of behavioral economic factors related to drug abuse (e.g., effort, cost/reinforcement) are also considered. Pharmacological factors have also been found to influence acquisition, such as prenatal exposure to drugs, and potential treatment drug can reduce the rates of acquisition in animal models. Interrelations among factors are described, and their implications are summarized. This review adds to previous accounts of acquisition by shifting the emphasis from analysis of the process of acquisition of drug-taking to an assessment of the major factors that are influential in the initiation and acceleration of this process. The goal is to present translatable findings from animal research that are useful for informing prevention of drug abuse in humans.

Key words: Acquisition/initiation, Drug self-administration, Reinforcing effects, Animal models, Route of administration, Biological determinants, Environmental determinants, Pharmacological influences

1. Introduction

Acquisition of drug self-administration (SA) is defined as initial use of a drug, a transition from early sporadic sampling to an increase over a period of hours, days, or weeks to a steady rate of intake over time. The use of animal models to represent this phase of drug abuse in humans has many advantages, such as the ability to study (1) behavioral and biological processes underlying acquisition of
drug SA; (2) factors that control acquisition, either alone or in combination, over the lifespan of an animal, and (3) behavioral and pharmacological approaches to preventing or reversing acquisition. These studies may then provide information regarding the initiation of human drug abuse on factors regulating the initial occurrence of drug addiction, the underlying neurobiology, and how drug addiction can be prevented. This prospective approach is in contrast to the longitudinal epidemiological studies that can be done in humans and to the retrospective reports that can be analyzed over a longer addiction cycle in humans. The methods used to accomplish these objectives are described below.

The focus of this review will be on newer developments in the last decade since acquisition of drug SA was last reviewed (e.g., (1–3)). The main acquisition procedures that have been used, with the oral, pulmonary, and intravenous (i.v.) routes, as well as parenteral and intracranial methods will be discussed in terms of traditional and updated methods and the findings that have been generated. One major finding is that there has been less emphasis on methodology and more emphasis on application of these models to better understand the increasing prevalence of drug abuse. Thus, the majority of this review will emphasize current findings on the main factors influencing acquisition, such as biological/genetic, environmental, developmental, and pharmacological.

2. Acquisition Procedures

The routes of drug intake most commonly studied in the preclinical laboratory are those most commonly used by humans, i.v. and oral; however, other routes have been used, and they are close approximations of the routes of administration that are used in human drug abusers. Drug reinforcement in laboratory animals was first convincingly demonstrated with the i.v. route (4). However, the successful use of other routes extends the generality of drug-reinforced behavior and permits examination of the role of route of administration. That drugs can be established as reinforcers by these multiple routes extends the parallel with human behavior. Moreover, the successful establishment of many drugs as reinforcers by multiple routes also broadens the domain of drugs as reinforcers and the conditions under which drug reinforcement can be studied.

2.1. Intravenous (i.v.)

A study of intracranial electrical stimulation demonstrated that delays of even 1 s markedly impair acquisition of brain self-stimulation (5). Nevertheless, it takes approximately 10 s for intravenously delivered cocaine to affect the brain (6). How can acquisition of intravenous cocaine reinforcement develop and be
Acquisition of Drug Self-Administration

effective under such conditions of delay? The answer probably lies in the initial peripheral stimulation that immediately follows cocaine infusion (6). This interpretation is supported by the use of a peripherally, but not centrally, acting cocaine derivative, cocaine methiodide. In rats with experience with cocaine SA, but not in naïve rats, the intraperitoneal (i.p.) administration of cocaine methiodide resulted in ventral tegmental glutamate release and reinstatement of responding. These results are important for they suggest a mechanism for bridging the delay between onset of drug infusion and onset of CNS effects, namely rapid onset of peripheral drug effects that come to function as conditioned reinforcers. The methods used to establish i.v. SA attempt to capture this process.

2.1.1. Procedures to Facilitate i.v. Acquisition

Drug SA studies have increased dramatically in the last 10 years, and a high proportion of them have used the intravenous route. In the majority of these studies, rapid acquisition was used as a means to subsequently study other phases of drug abuse, such as escalation or reinstatement, and two methods were commonly used. First, experimenter-administered priming injections of the SA drug are given at the start of the sessions to stimulate exploratory behavior on the response manipulandum. A second approach is to train a response for food reward, and then substitute drug. Another form of drug substitution has been used occasionally, whereby a new drug of interest replaces one that is strongly maintaining SA. The substituted drug is given for several days, and doses are varied. The original drug may be reinstated between doses of the new drug. A disadvantage of this method is that extinction can take 2 weeks or more; thus it is not clear whether the behavior is being reinforced by the substituted drug or is the result of extinction from the first drug.

2.1.2. Autoshaping

An autoshaping method has been used to establish i.v. drug SA, as it allows for a gradual and controllable acquisition process that can be standardized across animals, and it permits a quantitative analysis of the process (7). During autoshaping, 10 drug infusions are delivered under a random-interval (90 s) schedule during each 1-h interval for 6 h. For each of the 10 noncontingent infusions, a retractable lever is extended, the lights over the lever are illuminated, and an infusion is delivered after 15 s, and then the lever retracts. If there is a response on the lever before the 15 s elapses, the lever immediately retracts and an infusion is delivered. During a subsequent 6-h SA component, the lever remains extended, and infusions are available contingent upon a fixed-ratio 1 (FR 1) schedule. Autoshaping produces a gradual, negatively accelerating function for the percent of animals in the group that meet the acquisition criteria (8). It is sensitive to many manipulations (e.g., dose, schedule, feeding condition, sex, hor-
monal differences, treatment medications). Measures of acquisition are percent of group and number of days to meet acquisition criteria, which was defined as a mean of 100 infusions per 6-h session for 5 days for a 0.2 mg/kg dose, or 50 infusions/day for 0.4 mg/kg. The use of autoshaping as an animal model of the acquisition of drug SA has face validity because initial exposure to drugs and associated cues in humans is often without response cost. For example, in humans, the initial exposure to drugs may be from a dealer requiring little or no cost, and then the price is increased with frequency of subsequent use. Both the establishment of stimulus-reinforcer associations and initial low response cost are important in the establishment of the reinforcing effects of drugs.

2.1.3. Growth Curve Analysis

Growth curve modeling has been used to capture the process of acquisition and to add more quantitative indices than those provided by the time to acquire and number in the group meeting criteria (9, 10). The growth curve, modeled after human growth curves, describes individual patterns of increasing drug intake over time with a quadratic equation that yields several measures: y-intercept, slope, and an acceleration parameter. While the model is a promising approach and offers several dependent measures, it was not sensitive to differences in dose or FR requirement (11). Use of the model with i.v. nicotine SA described acquisition as an initial rapid acceleration in drug consumption, with an increase that waned over time (9, 10). Although the Donny et al. (9, 10) method has not been applied to other drugs or the oral route, descriptions of individual acquisition patterns from studies in other laboratories using oral (12) or i.v. (8) SA demonstrate similar acquisition functions.

2.2. Oral Methods

In humans, the oral route (and absorption in the stomach) is used with ethanol drinking and with the nonmedical use of prescription drugs; oral ingestion of nicotine products (e.g., chewing tobacco, gum) is through the mucosal lining of the mouth. Drugs such as phencyclidine (PCP), cocaine, marijuana, and others can also be consumed orally. In studies with animals, there are two impediments to establishing orally delivered drugs as reinforcers. One is the delay between ingestion and the onset of CNS effects, and the other is the aversive taste of most drug solutions when they are within the range of concentrations that can be reinforcing. Procedures have been developed to deal with these problems (for detailed reviews see (3, 13)).

With the i.v. route, the immediate onset of peripheral drug effects mediates the delay between the infusion and the onset of CNS effects. However, with the oral route, the delay between drug delivery and CNS actions is substantially longer. Therefore, how is it possible to establish orally delivered drugs as reinforcers?
Exteroceptive stimuli that accompany drug SA (e.g., physical properties of the drug and drug seeking and taking stimuli associated with the environment) serve as conditioned cues that maintain the behavior. Taste of drug preparations can come to serve as a conditioned reinforcer, and this has been evident in animal studies. For example, with pentobarbital, the range of unit drug doses that maintain oral drug SA is below the range of i.v. doses (see Table 1). The oral dose is calculated as the amount of drug delivered (mg) upon schedule completion divided by the monkey’s body weight. The amount of drug is the volume delivered (e.g., 0.5 mL) multiplied by the drug concentration (e.g., 1 mg/mL). These low drug doses suggest that it must be the taste of the drug solutions that mediates the delay between drinking and the initiation of CNS effects. A related finding is that a quinine solution, when substituted for PCP, maintained responding for 7 to over 30 sessions before values fell to control levels (21). Thus, the quinine solution was serving as a conditioned reinforcer. Support for the role of taste comes from studies of taste aversion conditioning that have demonstrated learning can occur when there are long intervals between ingestion of a substance and subsequent effects.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of oral (p.o.) and intravenous (i.v.) pentobarbital doses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pentobarbital doses (p.o.)</strong></td>
<td></td>
</tr>
<tr>
<td>Lightest monkey 7.2 kg</td>
<td>Heaviest monkey 11.8 kg</td>
</tr>
<tr>
<td>Pentobarbital (mg/mL)</td>
<td>Dose (mg/kg)</td>
</tr>
<tr>
<td>0.125</td>
<td>0.009</td>
</tr>
<tr>
<td>0.25</td>
<td>0.017</td>
</tr>
<tr>
<td>0.5</td>
<td>0.035</td>
</tr>
<tr>
<td>1</td>
<td>0.069</td>
</tr>
<tr>
<td>2</td>
<td>0.139</td>
</tr>
<tr>
<td>4</td>
<td>0.278</td>
</tr>
<tr>
<td>References</td>
<td>Pentobarbital doses (i.v.)</td>
</tr>
<tr>
<td>(14)</td>
<td>Dose: 3 mg/kg</td>
</tr>
<tr>
<td>(15)</td>
<td>Doses: 0.25, 0.5, 1, and 2 mg/kg</td>
</tr>
<tr>
<td>(16)</td>
<td>Doses: 0.1 to 3 mg/kg</td>
</tr>
<tr>
<td>(17)</td>
<td>Doses: 0.25, 0.5, 1, 2, and 4 mg/kg</td>
</tr>
<tr>
<td>(18)</td>
<td>Dose: 4 mg/kg</td>
</tr>
<tr>
<td>(19)</td>
<td>Dose: 5 mg/kg</td>
</tr>
<tr>
<td>(20)</td>
<td>Dose: 5 mg/kg</td>
</tr>
</tbody>
</table>
If animals do not drink a drug solution, acquisition of drug-reinforced behavior will not occur. The problem is that often drug solutions have an unpleasant taste; thus, sufficient drinking will not take place to allow the CNS reinforcing effects to occur. One solution is to induce drinking. Several methods have been used. One is to employ schedule-induced drinking, which results in excessive water intake when animals intermittently receive small pellets of food (22). Low drug concentrations are substituted for water, and over time the drug concentrations are gradually increased (23, 24). Once significant drug effects occur, such as changes in the animal’s overt behavior, the pellet deliveries can be discontinued (25). If the drug has been established as a reinforcer, drinking persists at levels that exceed vehicle (usually water) levels (25, 26). By having an initial session component free of inducing conditions, one can monitor the development of reinforcing effects within individual animals across sessions (27). Following the initial component, the inducing conditions can be introduced to insure that drug consumption occurs. A related method to induce drinking is to give the animal food during the session. Drinking of water and drug solutions (postprandial) reliably occurs following eating (12, 28, 29).

A third induction method is to use a liquid such as a low ethanol concentration that is readily consumed, probably due to a positive taste (30–32). Fading procedures are used to introduce the new drug and then remove the original liquid. A low concentration of the drug to be established as a reinforcer is added to the liquid solution (usually 2% ethanol), and across sessions the drug concentration is gradually increased. Subsequently, the ethanol concentration is reduced in steps to zero. If consumption of the drug solution persists above vehicle levels, then the drug has been established as a reinforcer. These induction methods have been successfully used with mice, rats, and rhesus monkeys. An additional strategy is to use animals that have high baseline levels of water intake. Low drug concentrations can be substituted for water and subsequently be increased across sessions (29, 31, 33, 34).

Once one drug has been established as an orally effective reinforcer for rhesus monkeys, other drugs can be substituted for the first drug and responding will be maintained (21). Usually, the drugs are from the same pharmacological class, such as PCP like (21, 35), barbiturates (36), and benzodiazepines (37). However, this is not always the case, for d-amphetamine was successfully substituted for PCP (35). The ability to establish orally delivered drugs as reinforcers by simple substitution for another drug expedites research and is theoretically interesting.
2.3. *Intragastric (i.g.)*

Rats and rhesus monkeys will self-administer a variety of drugs via the i.g. route, which is a variation of the oral route that bypasses taste and orosensory factors (38). However, it has been difficult to establish and maintain drug reinforced behavior using the i.g. route (see (39)). The reasons are probably due both to the delayed onset of drug effects relative to some other routes and to the delayed onset of peripheral cues. For example, with the i.g. route, there are no immediate taste cues such as those that occur with the oral route, and no rapid onset of drug effects found with the i.v. route. Acquisition is much slower than with the i.v. route, and the delayed onset of drug effects increases the possibility of overdose (39). Thus, with the i.g. route, large drug doses are necessary to sustain drug taking, and responding is not maintained under high-value intermittent schedules (39). These findings illustrate the importance of the immediacy of onset of either conditioned or unconditioned drug effects.

2.4. *Smoking*

A laboratory model of cocaine-base smoking is another oral procedure that was developed around 1990, and this laboratory method has been used to conduct parallel studies with rhesus monkeys (40) and humans (41). Monkeys were first trained to self-administer PCP and water, as described in the oral methods. They were then trained to make sucking responses on a hollow steel tube with a replacable, coiled nichrome wire that fits into the inside of the tube. The drug was dissolved in 95% alcohol, placed on the coiled wire with a syringe, then alcohol was allowed to evaporate. Before each smoking trial, a drug-coated coil was placed into the smoking tube and connected with the circuitry on the outside of the cage. After the response requirement (five sucking responses) on the smoking spout is completed, the coil was heated, the cocaine was volatilized, and the monkey inhaled the drug. A similar apparatus (and verbal instructions) was used with humans, and results indicated that nearly all of the drug was inhaled and absorbed by both monkeys and humans, and similar physiological measures (heart rate, blood pressure, drug level in plasma), and time courses resulted (41).

The smoking procedure has continued to provide valuable information over the last 18 to 20 years. Studies have shown that nondrug incentives and medications reduce cocaine smoking, and when both treatment approaches were combined, cocaine smoking nearly stopped (42). Other drugs such as heroin, heroin–cocaine (speedball) combinations, and THC have been studied, and extensive behavioral economic analyses of smoking-rewarded behavior have been conducted regarding the effect of sex and availability of nondrug reinforcers on the acquisition of smoking cocaine (43). The smoking model is labor intensive, but the results are highly consistent with human behavior, and tests of treatment attempts are predictive of clinical results (see (41)).
In addition to the i.v., oral, and inhalation routes, acquisition of drug-reinforced behavior can occur with other routes such as intramuscular (i.m.), i.p., and subcutaneous (s.c.) (see Table 2). For example, lever pressing by rhesus monkeys can be maintained by response-contingent i.m. injections of morphine or cocaine (44). The injection is administered by the experimenter upon completion of the schedule requirement. The advantages of this procedure are the lack of need for surgery, catheters, and infusion pumps. In addition, the i.m. route can be used over long periods of time. The disadvantages are the need for the experimenter to give the injection when the schedule is completed and the training required to establish i.m.-injected drugs as reinforcers.

A similar procedure has been developed for rats (54, 56). Initially the rats were trained to respond under a fixed-interval (FI) schedule with food as the reinforcer. Next, a fading procedure was used. After food was obtained, the rat was given either an i.p. or s.c. injection of the potent opioid, etonitazene. The dose was gradually increased across sessions, and subsequently the amount of food was systematically decreased. Responding persisted in the absence of food, and the substitution of saline for drug resulted in a drop in response rate below drug values. Reintroduction of the drug produced a return to the previous levels of responding. Thus, the drug clearly functioned as a reinforcer. The advantages and disadvantages are the same as with the i.m. route. With all three routes, all behavior leading up to the initial injection is reinforced by the drug without the behavior being influenced by direct drug effects. These experimental methods

<table>
<thead>
<tr>
<th>Route</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intramuscular (i.m.)</td>
<td>Rhesus monkeys</td>
<td>(44)</td>
</tr>
<tr>
<td></td>
<td>Squirrel monkeys</td>
<td>(45)</td>
</tr>
<tr>
<td>Intragastric (i.g.)</td>
<td>Rats</td>
<td>(46)</td>
</tr>
<tr>
<td></td>
<td>Rhesus monkeys</td>
<td>(20)</td>
</tr>
<tr>
<td>Intracerebroventricular</td>
<td>Rats</td>
<td>(47, 48)</td>
</tr>
<tr>
<td>Intracranial</td>
<td>Rats</td>
<td>(49, 50)</td>
</tr>
<tr>
<td></td>
<td>Rhesus monkeys</td>
<td>(51)</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>(52)</td>
</tr>
<tr>
<td>Intrathecal</td>
<td>Rats</td>
<td>(53)</td>
</tr>
<tr>
<td>Intraperitoneal (i.p.)</td>
<td>Rats</td>
<td>(54)</td>
</tr>
<tr>
<td>Subcutaneous (s.c.)</td>
<td>Rats</td>
<td>(55, 56)</td>
</tr>
</tbody>
</table>
make possible the study of drug-reinforced behavior over long time periods and in laboratories that lack the equipment and facilities for intravenous studies.

3. Factors Affecting Acquisition

A common goal of drug addiction research is to identify factors that contribute to the acquisition of a habitual drug-abuse pattern in humans, so that at-risk individuals can be targeted for prevention or treatment attempts that are focused on their specific vulnerabilities. Phenotypic variations in acquisition of drug SA are strongly influenced by individual differences in genotype, physiological state, drug history, interactions with the environment, as well as the pharmacological and neurobiological aspects of the drugs and the SA process. In the following section, these factors are divided into biological, environmental, and pharmacological/neurobiological determinants, and each is briefly discussed; however, these factors do not influence acquisition of drug SA independently, and many interactions exist among them (see Table 3; (57)).

Table 3
Interaction of risk factors for drug abuse

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Adolescents &gt; adults</th>
<th>Sex/hormonal</th>
<th>Impulsivity</th>
<th>Sweet liking</th>
<th>Novelty reactivity</th>
<th>Exercise</th>
<th>Environment</th>
<th>Stress reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex/hormonal F &gt; M</td>
<td>+</td>
<td>Sex/hormonal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Impulsivity HiI &gt; LoI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Sweet liking</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sweet liking HiS &gt; LoS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Novelty reactivity HR &gt; LR</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Exercise HiR &gt; LoR</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>O</td>
<td>Exercise +</td>
<td>+</td>
</tr>
<tr>
<td>Environment Poor &gt; rich</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stress reactivity High &gt; low</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Positively related, – negatively related, O no correlation, ? unknown
Research with animal models over the last decade has shown that female rodents and nonhuman primates acquire drug SA faster, in greater numbers per group, and at lower doses than males (58). This finding has consistently been reported with different routes of administration, several species, and with a wide range of drugs including amphetamine, caffeine, a cannabinoid receptor-1 agonist (59), cocaine, ethanol, fentanyl, heroin, methamphetamine (METH), morphine, nicotine, and phencyclidine (see reviews by (59, 60)). Most acquisition studies were conducted with rats; however, female monkeys exceeded males on acquisition measures (58). The sex differences (females > males) in acquisition also extend to other critical transition phases of drug abuse such as escalation, binge use, dysregulation, compulsive intake (61, 62), and reinstatement of behavior previously reinforced by cocaine (63). Also, chronic exposure to a cannabinoid agonist (CP 55,940) during adolescence increased acquisition of cocaine SA in females but not males (64). Females also respond better than males to behavioral and pharmacological treatment for drug SA (58); however, males exceed females in withdrawal effects (e.g., (65–67)).

The underlying basis for the most consistent sex differences (females > males) in various aspects of drug abuse is the ovarian hormones. Specifically, estrogen increases and progesterone decreases the rewarding effects of drugs. Studies with ovariectomized (OVX) estrogen-replaced animals indicate that drug SA occurs more rapidly and in greater numbers of rats than vehicle-treated OVX controls or sham-operated rats treated with the estradiol antagonist, tamoxifen. The facilitating effects of estrogen on acquisition have been shown with cocaine (60) and heroin (68). In contrast, progesterone counteracts the facilitating effects of estrogen in female rats, and male rats are not affected by these ovarian hormones (60). Estrogen and progesterone have similar opposite effects during other phases of the addiction process (69–71). Results of the animal studies are in close agreement with reports in humans regarding positive subjective effects of drugs when estrogen peaks (follicular phase) and negative effects when estrogen is opposed by high levels of progesterone (72). Thus, sex and hormones associated with the phase of the menstrual cycle may be important determinants in the initiation of drug abuse in humans.

### 3.1.2. Age

Animal models are essential for studying the influence of age on the acquisition of drug abuse, as it is difficult to experimentally study human drug abuse at the early age at which it begins. Animal studies have focused on comparisons of adolescent versus adult onset of drug SA to parallel human conditions (57). Two basic questions are most commonly addressed: (1) does drug exposure during adolescence alter acquisition of SA of the same or different drugs in adults, and (2) does the rate of acquisition of
Acquisition of Drug Self-Administration

Drug SA differ in adolescent versus adult animals? Rodents offer an ideal model for the acquisition phase because the length of their adolescence (~30 days) allows adequate time to initiate drug SA and to conduct subsequent tests during adulthood. For example, exposure to ethanol during adolescence resulted in greater acquisition of ethanol SA in adults (73). Work with rats also showed that nicotine exposure during adolescence (compare with exposure as adults) increased the acquisition of cocaine SA when both groups were tested as adults (74).

In studies that compared adolescents and adults during acquisition of drug taking, rats exposed as adolescents (vs adults) exhibited faster acquisition of cocaine SA and in greater numbers per group (75, 76). Adolescent rats also acquired SA of nicotine (77) and ethanol (71) faster than adults. Adolescent rats also showed greater intake of saccharin (vs water) than adults (74, 78). These results are consistent with greater sweet preference in younger versus older humans (79), and it is related to drug reinforcement because sweet preference predicts acquisition of drug SA (80). Overall, adolescent drug (and other reward) exposure increases the likelihood and rate of acquisition of drug use as adults.

3.1.3. Sweet Preference

Rats selected for their avidity for sweet tastes rapidly acquire SA of drugs such as amphetamine, ethanol, and morphine compared to animals with lower sweet preferences (see (79–80)). In rats that were selectively bred for a high (HiS) and low (LoS) saccharin intake, HiS rats exceeded LoS rats in free-choice and forced-choice ethanol drinking using a two-bottle choice (81), and in acquisition of i.v. cocaine and heroin SA (see (79–80)). Rats that self-administered cocaine had saccharin preference scores proportional to the speed of acquisition.

Recent studies indicate that HiS rats are more impulsive than LoS rats, and impulsivity is another behavioral factor that predicts drug SA. For example, HiS rats exceeded LoS rats in the acquisition of i.v. cocaine (0.8 mg/kg) SA under a Go/No-go procedure (69). Drug preference also correlates with sweet intake, as rats that were selectively bred for high or low ethanol intake showed corresponding high or low sweet intake, respectively (82). Thus, saccharin intake is a strong predictor of the acquisition of ethanol and cocaine SA, as well as other phases of drug seeking and taking such as escalation, extinction, and reinstatement (80).

3.1.4. Impulsivity

Impulsive behavior is another major predictor of acquisition of drug abuse (see reviews by (83, 84)). There are several operational definitions of impulsivity, including choosing a smaller-immediate reward over a larger-delayed reward, inability to stop a behavior that has no or negative consequences, and impatience, short attention span, or engaging in risky behaviors. These aspects of behavior and their relationship to drug abuse have been modeled
in animals and humans, and mainly two measures of impulsivity have been studied: **impulsive choice** – choice of a smaller immediate over a larger delayed reward (83–85), and **impaired inhibition** – inability to withhold a prepotent response (86). Impulsive choice is modeled in animal and human preclinical studies using a delay discounting procedure. These forms of impulsivity will be discussed with respect to their influence on acquisition of drug SA.

**Delay discounting.** Initial studies in rats using a delay discounting procedure (see (84)) have used this task to identify high (HiI) and low (LoI) impulsive animals that were subsequently evaluated for their rate of acquisition of drug SA. In one of the first studies (87), rats chose between two immediate pellets or 12 delayed (15 s) pellets in a Y-maze. When their subsequent consumption of 12% ethanol was measured, the HiI rats that selected the immediate pellets on at least 75% of trials consumed more ethanol than those that were less impulsive. A second study extended this task to operant conditioning measures of delay discounting for one immediate versus three delayed food pellets and examined subsequent i.v. cocaine SA in HiI and LoI rats (88). Rats selected as HiI showed more rapid acquisition of cocaine SA, and at greater numbers than that of LoI rats. Subsequent work with the HiI and LoI rats indicated that impulsivity was similar in males and females (~70%, and ~24% for HiI and LoI respectively) (63). Also, high impulsivity predicted escalation and reinstatement of cocaine-seeking behavior, but LoI rats exceeded HiI rats during extinction.

**Impaired inhibition.** The Go/No-go (89), stop signal reaction time (SSRT) (90), and 5-choice serial reaction time (5CSRT) tasks (91) have been used to measure impaired inhibition (see (84)). For example, rats selected as HiI based on the 5CSRT task made more nose-poke responses to initiate i.v. nicotine SA than LoI rats (92). In another study using this task, rats screened as HiI subsequently self-administered larger amounts of i.v. cocaine than LoI rats, and HiI rats (vs LoI) also had fewer D2 dopamine receptors in the ventral striatum (neurocircuitry related to reward, novelty response, and movement) (93). Interestingly, both this study using a 5CSRT task and the Perry et al. (88) study using the delay-discounting method demonstrated that impulsivity measures were predictive of cocaine SA, but they were not correlated with locomotor activity in a novel environment that has also been shown to predict acquisition of drug SA (94).

### 3.1.5. Novelty Reactivity

In humans, there is a correlation between drug abuse and behaviors such as sensation seeking, novelty seeking, and risk taking (95). Similarly in rats showing a propensity to explore a novel environment, such as rats that are high responders (HR) in a novel environment, showed greater drug-induced locomotor activity, and more readily acquired drug SA than low responders (LR) in the novel environment (96–98). For example, HR rats, compared
Acquisition of Drug Self-Administration

with LR rats, showed faster acquisition of amphetamine (94, 99), cocaine (100), and nicotine (101) SA, particularly when exposure to a novel environment was forced (e.g., 101) and not a choice (e.g., 99). Also, rats selectively bred for HR acquired cocaine SA more rapidly than LR-bred animals (102). In these studies, exposure to the novel environment was forced. In contrast, when free-choice novelty procedures were used (96), there were negative findings on the relationship between HR versus LR rats and acquisition of SA.

There is also evidence that a proclivity for physical activity predicts drug abuse, and this “exercise preference” is independent of novelty-related locomotor activity (103). For instance, wheel running is a nondrug, noningestive positive reinforcer for rats, as they lever press to gain access to a wheel (104). Rats escalate their revolutions when given unlimited (105) or long (103) access, and wheel running is chosen over food under some conditions (106). Thus, wheel running has several features in common with drug addiction. In a study using female rats, the animals were allowed access to a running wheel for 6 h daily for about 3 weeks until behavior stabilized, then groups were selected for high (HiR) and low (LoR) running based on a median split of revolutions. By the time the groups met the acquisition criteria, the HiR rats had significantly more cocaine infusions than the LoR rats, and that pattern continued for 14 days of maintenance. There were no differences in extinction between groups, but the HiR group exceeded the LoR group in reinstatement responses on the previously active lever after an i.p. cocaine-priming injection (103).

Animals in this study were initially tested for locomotor behavior in an open field apparatus for novelty reactivity (Day 1) and locomotor activity (Day 2). Neither measure revealed group differences correlated with wheel running. In another study, rats that were experienced with chronic exercise showed a greater conditioned place preference (CPP) for cocaine than a group exposed to sedentary conditions before the CPP procedure (107). Thus, individuals that experience more exercise, either because they are genetically predisposed or they have more opportunities in their environment, show greater sensitivity to the rewarding (SA) and conditioned-rewarding effects (CPP) of cocaine than those inclined toward or exposed to less exercise. Overall, avidity for wheel running (HiR) was a predictor of drug seeking and taking, and this was similar to other high-responding phenotypes such as HiS, HiI, and HR.

Roman high- (RHA) and low-avoidance (RLA) rats have been selectively bred respectively for rapid or poor acquisition of a two-way active avoidance in the shuttle box apparatus, initially in Rome and later in Sardinia, Italy (see (108)). When the RHA and
RLA rats were compared on acquisition, maintenance, extinction, and reinstatement of cocaine-seeking behavior (108), over 4 weeks of training at doses of 0.1, 0.2, 0.4, and 0.4 mg/kg, respectively, the RHA line showed significantly more infusions than the RLA line in weeks 3 and 4. The RHA line also showed higher extinction responding (resistance to extinction), and cocaine-induced reinstatement responding than the RLA line. This is another example of the effect of genetic differences in selected rat lines on their initial vulnerability to drug SA. Other genetically divergent lines show differences in drug SA (e.g., Lewis>Fischer 344), but acquisition studies are lacking.

3.1.8. Ethanol Intake

In other genetic studies, rats were bred to prefer (AA) or not prefer (ANA) alcohol, and the drug-naive AA rats showed greater intake of cocaine and etonitazene, a potent agonist at the mu opioid receptor, than ANA or outbred Wistar rats (109). The AA rats also acquired i.v. heroin SA more rapidly than the ANA rats, and when i.v. ethanol was substituted for heroin, the AA rats self-administered more than the ANA rats (110). With other lines, selective breeding for ethanol intake has been shown to result in rapid acquisition of ethanol SA (111) as well as acquisition of behavior maintained by other drugs (82) and nondrug substances (112). For example, Le et al. (82) examined the acquisition of i.v. nicotine and oral (10%) sucrose SA in alcohol-prefering (P) and nonpreferring (NP) rats. The P rats’ intake exceeded NP rats’, and there was a vertical upward shift in the dose (nicotine) – and concentration (sucrose) – response functions. There were also changes during extinction and reinstatement in which the P line exceeded the NP line. On the other hand, P versus NP line differences were not observed in cocaine acquisition or maintenance; however, higher doses were used that do not always reveal individual differences during acquisition (8). Rats bred for high and low ethanol intake also showed respective high and low intake of sweet substances (112) indicating that selective breeding may have pleiotropic effects. Genetic influences similar to those shown in animals are also clearly represented in humans (113). The findings suggest that there is a genetic correlation between ethanol preference and acquisition of not only ethanol but other drug and sucrose intake in drug-naive rats.

3.1.9. Neurobiology of Acquisition

Possible neurobiological mechanisms for the acquisition of drug SA have been discussed extensively (e.g., (114, 115)). There have been few studies investigating the neurobiological basis of the acquisition process, possibly because it is difficult to study behavior that is confounded with the direct effects of the drugs that are self-administered.

One approach would be to examine the role of different neurotransmitters involved with acquisition of SA of specific
Acquisition of Drug Self-Administration

drugs. For example, evidence suggests a critical role for dopamine in the reinforcing effects of cocaine. Genetic knockout studies would yield useful information, but they are done in mice, and SA models mainly exist in rats and primates, as i.v. SA is difficult to accomplish in mice. Nevertheless, in studies with mice dopamine receptor 1 subtype (D1) knockout mice did not reliably acquire SA of cocaine (and other D1 and D2 agonists) (116), while D2 knockout mice were no different than wild-type controls (117). Thus, D1 receptors are necessary for establishment of the reinforcing effects of cocaine and other dopamine-related drugs. Another option would be to examine neurobiological differences in rats selected or bred to be addiction-prone or -resistant, such as Lewis and Fischer 344, AA and ANA, P and NP, HiS and LoS, HiI, and LoI using drug-naive representatives of these populations.

3.2. Environmental Factors

Natural rewards such as eating, drinking, exploring novel environments, exercising, and socializing, activate the same brain reward circuits as drugs of abuse (118). This section illustrates how acquisition of drug SA is affected by environments that are either enriched or impoverished by these natural rewards. Other environmental conditions to be discussed are economic factors involved with acquisition such as access conditions and cost per unit dose, and the influences of physical and social stress.

3.2.1. Enriched versus Impoverished Environments

Environments that are enriched with nondrug rewards prevent and reduce drug abuse in humans under maintenance conditions (119) and in laboratory animals during acquisition, maintenance, and other phases of drug abuse (42). Interestingly, many of the biological factors that predict acquisition of drug SA (e.g., sweet preference, novelty seeking, physical exercise) function as alternative rewards and reduce drug abuse. When initial studies were conducted using unlimited or palatable food as enrichment, acquisition of both i.v. drug SA in rats and oral drug SA in monkeys (see (42)) was markedly reduced. Acquisition of drug SA was also prevented in rats by enrichment with noningestive, nondrug rewards such as novel play objects and group housing (96, 120), or exercise (107). The animal models of nondrug rewards have parallels in human studies. For example, in one study of prevention of the initiation of drug abuse, self-programmed social activities (“amazing alternatives”) were used as environmental enrichments with middle school children in addition to education and parental participation (121). The results indicated 20% less “last month” and 30% less “last week” alcohol and cigarette use compared with students in the control groups. Students in the intervention groups had significantly lower scores on the Tendency to Use Alcohol Scale by the end of eighth grade than the control groups. There was also lower first drug use in those who had not started drinking yet in the treated groups compared to the demo-
Graphically matched controls. In other studies, Higgins, Bickel, and coworkers found similar interference with maintenance of drug SA in the laboratory when money was concurrently available with heroin or cocaine, and SA decreased (see (42)). Similar outpatient clinical results have been reported (119).

**Palatable substances.** Access to palatable substances consistently reduces the acquisition of SA of drugs such as ethanol, opioids, stimulants, and PCP (see (3, 8)). There are many similarities in the neurobiology of reward for drugs, palatable substances, and food (80, 122). In an early study, Carroll et al. (123) reported that a glucose–saccharin (G + S) combination prevented acquisition of cocaine SA. In contrast, cocaine prevented acquisition of oral G + S intake resulting in a negative correlation between cocaine infusions and G + S intake over acquisition days. In a subsequent study, G + S or water was available 3 weeks before and/or during autoshaping for i.v. cocaine SA. In the groups with access to G + S or water only 3 weeks prior to acquisition, 100% of the rats acquired, but in the group exposed to G + S before and during cocaine acquisition, only 50% acquired. Only 75% acquired in the group having G + S only during acquisition. Thus, both previous plus concurrent G + S or concurrent G + S were effective in preventing the acquisition of cocaine SA, but previous plus concurrent access had the greatest effect.

In a later study (7) when a noncaloric sweetener, powdered saccharin, was added to ground lab chow and compared to the chow-only condition in rats, only 38% of the saccharin group cocaine acquired SA compared to 77% of the chow-only group; thus, the effect of sweeteners is not calorie-dependent. This work was later extended to two groups of rhesus monkeys that had access to either saccharin + water or only water. Only 43% of the monkeys exposed to saccharin acquired, whereas 86% of those with only water acquired (154). In a recent study, a choice between sucrose pellets and i.v. METH was examined in a group of rats and compared to a group receiving METH only during acquisition and other phases. The METH + sucrose group self-administered less than the METH-only group during acquisition, and their behavior was also reduced during maintenance, extinction, and reinstatement (124). Impairment of acquisition by dietary manipulations is not limited to sweet substances; exposure to a high-fat diet also diminished acquisition of cocaine SA in rats (125).

**Exercise.** Wheel running in rodents is another behavior that has similar neurobiological-behavioral impact as sweet substances and addictive drugs (119). As with i.v. cocaine infusions, rats can be trained to lever press for access to the running wheel (106). Rats recently allowed to run in a wheel subsequently showed increased ethanol intake (126), and they exhibited escalation of wheel running when given extended access (6 h) (105).
Acquisition of Drug Self-Administration

similar to the escalation reported for i.v. cocaine SA (127). Rats that run in running wheels also show a conditioned place preference to the environment that is associated with running (128), and genetically modified mice have similar responses to wheel running and ethanol SA (126). Thus, there is likely a common mechanism for the development of compulsive drug intake and a proclivity for running in rats and humans (119). The significance of this interaction is that providing access to exercise may prevent initiation, escalation, and reinstatement of drug seeking in humans.

Once established, cocaine SA was reduced by running wheel access in female rats (129). Similar findings have been reported regarding the effects of wheel running on oral intake of amphetamine (130) and ethanol (131). In contrast, rats that were allowed to exercise on a running wheel in their home cage did not differ in their time to acquire i.v. cocaine SA, compared to those from a sedentary environment (107). However, the rats with running wheel access subsequently had lower break points on a PR schedule for cocaine, indicating that running reduced the reinforcing effectiveness of cocaine.

Novelty/social access. The events discussed above (palatable substances and exercise) may also suppress drug-reinforced behavior via their novelty features. Novelty and drugs of abuse are also thought to share similar neural substrates (132), and in fact, exposure to drugs, sweet substances, exercise, and novel stimuli activate common mesolimbic dopamine reward systems (121). There are many examples of preference for novel over familiar objects/environments (e.g., (99)). Rats reared and maintained in novelty-enriched conditions (EC) containing novel objects and housed with conspecifics, self-administered less amphetamine (at low doses) than rats raised in isolated conditions (IC) (133). Novel objects also decreased acquisition of i.v. amphetamine SA in rats (99). Furthermore, novelty reduced amphetamine infusions over four repeated presentations; the reduction was more robust at low drug doses, and HR rats’ drug intake was more disrupted by exposure to novelty than LR rats (96). In monkeys, social stimuli may serve as reinforcing stimuli and can substitute for consummatory reinforcers (see (134, 135)). For example, monkeys will lever press for visual observation of another monkey, and male monkeys will relinquish liquid deliveries in a choice procedure to view pictures of female monkeys, but it is not clear whether the decrease in drug intake was due to substitution of a competing, rewarding substance or event or a trade of a newer novel condition for an existing, novel condition. To examine this question, Cain et al. (136) examined the effect of environmental enrichment (EC vs IC) on responding to the effect of a novel visual stimulus (light onset). EC rats responded less for the con-
tangent light stimulus than IC rats suggesting a decrease in the incentive value rather than a novelty substitution.

**Feeding conditions.** Food also affects central reward pathways that are activated by most commonly abused drugs (137). Food restriction was initially used to motivate drug-maintained responding, just as it had been used to study food-reinforced operant behavior; thus, it was customary to reduce food access to motivate drug-reinforced operant behavior. An early study found that food restriction elevated ethanol SA (27). In subsequent studies, food restriction increased oral etonitazene intake and all forms of i.v. drug self-administration in rats, and SA of orally delivered PCP and other drugs in monkeys (see (26, 33)). Later, studies evaluated the magnitude of food restriction on acquisition of cocaine SA by offering rats ad libitum, 20 g, or 10 g amounts of food each day. The groups acquired in 16.1, 9.5, or 6 days, respectively. Also, while 100% of the food-restricted rats acquired, only 77% of the ad libitum group acquired (8). A history of brief food restriction also increased subsequent i.v. cocaine SA (138). Acquisition of cocaine SA occurred faster in those that had a history of three brief forced dieting episodes compared with free-fed controls.

As a result of these early studies, a mild level of food restriction is commonly used in many studies of acquisition and other phases of drug abuse. This allows acquisition to occur in most animals and reduces intrasubject variability. However, dietary restriction is not uniformly accepted, and it continues to be evaluated for its suitability in behavioral paradigms. Bongiovanni (see (139)) recently concluded that food restriction is most useful in the first few days of acquisition, but continued use through extinction and reinstatement may confound intrinsic motivation for food (hunger) with drug-related cues that promote relapse, motivating events that may be comingled in the recovering addict. Another consideration is that food restriction is a stressor that motivates drug seeking and taking through stress hormones (140).

**3.2.2. Stress**

Stress is an environmental condition that generally increases acquisition of drug SA and accelerates drug seeking during other phases of drug abuse (see reviews by (115, 141–143)). Stress has been widely studied in animal models, and these reviews highlight several types of stressful events that increase acquisition of drug SA, such as physical stress (e.g., restraint, pain, dietary restriction) and various forms of social stress (isolation, defeat, repeated maternal separation).

**Physical stress.** Physical stressors such as tail pinch, footshock, and restraint increase the acquisition ofamphetamine, cocaine, fentanyl, and morphine SA in rats (144), but not METH (145). Forms of social stress such as aggression, competition, and isolation also respectively increase acquisition of cocaine, amphetamine,
Acquisition of Drug Self-Administration

opioid, and alcohol SA. Even witnessing stress in other animals or prenatal exposure to restraint stress in the dams increases acquisition of cocaine SA (146).

Stress (e.g., noncontingent footshock)-induced increases in cocaine SA are positively correlated with increases in plasma corticosterone (CORT), and drug SA does not occur unless plasma CORT reaches a critical threshold (see (142)). Exogenous injections of CORT increase acquisition of cocaine but not METH (146) SA in rats. Rats selected for a high level of exploration in a novel environment HR and a corresponding high initial CORT response were more likely to self-administer amphetamine than rats selected for LR (144). Thus, either exposure to stress or exogenous administration of stress hormones can increase acquisition of drug SA in rats. The effects of stress are more apparent at low doses or on the ascending limb of the dose-response function.

Manipulation of stress hormones results in predictable changes in acquisition of drug SA. For example, rats injected with CORT acquired cocaine SA at a lower dose than rats injected with vehicle (147). Blockade of CORT decreased the rate of acquisition of cocaine SA and the number of rats per group meeting acquisition criteria (148). Sex differences have also been noted in response to stress suggesting hormonal interactions (149). For example, pretreatment with the CORT synthesis inhibitor, ketoconazole, reduced the rate of acquisition and number of rats reaching acquisition criteria for heroin SA, and females were more affected than males (150). The mechanism by which stressors increase vulnerability to drug SA has been hypothesized to occur by a sensitization process involving stress hormones and dopamine (141). Thus, individuals more prone to stress, and/or having inadequate control over stress, may be more vulnerable to substance abuse.

Social stress. Social stimuli can also induce a stress response that increases the reinforcing effects of drugs (see (141, 142)). In humans, social behavior and related stress influence the acquisition of drug SA. This area of research has been extensively studied in the animal laboratory (see (142)); thus, only brief examples will be given. However, while there is a substantial amount of high-quality work on stress and its relation to drug abuse, more studies are needed on the role of stress in acquisition of drug SA. For example, stress is known to be a major stimulus in relapse models of drug abuse (see Chap. 17 for details), but its role in the initiation of drug SA has not been well studied. Two forms of social stress studied most frequently are social threat/defeat and social isolation. Acquisition of drug SA behavior was increased by aggressive attack or threat and social defeat (see (142)). In adult male rhesus monkeys, pair housing increased PCP SA and raised cortisol levels in most monkeys compared to when the same
monkeys were singly housed in the same cages with a solid partition (135).

In socially cohesive species such as rats and primates, social isolation is a stressor, and isolation results in elevations in drug intake. In other studies described above that examined the environmental effects of novelty (133), rats that lived (8–10 per cage) socially (SC) were compared to those that lived (1 per cage) in isolation (IC). They found that SC-housed rats had fewer amphetamine infusions during acquisition than IC rats, similar to the combined effects of novel objects and social housing EC. In another study, rats reared in isolation acquired cocaine SA more rapidly than age-matched rats reared in groups (151).

Furthermore, in colonies of male and female adult squirrel monkeys, extended social isolation increased alcohol intake, but not intake of a control liquid, and it also increased salivary cortisol levels (152). However, in the same study, acute separation reduced intake of alcohol and the control liquid. Thus, social stressors may have opposite effects on drug SA depending on temporal and contextual conditions.

There are two major behavioral economic factors that are relevant to the rate of acquisition of drug SA. First is the cost of the drug or unit price, defined as the effort expended (responses) per magnitude of the reinforcer (mg of drug) or responses/mg. Unit price can be varied by changing the number of responses or dose per delivery, and within limits, consumption will be constant at a given unit price regardless of whether responses or dose are varied (see (1, 153)). In studies of acquisition, a general finding is that as the unit price decreases (lower FR or increased dose), both a greater percentage of rats per group acquired cocaine SA, and they required fewer days to meet the acquisition criteria for cocaine and heroin (1). Similar results were obtained in monkeys trained to acquire SA of a low dose of PCP versus a higher dose (154). Generally, more rapid and consistent acquisition occurred with higher doses.

The second behavioral economic factor influencing acquisition is income, or the amount of resources (access, time) to obtain drug over a specific time period (155). When rats have long daily access to drug SA, they acquire SA faster and consume more drug leading to escalation of intake over time, especially at lower doses, compared with animals that have short access. This has been demonstrated with several drugs such as cocaine (127, 156), METH (157), and PCP (see 1 for details). Thus, access to the drugs in terms of time and dose, and the effort required per unit dose, are important determinants of the rate and success of acquisition and subsequent drug seeking and taking behavior.
Exposure to drugs during gestation can result in enhanced acquisition of drug taking in adults, although there have not been many studies of this nature, particularly with cocaine, which would have relevance considering the prevalence of cocaine use in pregnant women. However, a few studies with other abused drugs show that prenatal exposure enhances subsequent acquisition of drug abuse. For example, exposure to morphine from the 7th day of gestation until parturition increased the rate of acquisition of cocaine and heroin SA in adult rats (146). Also, perinatal exposure to ∆9-THC later increased morphine SA in adult rats (158). Female rats were exposed to nicotine and ethanol throughout pregnancy (159). When the rat pups of drug-exposed dams were allowed to acquire SA of i.v. nicotine during postnatal days 60–90, they acquired more rapidly and at a higher percentage per group than control groups whose mothers were pair-fed but not drug exposed or were exposed only to nicotine or ethanol. Sensitization may play a role when acquisition is accomplished by substituting one drug for another with similar pharmacological actions (see (2) for details). Other examples of drug history influencing acquisition of drug SA involve preexposing rats to a drug before establishing acquisition of i.v. SA. Experimenter-administered drug pretreatment has facilitated SA of amphetamine, caffeine, cocaine, and nicotine (see (8) for review). Enhanced acquisition after exposure to psychomotor stimulants has been attributed to a sensitization process involving the mesocorticolimbic dopaminergic system (e.g., (160)). Cross sensitization between pretreatment and SA drugs has also been demonstrated in rats and nonhuman primates (see (8)).

In addition to drug treatment prior to acquisition, drug treatment during acquisition has been studied as a means of prevention, early intervention, or to understand the neurobiological basis of drug reinforcement. While a wide variety of treatment agents have been tested during later phases such as maintenance, escalation, and reinstatement, there have been relatively fewer drug treatment studies during acquisition of drug SA. Practically speaking, there is little motivation to develop medications for this purpose, as acquisition occurs in the early teens, and a medication for prevention might not be feasible for that age group.

However, animal studies have provided some information on several medication models for prevention of acquisition. For example, in terms of understanding receptor systems involved with acquisition of drug SA, Schenk et al. (161) found that rats pretreated with the NMDA antagonist MK-801 failed to acquire reliable cocaine SA, indicating that the glutamatergic NMDA receptors are important in the establishment of cocaine as a reinforcer. In an attempt to interfere with the accelerating effect of stress on...
acquisition, a drug that reduces CORT synthesis, ketoconazole, was used, and it reduced acquisition (autoshaping) of cocaine SA at a dose that had no effect on food-maintained behavior (148). In another autoshaping study with cocaine, baclofen, a GABA<sub>B</sub> agonist (with potential for interfering with impulsive behavior), reduced acquisition of cocaine SA, and the effect was greater in females than males (162). Also, bupropion which is prescribed for multiple disorders reduced acquisition of METH SA (163).

4. Discussion

This review of acquisition of drug SA in animals and its application to the initiation of drug abuse in humans discussed a variety of methods and routes of administration. Since most drugs are abused by humans via the oral (including smoking) and intravenous routes, it is fortunate that these animal models provide close approximations to human behavior and a wide range of results. Studying initiation of drug abuse in humans (outside of longitudinal epidemiological studies) is impossible because it is unethical to introduce drugs of abuse to drug-naïve individuals. Initiation occurs in juvenile or adolescent years which add to the difficulties of studying development of drug abuse in humans. Overall, the findings from this review indicate that acquisition reliably occurs with (1) a wide range of laboratory animals, including males and females, (2) a broad range of drugs from at least six pharmacological classes, (3) many routes of administration, and (4) multiple behavioral procedures. The validity of these preclinical studies is supported by the finding that acquisition occurs with drugs that humans abuse and not with drugs that are not abused.

An important finding of this review is that there are several biological determinants that are highly predictive of acquisition of drug SA in animals. These include age, sex, hormonal status, innate sweet preference, reactivity to novelty, avidity for exercise, social rank, impulsivity, and genetic propensities to consume excessive amounts of alcohol and nondrug substances. Research over the last decade has highlighted how closely these biological determinants are interrelated with the propensity to abuse drugs, and how connected they all are with each other, forming a cluster of vulnerability factors (see Table 3). Another value of obtaining this knowledge from the acquisition research is that those having the opposite characteristics (e.g., less impulsive, less reactive to novelty, more socially competent) are protected from initiation of drug abuse, and these behaviors can be nurtured as part of the prevention process to immunize against development of drug abuse.

Along with the endogenous determinants, there are many environmental factors that have been studied in the last decade
along with pharmacological factors related to the initial drug exposure that offer important insights into prevention. These include enriched (vs impoverished) environments, which can be accomplished by offering nondrug alternatives to drug abuse, such as preferred dietary substances, exercise, social access, and novel opportunities. Stress is another major environmental variable that can take the form of physical or social difficulties that enhance the initiation of drug abuse, especially during the critical period of adolescence. Finally, factors related to the drug use itself are strong determinants. Much work has been reported on the influence of behavioral economic variables such as cost, drug access, and drug history, for example, prenatal exposure, or use of psychoactive medications. There is also an issue of concurrent drug use, whereby use of one drug facilitates use of the other (e.g., alcohol and cigarettes). Attempts to interfere with the initiation process in animal models have yielded a number of effective medications that are promising in animals, but there is the difficulty of using treatment drugs in preteens that makes this approach much less feasible than a behavioral approach.

Overall, the data that were reviewed in this chapter show a shift from the earlier emphasis on the science of capturing the acquisition process in animals, to the biological, environmental, and pharmacological factors that strongly influence acquisition in animals. Studying these factors is critical because drug abuse is increasing and beginning at earlier ages in humans. Unfortunately, there have been few successful treatments for drug abuse, including the most prevalent forms: nicotine, alcohol, and stimulant drug abuse. Thus, prevention becomes even more important, and understanding the factors that drive initiation of drug abuse is necessary for the development of effective prevention strategies, such as use of nondrug incentives (119). The recent research reviewed here on acquisition of drug abuse suggests that the next generation of research in this area should emphasize the genetic, biological, developmental, and environmental interactions that control the establishment of reinforcing effects. Such knowledge will be the basis for developing rational prevention methods that can be applied to humans.

References


Acquisition of Drug Self-Administration


Chapter 10

Escalation of Drug Use

Serge H. Ahmed

Abstract

Among the different behavioral criteria used to discriminate substance dependence (or drug addiction) from other non-disordered forms of drug use, drug intake escalation presents a number of unique features that makes it particularly suitable for modeling in nonhuman animals. This criterion has stood the passage of time despite major revisions of diagnostic systems, it is common to all known drugs of abuse and it can be readily and unambiguously operationalized in laboratory animals. Here I exhaustively review evidence showing that escalation to heavy consumption of different drugs (except perhaps nicotine) can be rapidly induced in the majority of individual animals (i.e., rats) by increased drug availability. Such an escalation of drug use is probably paralleled by an authentic escalation to drug addiction, as it is associated with the co-occurrence of other addiction-like changes (i.e., increased motivation for drug use; increased difficulty to abstain from drug use; decreased sensitivity to negative consequences). In addition, during escalation of drug intake, most individual animals become increasingly responsive to drug- and stress-primed, but apparently not cue-primed, reinstatement of drug seeking after extinction. Finally, following increased drug use, most individuals present selective cognitive dysfunctions (e.g., deficits in executive functions) that may contribute to the establishment and/or persistence of addiction. Thus, the study of individuals with escalating patterns of drug use should provide a unique and valid approach to investigate, experimentally, the behavioral and neurobiological mechanisms that underlie the progression to addiction.

Key words: Cocaine, Heroin, Nicotine, Tolerance, Compulsion, Self-regulation, Reward, Punishment

1. Introduction

Psychiatrists have long been, and still are, striving to fine-tune their diagnostic criteria to distinguish substance dependence or drug addiction from other, non-disordered forms of substance use (1–4). Such a distinction has just begun to be imported into research on animal models of addiction (5–12). It is now acknowledged by a growing number of researchers that mere drug self-administration and/or drug reinforcement (i.e., in Skinnerian
terms, the process by which a drug acts as a response-contingent stimulus that increases and/or maintains responding) is no longer sufficient evidence for an addiction-like profile in nonhuman animals. Animal “drug users” must also develop or present other behavioral signs to be considered a valid model of addicted humans. Among the different behavioral criteria of substance dependence that have been considered, escalation of drug use presents a number of unique features that makes it particularly suitable for modeling in nonhuman animals. It is indeed one of the rare behavioral criteria of dependence that has remained unchanged across successive revisions of diagnostic classifications (and thus probably has the least chance to be modified in future revisions), it is common to all known drugs of abuse and, finally, it can be readily and without ambiguity operationalized and induced in laboratory animals. In fact, as argued below, escalation to heavy drug consumption is probably an authentic escalation to addiction (or dependence), as it is associated in the majority of animals with the co-occurrence of other addiction-like changes. The study of individuals with escalating patterns of drug use should thus provide a unique and valid approach to investigate, experimentally, the behavioral and neurobiological mechanisms that underlie the progression to addiction.

2. Escalation of Drug Consumption as a Hallmark of Addiction

Drug intake escalation can be defined as a progressive increase in individual drug consumption over time that becomes excessive, overwhelming, and difficult to control. Everything happens as if drug use begets further drug use, a phenomenon dubbed “adjacent complementarity” by prominent economists interested in addictive behaviors (13). There is a large and long-standing consensus across many scientific disciplines for considering escalation of drug consumption as a core feature of addiction or even constitutive to this behavioral disorder. There are several reasons for this consensus. First, it is simply difficult to conceptualize the transition to addiction without postulating a progression toward heavy drug use. For instance, it would be odd to speak of tobacco addiction in so-called tobacco “chippers” who continue to smoke a few cigarettes a week as during their first encounters with cigarettes (14). Indeed, without escalation to exaggerated levels of drug use, there would be no harm (except those accidentally caused by acute intoxication) and no motivated attempts to stop or to reduce drug use. Second, escalation of drug use is one of the few diagnostic criteria common to all known forms of addiction, including addictions that involve licit or illicit drugs and so-called behavioral addictions that do not involve a substance (e.g., pathological gambling).
In contrast, other symptoms of addiction are specific to some drug categories and/or legal status (i.e., licit or illicit). For instance, the criterion “a great deal of time spent in activities necessary to obtain the substance, use the substance or recover from its effects” is not currently applicable to tobacco addiction, though this will probably change with the application of more strict regulation (15). Finally, among the different behavioral criteria being used to define or diagnose addiction, as opposed to recreational drug use or drug abuse, escalation of drug intake has stood the passage of time despite several successive major revisions in diagnostic classifications. This formidable resistance to change suggests that this criterion is unlikely to change in future diagnostic revisions, except perhaps to further increase its operationality (4).

To prevent confusion, it is important from the outset to establish a clear conceptual distinction between escalation of drug use and drug tolerance. For historical reasons, drug intake escalation has always been associated with drug tolerance in diagnostic systems. In fact, escalating drug use is often presented as one of the definitions of, or a surrogate for, drug tolerance. In the current edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM), tolerance is defined as either a “markedly diminished effect with continued use of the same amount of the substance” or a “need for markedly increased amounts of a substance to achieve intoxication or desired effect” (3). However, as defined above, escalating drug use is an individual behavioral process that can and should be conceptually separated from pharmacodynamic tolerance (16). Indeed, depending on the theoretical framework considered, one can conceive of a wide array of different underlying mechanisms – not necessarily mutually exclusive. Certainly, under some conditions, escalation of drug use can directly arise from tolerance to some of the effects of the drug (e.g., tolerance to the drug’s rewarding or aversive effects) brought about by molecular adaptations within drug receptor signaling pathways and other cellular mechanisms. However, many alternative mechanisms operating at different, higher levels of neurobiological organization can also be envisioned. Here is a short, certainly not exhaustive, list of potential mechanisms that could account for escalating patterns of drug use. First, within an affective self-medication or self-regulatory framework, drug use is maintained by relief of a preexisting affective condition. Escalation of drug use then arises, not because the drug progressively loses its efficacy in ameliorating the affective condition, but because this condition
worsens with extended drug use, either as a direct or indirect consequence of drug use (e.g., through “reward allostasis” or “disuse atrophy”) (16, 17). Second, in a multisystems learning framework, escalation of drug use could result from a shift from goal-directed to habitual drug-taking behavior (18). Specifically, goal-directed drug use is intrinsically limited by deliberation over potential alternative courses of actions. With extended experience, however, drug use becomes habitual and thus less limited by time-consuming deliberative decision-making processes. One can predict, however, that such a mechanism will only account for drug intake escalation in stationary and highly predictable environments (e.g., drug self-administration chambers for laboratory animals). In more variable, uncertain environments that require strategic decision-making, such a mechanism is unlikely to be recruited. Third, in a behavioral economics framework, drug consumption is influenced by microeconomic factors, including drug availability, prices and accessibility to substitutes or alternative commodities (19). Thus, escalating drug use can result from increased drug availability, decreased drug price and/or reduced accessibility to nondrug substitutes. Finally, from a psychosocial perspective, drug use is limited in a specific society or culture by cultural norms, taboos, and/or overarching value systems (20). Here escalation of drug consumption can result from a progressive loss of influence of prevailing social or cultural norms on drug taking due, for instance, to social exclusion or marginalization processes that are directly or indirectly related to drug use. Within the same framework, escalating drug use can also arise from a progressive depletion in the individual’s social capital brought about by extended drug use. Thus, to sum up, the historical link established between drug intake escalation and tolerance has no apparent conceptual necessity. It is an empirical question to determine whether escalation of drug use is more often than not driven by drug tolerance and/or other mechanisms. In the remaining text, I will focus on drug intake escalation as a behavioral process without theoretical commitment to a specific underlying mechanism.

4. Experimental Induction of Drug Intake Escalation

As suggested above, several different factors can precipitate – at least in principle – escalation to higher levels of drug consumption. Despite such potentially diverse etiology, however, research on laboratory animals (i.e., rats) has so far been limited, mainly, to investigating the role of increased drug accessibility in promoting the progression toward excessive drug use (see Sect. 9, for additional information regarding this limitation). Specifically,
escalation of drug consumption is induced reliably and rapidly by increasing the length of daily sessions of drug self-administration, as compared to much shorter daily sessions. The aim of this section is to summarize this research, but before proceeding, some comments on several relevant methodological issues are in order.

A first, obvious issue concerns the method for distinguishing the escalation process per se from the increase in behavior related to the acquisition of the instrumental response (i.e., positive reinforcement). At least two different approaches can be used to achieve this goal. In a within-subjects design, subjects can be pretrained before having long access to drug self-administration. For instance, in some published studies, animals were first trained to acquire the drug self-administration response before increased drug availability. In this approach, escalation of drug self-administration manifests as a gradual increase in drug consumption over time above the pre-escalation level of consumption. In a between-subjects design, at least two groups of untrained animals are tested: one group has a short, daily access (ShA) to drug self-administration while the other group has a much longer daily access (LgA). In this approach, drug intake escalation appears in the LgA group as a gradual increase in the rate of drug consumption above the control level of the ShA group. This between-subjects design is less time consuming than the first approach because it does not require pretraining. Of course, these two approaches can be combined to better assess the development and maintenance of drug intake escalation following increased access time to the drug.

Another important methodological issue concerns the minimization of potential experimental restrictions on drug consumption. Obviously, to assess changes in drug consumption as a function of drug availability, individuals should be relatively free to regulate their drug intake (21). Thus, the response ratio requirement to obtain a fixed drug dose should be reduced to the minimum as in the continuous reinforcement (CRF) schedule. In addition, the postinjection time-out period should be selected to allow for the highest possible number of injections while protecting animals from the risk of overdose (which increases with the unit dose available). Finally, limiting the maximum number of injections per session should be avoided as this may interfere with the escalation process (22–24).

A final issue concerns the choice of the dependent variable for assessing the effects of drug access time on drug self-administration in the between-subjects design (i.e., ShA versus LgA). Specifically, the problem is to select a dependent variable with the highest degree of between-subjects comparability (obviously, this choice is not a problem in the within-subjects design because the assessment of drug intake escalation only involves within-subject comparisons). The most comparable dependent variable is the number
of drug injections during the time period common to all access durations (e.g., during the first hour when comparing 1-h versus 6-h sessions of drug self-administration). Inversely, the least comparable variable is the total number of injections simply because it is directly influenced by the independent variable. Another potential dependent variable could be the average hourly number of injections obtained by dividing the total number of injections by the length of the self-administration session. However, this variable may be a source of inaccurate comparisons when there are pronounced within-session variations in drug self-administration – which is often the case for intravenous cocaine or heroin self-administration (e.g., initial drug loading). Thus, whenever possible, it will be preferable to compare drug use during the time period common to all access durations.

4.1. Stimulants

Steven Dworkin and his colleagues (25) were the first to suggest that different access time to intravenous cocaine self-administration is associated with different levels of intake in rats. In their initial experiment, rats had daily access to cocaine (0.5 mg/kg) during 12 (\(N=4\)) or 24 (\(N=7\)) hours for several days. Rats with the longest access time to the drug self-administered more cocaine than the other rats (3.5 versus 1.7 infusions/h). However, this early experiment involved a very limited number of rats and provided little information about initial levels of drug intake and how they eventually changed over time. Thus, from this study alone, it is not possible to determine whether the differences in cocaine consumption observed, as a function of drug access time, directly resulted from differential drug intake escalation or from some other, uncontrolled factor (e.g., preexisting individual differences) (26).

More recently, controlled experiments have addressed these shortcomings. It is now clearly established that the length of daily access to cocaine can profoundly alter the rate and pattern of drug self-administration. In one series of independent experiments (7, 21, 27), rats were first trained to self-administer food and then intravenous cocaine under a CRF schedule during several daily 1-h sessions. After stabilization in the rate of cocaine self-administration, rats were assigned in a counterbalanced manner to two access conditions: in the ShA condition, rats continued to have access to cocaine during daily 1-h sessions while in the LgA condition, rats had their daily access to the drug increased to 6 h. As expected, ShA rats maintained the same rate of cocaine self-administration throughout the duration of the experiment, though they were free to take more (Fig. 1a). In one study, this stability of intake was shown to last at least 5 months (21). In contrast, with longer daily sessions to the drug, rats steadily escalated both their first hour and total intake of cocaine (Fig. 1a, c). Importantly, during the first hour of drug access, cocaine
self-administration by LgA rats rose to a level much higher than the stable level of intake maintained by ShA rats (Fig. 1a).

These basic findings were reproduced in a separate series of experiments in rats with a different behavioral history (28–31). In these experiments, rats were first trained to self-administer food under a CRF schedule after which they were tested during one single 1-h session of cocaine self-administration. The goal of this screening phase was to measure the initial level of operant responding before assignment of individuals to the ShA or LgA condition. Thus, in these experiments, rats had minimal experience
with cocaine before differential drug exposure. Under these circumstances, the day-to-day pattern of cocaine self-administration is typically biphasic: cocaine intake slightly decreased within the first 5 days, reached a minimum and then began to increase to eventually level off at a stable level (Fig. 1b). The origin of the initial decrease in cocaine consumption has not been directly studied, but it probably corresponds to a transitional period during which initially cocaine-naïve animals progressively adapt to the change of reinforcers (from food to intravenous cocaine) and learn to titrate the effects of cocaine. This biphasic pattern was equally observed in both ShA and LgA rats. In LgA rats, however, the initial decrease in consumption was briefer and milder than in ShA rats and was followed by a much steeper increase in cocaine self-administration. As a result, the first hour of cocaine intake by LgA rats escalated within a few days above that of ShA rats (Fig. 1b). In addition, regardless of behavioral history, there was also a robust and parallel escalation in total cocaine consumption (Fig. 1d). Thus, regardless of initial training and pharmacological history, short versus long access time to cocaine self-administration induces two distinctive patterns of cocaine consumption: a stable pattern of moderate cocaine use versus an escalating pattern of excessive cocaine use.

Over the past 10 years, this basic observation has been reproduced several times by other teams or laboratories. Currently, these two patterns of drug use have been replicated in dozens of independent experiments (7, 21, 27–61). The metaanalysis of these studies clearly demonstrates that the relationship between increased drug access time and cocaine intake escalation holds true across a wide range of subjects and experimental conditions (Fig. 2), despite the occurrence of negative data from some laboratories (33, 34, 46, 47, 55). Furthermore, this relationship also extends to other stimulant drugs (Fig. 2). Increased access time to the drug precipitates an escalation of both amphetamine and methamphetamine self-administration (23, 24, 62–64) (Fig. 2).

There is, however, one intriguing exception: nicotine. Increased access time to nicotine decreased, rather than increased, drug intake (Fig. 2) (Shelley Watkins, Serge Ahmed, George Koob and Athina Markou, 1999, unpublished data) (53, 65). This decrease in nicotine intake is paradoxical and may point to some unsuspected differences in abuse liability between nicotine and other stimulant drugs. Consistent with this interpretation, Kenny and Markou (65) recently reported that extended exposure to nicotine self-administration induced opposite changes in brain reward function compared to those seen following escalation of cocaine (29) or even heroin self-administration (66). Alternatively, the lack of escalation in nicotine self-administration may also reflect inappropriate access conditions. For instance, dramatic escalation to high levels of ethanol consumption can be
Escalation of Drug Use

Fig. 2. Metaanalysis of published studies on drug intake escalation in rats. A total of 49 separate studies have looked at the effects of drug access time on the pattern of self-administration. These studies amount to a total of 74 independent experiments (cocaine, 55; amphetamine, methamphetamine, 7; nicotine, 2; heroin, morphine, fentanyl, 11). Most, though not all, experiments compared the effects of access to 1 (ShA) versus 6 h (LgA) to the drug on subsequent changes in drug use. For each experiment, changes in drug use over the first 2 weeks were estimated using the digitizer software XYit3.1.4 (Geomatix Ltd, UK) and were expressed as percent change from the first day of drug access. The number above each bar corresponds to the number of experiments performed for each drug and each access condition (ShA versus LgA). In this set of experiments, the LgA condition was sometimes tested alone, which explains why the number of experiments corresponding to this access condition is generally higher than that corresponding to the ShA condition. Stimulant drugs (i.e., amphetamine and methamphetamine).

rapidly triggered by forced periods of withdrawal between LgA sessions (67–69), whereas it takes dozens of weeks for a minority of animals to escalate their ethanol consumption with unlimited, continuous access to the drug (5, 6). Thus, it is possible that a more intermittent access to LgA sessions of nicotine self-administration would be more favorable to nicotine intake escalation, as suggested by recent studies on the nicotine deprivation effect (70, 71).

4.2. Opiates

Previous research has clearly established that continuous access to opiate self-administration is associated with a gradual escalation to higher levels of drug consumption in rats (72–74). However, until very recently, little research has been conducted to systematically assess the relationship between the length of drug access and the pattern and rate of opiate self-administration (66, 75–78). Nevertheless, the few studies published to date consistently extend most of the findings obtained with stimulant drugs to heroin, apart from the exception of nicotine (but see (79)). As with cocaine and regardless of initial training and pharmacological history, short versus long access time to heroin self-administration induces two
Ahmed

276

Ahmed

different patterns and levels of consumption: a stable pattern of moderate heroin use versus an escalating pattern of excessive heroin consumption (Fig. 3a–d). Intriguingly, as revealed by a metaanalysis of the literature (22, 73–79), the severity of drug intake escalation appears to be higher with opiates (heroin, morphine, fentanyl) than with stimulants (Fig. 2). This relatively unexpected finding may suggest that opiates have a higher “dependence liability” than stimulants. Further comparative research is needed to confirm this interpretation.

Fig. 3. Effects of access time to heroin on the pattern of self-administration. Data represent the mean number ± SEM of heroin injections during the first hour of the session (top panels) or during the whole session (bottom panels). Rats had access to heroin (unit dose = 0.015–0.02 mg, i.v.) for either 1 h (ShA rats) or 6 (or 11) h/day (LgA rats). In the first experiment (a and c), rats were first allowed to self-administer heroin during 2 h/day for at least 10 days before having differential access to the drug (for additional information, (75)). In the second set of experiments (b and d), rats had no prior training history before having differential access to heroin self-administration (for additional information, see (77, 78)). In these experiments, the unit dose of heroin available during the last 5 h of each LgA session was increased to 0.06 mg. Data in (d) represent the mean number ± SEM of heroin injections during the last 5 h. The horizontal gray box indicates the mean total number ± SEM of drug injections during the first day.* Different from ShA rats or from the first day (P<0.05).
4.3. Ethanol

It is notoriously difficult to incite naïve animals to spontaneously drink high amounts of ethanol to become dependent (80). For instance, as mentioned earlier, with unlimited access to ethanol in the home cage, it takes dozens of weeks for a minority of rats to escalate their ethanol consumption (5, 6). However, following the lead of a seminal, though neglected, study by Roy Wise in the early 1970s (68), a recent series of experiments from different laboratories has now clearly demonstrated that most rats can rapidly escalate their ethanol consumption to high levels if given a long and intermittent access to a highly-concentrated ethanol solution (i.e., 24-h access every other day to 20% ethanol) (67, 69). Note that in those studies, rats also had access to water, so ethanol drinking was not forced or driven by thirst. In addition, as with cocaine or heroin intake escalation, ethanol intake escalation was associated with a dramatic increase in ethanol loading early in the 24-h session (i.e., within the first hour). It remains to be seen, however, whether short versus long access to intermittent ethanol drinking can induce two patterns of ethanol consumption, as seen with many other drugs of abuse.

5. Drug Cross-Escalation

As described above, drug intake escalation is observed across a broad spectrum of drugs of abuse, including stimulants, opiates, and ethanol. Whether there is cross-escalation between different drugs of abuse or within the same drug class (e.g., the opiates) has not yet been tested systematically. Drug cross-escalation is defined here as an increase in the consumption of a newly encountered drug after intake escalation of a different drug. The systematic study of the phenomenon of drug cross-escalation may provide critical information, not only about neuropsychopharmacological commonalities in the escalation process, but also for better understanding polydrug abuse and addiction. To begin to address this issue, it was determined whether there is a cross-escalation between cocaine and heroin consumption – two highly addictive drugs with both common and different neuropsychopharmacological effects. Two separate groups of rats were first allowed to self-administer either cocaine or heroin for 1 h/day and then, after stabilization of drug intake, escalation of cocaine or heroin self-administration was precipitated by increasing drug access time to 6 h/day (Lenoir and Ahmed, submitted). For each group, a dose-injection function for drug self-administration was generated both before and after increased drug access time. As shown in Fig. 4, there was virtually no cross-escalation between cocaine and heroin. Specifically, as shown previously, after prolonged access to one drug (cocaine or heroin), rats escalated their consumption of...
Ahmed

this drug, which resulted in a characteristic vertical shift of the corresponding dose-injection function. However, the same rats did not increase their intake of the alternative drug, regardless of the available dose. The lack of cross-escalation to heroin self-administration in cocaine-escalated rats was confirmed in another study. In this study, rats \((N=8)\) were initially trained to self-administer both cocaine and heroin during 1 h on alternate days until stabilization of cocaine and heroin intake. Then, access to cocaine was increased to 8 h/day during several days. As expected, rats escalated their cocaine intake during extended access to cocaine but again, regardless of the dose available, there was no cross-escalation between cocaine and heroin. In fact, there was even a nonsignificant trend for decreased heroin consumption compared to prior cocaine intake escalation (Guillem and Ahmed, unpublished data). The lack of cross-escalation to cocaine self-administration in heroin-escalated rats is consistent with previous research showing no change in cocaine intake in rats with frequent heroin use under a discrete-trials procedure of drug self-administration (81, 82). Future research is clearly required to better assess the generality of these findings across a wider range of drugs of abuse, both between and within drug classes.

6. The Dynamics and Stability of Drug Intake Escalation

As illustrated above, escalation of drug consumption is clearly a transient and negatively accelerating process, which eventually levels off at a new, higher level of consumption. This process can
thus be characterized by different dynamic and state parameters, such as, its shape, speed, and final, post-escalation steady-state. So far, comparatively little research has been conducted to study how these different features systematically vary as a function of drug access condition. In one systematic study, Wee and colleagues (57) showed that the final, post-escalation level of cocaine intake increases with the length of daily sessions of cocaine self-administration: the longer the self-administration session, the greater the steady-state level. Whether the final, post-escalation level of drug consumption can also be influenced by other factors is less clear. In two studies from the same laboratory, the level of escalated cocaine or methamphetamine intake decreased with the training dose (57, 62) while in another study, the level of escalated cocaine self-administration remained unchanged (61). Clearly, additional parametric research is needed to better quantify the dynamics of the escalation process. This research will eventually prove useful for future pharmacological and neurocomputational modeling approaches to drug addiction (83).

Knowledge about the maintenance of escalated levels of drug consumption is even more limited. In one study, the maintenance of escalated levels of cocaine self-administration was shown to depend on the session duration (21). In this experiment, after inducing cocaine intake escalation, the daily session duration was reduced from 6 to 1 h. As a result, escalated levels of cocaine intake significantly decreased over time and progressively, though very slowly, returned toward pre-escalation levels of consumption. Indeed, even after 2 months of reduced drug access, the de-escalation of cocaine self-administration was still incomplete. In contrast, in another, related experiment, escalated levels of cocaine self-administration completely de-escalated to pre-escalation levels after only 1 month of forced abstinence (7). This differential outcome shows that compared to total abstinence, intermittent, brief exposure to cocaine self-administration can considerably retard, without preventing, drug recovery. This finding may have important clinical implications.

7. Association of Drug Escalation with Other Addiction-like Changes

There is now considerable evidence showing that increased access time to a variety of drugs of abuse can precipitate a rapid escalation to higher levels of drug consumption – a hallmark of addiction. More recent research now indicates that animals with escalating drug use also present other major behavioral signs of addiction, compared to animals with a more stable pattern and moderate level of drug use. These behavioral signs of addiction include: (1) an increased motivation to self-administer the drug,
greater difficulty in abstaining from drug use, and (3) a relative indifference to negative consequences. Finally, converging evidence from different laboratories shows that animals with increased drug use also become more sensitive to drug-induced and stress-induced reinstatement of drug seeking after extinction. Reinstatement of extinguished drug seeking is a well-validated animal model of craving and/or relapse. Though drug-induced craving is not a current diagnostic criterion of addiction, it nevertheless represents a selective marker of addiction since it is not seen in nondependent individuals. Thus, as a whole, the differences between stable/moderate and excessive/escalating patterns of drug self-administration induced by differential drug access time in rats recapitulate the major behavioral differences between controlled and compulsive drug use in humans.

7.1. Association with Increased Drug Value and/or Motivation

Post-escalation upward shifts in the dose-effect function for cocaine self-administration first suggested an increase in the reinforcing value of and/or motivation for the drug following extended drug use. To understand why, the dose-effect function for cocaine self-administration must be reinterpreted within a behavioral economic framework. Within this framework, the drug dose is inversely equivalent to the response requirement (or price) for maintaining the drug effects within the preferred range of the individual: the lower the dose, the higher this requirement. Thus, an upward shift in the peak rate of self-administration, as observed following drug intake escalation, reflects an increased willingness to pay a higher maximum price to defend drug consumption (i.e., a more inelastic drug demand), which betrays an increase in drug value and/or motivation. Christensen and coworkers have reached the same conclusion using a more general mathematical approach for assessing the inelasticity of drug demand from dose-intake curves. Finally, an increase in the inelasticity of drug demand was directly observed following heroin intake escalation. As a whole, these studies suggest that escalation of drug consumption is associated with an increase in the reinforcing value of and/or motivation for the drug (but see (52)).

More conventional evidence for increased drug value and/or motivation following drug intake escalation was also obtained using the progressive ratio (PR) procedure. Paterson and Markou reported that following cocaine intake escalation, LgA rats maintain a higher breakpoint than ShA rats. This initial observation was later confirmed by other teams or laboratories and was recently extended to other drugs of abuse, including methamphetamine and heroin. Two independent studies, however, failed to reproduce this finding. In fact, in one of these studies, escalation of cocaine self-administration — which was induced by passive
administration of cocaine – was associated with lower, not higher, breakpoints (94). The origin of this discrepancy is currently unknown.

Finally, using the operant runway procedure as an alternate measure of motivation, Ben-Shahar and colleagues (39) showed that, following cocaine intake escalation, LgA rats run faster than ShA rats to reach a goal box to obtain intravenous cocaine. Thus, overall, the bulk of evidence indicates that escalation of drug consumption is associated with an increase in the reinforcing value of and/or motivation for a variety of drugs of abuse, including cocaine, methamphetamine, and heroin. However, additional research is needed to extend this conclusion to other drugs of abuse (e.g., nicotine and ethanol) and to resolve some of the contradictions found in the literature.

Wolffgramm (5, 6) was perhaps the first to suggest that a relative resistance of drug self-administration to punishment (i.e., the process by which an aversive, response-contingent stimulus suppresses responding for the drug) could represent an operational measure of compulsive drug use in laboratory animals (see also (21)). Accordingly, if drug use by laboratory animals becomes compulsive, then it should continue to some extent despite immediate or delayed negative consequences. In other words, if animals become compulsive drug users, they should take more risk while seeking and/or taking the drug. In support of this, Wolffgramm (5, 6) showed that rats with escalated levels of ethanol consumption following continuous access to ethanol continued to drink more than non-escalated rats despite making the ethanol solution bitter with the addition of quinine (bitter taste is a natural deterrent that is ecologically correlated with poisonous food plants). Though this observation suggests that escalated ethanol consumption is indeed compulsive, it could also be explained by other, unrelated, factors, such as, unmeasured changes in bitter taste sensitivity.

Subsequently, Vanderschuren and Everitt (10) showed in a heterogeneous seeking-taking chain schedule that following extended access to cocaine self-administration, rats become more resistant to conditioned suppression of cocaine seeking. Importantly, this outcome was not attributable to a decrease in pain sensitivity or in aversive conditioning, as rats with extended cocaine use showed the same level of conditioned freezing following footshock stress than rats with moderate cocaine use. Decreased sensitivity of cocaine seeking to conditioned suppression of cocaine seeking can be explained, however, by a variety of relevant behavioral mechanisms, including aberrant behavioral automatization, attentional bias away from nondrug cues and/or generalized increase in risk taking. The latter explanation is consistent with a recent study showing that LgA rats with escalated
levels of cocaine intake explore more often the open, more risky arms of a plus-maze apparatus than ShA rats (49).

Finally, in a modified punishment procedure, LgA rats appeared much more resistant than ShA rats to the delayed effect of punishment on cocaine self-administration (Ahmed, 2004, unpublished observation). Briefly, following 78 sessions of differential access to cocaine self-administration, both ShA and LgA rats had access to cocaine during one single 2-h session. During the first hour, responding (i.e., lever pressing) for cocaine was not punished while during the second hour, each reinforced response was punished by a brief electric footshock administered through the grid floor of the operant chamber (1.2 s, 1 mA). Changes in response between the first and second hour allowed assessment of the unconditioned punishing effect of footshock on cocaine self-administration. On the days following the punishment day, rats were allowed to self-administer cocaine without punishment during three consecutive 1-h sessions. Changes in cocaine intake during these days (compared to pre-punishment first hour intake) allowed assessment of the delayed effect of punishment on cocaine self-administration. As expected, LgA rats (N=6) self-administered much more cocaine than ShA rats (N=7) on the last 3 days before the punishment day (Fig. 5a). However, response-contingent footshock during the second hour profoundly and equally suppressed cocaine self-administration in both ShA and LgA rats, showing that both groups were equally sensitive to the unconditioned

Fig. 5. Effects of access time on punishment-induced suppression of cocaine self-administration. (a) Mean number of first hour cocaine injections averaged over the last three baseline sessions of self-administration preceding the punishment day. (b) Unconditioned punishment-induced suppression of cocaine self-administration expressed as percent change from the first, non-punished hour. (c) Delayed effects of punishment on cocaine self-administration expressed as percent change from the pre-punishment baseline. * Different from ShA rats; # different from the pre-punishment baseline (P< 0.05).
punishing effects of footshock (Fig. 5b). However, on the following days, cocaine self-administration by ShA rats remained partially suppressed despite the discontinuation of punishment while cocaine intake by LgA rats returned to the pre-punishment baseline (Fig. 5c). In fact, 100% of LgA rats were resistant to the delayed effect of punishment on cocaine taking compared to only 28% of ShA rats (a rat was considered resistant if the average suppression over the three post-punishment days was lower than 30%). Thus, taken together, the available evidence indicates that rats with escalated drug consumption are more resistant to punishment and more prone to engage in risky behavior during drug taking.

The difficulty of abstaining from drug use can be operationalized in laboratory animals by persistent drug seeking even when the drug is no longer available (i.e., resistance to extinction) (75). Using this operational definition, LgA rats with escalated heroin consumption were found to experience more difficulty in extinguishing heroin or morphine seeking than ShA rats (75, 77, 79). In two studies, this resistance to extinction was present during several days (75, 79). More recently, Zhou and colleagues (95) showed in a systematic study that the degree of resistance to extinction is both a function of the amount of heroin self-administration and of the length of drug withdrawal. Surprisingly enough, however, no resistance to extinction is observed following escalation of cocaine (32, 46, 47, 50, 61, 96) or methamphetamine self-administration (63). Interestingly, in these studies, the length of drug withdrawal preceding extinction was relatively short (i.e., 24–72 h). When the withdrawal interval is longer (i.e., 3 weeks), LgA rats with escalated levels of cocaine intake responded more during extinction than ShA rats (42). This observation may also suggest that cocaine craving incubates more rapidly and intensely in LgA rats than in ShA rats (97) (but see (96)). In summary, escalation of drug consumption is associated with an increased difficulty in abstaining from drug use, as operationalized by an increased resistance to extinction. However, in the case of stimulant drugs, the expression of this association seems to require a relatively long incubation period. Future research is needed to test this issue.

7.3. Association with Difficulty of Abstaining from Drug Use

In addicted individuals, drugs of abuse do not only evoke intense rewarding sensations but also induce craving for more drugs (85, 86). Drug-primed craving can be studied in animals in the reinstatement model (84). Briefly, in this model, responding for the drug is first extinguished by discontinuing drug delivery and then reinstated by a passive (i.e., response-independent) reexposure to the drug. Importantly, during reinstatement testing, responses continue to be unrewarded as during extinction and, therefore,
reflect genuine drug-seeking behavior. Using this model, Mantsch and coworkers (61) showed that escalated cocaine self-administration was associated with an increase in cocaine-primed reinstatement of drug seeking. This finding has now been reproduced several times (31, 46, 47, 50) and extended to other drugs of abuse, including heroin (77) and methamphetamine (63). Using a within-session extinction procedure, it has been estimated that about 80% of LgA rats are consistently sensitive to intravenous cocaine- or heroin-primed reinstatement of drug seeking compared to about 20% of ShA rats. Finally, drug intake escalation is also associated with an increased sensitivity to stress-primed reinstatement (50, 75) but apparently not with increased responsiveness to conditioned cue-induced reinstatement (63, 79, 95).

8. Individual Variation in Escalation to Dependence

In humans, not all drug users become addicted, a phenomenon that has been recognized since the beginning of addiction research (98). Likewise, in laboratory animals, not all individuals show escalation of drug self-administration and other associated signs of addiction following extended drug access (5, 6, 54, 99–107). What is less clear and subject for debate, however, is the extent of this individual variation and whether it is influenced by drug availability and other factors (108). According to a recent series of studies, only a minority of individual rats (about 20%) would become dependent on cocaine, even following several months of exposure to cocaine self-administration (8, 9). Though this relatively low estimate is very close to the proportion of cocaine addiction in humans, its interpretation should nevertheless be made with caution. First, in these studies, rats had a short, not a long, daily access to cocaine (i.e., 2 h/day) which probably explains the low estimate of rats with addiction-like behavior. In fact, as amply reviewed here, the proportion of individuals that ultimately develop cocaine intake escalation and other addiction-like changes following extended drug access can considerably increase with the length of daily self-administration sessions (cocaine intake escalation: 70% of LgA rats versus 12% of ShA rats; decreased sensitivity to punishment: 100% of LgA rats versus 28% of ShA rats; increased responsiveness to drug-induced reinstatement: 80% of LgA rats versus 20% of ShA rats) ((31, 77, 108); present chapter). Second, the statistical method used to estimate the frequency of addiction-like behavior in rats in (8, 9) is circular and presupposes what it seeks to demonstrate, that is, drug addiction is essentially a statistically deviant behavior that would only affect few vulnerable individuals. Indeed, the statistical threshold chosen to consider an individual positive for one
specific behavioral criterion was limited a priori to only 33% of the maximum possible frequency of cases. The actual estimate of 20% of addiction-like cases was lower than the theoretical minority of 33% simply because an individual had to conjointly satisfy several imperfectly correlated criteria to have an addiction-like profile. Obviously, this method of estimation is strongly biased toward finding what it eventually found, that is, a small minority of individuals with addiction-like behavior. A critical challenge for future research will be to devise other, less biased, criteria for addiction models. These criteria should not arbitrarily postulate that drug addiction is a statistically deviant behavior to which the majority of individuals would be inherently or constitutively resistant. Ideally, they should even authorize a maximum possible frequency of 100% of addiction cases in laboratory animals. Whether this prevalence can be observed or not is an empirical question.

9. Conclusions, Perspectives, and Limitations

The research reviewed above clearly shows that, with extended daily access to drug self-administration, as opposed to shorter drug access, animals are considerably more likely to escalate their consumption and to develop other addiction-like changes. Thus, the comparative study of individuals with differential drug access may provide a unique approach to experimentally studying drug addiction and to resolving some long-standing issues in drug addiction research. First, this comparative approach should help to determine the direction of causality between patterns of drug use and some behavioral (or neurobiological) deficits seen in human drug addiction (i.e., the famous chicken-and-egg dilemma). For instance, recent research suggests that the causality between extended drug use and some higher-order executive dysfunctions is probably bidirectional (9, 40, 41, 63, 103, 106, 109–113, 122). Second, this approach should also assist in the study of the sequences of occurrence of different symptoms during the transition from first drug use to addiction (114). For instance, there is some evidence showing that increased sensitivity to drug-induced reinstatement can occur independently of drug intake escalation (46, 47). Third, this approach should facilitate research on the factors that protect from the development and/or persistence of heavy drug consumption – the latter being particularly relevant for new treatment development. For instance, it was recently found that access to an alternative reward following extended access to cocaine or heroin self-administration alone can lead many rats to reduce or even stop escalated drug use (76, 78, 115). Finally, the comparison of individuals with short versus long access to drug self-administration should also hopefully
promote the search for the specific brain dysfunctions that cause and underlie drug addiction. For instance, recent research found a selective and persistent decrease in lateral hypothalamus reward processing only in rats with escalating cocaine or heroin use \( (29, 66) \). This selective decrease in reward processing would result in large part from the recruitment of brain anti-reward neurotransmitter pathways \( (116) \).

These promising perspectives should not, however, overshadow some important caveats that may limit the generality and applicability of the model of differential escalation of drug use. First, the model relies heavily on the postulate that the pattern of drug self-administration displayed by individuals with limited drug access models non-disordered forms of substance use in humans \( (i.e., \) occasional drug use, recreational drug use, controlled drug use). The absence of drug intake escalation and other addiction-related changes in most of these individuals are consistent with this postulate \( (8, 9, 108) \). But, obviously, more comparative research between drug naïve animals and ShA rats is needed to better establish its empirical validity. Also, this research will help to better define the specific drug access factors that maintain drug use without promoting the progression toward addiction. Second, escalation of drug use has so far been induced by a very specific environmental factor, that is, increased drug availability. As mentioned earlier, the etiology of drug intake escalation in humans is probably more diverse and complex. Future research should determine whether escalation of drug use precipitated by increased drug availability is representative of escalation processes precipitated by different etiological factors, environmental \( (e.g., \) chronic stress, \( (107, 117) )) \) or not. Third, escalation of drug use has been documented, primarily, in specific nonhuman animals in specific situations, that is, in male, young adult rats, growing and living in a stable, impoverished and artificial environment, and having access to drugs with little or no alternative choices. It remains to be seen whether drug intake escalation can be observed in other individual animals and in other situations. For instance, evidence for drug intake escalation and associated addiction-like changes is currently equivocal in nonhuman primates \( (99, 118, 119) \). Because of their phylogenetic, genomic, and behavioral proximity to humans, demonstrating escalation of drug use in nonhuman primates represents an important challenge for further validation of the model. Finally, it will be important to extend research on drug intake escalation to other potentially addictive, nondrug rewarding activities. For instance, recent research suggests that extended access to a running wheel or food can also lead to an escalation in daily running \( (120) \) or food consumption \( ((121); \text{but see} (89)) \). Further research is needed to explore the commonalities and differences between escalation of drug use and other forms of behavioral escalation.
Acknowledgments

This work was supported by grants from the French Research Council (CNRS), Université Victor-Segalen Bordeaux 2 and Mission Interministérielle de Lutte contre la Drogue et la Toxicomanie (MILDT). I thank Drs. Magalie Lenoir, Karyn Guillem, and Kelly Clemens for their comments on a previous draft of this book chapter. I also thank the reviewer and the editor for their constructive comments. I dedicate this book chapter to my wife, Dr. Saloua Aidoudi.

References

Ahmed

self-administration after escalation in rats. Psychopharmacology 146:303–312
38. Ben-Shahar O, Keeley P, Cook M et al (2007) Changes in levels of D1, D2, or NMDA receptors during withdrawal from brief or extended daily access to IV cocaine. Brain Res 1131:220–228
289 Escalation of Drug Use


52. Oleson EB, Roberts DC (2009) Behavioral economic assessment of price and cocaine consumption following self-administration histories that produce escalation of either final ratios or intake. Neuropsychopharmacology 34:796–804


68. Wise RA (1973) Voluntary ethanol intake in rats following exposure to ethanol on various schedules. Psychopharmacologia 29:203–210
Chapter 11

Environmental Modulation of Drug Taking

Aldo Badiani, Daniele Caprioli, Arianna Testa, Maria Teresa De Luca, and Michele Celentano

Abstract

A variety of animal models have been developed to mimic the interactions between drugs and environment that are thought to play a crucial role in human addiction. A history of exposure to stress, for example, facilitates the development of drug addiction and drug relapse. Furthermore, there is solid evidence that drug-related contextual cues (i.e., environmental stimuli paired with drug taking that have acquired conditioned stimulus properties) can precipitate drug seeking in both humans and animals, indicating the importance of associative learning processes. Finally, there is some evidence (mostly of anecdotal nature) that the circumstances immediately surrounding drug taking can modulate drug intake in ways that are not easily reducible to conditioning or stress. In the past few years some effort has been made to investigate this latter type of drug-environment interaction using animal models. Most importantly, we have recently shown that the context can modulate the reinforcing effects of addictive drugs independently of its physical characteristics. In these studies, some animals were transferred to the test cages immediately before the treatment (Non Resident group), whereas other animals were kept at all times in the test cages (Resident group). Some studies were conducted using a single drug, whereas others employed a polydrug taking procedure. In the present chapter, we will review not only the results obtained using this animal model but also those yielded by translational studies conducted in human addicts. Finally, we will discuss the implications of these findings for the study of drug addiction in humans and animals.

Key words: Conditioning, Stimulus properties, Contextual learning, Associative learning, Relapse, Opiate, Stimulant, Polydrug use

1. Introduction

In the past 2 decades, a variety of animal models have been developed to mimic the interactions between drugs and environment that are thought to play a crucial role in human addiction (1). A history of exposure to stress, for example, is known to facilitate the development of drug addiction and drug relapse (2–7). Furthermore, there is solid evidence that drug-related contextual
cues (i.e., environmental stimuli paired with drug taking that have acquired conditioned stimulus properties) can precipitate drug seeking in both humans and animals (8–12), indicating the importance of associative learning processes. Finally, there is some evidence (mostly of anecdotal nature) that the circumstances immediately surrounding drug taking can modulate drug intake in ways that are not easily reducible to conditioning or stress (13–18). In the past few years, some effort has been made to investigate this latter type of drug-environment interaction using animal models. It has been shown, for example, that drug taking in rodents can be altered by changes in the physical characteristics of the drug environment, as indicated by reports that exposure to novel objects can reduce amphetamine intake (19) and that heat can enhance MDMA intake (20). Most importantly, we have recently shown that the context can modulate the reinforcing effects of addictive drugs independently of its physical characteristics. In these studies, some animals were transferred to the test cages immediately before the treatment (Non Resident group), whereas other animals were kept at all times in the test cages (Resident group). Some studies were conducted using a single drug whereas others employed a polydrug taking procedure. In the present chapter, we will review not only the results obtained using this animal model but also those yielded by translational studies conducted in human addicts. Finally, we will discuss the implications of these findings for the study of drug addiction in humans and animals.

2. Environmental Modulation of Cocaine, Amphetamine, and Heroin Self-Administration in Rats

An initial series of studies was devoted to investigating the effect of environmental context on cocaine, amphetamine, and heroin self-administration (21, 22). In these experiments, male Sprague–Dawley rats were implanted with a catheter in their right jugular vein and 1 week after the surgery were assigned to one of two conditions: Resident or Non Resident. The rats in the Resident groups were housed in the self-administration chambers (each equipped with two retractable levers) where they remained for the entire duration of the experiment. In contrast, Non Resident rats were transferred to the self-administration chambers only for the experimental session.

All experiments included 13 experimental sessions (3 h each). At the start of each session, the two levers were extended and remained extended for the entire duration of the session, except during the time-out periods. Only one of the two levers produced a drug infusion (active lever), whereas the other lever had no direct consequences on drug infusion but did reset the counter of
Environmental Modulation of Drug Taking

the active lever. Independent groups were trained with different drug doses (delivered in a 40-μl volume over a 3-s period): 200, 400, or 800 μg/kg per infusion for cocaine; and 12.5, 25.0, or 50.0 μg/kg per infusion for amphetamine and heroin. Some rats were also tested for saline self-administration.

During sessions 1–7, the fixed ratio (FR), i.e., the number of consecutive lever presses required to obtain a single infusion, was raised from FR1 (sessions 1–2) to FR2 (sessions 3–5) and then to FR5 (sessions 6–7). Upon completion of the task, both levers retracted and were extended again after a time-out period of 40 s. During the subsequent sessions, the rats were allowed to self-administer additional drug doses and were then tested in a progressive-ratio procedure during which the number of responses required to obtain a single infusion was increased within the session according to the following progression: 5, 10, 20, 30, 50, 70, 100, 150, 200, 300, 500, and so on. The number of lever presses emitted during this session was used as an index of the motivation for heroin taking.

The following is a summary of the characteristics of the Resident versus the Non Resident condition.

1. Both Resident and Non Resident rats were single-housed after surgery.
2. The self-administration environment was physically identical for all rats, but for some animals this was also the home environment (Resident group), whereas for other animals it represented a distinct and, at least initially, novel environment (Non-Resident group).
3. During testing, the self-administration chambers contained no food or water. The rest of the time the animals had free access to food and water.
4. The distance traveled by the Non Resident rats during the transfer to the self-administration chamber was about 1 m (all animals were kept in the same dedicated testing rooms for the entire duration of the experiments, and therefore there was no transport from one room to another).
5. Immediately before the start of each session, the Resident rats were briefly handled to remove food and water from the chamber. When necessary, both Resident and Non Resident rats were briefly handled to deliver priming infusions.
6. All testing took place in the dark phase (between 13:00 and 17:00 h) 7 days a week.
7. All other husbandry routines were identical in the two groups.

Figure 1 summarizes the results of the data concerning the acquisition of drug self-administration. In particular, it shows the number of lever presses on the last training session conducted on an FR5 schedule of reinforcement (session 7). It can be seen that cocaine and amphetamine self-administration was greater in the Non Resident rats than in the Resident rats. Indeed, it appears that the dose–effect curve for the acquisition of cocaine and amphetamine self-administration was shifted to the right in the Resident versus the Non Resident condition. In contrast, heroin self-administration was greater in the Resident rats than in the
Non Resident rats, with an upward shift of the dose–effect curve. The results of the progressive ratio sessions confirmed that the motivation for heroin was greater in Resident than in Non Resident rats, whereas the motivation for cocaine was greater in Non Resident than in Resident rats (21).

These findings were quite surprising for at least two reasons. First, they were partly at odds with previous studies, conducted by us and other authors, concerning drug-induced psychomotor sensitization (a phenomenon consisting of a progressive increase in locomotor activity following repeated administrations of drugs, such as cocaine, amphetamine, heroin, etc.). In particular, it had been had reported that Non Resident rats exhibit greater sensitization than Resident rats when repeatedly treated with cocaine (23, 24), amphetamine (25, 26), morphine (27, 28), or heroin (29). The existence of a close relationship between the psychomotor and the rewarding effects of addictive drugs is widely accepted in the literature (30). It has even been hypothesized that the neuroadaptations associated with psychomotor sensitization are somewhat similar to those responsible for the development of drug addiction (31). Thus, it would be expected that any manipulation capable of facilitating drug-induced psychomotor sensitization would also facilitate drug self-administration. Indeed, this is what we found with cocaine and amphetamine. By contrast, heroin reward was substantially greater in Resident rats, relative to Non Resident rats, indicating unforeseen dissociations between opioid and psychostimulant reward and between opioid reward and opioid-induced activity.

The second reason the findings summarized above were surprising is represented by the unforeseen dissociation in the effects of drug taking context on psychostimulant versus opioid reward. Indeed, research in the last 2 decades has stressed the existence of
shared neural substrates for the rewarding effects of addictive drugs (32). Also from a theoretical point of view, the current trend is to emphasize the similarities among various types of natural and pharmacological rewards. Thus, the importance of better characterizing our animal model became obvious. To this end, we employed polydrug taking procedures rarely used in the experimental setting.

3. Environment and Polydrug Taking

Drug abuse is rarely limited to a single substance, polydrug abuse being the norm rather than the exception. Most heroin addicts, for example, also abuse psychostimulants, especially cocaine (33, 34), and vice versa. The pattern of drug taking and drug preference is widely thought to be a function of local availability, street price, lifestyle, and other sociocultural factors (35–37). As a consequence, very little laboratory research has been devoted to this type of issue. However, the findings described in the previous section suggested that the environmental context could differentially modulate the intake of opioid and psychostimulant drugs in a much more basic manner. In the following sections we will discuss the results obtained using three different procedures in which the same rats were given the opportunity to self-administer both heroin and a psychostimulant drug (amphetamine or cocaine).

In this study, Resident and Non Resident male Sprague–Dawley rats that had been trained to self-administer amphetamine were given the opportunity to self-administer heroin (22). The experimental design of the first part of this study was similar to that described for the experiments in the previous section, but after session 12, the rats were given a 7-day period of rest (during which they were kept in their respective home cages) and were then challenged with a noncontingent i.v. infusion of 100.0 µg/kg of heroin (session 13) to assess their psychomotor response over 60 min. On the following days (sessions 14–25) the rats were switched to heroin self-administration (25.0 µg/kg per infusion) following procedures similar to those used for amphetamine in sessions 1–12.

As shown in Fig. 2, environmental context modulated heroin and amphetamine self-administration in opposite directions. Thus, the results illustrated in Fig. 1 could be reproduced even with a within-subject design. We also found a dissociation in the effects of environment on heroin self-administration versus heroin-induced locomotor activity; that is, the psychomotor response to heroin was greater in Non Resident than in Resident rats (22).
In this study, we used an experimental procedure in which Resident and Non Resident male Sprague–Dawley rats were given the opportunity to self-administer both cocaine and heroin on alternate days, under different schedules of reinforcement (38). Although we did expect that the acquisition of cocaine self-administration would affect that of heroin (and vice versa), somewhat blunting the effect of environment, we predicted that Non Resident rats would take more cocaine relative to heroin than Resident rats. More specifically, we predicted that the ratio of cocaine to heroin taking in individual rats would be greater in Non Resident than in Resident rats.

Throughout the experiment, heroin (25.0 µg/kg) and cocaine (400.0 µg/kg) were made available to the rats on alternate days, the starting drug being counterbalanced within groups. These doses were chosen on the basis of the results illustrated in Fig. 1. Given that the half-life of both cocaine and heroin is shorter than 1 h (39–41), it was safe to assume that the 21 h separating consecutive sessions were sufficient to achieve the complete clearance of each drug. Right and left levers were paired to either heroin or cocaine, in a counterbalanced manner. The FR was raised from FR1 (sessions 1–4) to FR2 (sessions 5–10) and then to FR5 (sessions 11–14). At the end of session 14, the rats were assigned to two different experimental procedures. Some rats underwent, on sessions 15 and 16, a progressive-ratio procedure, for heroin on 1 day

\[ \text{SA sessions (FR)} \]

![Amphetamine SA -- Heroin SA](image)

Fig. 2. Number of lever presses (means ± SEMs) on the active lever in animals trained first to self-administer amphetamine (acquisition dose: 25 µg/kg per infusion) and then heroin (acquisition dose: 25 µg/kg per infusion) at a progressively higher schedule of reinforcement. The rats were either transferred to the test cages immediately before the treatment (Non Resident group) or kept at all times in the test cages (Resident group) (Data from (42). With kind permission).

### 3.2. Alternating Cocaine and Heroin Self-Administration
and cocaine on the other, in a counterbalanced manner. The other rats continued to alternate heroin and cocaine self-administration for 12 additional sessions (sessions 15–26), during which the FR was raised from FR10 (sessions 15–16) to FR20 (sessions 17–18), FR 30 (sessions 19–20), FR50 (sessions 21–22), FR70 (sessions 23–24), and, finally, FR100 (sessions 25–26).

Figure 3 illustrates the data concerning the rats that were tested over 26 sessions. It can be seen that Resident rats took much more cocaine relative to heroin than Non Resident rats and that these differences became larger by increasing the FR. For example, on FR50, Resident rats took on average about 4 times more infusions of cocaine than of heroin while Non Resident rats took on average 18 times more cocaine than heroin. The results of the progressive ratio session confirmed that the reinforcing effects of cocaine and heroin are a function of the context of drug taking (38).

3.3. Choosing Heroin Versus Cocaine

Although the combined abuse of cocaine and heroin by addicts is the rule rather than the exception, very little is known about the variables that regulate cocaine versus heroin taking. As discussed above, rats adjust their intake of these two drugs as a function of the environment of drug taking, Non Resident rats taking more cocaine relative to heroin than Resident rats. Thus, we wondered whether environmental context could exert its modulatory influence even on drug preference, that is, on drug choice when the two drugs are made available simultaneously (42).

To test this hypothesis, male Sprague–Dawley rats with a double-lumen catheter were trained in ten consecutive daily 3-h
sessions to self-administer cocaine (400 μg/kg/infusion) and heroin (25 μg/kg) on alternate days (i.e., there were five sessions for each drug). Cocaine and heroin were each paired with one of the two retractable levers and one of two cue lights (red or green). The assignment of levers and cues was counterbalanced for heroin and cocaine. At the start of each session, only the lever associated with the drug to be self-administered on that session was extended and the appropriate cue light was turned on. As in the studies described above, the number of consecutive lever presses required to obtain a single infusion was raised from FR1 to FR5 across test sessions. After each infusion, the cue light turned off and the lever retracted. The cue light turned on and the lever extended again after 40 s. The rats were then given simultaneous access to both cocaine and heroin for seven consecutive daily sessions. We predicted that a larger proportion of Non Resident rats would choose cocaine over heroine relative to Resident rats, and vice versa.

Indeed, as shown in Fig. 4, 76% of rats exhibited a preference for either cocaine or heroin (as indicated by bootstrapping analysis, \( P<0.001 \)) but with important differences between the Resident versus the Non Resident condition. The rats that preferred heroin to cocaine were six times more numerous in the Resident group than in the Non Resident group (47% vs 8%), whereas the rats that preferred cocaine to heroin were two times more numerous in the Non Resident group than in the Resident group (67% vs 33%).

![Fig. 4. Proportion of rats expressing a preference for cocaine versus heroin (based on the results of bootstrapping analysis) after being trained for both cocaine (400 μg/kg per infusion) and heroin (25 μg/kg per infusion) self-administration and then given the opportunity to choose between the two drugs over seven consecutive choice sessions, during which both drugs were made simultaneously available. The 50/50 label refers to the rats that self-administered about the same amount of the two drugs. The rats were either transferred to the test cages immediately before the treatment (Non Resident group) or kept at all times in the test cages (Resident group) (Data from (42). With kind permission).](image-url)
Interestingly, we found no correlation between the amounts of cocaine and heroin self-administered during the training phase and the amounts of cocaine and heroin self-administered during the choice phase, as indicated by linear regression analysis.

As discussed above, rats that were given the choice between cocaine and heroin self-administration expressed different drug preferences depending on environmental context: cocaine being preferred by most rats not residing in the self-administration chambers and heroin by most rats living in the chambers. The possible implications of these results for human addiction were obvious but, to the best of our knowledge, we could find no reports on the context of heroin versus cocaine taking in human co-abusers. Thus, we decided to adopt a translational approach to investigate the ambience selected by intravenous drug users to inject heroin and cocaine intravenously (42). We predicted that heroin would be injected more often at home than outside and vice versa for cocaine.

The participants in the study were recruited among the outpatients of Villa Maraini Therapeutic Community (an addiction clinic affiliated with the Italian Red Cross) who had a fixed address and met DSM-IVR (43) Drug Dependence criteria for cocaine and/or heroin but were not affected by other major psychiatric disorders. One hundred and four individuals completed the interview. Seventy-nine of the participants reported using both heroin and cocaine whereas 21 reported using only heroin and 4 using only cocaine. During the interview, the participants were asked where they had usually taken heroin and/or cocaine in the last 3 months. Follow-up questions were aimed at assessing, for each drug, whether it was taken: (1) always at home, (2) mostly at home, (3) sometimes at home sometimes outside (50/50), (4) mostly outside, and (5) always outside. The participants were also asked whether the context of drug taking represented a real preference or was the result of constraints.

Figure 5 illustrates the setting of cocaine versus heroin taking for the 79 individuals who co-abused the two drugs, 24 of whom used both drugs intravenously and 13 both drugs intranasally. Approximately 74% of all co-abusers preferred to inject heroin at home (60.8% always and 12.7% mostly) whereas about 22% preferred to take it outside (16.5% always and 5.1% mostly). The opposite was true for cocaine: approximately 25% preferred to take it at home (20.3% always and 5.1% mostly) versus 67% outside (58.2% always and 8.9% mostly). Similar results were obtained when the analysis was limited to the individuals taking both cocaine and her-
oin intravenously or intranasally. The pattern of drug taking for the 13 individuals who reported occasionally injecting a combination of heroin and cocaine (speedball) was identical to that of heroin alone: 70% of them preferred to inject the speedball exclusively at home whereas 23% preferred to take it always outside. It is important to note that none of the demographic variables (including employment status, age, amount of drug taking, replacement treatments, etc.) had any significant influence on setting preference for either cocaine or heroin (42).

It is remarkable that the findings from the human study coincided so closely with the findings from the animal studies, even though the designs were, of necessity, different in the two species.

5. Neurobiological Basis for the Dissociation in the Environmental Control of Cocaine Versus Heroin Reward

There is widespread consensus that the neural substrates underlying the addictive and abuse potential of major addictive drugs (32) are similar, if not identical. Virtually all drugs of abuse can increase dopamine levels in the terminal regions of the mesotelencephalic dopaminergic system (44), albeit through an action at different binding sites. Cocaine, amphetamine, and other psychostimulants induce dopamine overflow by binding the dopamine-reuptake transporter (45, 46), whereas heroin and morphine facilitate dopaminergic transmission by activating mu-opioid receptors located on GABAergic neurons in the ventral tegmental area and substantia nigra, hence disinhibiting mesotelencephalic dopamine neurons (47–50). In turn, dopaminergic transmission has been implicated in both the psychomotor and
Environmental Modulation of Drug Taking

the rewarding effects of addictive drugs (51). In agreement with
the notion of shared substrates for activating effects of psycho-
stimulant and opioid drugs, psychomotor sensitization to cocaine,
amphetamine, morphine, and heroin are facilitated in rats that
are exposed to the activity chambers only for the treatments (as
the Non Resident rats in the present study) relative to rats that
are kept in the activity chambers at all times (as our Resident rats)
(23–29).

In contrast, the studies reviewed in the present chapter indi-
cate that the drug taking context can exert opposite effects on
cocaine versus heroin self-administration and that this dissocia-
tion can be observed even in the same animals. It is obvious,
however, that the modulatory actions of environment on heroin
versus amphetamine self-administration cannot be explained by
invoking the shared ability to enhance dopamine transmission.
Consistent with this logical deduction, we have previously found
that the psychomotor activating effects of amphetamine can be
modulated by environmental context without altering amphet-
amine-induced dopamine overflow in the caudate and in the
nucleus accumbens (52, 53). Clearly, the mechanisms responsible
for the rewarding effects of psychostimulant and opioid drugs
must differ in some critical aspect.

Indeed, it has long been known that lesions of this system
can impair cocaine and amphetamine self-administration while
having little effect on heroin and morphine self-administration
(54–60). Additional important differences between the central
effects of psychostimulants and opioids are known. For example,
the “hedonic” effects of opioid agonists but not those of psycho-
stimulant drugs have been related to the ability of the former to
impinge directly onto striatal neurons, independent of dop-
aminergic transmission (61). Cocaine and heroin have been
shown to differ also in their ability to induce long-term neuro-
adaptations in the reward circuitry of the brain. The repeated
administration of high doses of cocaine was found to increase
dendritic arborization in the nucleus accumbens and in the pre-
frontal cortex whereas repeated morphine produced the oppo-
site effects (62). Furthermore, Chang and colleagues have
reported that cocaine and heroin self-administration engage
distinct, albeit overlapping, subpopulations of mesolimbic neu-
rons (63). These and other lines of evidence suggest that the
neural substrates of psychostimulant reward differ from those of
opioid reward (64–66) making it somewhat less surprising that
the two classes of drugs would exhibit different interactions
with the environment.

Our findings confirm the importance of focusing on the non-
overlapping central effects of opioids and psychostimulants.
Opioid agonists, for example, can impinge on the striatal complex
independently of dopamine by acting at mu-opioid receptors
located on striatal projection neurons (67). Interestingly, the striatopallidal neurons of Non Resident rats have been shown to be affected by the intraperitoneal administration of morphine (68) in a direction opposite to that produced by amphetamine (69, 70) and cocaine (24, 70). Fos mRNA expression in these neurons was increased by amphetamine and cocaine, relative to saline-injected animals, whereas it was reduced by morphine. In contrast, Fos mRNA expression in striatonigral neurons of Resident rats was little or not affected by amphetamine, cocaine, or morphine. We also found, regional differences in the pattern of Fos mRNA expression induced by cocaine and amphetamine versus that induced by heroin (29, 52, 70). These earlier studies were conducted using intraperitoneal injection of medium to high doses of drugs. Most importantly, we found a regional dissociation in the modulatory effect of environment with intravenous infusions of very low (“self-administration”) doses of cocaine (400 µg/kg) and heroin (25 µg/kg). As illustrated in Fig. 6, we found that cocaine induced Fos mRNA expression in the posterior caudate under both Resident and Non Resident conditions (particularly in the dorsal portion), whereas heroin had a significant effect only in the Resident group (particularly in the ventral portion) (38). Overall, these studies suggest that the posterior caudate plays an important role in the interaction between drug and environment.

Although these neurobiological results are not immediately transferable to the behavioral model used here and in previous studies, they clearly represent an important starting point for fur-

![Fos mRNA density (arbitrary units)](chart)

Fig. 6. Fos mRNA expression in the posterior caudate following a single noncontingent i.v. infusion of saline, cocaine (400 µg/kg), or heroin (25 µg/kg). The rats were either transferred to the test cages immediately before the treatment (Non Resident group) or kept at all times in the test cages (Resident group) (Data from (38). With kind permission).
ther investigations aimed at explaining the opposite effects of drug taking context on cocaine versus heroin self-administration, as well as on heroin-induced psychomotor sensitization versus heroin self-administration. Lesions studies are necessary to verify if the integrity of the posterior caudate is required to produce these dissociations.

6. Summary and Conclusions

The findings reviewed in the present chapter indicate that rats having access to cocaine and heroin take these two drugs in different amounts, as a function of context. Although it is generally accepted that the circumstances associated with drug taking can affect the propensity to abuse one drug or the other, there is a dearth of evidence for this interaction because of the extreme difficulty of manipulating, in a controlled fashion, the context of drug taking in humans and the belief that the environmental variables implicated in drug abuse are paramount with cultural or economical variables (1). Our translational study indicates that even in the laboratory rat the context can play a powerful role in modulating drug taking and drug preference (42). It is tempting to speculate that one way environmental context may affect drug choice is by providing an ecological backdrop against which drug effects are rated as more or less adaptive. The fact that each addictive drug produces a distinctive constellation of effects, which may or may not partly overlap with that of other drugs (e.g., psychostimulant and opioid drugs share the ability to induce a state of euphoria), is not an obstacle to our hypothesis. Indeed, while some effects may be largely “indifferent” to environmental context (e.g., euphoria), it is reasonable to assume that other effects may be appropriate only to certain settings. The sedative, inward-looking effects of heroin, for example, would be experienced as suitable to safe, non-challenging environments, like home, whereas the activating, performance-enhancing effects of cocaine would be more appropriate to arousing, exciting contexts.

The geographical distribution of heroin, cocaine, and amphetamine abuse has never been homogeneous, despite the fact that these two drugs have been available on the global market for more than 100 years. Indeed, each of them represents the drug of choice of some individuals in some countries, cities, communities, and neighborhoods, but not in others (71, 72). Furthermore, their use changes over time, with popularity changing with lifestyle changes. Yet very little is known about how the context in which drugs are experienced influences the propensity to use the same drug again and even less about the neural bases of this influence, attempts at explaining the role of environment in drug preference having
come mainly from disciplines such as economics, anthropology, and sociology. The animal model described in the present chapter represents a starting point to begin investigating the neural basis of drug choice and exploring at a preclinical level more specific therapies for different types of addiction.

References

Environmental Modulation of Drug Taking


Chapter 12

Craving

Jeffrey W. Grimm

Abstract

The thesis of this chapter is that the unconscious (basic) craving process, consisting of activation within limbic incentive motivation and memory systems, is ultimately responsible for relapse behaviors. The chapter first includes a brief discussion of current clinical conceptions of craving. This is followed by an argument for what aspect of human craving animals may experience. This leads to a description of various animal models (primarily using rats) that indirectly measure activity of a basic craving mechanism. A final section is provided with examples of the utility of animal models of craving illustrated with translational evidence. It is argued that basic craving is amenable to study by animal models of craving that measure motivated drug seeking behavior. Furthermore, reflection on a distinction between conscious (subjective) craving and basic craving leads to the following possible conclusions: one could treat the conscious craving and this would provide some benefit to the addict. But what would remain is the basic craving response to drug-paired stimuli, situations, and even drug-focused thought processes. Reducing basic craving using pharmaco- or behavioral therapies based upon animal model findings may ultimately be more effective at reducing relapse behaviors.

Key words: Craving, Incubation, Reinstatement, Motivation, Habit, Automaticity, Drug seeking, Relapse

1. Introduction

The purpose of this chapter is to review animal models of craving. Since the definition and usefulness of craving as a construct in humans has been challenged (1, 2), the chapter first includes a brief discussion of current clinical conceptions of craving. This is followed by an argument for what aspect of human craving animals may experience. This leads to a description of various animal models (primarily using rats) that indirectly measure activity of a basic craving mechanism. A final section is provided with examples of the utility of animal models of craving illustrated with translational evidence.
2. What Is Meant by “Craving”?

Craving is an often used, but inconsistently defined, construct. For example, the World Health Organization (WHO) ICD 10 lists “strong desire to take the drug” as a possible symptom of dependence syndrome, yet does not specifically list craving (3). A subsequent WHO expert meeting on the “craving mechanism” provided a now often-cited statement from 1992:

Drug craving is the desire for the previously experienced effects of a psychoactive substance. This desire can become compelling and can increase in the presence of both internal and external cues, particularly with perceived substance availability. It is characterized by an increased likelihood of drug seeking behaviour and, in humans, of drug-related thoughts (4, p. 5).

A less clear statement arose from a meeting of experts brought together by the National Institute on Drug Abuse. In this statement (2), the group agreed that drug-paired stimuli were one cause of craving and there was consensus that:

...craving is a subjective state in humans that is associated with drug dependence...the majority of measurements involve self-report questionnaires which range in complexity from endorsements of simple yes-no statements (‘I crave drug x’) to questionnaires that have attempted to analyze multiple aspects of craving...(e.g. urges, desires, wanting) (p. 128).

Such flexible definitions of craving have been criticized (rather tongue-in-cheek) by Stephan Tiffany and colleagues (5) in a summary of consensus statements appearing in the literature in the 1990s:

Although we do not know what craving is and we can establish no consensus about the best way to measure it or manipulate it, we certainly believe that more research should be conducted on this possibly, but not necessarily, important construct (p. S178).

While self-report is a typical procedure to measure craving, it is important to distinguish self-reported craving from other measures that, along with self-reported craving, are subsumed under the broad categorization of “cue-reactivity.” Some of the confusion expressed by consensus groups likely stems from use of this term interchangeably with subjective craving when, in fact, there are critical distinctions. Cue reactivity might be assessed as a change in a physiological measure, a change in self-reported mood or craving, or relapse itself.

Typical physiological measures in response to drug-paired cues include changes in galvanic resistance and heart rate (6). Less typical are measures of facial EMG responses (7). Physiological changes do not always covary with craving self-report (8), and the
effect sizes for these measures are relatively modest compared to subjective reports (6). These apparent weaknesses have been used as to criticize the utility of physiological measures of cue reactivity as indicators of craving (6).

Subjective assessments of craving are complicated by a host of issues. In survey studies, craving is sometimes used to define itself (e.g., “are you craving cocaine right now”) or is assumed to be expressed as a desire, or an urge (9). Even more, urge is sometimes measured as an “intention” to take drug vs. as an “expectancy of a positive outcome” (10–12). These differences in assessment and theoretical orientation are also, by nature of subjective report, complicated by reactivity problems (e.g., demand characteristics) (2). For both physiological and self-report measures in the laboratory, there also exists the dilemma that the addict is aware that any craving induced in the laboratory will most likely not be satisfied with actual drug availability (11, 13, 14). And even if this were so, the abstinence-focused patient would likely experience and report distress when confronted with a relapse-provoking situation. These anxiety-provoking effects likely distort the emotional valence of self-report outcomes. Finally, there is the problem of distorted memory when an addict reports craving that has preceded a lapse or relapse. For example, smokers overreported stress antecedent to a lapse when comparing recall of stress over the past several weeks compared to a computerized diary they had kept (15).

Despite these complications in defining craving, it is clear that craving of some sort is experienced by many addicts and this state causes suffering (16). What is less clear, however, is how craving relates to actual relapse to drug taking. Review of some well-cited clinical literature on relapse can easily leave one with the impression that craving is the key to understanding relapse, especially protracted relapse. For example, from detailed observation and survey evaluation of 30 outpatient cocaine abusers attempting prolonged abstinence, Gawin and Kleber (16) created a three-phase model of cocaine abstinence that relies heavily on craving as an antecedent to relapse. In this model (Fig. 1), craving increases over the course of the first 10 weeks of withdrawal and functions as a trigger to initiate relapse behaviors that precede a return to binge cocaine self-administration. Protracted withdrawal (abstinence) is punctuated by craving that may be activated by conditioned cues. Thus, the addict is always at “risk” for relapse due to the risk of craving.

In contrast to this popular model of relapse, Tiffany and Carter (17) provide several examples of published studies indicating a potentially spurious relationship between self-reported craving and relapse. Even more, in one study, urge to drink in alcoholics actually predicted less relapse (18). How might the presumed importance of craving in relapse be reconciled with the fact that
self-reported craving does not reliably predict relapse? Tiffany proposed a model that, in a sense, moves subjective craving to a cognitive epiphenomenon (1). This cognitive processing model supposes that actual drug relapse is due to unconscious, “automatic” behavioral responses that were developed during the establishment of the addiction. This automatic relapse is proposed to be “mindless” and resembles the theoretical construct of habit described by William James 100 years before as:

In a habitual action, mere sensation is a sufficient guide, and the upper regions of brain and mind are set comparatively free (19, pp. 115–116).

In the cognitive processing model, it is the recognition, in cognitive awareness, of this automatic response that is the subjective craving reported by addicts (Fig. 2). Tiffany and others have provided some evidence to support the concept that this subjective craving, and the thinking required to either combat it or go with it, requires a degree of cognitive processing that disrupts other processing. It is suggested that this interference may produce the psychic distress experienced by many addicts experiencing craving (20).
From a systems perspective to understanding addiction, it would then seem critical to identify the system(s) underlying automaticity. The term automaticity is limited, however, in that it does not encompass other factors relevant to the sensitivity to reward. For example, drug seeking behavior is not simply a habit. Craving has clear motivational components. Motivational state can, in turn, be regulated by drive state and motivation can become attached to incentive stimuli. As drug seeking behaviors are guided and driven by stimuli imparted with incentive by previous association with drug taking, the drug seeking would be largely a manifestation of activity of the limbic circuitry underlying drive, motivation, and incentive salience (21–24). An analogous phenomenon could be situational-invoked fear in post-traumatic stress disorder as a manifestation of activation of central amygdala and central stress systems (25). Perhaps the system that produces drug seeking should be referred to as the “craving mechanism” (4). Within this framework in the present chapter, basic craving will be used to refer to this mechanism in part to separate it from the subjective craving typically assessed in clinical studies. As will be outlined below, activation of this basic system by drug-paired cues (exteroceptive or interoceptive) arguably
serves as the beginnings of behavioral, and ultimately cognitive, manifestations of craving. It will be argued that subjective craving could be considered an epiphenomenon of engaging the basic craving system.

The basic craving system is essentially the brain reward system (21) interacting with memory systems that signal, for example, current reward valuation and contextual or discrete cue salience due to previous association with a reward (26). As such, reward is a construct entangled with incentive motivation and memory and has been defined by Wise as:

“an object or event that elicits approach and is worked for; its analogue is ‘a reinforcer’…In addition to their reinforcing effects, rewarding and reward-associated stimuli have proactive, drive-like effects. Such stimuli cause motivational arousal and increase the probability of response initiation when the primary reward has not yet been earned or directly sensed” and “the common term ‘reward’ is often used to denote the undifferentiated effects of reinforcement and motivational arousal” (27, pp. 2–3).

From a systems perspective, the study of reward and incentive motivation provides a strong framework for studying appetitively motivated behaviors such as drug seeking driven by craving. Craving is essentially incentive motivation for drug (28). This definition invokes the reward system, but also memory systems. These memory systems include those embedded in reward including reinforcement (see (29)) and systems that support interoceptive cue–drug associations (see (30)) as well as exteroceptive contextual and discrete (exteroceptive or interoceptive) cue–drug associations. It is clear that some of the memory components are involved more at a level of indicating when an incentive stimulus has been recognized (e.g., basolateral amygdala) and in which context (e.g., hippocampus) (31, 32). Other systems appear to be involved in evaluating the remembered reward valence of the stimulus (e.g., orbitofrontal cortex) (33).

Overall, the result of activation of the basic craving system is to mediate drug seeking in the presence of drug-paired stimuli as well as the reinforcing efficacy of a drug when the drug is present. In the latter case, interoceptive effects of the drug could stimulate craving (34). Therefore, for the addict, one can describe the likelihood of relapse as the perceived (perhaps unconsciously) reinforcing efficacy of the drug when the drug is available. For Gawin:

“Initiation of a cocaine binge thus depends on an interaction between drug availability, environmental stimuli (conditioned cues), and the withdrawal status of the dependent abuser.” and “such (conditioned cocaine) cravings appear after the appearance of varied, idiosyncratic objects or events that were temporally paired with prior cocaine intoxications, and these appearances are experienced as partial memories of cocaine euphoria.” (italics added) (35, p. 1582).
Animal models are an obvious choice for exploring behavioral and neurobiological substrates of basic craving due to the level of experimental control and the fact that most laboratory species (e.g., rats) are without the level of conscious reflection of humans. In fact, animal models might best serve to model the various aspects of craving, rather than provide a comprehensive model of the human addict (35, 36). Recently Miczek and de Wit echoed this limitation in a paper on translational issues and requirements for an animal model. Eight principles for effective animal models are listed – the first suggests that the scope of a model be restricted to a “core symptom” of the clinical disorder (37). Basic craving could be argued to be a core symptom of addiction.

So what aspects of basic craving can be examined with animal models? Several theoretical models for relapse have been presented over the years and basic craving could be argued to be a component of each. The models can be organized into two groups according to whether they frame relapse as driven by factors associated with drug withdrawal (dependence) or by factors that are drug-like (reward). It has been demonstrated, at least for morphine, that the neuronal substrates of dependence and reward are dissociable (38). For this “duality of craving” (39), dependence mechanisms are sometimes referred to as opponent processes while reward mechanisms are proponent processes (40).

Opponent processes driving a craving mechanism include conditioned withdrawal and conditioned compensatory responses. Conditioned withdrawal-induced craving was first presented as a situation where an individual would experience withdrawal symptoms if they were to return to the context or to confront cues initially paired with drug intake. In Wikler’s formulation, this conditioned withdrawal state would then induce drug seeking and relapse (41). While a compelling hypothesis, it has not been borne out by research studies (e.g., (42)). A more likely drug conditioning scenario would be that stimuli paired with drug taking could come to produce conditioned compensatory responses to the unconditioned effects of the drug. This is a withdrawal model that follows from Siegel’s findings that rats experience drug-opposite responses when placed in an environment where drug has previously been delivered. These conditioned compensatory responses have a large effect of producing drug tolerance (30), but their role in craving and relapse has not been substantiated. There is evidence that spontaneous or precipitated withdrawal can raise brain stimulation reward threshold (43, 44) and slightly increase drug self-administration (44), but such a change in threshold has not been linked to craving in the absence of drug. Even more, spontaneous or precipitated withdrawal from heroin
Grimm does not lead to reinstatement of extinguished heroin seeking in rats (45, 46) and while naltrexone-induced withdrawal was found to increase cue-induced heroin seeking when given immediately following heroin self-administration, a similar pretreatment was not effective at altering cue reactivity 2 weeks following heroin self-administration (47). Likewise, early withdrawal from several drugs of abuse actually appears to better predict relatively low levels of craving in both humans (16) and rats (48). Overall, opponent processes do not clearly account for craving behaviors.

A proponent process, however, has been presented as a more likely mediator of craving. The motivation to experience drug-like effects has been identified as a key factor in relapse (10, 35) and has been most clearly identified as a predictor of drug seeking in animal models of addiction (48, 49). The basic craving mechanism described in the earlier section of this chapter is proponent and is derived from the wealth of theoretical considerations and empirical findings on incentive motivation published over the past several decades. Incentive motivation, simply stated, is the reward valence attributed to an object or place due to its pairing with a reward in the past (27). Exposure to that object or place will produce approach behaviors, indicative of activation of a reward mechanism (21). It is important to recognize that these conditioned behaviors are not simply conditioned reflexes, but conditioned behavioral responses heightened by drive and motivational factors. For example, hunger can increase drug seeking (50) and responding for the presentation of a drug-paired stimulus is taken as a measure of the conditioned reinforcing properties of the stimulus (51).

Figure 3 incorporates a proponent-driven basic craving mechanism into a relapse model that acknowledges both conscious and unconscious dimensions of craving. From this basic craving model, it is suggested that for humans, drug-craving actions could occur independent of conscious awareness akin to the mindless relapse of Tiffany. Conscious craving would then be a conscious reflection of the underlying mood/motivational state and behavioral activation; a “cognitive interpretation” of the activation of the basic craving mechanism (52).

Understanding these basic craving processes could then arguably be the critical focus for craving research. Behavioral and pharmacological interventions would be aimed at these underlying processes rather than targeting the cognitive symptoms. For example, one approach to managing addiction is to reduce the relative reinforcing effect of a drug by contingency management. Contingency management involves decreasing the relative value of the drug by increasing the value of remaining abstinent (53). This isn’t to say that conscious craving would not take on a life of its own in humans. Obsessive thoughts are a debilitating aspect of addiction and they could be disconnected from actual motivation.
Craving

to take the drug – akin to cravings vs. urges in Marlatt’s cognitive behavioral model of relapse (10). It is also possible that these drug memories could prime the subconscious basic craving system (Fig. 3). Activation within this system would then result in the increased probability of drug seeking and increased reinforcing efficacy of drug when it is available.

Animal models are well-suited for identifying core behavioral components, and underlying neurobiological substrates, of the basic craving mechanism. An animal model “peels away” several layers of complexity that appear to confuse the measurement of craving in humans. A recent example of this is the observation that a “lapse” in rats increases the probability of a relapse (54). A current cognitive-based explanation for this effect in humans is that the abstinence violation of the lapse allows greater rationale for relapse by the addict (10). Since the same effect is seen in rats, the parsimonious explanation would be that there is

4. Assessing Craving Using Nonhumans

Fig. 3. An incentive motivational model of a basic craving mechanism. This model, its creation influenced by (28) and (121), supposes that drug-paired stimuli are detected and valued by memory systems (e.g., basolateral amygdala and orbitofrontal cortex) and drug seeking responses are initiated with their vigor determined by their incentive salience (e.g., via activity of meso-accumbal dopamine projections). The drug seeking responses are therefore an indirect measure of the activity of the basic craving mechanism. The model also allows the possibility of interaction between a basic craving mechanism and subjective craving and for subjective craving to produce drug seeking independent of the basic craving mechanism.
a change in reinforcing efficacy of drug or drug-paired stimuli due to the lapse – a higher-level cognitive explanation is not necessary.

In this section, several animal models for assessing the proponent aspects of craving will be identified and described in brief. These models could be argued to indirectly measure the basic craving construct outlined in the previous section (Fig. 3). Several of these models are discussed in detail in other chapters in this volume (see Chaps. 6, 13, and 17).

For the following section, the operational definition of basic craving is intentional, motivated approach to, and/or response for, a drug or a drug-paired stimulus.

Using this definition, a change in basic craving is inferred by an increase in drug seeking behavior in the absence of drug (55) or an increase in reinforcing efficacy of the drug when it is available (56). A key aspect of this definition is that it does not simply define automatic behavior or habit. The intent of the definition is to capture how craving is an acquired motivation (28). This operational definition implies a necessary role for a central motivational mechanism that interacts with feedback on the incentive value of stimuli in the environment. The strength of the models described below is in how they tap into key aspects of the basic craving mechanism. The models described are categorized according to whether the drug is present, and to the main conditioning features of the model (e.g., classical or operant or both). Not all models that measure craving are presented here due to space considerations. In addition, notably absent are models that assess how impulse control and higher-order memory systems modulate craving, including those that tap into the role of the prefrontal cortex and orbitofrontal cortex in regulating drug seeking behavior. Such models of impulsivity (57) and reward revaluation (33) are providing important insight into how cortical regions can ultimately “decide” (or fail to decide) on how basic craving will manifest as overt behavior.

One consistent finding from the models described below is that key elements and projections of the mesolimbic dopamine pathway mediate the craving-related behavior being modeled (drug seeking). Relevant examples of this corroboration are provided for each model. Excellent reviews of the validity levels of most of the following models may be found in (28, 58).

Self-administration of a drug is a hallmark of the rewarding effects of the drug (see Chaps. 2–5). Motivational aspects, aspects central to basic craving, can be measured by rate of self-administration but also rate of responding in the absence of the drug (i.e., extinction) (59) including responding in the initial drug-free components of fixed interval and second-order schedules of reinforcement
(51, 60). Procedures that assess responding in the absence of drug are discussed in paragraphs below. Interpretation of responding for drug has been criticized for the dependence of the measure on rate of responding. Fixed ratio responding (fixed number of responses required for each drug delivery) is typically identified as the most problematic. That is, there may be confusion when interpreting a change in response rate (e.g., following pharmacological manipulation) as being due to a change in incentive motivation or due to a motor side effect of the drug (61). This criticism is less tenable in certain situations when considering that relatively low doses of a dopamine antagonist actually increase operant behavior reinforced by a psychostimulant (27). Despite this fact, a popularly employed alternative to the fixed ratio procedure that is argued to be less sensitive to the rate-dependency problem (62, 63) is the progressive ratio (PR) procedure (64). The typical procedure under the PR schedule is for the number of responses required for drug delivery to increase after each drug delivery; often the increases occur along a logarithmic scale (65). Use of the PR procedure with rats and nonhuman primates has revealed insights into mesolimbic brain regions involved in incentive motivation (61, 66, 67) and has provided a useful procedure for comparing the relative reinforcing efficacy of different drugs of abuse (64). In Fig. 4, individual response records are provided where a rat responded for different doses of cocaine on a PR schedule of reinforcement. Higher doses supported increased effort, suggesting increased motivation to obtain the drug.

Reinstatement is a general term to describe the reactivation of a previously extinguished behavior (55) (Relapse is explored in depth in Chap. 17). Priming-induced reinstatement describes a reestablishment of either drug taking or drug seeking following noncontingent delivery of drug. Similar to Davis and Smith (68) with morphine, de Wit and Stewart found that cocaine effectively reinstated extinguished lever responding initially supported by cocaine (49). In Fig. 5, rats that decreased lever responding due to non-reinforcement (extinction) reinstated lever responding following reexposure to cocaine. This “taste” of drug appears to function as a prime of the basic craving mechanism.

Furthermore, the reactivated responding reflects activation of dopaminergic mesolimbic circuitry (e.g., nucleus accumbens) (69), already identified as components of the reward and incentive motivation circuitry (23, 70). While self-administration and priming studies are central to addiction and craving research, procedural issues complicate interpretation of findings from these studies. In particular, the drug is present during the behavioral evaluation (except for prior to the first injection – see start of this section). Stimulative or depressive effects of the self-administered drug could confound interpretation of effects of other pharmacological manipulations (62, 63). Second, related only to
self-administration, is that these models do not model relapse after extended periods of abstinence.

4.2. Place Preference

A way around the problem of confounding effects of the self-administered drug on measures of basic craving is to use procedures where craving is assessed in the absence of the drug. *Conditioned place preference* (CPP) is one procedure (CPP is explored in depth in Chap. 6). CPP could be described as a motivated approach to a drug-paired environment due to previous association of that environment with drug. In its simplest form, a CPP suggests that a location has been associated with the rewarding effects of a drug. As stated above, the selection of this location would require both incentive motivation and memory. Response requirements from the subject for CPP are minimal, in part due to the fact that the learning is a stimulus–stimulus (Pavlovian) association and requires no behavioral output initially.

Fig. 4. Event records of progressive ratio responding for cocaine by a rat. Downward arrows indicate self-infusions of cocaine. Higher doses of cocaine support higher ratios (number of responses required for cocaine delivery) of responding. This is taken as an indication that the higher doses have greater reinforcing efficacy. Achieving higher break points (final ratio that supports sustained responding) is also taken as a measure of the motivation to self-administer the drug (Reprinted from (65) (Fig. 1) with kind permission of Dr. Roberts and Springer Science + Business Media).
It is interesting that CPP has been demonstrated not only in mammals, but also in the invertebrates Drosophila (71) and planaria (72). Figure 6 depicts a methamphetamine-produced CPP in a planaria. The figure indicates locomotor path during training and testing. An initial bias to a quadrant is lost and preference to another quadrant is created when that quadrant was paired with methamphetamine application.

Both Drosophila and planaria have rudimentary dopamine systems and have qualitatively similar locomotor responses to several drugs of abuse as mammals (73). While the CPP and the basic craving it suggests in these species might be debated to be more homologous than analogous compared to mammals, it is a thought-provoking example of how simplistic animal models might be used in addiction research. In addition, from an evolutionary perspective, the putative conservation of the basic learning and underlying neurochemistry of basic craving suggests a remarkable degree of adaptive fitness.

As noted above, and in contrast to self-administration procedures, testing for craving in CPP is done in a drug-free state. In addition, variations of the CPP procedure have been created making it amenable to study as a “relapse” model – the CPP reinstatement procedure (74). Most CPP in rats is dependent upon mesolimbic dopamine (75, 76).

4.3. Discriminative and Pavlovian Stimuli

Pavlovian associations also can gate drug-craving behavior by functioning as “occasion setters” and by enhancing or reinstating
drug seeking behaviors (77). These stimuli, sometimes discrete and sometimes contextual, come to discriminate for the subject when drug is available. In various procedures, they have been found to increase drug seeking – an indication of basic craving. Discrete stimuli may become discriminative stimuli if they are consistently present during drug availability and consistently absent when drug is not available. Weiss and colleagues have used such a model to demonstrate that rats will respond on a lever in the absence of drug when a stimulus that previously indicated drug availability is presented. Figure 7 depicts the results of one of their studies (78) wherein rats responded in the presence of a cocaine-predictive tone (S+) but not in the presence of a saline-predictive light (S−). S+ responding was attenuated by pretreatment with the dopamine D1 receptor antagonist SCH23390.

Models using contextual stimuli (including salient odors) include the runway model and contextual reinstatement. In the runway model, drug availability by running to the end of an alley is signaled by a specific smell (79). In contextual reinstatement, drug self-administration is allowed in one environment characterized by salient visual cues (e.g., striped walls) and odor (e.g., vinegar), while extinction of lever pressing occurs in another (e.g., plain walls and menthol odor). Reinstatement occurs when the subject returns to the drug self-administration context (80). Studies using these various measures to examine craving induced by discriminative...
and contextual stimuli have invariably found a critical role for the mesolimbic dopamine system (81, 82).

One other procedure for examining the effect of a conditioned stimulus on reward seeking behavior is the Pavlovian to Instrumental Transfer (PIT) procedure. In this procedure, a stimulus is always presented along with access to the reinforcer (Pavlovian association). The subject then learns to make an operant response to receive the reinforcer in the absence of this conditioned stimulus. Finally, the conditioned stimulus is presented while the subject is performing the operant (typically in extinction). PIT is observed as an increase in operant responding when the conditioned stimulus is present. PIT is intriguing as an animal model of craving as it has been argued that, for addicts, conditioned stimuli often appear not contingent upon their behavior, and that these stimuli drive drug craving (55). PIT has been found to be dependent on components of a mesolimbic basic craving mechanism (83, 84). More discussion of the effects of environmental modulation of drug addiction may be found in Chap. 11 of this volume.
While craving by addicts may be activated by presentation of drug-paired stimuli not contingent upon their behavior (Pavlovian stimuli), drug craving manifest as drug seeking may be controlled by a combination of Pavlovian and operant (instrumental) associations. For example, craving for heroin is reportedly higher when the “stimulus” opportunities are greatest: interacting with a home environment was more likely to induce craving compared to a laboratory environment that included drug-paired stimuli (8). A home environment, where drug taking occurs, would provide a rich conditioning environment where previously neutral stimuli as well as behaviors become paired with the drug taking experience and may collectively function as compound conditioned stimuli. Bindra argued for a unification of Pavlovian and operant learning (85) and such a formulation may eventually contribute to a better understanding of drug craving. To this end, conditioned reinforcement (or reward) models allow measurement of both Pavlovian and operant learning.

A relatively simple conditioned reinforcement model is cue-induced reinstatement. In this model, rats learn to self-administer drug along with the presentation of a discrete stimulus (e.g., tone + light). Responding is then extinguished in the absence of both drug and this stimulus. Reinstatement occurs when the subject is allowed to respond for presentation of the stimulus in the absence of drug. A critical feature of this model is that robust reinstatement requires that the subject has contingent access to the stimulus – noncontingent presentations of the stimulus do not reinstate responding (86). Subjects are responding for the presentation of the stimulus indicating it has rewarding effects of its own. In effect, they are “craving the stimulus” due to its previous association with drug. There are several variants of this approach in use and each model demonstrates that stimuli consistently presented with drug taking come to be effective conditioned reinforcers (54, 87). Other well-established conditioned reinforcement procedures include requiring a subject to learn a novel response to produce a stimulus previously associated with drug (88), and chaining long sequences of conditioned reinforcement responding with an ultimate payoff of a drug delivery (second-order schedule of reinforcement; (51)). Figure 8 represents one of the first published studies with rats responding on a second-order schedule for cocaine. As indicated on the response records, rats with intact mesolimbic dopamine systems, but not with bilateral lesions of the basolateral amygdala, responded vigorously for a cocaine-paired stimulus in the absence of cocaine.

Similar to findings with discriminative and Pavlovian stimuli, and indicated by the results of Whitelaw et al. (51), conditioned reinforcement responding is mediated by mesolimbic dopamine terminal regions (88).
Craving

The utility of animal models of craving is only realized when they can inform the human problems of craving and addiction. As noted early in this chapter, animal models may be most useful when they clearly model one core aspect of the human disorder and also if a model of a supposed cognitive phenomenon uses behavioral and/or neural measures (35, 37). This section briefly identifies three examples of correspondence between findings in animal models of craving and human studies of craving following these guidelines. In general, most “translational” evidence supports a critical role for limbic dopamine structures in basic craving. This is not surprising, as this is precisely what is predicted by incentive motivation theory (21, 22, 83).

The animal models of craving described above are limited in their value to clearly portray craving in humans due to the fact that variables leading to craving in humans are not easily reproduced in the laboratory (55). In addition, as described earlier, animal models might best only assess certain aspects of a human condition (37). However, craving assessed with the reinstatement model appears to be a decent model of human craving in that cues originally paired with drug can come to drive drug seeking behavior (55). This model has been useful for identifying brain structures involved in craving, for screening the potential utility of anti-craving compounds (48), and has also provided direction for more research on how cue-induced craving comes to invigorate...
drug seeking and relapse long after the cessation of drug taking. “Incubation of craving” describes a time-dependent increase in cue-induced drug seeking. In rats, craving for a cocaine-paired cue is dramatically higher 2 months into forced abstinence from cocaine self-administration compared to 1 day or even 7 days of forced abstinence (Fig. 9 top) (89). A similar time-dependent increase in craving has been observed following forced abstinence from heroin, methamphetamine, sucrose (90), and alcohol (91).

While these findings are exciting as a basic research demonstration of plasticity of behavior following drugs and food, there are now clinical research studies indicating that incubation of

![Graph a](image)

### Top panel

**Incubation of cocaine craving.** Rats respond more for a cocaine-paired stimulus as the number of days of abstinence progresses. Means ± SEMs are indicated on the figure. (Top panel) Evidence for incubation of craving for cigarettes. In this subgroup from a larger study, urge to smoke increased over a month of abstinence from smoking. Means are indicated on the figure. B1 and B2 are baseline measure prior to abstinence and QD is quit day. **Top panel** redrawn from (89). **Bottom panel** redrawn from (93) with kind permission of Dr. Niaura.
Craving occurs in humans. For example, Piasecki et al. (92, 93) identified a subgroup of abstaining smokers who reported their urge to smoke increased over 1 month following quitting (Fig. 9 bottom).

Incubation of craving in humans is also supported by preliminary data from a study where smokers being paid to abstain reported greater cue-induced craving at 1 month vs. 1 day of abstinence from smoking (Dr. Harriet de Wit, personal communication). Even more, there are two studies that have reported incubation of cocaine craving in abstaining cocaine addicts (94, 95) and one reporting incubation of heroin craving in abstaining heroin addicts (96). These findings appear to validate the model proposed by Gawin and Kleber (16) wherein craving induced by drug-paired cues comes to be a major determinant of drug seeking in protracted abstinence. It remains to be seen how general incubation of craving is with humans. For example, if incubation occurs with food cues, it could be a large mediator of diet recidivism. Even more, the incubation phenomenon suggests that information gained from preclinical studies that identify brain and behavioral changes at later time points in abstinence may have more relevance for development of treatments for addiction compared to treatments derived from studies that have focused on acute withdrawal.

The effectiveness of targeting the dopamine system in treating craving is illustrated by translational progress in the pharmacological treatment of craving in alcoholism. For example, Weiss and colleagues found that both dopamine D1 and D2 antagonists attenuated alcohol craving measured as alcohol seeking in the presence of an alcohol self-administration-paired conditioned stimulus (97). Olanzapine, an antipsychotic drug that antagonizes dopamine D4 receptors (of the D2 receptor family) reduces cue-induced craving in some alcoholics (98). This is an exciting translation as there exist results from several craving studies in animals indicating anti-craving effects of DA antagonists for other drugs of abuse (e.g., cocaine (78, 99)).

Opioid antagonists offer another promising target for alcohol craving and this is also supported by translational evidence. Animal models of craving using discriminative or contextual cues revealed a reduction in alcohol craving-related behaviors following acute naltrexone (100, 101). Naloxone has now been shown to reduce craving for alcohol by alcoholics (102, 103). Opioid antagonists have also been found to reduce craving for other drugs (e.g., amphetamine (104)) and for food by binge eaters (105, 106). Complementing this latter finding, we have recently reported that naloxone reduces craving for sucrose in an animal model of craving (107).
Several other neurotransmitter receptor targets for potential translation to human craving therapy have been suggested by animal models including glutamate (108), cannabinoid (109), and GABA receptors (110) as well as multi-receptor-targeted compounds (111). Some clinical success for treating alcohol craving has been reported targeting glutamate receptors (N-acetylcysteine (112)) and a multi-receptor mixed agonist (modafinil) has been found to reduce cocaine intake (113). It remains to be demonstrated whether specifically targeting cannabinoid or GABA receptors will be as effective.

There appears to be good overlap between the brain regions identified in animal models as being part of incentive motivational systems and brain regions identified as changing activity during human brain imaging of craving (114). These studies with humans consistently find that mesolimbic structures change activity during craving episodes (115). A causal role for some of these structures in craving has been determined in experiments using animal models of craving (69, 114). The amygdala is one such structure. Figure 10 (top) depicts findings from a cue reinstatement procedure in which the basolateral amygdala was bilaterally inactivated, in some rats, prior to the test. Craving, assessed as responding for a cue previously associated with cocaine self-administration, was attenuated in these animals (86). The lower panel (Fig. 10 bottom) in the figure illustrates activation of the amygdala in cocaine addicts that viewed a cocaine-focused video and reported craving for cocaine (116).

The nonhuman and human craving literature regarding brain substrates of craving appear to be converging, and this will likely lead to validation of the systems underlying basic craving, and to better treatments for craving. For example, human brain imaging has identified decreased striatal dopamine D2 receptor occupancy in methamphetamine addiction and obesity (117) and increased dopamine overflow in the striatum during cocaine craving (118). These results, along with the craving-reducing effects of dopamine antagonists (above) on craving in both animal models of craving and in human addicts, strongly implicate midbrain dopamine systems in craving. These findings are predicted by the basic craving model. An exciting study recently published supports this conclusion. Childress et al. found that “subliminal” exposure to either drug or sex image–paired stimuli activated basic craving structures including the nucleus accumbens and amygdala (119). Exposure to these stimuli later related to improved valuation of the stimuli despite the participant not recalling having seen the stimuli previously. These findings would be expected if appetitive stimuli can be processed subconsciously as incentives that come to have a stronger effect on incentive motivation in the future.
6. Conclusions

The thesis of this chapter is that it is the unconscious (basic) craving process, consisting of activation within limbic incentive motivation and memory systems that is ultimately responsible for relapse behaviors. It was argued that basic craving is amenable to study by animal models of craving that measure motivated drug seeking behavior. Reflection on a distinction between conscious (subjective) craving and basic craving leads to the following possible conclusions: one could treat the conscious craving and this would provide some benefit to the addict. But what
would remain is the basic craving response to drug-paired stimuli, situations, and even drug-focused thought processes. Reducing basic craving using pharmaco- or behavioral therapies, based upon animal model findings, may ultimately be more effective at reducing relapse behaviors.

References

4. WHO (1992) Informal expert group meeting on the craving mechanism. World Health Organization, Vienna
54. Leri F, Stewart J (2002) The consequences of different “lapses” on relapse to heroin...


84. Lex A, Hauber W (2008) Dopamine D1 and D2 receptors in the nucleus accumbens core and shell mediate Pavlovian-instrumental transfer. Learn Mem 15:483–491


Chapter 13

Habit Formation and Compulsion

David Belin, Daina Economidou, Yann Pelloux, and Barry J. Everitt

Abstract

Our increasing understanding of the psychological mechanisms involved in the transition from controlled to habitual compulsive drug use, the hallmark of drug addiction, relies on animal models in which the underlying behavioral construct reflects some of the main features of drug addiction in humans, such as foraging for the drug during extended periods of time, habitual drug seeking behavior and drug seeking or drug taking behaviors that are maintained despite adverse consequences. We have placed great emphasis on the development of behavioral procedures whereby animals not only self-administer drugs, but pathologically seek and take drugs in a way that resembles the clinical condition in human drug addicts. Thus, over the last 10 years we have developed models in rats that specifically address the development of habitual drug seeking behavior, compulsive cocaine seeking and taking behavior, and even addiction-like behavior. In this chapter, we review the behavioral procedures, namely second-order schedules of reinforcement, two-link heterogeneous chained schedules of reinforcement and the “three addiction-like behavioral criteria selection procedure” that we have used in rats to model habitual drug seeking behavior, compulsive drug seeking and taking behavior and addiction-like behavior. Although not yet widely adopted, these models have already contributed to the identification of some neurobiological and psychological mechanisms involved in the vulnerability to drug addiction and the transition from controlled to compulsive drug use, thereby emphasizing their great heuristic value in attempts to understand drug addiction.

Key words: Cocaine, Striatum, Dopamine, Prefrontal cortex, Habits, Compulsivity, Rats, Second-order schedules of reinforcement, Seeking–taking schedules of reinforcement

1. Introduction

All drugs abused by humans have reinforcing properties in many species, including planarians (1) and flies (2, 3), and they are readily self-administered by vertebrates such as mice (4–8) or rats (9–13), dogs (14), and nonhuman primates (15–21). Thus, animal models of sensitivity to the reinforcing properties of addictive drugs as well as the initiation and maintenance of drug taking (see previous chapters)
have great heuristic value for the pharmacology of drug addiction since they show both face and predictive validities.

These animal models have increased our understanding considerably of the psychological, neural, cellular, and molecular mechanisms whereby addictive drugs exert their reinforcing effects (22–30), but they do not address the transition from controlled to habitual and compulsive drug use, the hallmark of drug addiction (Table 1). Indeed, among the individuals exposed to drugs, and there are many who occasionally drink only a glass or two of an alcoholic beverage, or smoke a cigarette or two, only 15–30% overall will switch from casual, “recreational” drug use to drug abuse and drug addiction (47) (Fig. 1). These drug addicts not only take drugs, they spend great amounts of time foraging for their drugs, compulsively take drugs, lose control over drug intake, and persist in taking drugs despite the many adverse consequences of doing so, including compromising their health, family relationships, friendships, and work. Many drug addicts resort to criminal behavior to obtain the funds necessary to sustain their compulsive drug use and the great majority eventually relapse to drug use even after prolonged periods of abstinence.

This negative behavioral picture illustrates how drug addiction is not merely a drug taking disorder, but is also defined as a chronic relapsing disorder characterized by loss of control over drug intake and compulsive drug seeking and taking that is maintained despite adverse consequences, as described by the seven diagnostic criteria in the DSM-IV (31) (Table 1). Therefore, it is important to separate two issues: why people take drugs and why people compulsively take drugs (48). There is increasing evidence suggesting that drug addiction results from gradual adaptation processes in the brain of vulnerable subjects in response to chronic drug exposure, that may ultimately lead to a shift in the psychological mechanisms that govern drug seeking and drug taking behaviors, including habits (49, 50), aberrant instrumental learning mechanisms controlled by Pavlovian cues, failure in behavioral control (51, 52), decision making, and self-monitoring processes (53). Similarly, we have argued previously that, during the development of drug addiction, drug seeking is initially goal directed but becomes habitual, and ultimately compulsive, thereby emphasizing the potential importance of maladaptive automatic instrumental learning mechanisms and their control by Pavlovian incentive processes in the emergence of compulsive drug use (51, 54–56). At the neurobiological level, we have hypothesized that this progressive transition in the psychological mechanisms that govern drug seeking behavior may reflect a progressive shift from prefrontal to striatal mechanisms paralleled by a progression from ventral to dorsal striatum as the locus of control over behavior (55). Therefore, the investigation of the transition from controlled
Table 1
Animal models of habitual and compulsive drug use in reference to the DSM-IV criteria (Adapted from (31))

<table>
<thead>
<tr>
<th>DSM-IV diagnostic criteria for drug addiction</th>
<th>Psychobiological dimension</th>
<th>Monodimensional animal models</th>
<th>Multidimensional animal models</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Need for markedly increased amounts of a substance to achieve intoxication or desired effect, or markedly diminished effect with continued use of the same amount of the substance</td>
<td>Pharmacological tolerance</td>
<td>Reinstatement [1-4] / relapse [5]</td>
<td>3-criteria model of drug addiction: inability to refrain from drug-seeking [14-16]</td>
</tr>
<tr>
<td>2. The presence of a characteristic withdrawal syndrome or use of a substance (or a closely related substance) to relieve or avoid withdrawal symptoms</td>
<td>Negative affect/mood, depression, anhedonia, anxiety</td>
<td>Escalation of drug intake [6-7]</td>
<td>3-criteria model of drug addiction: escalation of drug intake during long access to cocaine [14-16]</td>
</tr>
<tr>
<td>3. Persistent desire to use drugs or one or more unsuccessful efforts to cut down or control substance use</td>
<td>Impulsivity, behavioural control failure</td>
<td>Resistance to punishment [8], resistance to conditioned suppression [9]</td>
<td>3-criteria model of drug addiction: increase break points in a progressive ratio schedule of reinforcement [14-16]</td>
</tr>
<tr>
<td>4. Substance used in larger amounts or over a longer period of time than the person intended</td>
<td>Behavioural control failure</td>
<td>Progressive ratio, seeking-taking and second order schedules of reinforcement [10-13]</td>
<td>3-criteria model of drug addiction: resistance to punishment or adverse consequences [14-16]</td>
</tr>
<tr>
<td>5. Important social, occupational, or recreational activities given up or reduced because of substance use</td>
<td>Habits/compulsivity, behavioural control failure</td>
<td>Resistance to punishment, resistance to conditioned suppression</td>
<td>3-criteria model of drug addiction: inability to refrain from drug-seeking [14-16]</td>
</tr>
<tr>
<td>6. A great deal of time spent in activities necessary to obtain, to use, or to recover from the effects of substance used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Continued substance use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to be caused or exacerbated by continued use</td>
<td>Compulsivity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

to compulsive drug use, which is one if not the most fundamental issue for research on drug addiction, explicitly raises the importance of the construct validity of animal models of habitual and compulsive drug seeking and drug taking behaviors.

Recently developed animal models of habitual and compulsive cocaine seeking and taking (37, 39, 40, 44, 46, 57, 58) have indeed proven to be useful experimental tools for investigating the psychobiological mechanisms underlying the transition from controlled drug use to drug addiction and have provided evidence to support the notion that a ventral to dorsal striatum shift occurs in the control over well established, or habitual, cocaine seeking (58–60).

In this chapter, we will thus review the contribution of animal models of habitual and compulsive drug seeking and taking to the advances made in the understanding of the pathophysiology of drug addiction. More precisely, we describe experimental procedures currently developed to model compulsive drug seeking and drug taking behavior and review their contribution to the understanding of the psychological mechanisms involved in the development of drug addiction. Finally, we will focus on an animal model of addiction-like behavior in the rat that integrates both the compulsive feature of drug addiction and interindividual
differences in the vulnerability to develop drug addiction, thereby providing a powerful tool for the investigation of the behavioral and biological factors of vulnerability to drug addiction.


2.1. Theoretical Implication of Habits in Drug Addiction: Historical Background

Early theoretical arguments concerning the importance of “automatic processes” or “habits” in the development of drug addiction (50, 54, 61) were based on the observation that “drug-use behaviors tend to be relatively fast and efficient, readily enabled by particular stimulus configurations (i.e., stimulus bound), initiated and completed without intention, difficult to impede in the presence of triggering stimuli, effortless, and enacted in the absence of awareness.” These behavioral features resonate well with the five main characteristics of behavioral automaticity, often assumed to reflect habits that Tiffany extracted from a meta-analysis of the literature: speed, autonomy, lack of control, effortlessness, and absence of conscious awareness. Indeed, automatic processes are associated with an increased speed and decreased variability of performance. Thus in humans, habitual responses do not depend upon awareness but are directly triggered by conditioned stimuli without any recruitment of higher cognitive processes, such as intention or decision making, and are therefore difficult to inhibit in the presence of the eliciting stimuli. Additionally, habitual responses are relatively insensitive to variations in their consequences, as illustrated by William James’s description, in the late 19th century, of a clear dissociation between plans and actual actions in specific contexts: “very absent-minded persons in going to their bedroom to dress for dinner have been known to take off one garment after another and finally to get into bed, merely because that was the habitual issue of the first few movements when performed at late hours.” In this description, environmental stimuli (late hour, entering the bedroom) trigger an action that is dissociated from the initial goal, which can then be considered as devalued. Similarly, more recent studies have reported that driving habits are impervious to the value of the goal: individuals with a strong driving habit will, for example, continue using their car instead of shifting to public transportation to commute to work even when the highway they use for commuting is closed (62).

2.2. Drug Addiction as the “Bad Habit” of Drug Seeking and Drug Taking Behaviors

The aforementioned behavioral features of automation, or habits, show great construct validity when integrated into the psychobiological framework of drug addiction. Indeed, the development of habitual action schemata whereby drug seeking and taking are stimulus bound and beyond cognitive control may account for various behavioral features observed in human addicts, such as
stimulus-bound relapse even after protracted abstinence, and stimulus-maintained drug seeking over prolonged periods of time.

These habits may even encompass higher schemata, themselves controlling goal-directed sequences of behavior, but nevertheless triggered specifically by drug-associated stimuli. The stimuli that trigger drug seeking are multiple and varied, ranging from drug-associated paraphernalia, locations, internal states, such as withdrawal or anxiety, friends, or the drug itself. Therefore inflexible habitual drug seeking responses can be elicited by either external (environmental) or internal (thoughts or internal states) stimuli. These drug-associated stimuli may eventually play another important role in supporting drug seeking behavior in addicts who, in real life, spend a great deal of time foraging for the drug, often involving long sequences of behavior during which the drug-associated cues, acting as conditioned reinforcers, bridge delays between drug seeking and drug taking.

We have suggested that addictive drugs may subvert natural learning processes, such as action–outcome and stimulus–response (S–R) instrumental learning mechanisms, as well as Pavlovian–instrumental interactions, thereby facilitating the development of habitual control over drug seeking and drug taking (50). Thus, drug use that is initially a goal-directed action, controlled by the reinforcing properties of the drug, progressively becomes divorced from these reinforcing properties and more controlled by stimuli in the environment that have repeatedly been associated with the drug. The development of habitual drug use cannot alone account for the development of compulsive drug use, which is hypothesized to involve a failure in cortical top-down control mechanisms, but it may nevertheless play a central role in the development of drug addiction. We have thus developed the hypothesis that drug addiction results from progressive adaptations in the brain, which ultimately lead to a loss of executive control over maladaptive drug seeking and taking habits (55, 56).

It is now well established that the same instrumental response can be mediated by either a goal-directed (action–outcome, A–O) or a habitual (S–R) system (Fig. 2).

Behavioral procedures have been developed to investigate the nature of the psychological substrate that governs instrumental behavior in animals. When instrumental responding is habitual, the animal’s instrumental performance is no longer an action in that it is not under the direct control of the representation of the motivational value of the outcome (action–outcome, A–O), but is instead an automatic response triggered by the stimuli associated with the outcome (S–R). Thus, habitual instrumental performance is impervious to affective devaluation of the outcome—usually by pre-feeding to satiety or lithium chloride-induced malaise— or
contingency degradation, whereas these manipulations markedly reduce instrumental responding when it is controlled by an A–O process (Fig. 2). Thus, to demonstrate habitual control over instrumental performance requires a direct manipulation of the incentive properties of the reinforcer, a manipulation that has not yet been developed for non-ingestive reinforcers, such as intravenously self-administered drugs.
Dickinson and colleagues (63, 64) have defined the conditions whereby instrumental responding shifts from being controlled by a goal-directed to an habitual mechanism. Thus, overtraining under fixed-ratio schedules of reinforcement or limited exposure to interval schedules of reinforcement similarly result in the transition from A–O to S–R control over instrumental responding for natural rewards, that is, performance of the instrumental response is unaffected by devaluation of the outcome when tested in extinction (64).

At the neural systems level, A–O and S–R learning processes depend upon dissociable structures, as demonstrated by lesion or inactivation procedures in rats. Thus, lesions of the prelimbic cortex (65, 66) or glutamate receptor blockade in the dorsomedial striatum (67, 68) disrupt goal-directed responding, whereas lesions or inactivation of the infralimbic cortex (65, 66), the dorsolateral striatum (67, 69, 70), or its dopaminergic innervation (71) abolish S–R control over behavior, thereby rendering instrumental performance sensitive to the motivational value of the outcome in overtrained rats.

Although the potential implication of behavioral autonomy in the development of drug addiction was first suggested almost 20 years ago (61), it is only in the last 5 years that animal models of habitual drug seeking have been developed. Even though initially limited to oral drug self-administration, these animal models have provided the first clear experimental evidence that drugs of abuse such as cocaine and alcohol actually facilitate the development of the S–R process compared to natural rewards (72, 73).

In a series of studies, Dickinson and colleagues have developed animal models of habitual oral self-administration of alcohol and cocaine (72, 73). In these two experiments, rats were initially trained to respond on one manipulandum (lever or rod) for a food pellet or a lemon-sucrose solution and a second manipulandum (lever or rod) for an alcohol (10%)- or cocaine (0.1%)-sucrose (10%) solution. Responses on each manipulandum were reinforced under RI schedules, in which the contingency between the response and the outcome is not explicit, thereby favoring the development of the S–R process in the control over instrumental performance. After ten training sessions during which animals had similar access to the natural or drug reward, aversion conditioning took place whereby each reinforcer was specifically devalued without interfering with the other reinforcer. For this, each animal in the natural reward-devalued group
received noncontingent presentations of the natural reward followed by an intraperitoneal injection of an isotonic LiCl solution whereas rats in the drug-reward group received noncontingent presentations of the drug solution and then the LiCl IP injection. Animals were then subjected to a single (8 or 10 min) extinction session during which they had access to the two manipulanda and their instrumental responses were recorded.

Since the devaluation procedure took place without presentation of the manipulanda, instrumental performance during extinction was not related to any direct effect of the aversion conditioning upon instrumental performance. Figure 3a, b illustrates the results of the two studies, emphasizing that rats orally self-administering alcohol and cocaine showed facilitated resistance to reinforcer devaluation compared to natural rewards. Thus, after equivalent training, consumption of addictive drug-containing solutions resulted in habitual control over instrumental performance at times when responding for natural rewards remained goal directed.

---

Fig. 3. Oral drug self-administration facilitates the instantiation of habitual food seeking behavior. Rats orally self-administering alcohol and cocaine showed facilitated resistance to reinforcer devaluation compared to natural rewards. **Left panels:** Instrumental performance for oral alcohol self-administration following affective devaluation of the outcome. Data presented are mean number of responses on the lever associated with pellets or ethanol during the extinction session (see text for details). Whereas specific devaluation of the pellet reward produced a decrease of responding on the associated lever, thereby showing that instrumental responding for this natural reward was goal-directed, the same manipulation failed to influence lever presses for ethanol, revealing that ethanol seeking was habitual. **Right panels:** Lever pressing for oral cocaine self-administration following devaluation of the outcomes (see text for details). Whereas a devaluation effect was observed on instrumental performance associated with the lemon-sucrose, the same manipulation failed to diminish instrumental performance associated with the cocaine-sucrose solution, thereby demonstrating that self-administration of cocaine facilitated the establishment of S–R control over instrumental performance (**Left panels:** Adapted from (72); **Right panels:** Adapted from (73)).
Behavioral sensitization is a widely used animal model of drug addiction (see Chap. 7 in this book) that captures long lasting (74) pathological behavioral and biological adaptations to repeated exposure to addictive drugs. Thus, sensitization to cocaine or amphetamine develops in response to a sub-chronic treatment regimen and is characterized by potentiated locomotor and neurochemical responses (i.e., dopamine release) to a drug challenge after repeated experimenter-delivered infusions of this drug. These behavioral and neurochemical features have been suggested to reflect an increased sensitivity to the motivational, or “incentive” properties of drug-associated stimuli and the drug itself, as referred to in the “incentive sensitization” theory of drug addiction (75), thereby emphasizing the role of psychomotor sensitization in the development of drug addiction.

Behavioral sensitization has been related to alterations in dopamine release, synaptic plasticity, gene expression in both the ventral and the dorsal striatum (76), and especially the more lateral part of the dorsal striatum, the neurobiological locus of formation of S–R associations (67, 69, 70). Thus, on the basis of the neurobiological profile of cellular and molecular adaptations in response to repeated exposure to psychostimulants that includes the dorsolateral striatum, it has been hypothesized that behavioral sensitization may facilitate the development S–R habits. Using behavioral sensitization procedures, that is, daily IP injections of amphetamine (2–2.5 mg/kg daily, 5–7 days), the long-term (4–6 weeks) influence of drug exposure to the sensitivity to reinforcer-specific satiety and lithium chloride-induced nausea devaluation procedures has been measured (77, 78). In both cases, devaluation tests were performed after short-term lever-press training for two alternative reinforcers, one associated with each lever, under a random ratio schedule of reinforcement (30 or 60 s). After completion of the devaluation procedures, controlling for the specificity of the reinforcer, lever presses were measured during a 10 min extinction session. The results of these experiments are illustrated in Fig. 4.

Fig. 4. (continued) infusions following presentation of the other reinforcer. When tested under extinction, control animals show greater lever pressing suppression than amphetamine-treated rats after devaluation soon after stabilization of performance (left) but not after extended training (right). (c) Long–Evans rats were subjected to a cocaine sensitization regimen with one daily IP injection of cocaine for 14 days while a vehicle group received injections of equivalent volumes of vehicle. Twenty-one days following cessation of the sensitization regimen, rats were trained to acquire a light-food pellet Pavlovian association for eight daily sessions at the end of which procedure they were assigned to a devalued and non-devalued group. Devaluation was induced by taste aversion (IP injection of lithium chloride following noncontingent presentation of food). Rats were tested under extinction where the food-associated CS was presented in the absence of food delivery. As illustrated, cocaine-sensitized rats trained under a Pavlovian learning task did not reduce conditioned approach responses toward a CS after devaluation of its associated US unlike control animals (a: Adapted from Nordquist et al. 2007; b: Adapted from (77); c: Adapted from (79)).
Habit Formation and Compulsion

Fig. 4. Cocaine and amphetamine sensitization facilitates habit formation. (a) Lister hooded rats initially received one daily IP injection of amphetamine (2 mg/kg) (amphetamine group) or saline (vehicle group) for 7 days. Seven days after cessation of this sensitization regimen, rats were trained to enter a magazine to receive either a sucrose or maltodextrine solution (counterbalanced across treatment and devaluation group). Animals were then trained to respond on a lever, initially under continuous reinforcement and subsequently under a RI 30 s schedule of reinforcement. Animals’ exposure was equated to each of the two reinforcers prior to the devaluation test. Devaluation was performed by specific satiety (1 h access to the instrumental reinforcer) and both magazine entries and lever presses were measured under extinction (8 min). As illustrated, amphetamine-sensitized rats are resistant to this manipulation since they maintained lever pressing and magazine entries whereas vehicle-treated animals showed a marked decrease of their responses. (b) Rats initially received repeated injections of either saline or amphetamine and were subsequently trained to press two levers, each of which was paired with a specific liquid reward. Once stable behavioral performance had been established and rats discriminated well the two levers and their associated rewards, devaluation of one of the outcomes was carried out by three post-training injections of lithium chloride for one reinforcer, whereas non-devalued animals received saline
Nordquist and colleagues showed that after 12 sessions of training under a random-interval schedule, both amphetamine and saline-treated animals showed habitual control over instrumental performance since the satiety-devaluation procedure did not alter lever presses during the extinction session in each of these groups. However, after only six sessions of training, although saline-treated rats were sensitive to the devaluation procedure, amphetamine-treated rats maintained a level of responding for the devalued reinforcer that was similar to the non-devalued reinforcer, thereby revealing that instrumental performance was no longer under the control of the motivational value of the reinforcer. Similarly, Nelson and Killcross showed that amphetamine-treated rats did not diminish their active lever presses for the devalued reinforcer when tested under extinction after LiCl-induced nausea at a time when saline-treated rats were shown sensitive to devaluation.

These experiments strongly suggest that noncontingent exposure to addictive drugs, or at least psychostimulants, triggers neural mechanisms that facilitate the instantiation of inflexible habitual instrumental behavior.

Although interesting, these studies raise an important issue concerning the behavioral features of human drug addicts: drug addicts display habitual, inflexible, and compulsive behavior toward the drug, but not natural rewards. Thus, if addictive drugs facilitate habitual control over natural rewards in animal models, why is it that human addicts show compulsive habitual drug taking to the detriment of natural rewards? Interestingly, Nelson and Killcross showed that a post-training sensitization regimen did not affect the performance during extinction following the devaluation procedure compared to controls, suggesting that habit learning can be facilitated by exposure to addictive drugs, whereas performance of already acquired S–R habits are not. Although this experiment was initially designed to control for any effect of sensitization upon the expression, rather than learning, of goal-directed actions, these data may allow reconciliation of the animal and human literature. We speculate that, after the initial exposure to an addictive drug, only newly acquired drug-related activities, may be instantiated as inflexible habits.

Interestingly, cocaine sensitization also results in maladaptive perseverative responding after Pavlovian reinforcer devaluation (80) (Fig. 4c). Schoenbuam and Setlow showed that rats given daily IP cocaine injection (30 mg/kg) for 2 weeks and trained under a Pavlovian learning task 3 weeks after cessation of drug treatment did not reduce conditioned approach responses toward a CS after devaluation of its associated US unlike control animals that had received saline infusions. Thus, psychostimulant exposure not only enhances the development of rigid operant responses, but also the development of rigid Pavlovian approach responses.
However, in all the studies discussed above, all rats, including cocaine-sensitized rats, showed a consummatory aversion to the reinforcer after devaluation, revealing that drug exposure does not influence consummatory responses despite altering instrumental performance. It is therefore important to develop animal models that allow a dissociation between the instrumental, Pavlovian, and behavioral control mechanisms involved in voluntary, habitual, or compulsive drug self-administration. The experimental test of this hypothesis is dependent upon the ability to dissociate in animals preparatory from consummatory responses for addictive drugs, the latter being, at least for natural rewards, governed by Pavlovian mechanisms. We thus implemented procedures in rats that allow us to dissociate drug seeking from drug taking behavior, namely, second-order schedules of drug reinforcement (17, 41, 42, 81) and two-link heterogeneous chained schedules of reinforcement (39, 43, 82).

In trying to separate drug seeking from drug taking, schedules of reinforcement must be implemented in which operant responding for the drug during the drug seeking phase is not affected by the drug itself, that is, so that drug seeking behavior can be measured without interference by stimulant or sedative actions of the self-administered drug.

Two-link heterogeneous chained schedules of reinforcement aim to dissociate spatially, temporally, and instrumentally drug seeking from drug taking behavior. Second-order schedules of reinforcement allow the investigation of cue-controlled drug seeking over prolonged periods of time.

In this procedure, completion of the first link of the chain, designated the seeking link, results in access to the second, or taking, link which permits, once performed, the delivery of the reinforcer. Acquisition of the chain schedule is achieved through successive steps of increasing complexity which start with introduction of the taking lever. A lever press is then reinforced under a fixed-ratio (FR) 1 schedule so that each lever press produces drug reinforcement accompanied by the withdrawal of the taking lever. After several sessions of stable responding, the seeking lever is introduced while the taking lever is retracted. The first press on the seeking lever initiates a random interval (RI) schedule with the first seeking lever press occurring after the RI has elapsed, terminating the first link of the chain; this results in retraction of the seeking lever and insertion of the taking lever to initiate the second link. One press on the taking lever results in the presentation
of the reinforcer followed by a time-out period. Thereafter, the
seeking lever is reinserted to start the next cycle of the schedule.
The effects of experimental manipulation can thus be assessed
through measures of seeking responding (latency, number, or
response rate) as well as taking responding (latency). The interest
in dissociating seeking and taking behavior is obvious when con-
sidering that the two instrumental components are influenced by
dissociable processes since they are differentially sensitive to
devaluation, incentive learning, or Pavlovian manipulations (83).
In addition, cocaine seeking performance is monotonically related
to the dose of drug with a relatively long time-out (43), and is
profoundly affected by extinction of the taking link (82).

In the street, drug seeking behavior is stimulus bound in that
drug addicts forage for their drug under the control of stimuli in	he environment, acting as conditioned reinforcers that support
long sequences of behavior in the absence of the outcome. More
formally, conditioned reinforcers are stimuli that have themselves
acquired rewarding properties after repeated associations with
unconditioned rewards. Conditioned reinforcers bridge delays
between seeking and obtaining the drug. Psychostimulants, opi-
ares, speedball, cannabis, or nicotine-associated CSs act as power-
ful conditioned reinforcers since they greatly enhance drug
seeking behavior when presented contingently, but not noncon-
tingently, upon instrumental responding during, usually, interval
schedules of reinforcement (17, 41, 84–87). Conditioned rein-
forcers can also support the acquisition of a new instrumental
response (88). Such properties are clearly demonstrated in proce-
dures where animals work to obtain presentation of a conditioned
stimulus, often in the absence of the unconditioned reward.

In second-order schedules of reinforcement that we have
used, the CS is presented response-contingently usually under a
FR schedule, during an overall fixed interval or FR schedule for
the primary reinforcer, and markedly enhances and maintains
responding for long periods of time (Fig. 5). Thus, under a sec-

4.2. Second-Order
Schedule of Cocaine
Reinforcement

ond-order schedule of reinforcement, a strong contingency exists
between the instrumental response and the presentation of the
CS (under a FR) as well as the relatively weaker contingency that
is arranged between instrumental performance and the outcome
(the drug) that is reinforced only after completion of the first
ratio after each interval has elapsed. Such schedules therefore
facilitate the development of S-R control over instrumental
responding. In addition, it has been shown that omission of CS
presentation in second-order schedules of reinforcement disrupts
cocaine seeking more than food seeking behavior (85), suggest-
ing that prolonged psychostimulant seeking is particularly depen-
dent upon conditioned reinforcement. Thus, instrumental
responding during the first interval of a second-order schedule of
Habit Formation and Compulsion

reinforcement shows face and construct validities with regard to the behavioral features of drug seeking in humans: stimulus bound, somewhat dissociated from the unconditioned effects of the drug and long lasting.

Second-order schedules of cocaine and heroin self-administration were initially developed by Goldberg and colleagues in nonhuman primates to assess the influence of environmental stimuli upon drug self-administration (15, 17, 18). We have also established second-order schedules of drug reinforcement in rats (41). In the study by Arroyo and colleagues (41), rats were initially required to learn self-administration of cocaine under continuous reinforcement, that is, FR1. After stabilization of responding (5–7 daily 2-h sessions), a second-order schedule with FR components of the type FR₁(FRₓ:S) was introduced, with initial values of x and y set to 1, so that each active lever press resulted in the presentation of the CS and the delivery of 0.25 mg of cocaine. Then x and y values were progressively increased with increments in response requirements starting with x, that is,
FR5(FR1:S) and FR10(FR1:S), then y, that is, FR10(FR2:S), FR10(FR4:S), FR10(FR7:S), and FR10(FR10:S). After stabilization of responding under this FR10(FR10:S) schedule of reinforcement, which therefore requires 100 active lever presses and ten 1 s presentations of the CS to obtain a cocaine infusion, a final fixed interval schedule FI15(FR10:S) was introduced such that a cocaine infusion was delivered only following the tenth active lever press that occurred when the 15 min interval had elapsed. Finally, rats were allowed to perform cocaine seeking behavior under this schedule for 10 days. This acquisition procedure produces robust and stable CS-dependent rates of responding (41) and has been used extensively to probe the neural mechanisms involved in the acquisition and the performance of cue-controlled cocaine seeking (58–60, 89).

It is also possible to decrease the acquisition period to 11 days (90). In this case, the training phase consists of 3 days of FR1 training, 2-h daily sessions, 30 infusions (0.25 mg cocaine/infusion) followed by the introduction of interval schedules, with daily increments: FI1 min, FI2 min, FI4 min, FI8 min, FI10 min, and FI15 min. After 3 days of training under the FI15 schedule, contingent presentations of the CS are introduced under a FR10 schedule such that rats are now trained under a FI15(FR10:S) second-order schedule of reinforcement. This acquisition procedure provides a direct measure of the potentiation of responding during interval schedules by the contingent presentation of the CS since it is introduced only once the responding under fixed interval has stabilized. Thus, although the average response rate is 50–70 during the first interval of a FI15 schedule, it reaches 150–200 when the CS is contingently presented (Fig. 5), as described by Belin and Everitt in a study addressing intrastral mechanisms involved in habitual cocaine seeking (58). Indeed, short and long-term training under second-order schedules of reinforcement for cocaine have been very useful for investigating the neural mechanisms involved in the transition from newly acquired to well-established or habitual cue-controlled cocaine seeking.

The acquisition of cue-controlled cocaine seeking depends upon the basolateral nucleus of the amygdala (BLA) (91–93), the AcbC (89, 94), and also the orbitofrontal cortex (OFC) (95, 96) (Fig. 6a). Performance of cue-controlled cocaine seeking depends upon the VTA (97) and the interaction between the BLA and the AcbC (94) (Fig. 6). Finally, the nucleus accumbens shell mediates the dopamine-dependent potentiating effects of cocaine over cue-controlled cocaine seeking (89). When cue-controlled cocaine seeking becomes well established, or habitual, that is, after several weeks of training under a FI second-order schedule of reinforcement, contingent presentations of CSs increase extracellular
Habit Formation and Compulsion

353

Acquisition

Early performance

Habitual performance

Fig. 6. Neurobiological substrates of the acquisition, maintenance, and habitual performance of cue-controlled cocaine seeking behavior. **Acquisition:** The acquisition of cue-controlled cocaine seeking depends upon the BLA, the AcbC, and also the OFC. **Early Performance:** Performance of cue-controlled cocaine seeking depends upon the VTA and the interaction between the BLA and the AcbC. Finally, the AcbS mediates the dopamine-dependent potentiating effects of cocaine over cue-controlled cocaine seeking. **Habitual Performance:** When cue-controlled cocaine seeking becomes well established, or habitual, contingent presentations of CSs increase extracellular dopamine concentration in the dorsolateral striatum (DLS) but not in the AcbC or AcbS. Moreover, bilateral dopamine receptor blockade in the DLS selectively reduces cocaine seeking habits in rats. Thus, between the acquisition and the subsequent performance, or maintenance, of cue-controlled cocaine seeking there is an apparent shift in the locus of control from the Acb to the DLS, which, we have hypothesized, reflects the development of habitual drug seeking.

dopamine concentration in the dorsolateral striatum (DLS) but not in the AcbC nor in the AcbS (59). Moreover, bilateral dopamine receptor blockade in the DLS, but not in the AcbC, selectively reduces cocaine seeking habits in rats (58, 60).

Therefore, between the acquisition and the subsequent performance or maintenance of cue-controlled cocaine seeking there is an apparent shift in the locus of control from the nucleus accumbens to the dorsolateral striatum, which, we have hypothesized, reflects the development of habitual drug seeking (56) (Fig. 6). We have established that this progressive ventral to dorsal striatum shift depends upon the intra-striatal and serial dopamine-dependent connectivity, linking the AcbC to the DLS both in nonhuman primates (98) and in rats (99), that has been proposed to be an anatomical substrate for integrative mechanisms linking incentive motivation to cognitive processes (98, 100). We have recently demonstrated that disconnecting the AcbC and its regulation of dopamine transmission in the DLS impairs habitual cue-controlled cocaine seeking to the same extent as bilateral dopamine receptor blockade in the DLS alone (58) (Fig. 7). This asymmetric manipulation does not impair general operant responding
when instrumental performance for either a natural reward or cocaine is still under A–O control (Belin D., Besson M. and Everitt B.J., unpublished observations). On this evidence, we speculate that after extended training under the second-order of cocaine reinforcement, cocaine seeking becomes established as an incentive habit whereby the Pavlovian incentive influences exerted by the BLA over the AcbC, in turn, enhance the powerful dorsolateral striatal dopamine-dependent habit system (Fig. 8).

Although incentive habits play an important role in the pathophysiology of drug addiction, they do not account for the different behavioral aspects of the pathology, and especially compulsive drug use, that is, maintained drug use despite adverse consequences, which is a hallmark of drug addiction (see Table 1). Only recently have preclinical models of compulsive drug self-administration been developed, based on the premise that compulsive drug seeking or
Habit Formation and Compulsion

Fig. 8. Drug addiction conceptualized as a loss of executive top-down inhibitory control over an incentive habit. Exposure to addictive drugs triggers neurobiological, and hence, functional modifications, in neural networks involved in implicit subcortical, and declarative cortical, mechanisms. At the subcortical level, addictive drugs alter Pavlovian and instrumental learning mechanisms: they enhance the Pavlovian incentive influences from the BLA on the AcbC and alter the Pavlovian incentive processing between the BLA and the OFC thereby leading to increased incentive salience of drugs and environmental stimuli associated with them. Moreover, addictive drugs facilitate the instantiation of habitual responding, whereby drug seeking behavior is no longer under the direct control of the motivational properties of the drug itself, but instead is governed by stimuli in the environment. The development of habitual drug seeking and drug taking behavior may be related to a ventral to dorsal striatal shift in the locus of control over behavior dependent upon ascending, dopamine-dependent circuitry linking the ventral to the dorsal striatum via recurrent connections with the dopaminergic neurons of the ventral midbrain. Thus, maladaptive Pavlovian incentive processes that control “drug-oriented incentive impulses” in the AcbC are eventually channeled to the dorsal striatum-dependent habit system, thereby resulting in the emergence of incentive habits, which facilitate repetitive inflexible drug seeking and drug taking behavior. Nevertheless, incentive habits cannot account for the development of compulsive drug taking behavior, which, instead, may arise from the interaction between implicit subcortical mechanisms that tend to drive the addict toward drugs and drug-associated stimuli and declarative cortical mechanisms. Indeed, exposure to addictive drugs alters prefrontal cortical function, whereby top-down executive control over behavior is impaired. Drug addicts and drug exposed animals display cognitive inflexibility, impaired decision-making processes and high rates of impulsivity, suggesting impairment of prefrontal cortical function. Thus, once incentive habits develop and interact with impaired prefrontal executive function, drug use becomes compulsive.
taking can be operationalized as persistent instrumental responding despite aversive consequences such as punishment and that it only emerges after extended access to the drug.

5. Compulsive Drug Self-Administration: When Punishment Fails to Prevent Drug Seeking and Taking

5.1. Animal Models of Compulsive Drug Seeking and Drug Taking

As emphasized previously, addicted individuals not only consume large amounts of drugs but are also unable to repress their drug use regardless of its consequences. Thus, addiction shares common features with other compulsive disorders that are characterized by the uncontrollable and irresistible urge to performance of an act, often to relieve anxiety or stress, but regardless of the rationality of the motivation. The compulsive aspect of drug use in addicted subjects is even more obvious when similarities between addiction and obsessive compulsive disorder (OCD) are considered. Indeed, compulsive behavior in the 4th version of the DSM (31) as a criterion for OCD is defined by the repetitive behaviors or mental acts that the person feels driven to perform in response to an obsession, or according to rules that must be applied rigidly aimed at preventing or reducing distress or some dreaded event or situation, but are either not connected to the issue or are excessive. similarities between addiction and OCD have led, based on a modified version of the Yale–Brown Obsessive Compulsive Scale (Y-BOCS-hd; (101)), to the development of the Obsessive Compulsive Drinking Scale (OCDS), a self-rated questionnaire that is able to discriminate accurately between alcoholic outpatients and social drinkers with high sensitivity and specificity (102), suggesting that obsessionality and compulsion are key features of the heavily addicted individual (102).

The inability to inhibit prepotent responses observed in compulsive disorders is commonly associated with perseverative responding regardless of negative feedback. Everitt and Robbins (2005) have suggested that this reflects a state of “must do!,” that is, specific behavioral responses must be repeated – although this subjective response could arise post hoc as a rationalization of “out-of-control” habitual behavior rather than being the driving influence (55).

Signal attenuation is a theory driven model of obsessive compulsive disorder where perseverative responding is induced by simulating a deficit in feedback sensitivity (for review (103)). Subjects trained to lever press for food are given additionally an external stimulus feedback for the response. On the test day, the deficiency in response feedback is simulated by extinguishing the contingency between the response and the stimulus. Similarly, numerous preclinical models of addiction and relapse are often based on extinction procedures.
In addition to the frequent assessment of performance under extinction as an index of motivation, reinstatement procedures have been widely employed to study factors involved in relapse to drug seeking behavior (104). In this model, extinguished drug-reinforced behavior normally resumes after noncontingent priming injections of the drug, re-exposure to drug-paired cues, or exposure to stressors. Concordance between the events that induce reinstatement in laboratory animals and those that provoke relapse in humans confers predictive validity to this model.

Extinction-based procedures in rodents have been proposed to mimic drug cessation, or abstinence, consequent on the lack of drug availability. However, it is far from clear that instrumental extinction has either face or ecological validity as a model of abstinence, since addicts never, or rarely, undergo extinction of their instrumental drug taking responses, such as i.v. drug preparation and injection. Drug addicts might be confronted for different reasons by the temporary restriction of drug availability, but they commonly resume drug use as soon as drugs become available again. On the other hand, extinction/reinstatement models do have direct relevance for behaviorally based treatments if they focus instead on eliminating the conditioned effects of drug-related stimuli by presenting them in the absence of the drug (105). Cue exposure therapies, initially developed to treat phobic neurosis, have been applied to the treatment of drug addiction on the understanding that disrupting the relationships between the drug and environmental stimuli associated with it may have beneficial effects. However, unlike phobic neurosis (106, 107), extinction treatment trials have not yet proven to be effective to treat heroin and nicotine dependence (108, 109).

Moreover in rats, a newly acquired instrumental response supported only by the conditioned reinforcing properties of stimuli previously paired with either cocaine, heroin, or sucrose can persist in the complete absence of the primary reinforcer over months of repeated, intermittent testing (110). Thus, it is possible that conditioned reinforcers, through their acquired reinforcing properties independent of the mental representation of the primary reinforcer, may control habitual instrumental responses, which are resistant to extinction. In addition, a deficit in extinction of Pavlovian associations produced by repeated drug exposure (111) may cause relatively long-lasting impairments in the control of behavior and thus facilitate the compulsive features of conditioned stimulus-maintained drug seeking. In summary, although persistent responding under extinction may provide a model of compulsivity, we suggest that it is perhaps more relevant to assess compulsivity as the altered responsiveness to adverse, instead of omitted, reinforcement. Moreover, it remains unclear whether the omission of a reinforcer, as in extinction procedures, and presentation of an aversive stimulus, as in the punishment
procedure, are equivalent in terms of the psychological states they engender. Hence, it should not be assumed that there are commonalities in the underlying mechanisms when persistent responding is established using these two methodologies.

Limitations of extinction procedures in the context of drug addiction have led to the development of new animal models that reflect more ecologically valid influences in drug addicts. Clinical data on abstinence from cocaine use suggest that the negative consequences directly related to use are a major reason for cessation (112). Indeed, drug use is a high-risk behavior as it often compromises health, work, and social relationships (113–116). Preclinical models of drug addiction might therefore attempt to resemble in several respects the human conditions of compulsivity and fulfill some important features of the pathology in order to meet the necessary requirements of construct, face, and predictive validities essential for the clinical application of data obtained from animal studies (117, 118). Of course, in animals it is extremely difficult to exactly reproduce compulsive drug seeking and taking as seen in human drug addicts because of obvious limitations including the absence of direct personal costs such as family or society problems associated with drug abuse, or limited alternative reinforcement choices. However, despite such limitations, compulsivity in preclinical models of drug addiction should and must be defined as an inability to cease drug seeking and taking under conditions in which the drug is constantly available but its obtainment is associated with adverse consequences.

In recent years, progress has been made in an attempt to mimic human conditions of compulsive drug use. Since aversive consequences can originate from either the drug effect itself, the stimuli associated with drug use or the response for the drug, we will describe in detail how these features have been integrated in animal models of compulsive drug seeking and taking, referring to the potential advantages or disadvantages that each may present.

“Must do” despite adverse consequences

* “Must do” despite devalued consequences

In addition to their reinforcing properties, most addictive drugs have toxic effects, which after repeated use can lead to severe health complications. Such aversive properties would normally progressively devalue any reinforcer, and facilitate the engagement of the subject in alternative responses, incompatible with the pursuit of the initial reinforcer. However, despite often acknowledging the deleterious outcome of drug use, addicts rarely achieve spontaneous voluntary abstinence, and when they manage to do so, often relapse to compulsive drug use.

Similarly, as previously discussed, rats differentially respond to devaluation of drugs of abuse and natural reinforcers.
Performance for food is markedly affected by pairing its ingestion with illness produced by injection of lithium chloride. In contrast, devaluation of orally administered alcohol and cocaine does not greatly decrease drug seeking performance (72, 73).

However, in the aforementioned devaluation procedure, aversive conditioning and instrumental training are normally conducted in different contexts to rule out any inhibition of performance induced by the context and thus, directly assess the importance of the outcome representation in performance. Importantly, while instrumental performance for cocaine or alcohol in the training context remains insensitive to reinforcer devaluation, aversive conditioning successfully suppresses consumption. In contrast, addicts might experience the deleterious consequences of drug intake at any time during their regular use. That is, there is no differentiation between the contexts where the deleterious consequences occur and where they do not. Despite knowledge of the possible deleterious consequences of regular drug intake, abstinence is still difficult to achieve. The adverse consequences of drug self-administration are sometime delayed, rendering the contingency less discernable. However, even “deterrent” treatments such as disulfiram prescription have shown limited efficacy although the contingency between the aversive consequence of drug intake and drug intake itself is strengthened. Disulfiram inhibits aldehyde dehydrogenase and prevents the metabolism of alcohol’s primary metabolite, acetaldehyde, the rapid accumulation of which in the blood causes unpleasant “hangover” effects to occur when alcohol is ingested. The association of these symptoms with drinking should discourage further consumption of alcohol. However although disulfiram helps reduce drinking frequency, it fails to promote continuous abstinence or delay the resumption of drinking (119).

Similarly, extended access to free choice between drug solutions and water interrupted by periods of withdrawal in rats results in high levels of drug intake even when solutions are adulterated with bitter tasting quinine, evidencing the compulsive pattern of drug intake (120–122).

Until drug users explicitly experience the aversive consequences of drug use, drug taking is mainly moderated through warnings rather than actual punishment. Once experienced, aversive stimuli temporally distant from drug intake can appear, thereby rendering aversive contingencies less distinguishable. Moreover, the aversive consequences of drug use are counter-conditioned by previously extended drug presentation, which has been described as retarding the development of the conditioned emotional response (123). All these processes may facilitate the attribution of aversive consequences to irrelevant stimuli. Adding a stimulus previously associated with an aversive outcome to the training
context should normally reduce the frequency of a conditioned response. Indeed, although the aversive stimuli are not directly associated with drug use itself, a conditioned suppressor may be viewed as “devaluing” the drug reinforcer since subjects would be required to respond for the drug in a state of conditioned fear (40).

However, Vanderschuren and Everitt (2004) found that the presentation of a Pavlovian conditioned fear stimulus suppressed cocaine self-administration after a brief, but not an extended, period of cocaine taking. These data support the view that while instrumental behavior directed at obtaining drugs is initially a flexible, goal-directed form of behavior, following prolonged drug exposure, drug seeking becomes insensitive to signals of punishment, thereby indicating its compulsive nature. However, it remains unclear whether in the multi-operant environment that drug addicts are normally exposed to, presentation of aversive conditioned stimuli may favor avoidance rather than abstinence.

Aversive stimuli might eventually be perceived as directly associated with drug use. Punishment has often been debated as a treatment procedure, both in terms of its ethical acceptability and its efficacy. Nevertheless, it undoubtedly remains an important component of the everyday life of drug addicts.

In animals, response-contingent delivery of mild electric shock and time-out are the most frequent sources of punishment used (124). Since these punishers are quite different, further studies are required to determine whether the behavioral suppression they induce relies on the same construct. However, even though differing in many procedural parameters such as the locus or intensity of punishment, footshock-induced punishment has been used in several recent animal models of compulsive drug seeking and drug taking behavior. Thus, we will focus here on this punisher, although footshock-induced suppression may not easily be generalized to the human condition.

In most of the studies on drug taking despite adverse consequences, mild foot shocks, set at a constant intensity, are applied contingently on a response reinforced by a constant dose of drug. In this case, resistance to punishment is assessed through the persistence of the instrumental response despite contingent delivery of the punisher. Alternatively, the degree of response suppression is both dependent upon the magnitude of the reinforcer, the intensity of the punishment event, the schedule of their respective presentation and the delay between the instrumental responses and their consequences (125).

Consequently, Cooper et al. (126) increased daily by 0.04 mA the intensity of a shock that was initially set to 0.25 mA until rats stopped responding (lever pressing) during the 30 min daily sessions for 3 consecutive days. Whereas such a procedure has the advantage of assigning for each rat the final shock intensity that
led to self-imposed abstinence, it constrains the opportunity for repeated testing when required.

The punishment contingency has been used at different loci of the instrumental drug taking action. Thus, taking (127) or seeking (39) behavior has been punished specifically, but contingent punishment has also been applied to a manipulandum that does not distinguish seeking from taking behavior (44–46, 126).

Since preparatory and consummatory responses have been shown to be under the influence of dissociable processes (83), it is conceivable they are differentially sensitive to punishment. In order to assess the sensitivity of seeking and taking responses to punishment we used punishment in the seeking–taking task that spatially and temporally dissociates the “preparatory” and “consummatory” behaviors (43). Both types of punishment induced a progressive suppression in performance, but punishment of the taking response resulted in less suppression than punishment of the seeking response.

As previously mentioned, aversive stimuli may initially not be distinguished as explicitly contingent on the instrumental response, and instead may be attributed to environmental components that favor conditioned suppression instead of punishment-induced suppression. It is only after several pairings that specific inhibition of the punished response develops at the expense of general inhibition. Whereas the specificity of response-induced suppression is commonly seen, most preclinical experiments usually do not dissociate the specific from the general form of response inhibition. Separation of both components during punishment can be achieved either measuring a concomitant, but nonpunished response, or by extending punishment until performance has stabilized.

Finally, the efficacy of the punishment of drug seeking or taking depends on drug history. After short exposure to amphetamine or an opiate (remifentanil), punishment produces robust suppression of self-administration that resumed for the opiate in all subjects approximately 5 days after punishment was discontinued (128). However, the punishment effect obtained for amphetamine lasted much longer (1974) (127). After extended access to cocaine, punishment produced suppression of a seeking response except in a subgroup of animals (about 25%). Thus, compulsive drug seeking appears, as in humans, only after extended exposure to the drug in a small proportion of subjects conferring on these models good predictive validity.

- “Must do” despite the decrease in relative value of a choice option.

Whereas the aforementioned procedures may address some aspects of compulsivity they are often conducted in a context having a restricted response and reinforcer availability. In contrast, drug addicts are exposed daily to multi-operant environments (129, 130).
Persistence in the face of temporary or partial lack of reward, often proposed as an index of craving, is in most cases adaptive as it increases the probability of obtaining the reinforcer. In contrast, persistent responding for an omitted reward at the expense of other rewards in a multi-operant setting more obviously reflects maladaptive decision making. Subjects normally reallocate their behavior as soon as contingencies are reversed, reassigning their behavior from a discontinued element to a newly rewarding element. Similarly, when a subject is exposed to two alternative responses for the same reinforcer, punishment of only one response more profoundly influences the preferred one (125). In contrast, psychostimulant addicts, but also rats and monkeys exposed to cocaine, are impaired in learning to stop responding to previously rewarded cues, despite the loss of reinforcement (79, 131–138). Such procedures suggest the alteration of decision making after extended exposure to addictive drugs. However, because the reallocation is motivated by the same reinforcer, they obviously do not provide relevant models of abstinence which aim to help the reallocation of instrumental behavior from drug to natural rewards.

As emphasized by Ahmed (2005) (139), in contrast “to human drug users who have access to a large spectrum of social and non-social alternative reinforcers, experimental animals have no other choice during drug access but to take the available drug to obtain some level of satisfaction” alternative reinforcers greatly modify drug self-administration.

Providing alternative reinforcers for drug-free urine or blood samples in contingency management therapy has been shown to improve the ability of addicts to remain abstinent (for review see (140)). Similarly in rats, when drug and nondrug reinforcers are presented in discrete choice trials, for example, in which one choice excludes the others, all rats, even sensitized or dependent, were reported to prefer a natural reward over heroin (139) or cocaine (141).

Alternatively, only a few studies have been designed to determine the effect of a concurrent alternative reinforcer on drug-related behavior, for example, in a situation where both behaviors were not mutually exclusive. In monkeys, presentation of a concurrent alternative reinforcer during drug access reduces the proportion of animals that learn to self-administer the drug, and decreases cocaine intake when the behavior has already been acquired after moderate training (for review see (142)). Other nonfood alternatives have also been shown to reduce stimulant intake, such as a wheel for running (143, 144), a novel neutral stimulus (145), or pups (in dams) (146). Simultaneous presentation of a sucrose solution with different alcohol solutions results in a decrease in alcohol consumption in
short-term alcohol-exposed rats. In contrast, when similarly offered an alternative reinforcer after deprivation, long-term alcohol-exposed rats consume significantly higher amounts of alcohol than during baseline drinking (147).

In addition to their competing properties, alternative reinforcers might affect the response to alteration of reinforcement contingencies. Restricted presentation of food reward has been shown to result in aberrant water intake (148). Whereas this phenomenon, known as schedule-induced polydipsia, has been proposed to reflect the effects of motivational “excitement,” including stress (149), it has also been suggested that such aberrant responding is reinforced by releasing the frustration elicited by food restriction (148). Durham et al. (150), found that in a multitask setting where different behaviors of a rat are carefully registered before and after the application of a punisher, punishment of one behavior produced both a decrease in the punished response but also an increase in alternative responses. The two process theory of punishment-induced suppression stipulates that any other behavior than the punished one that competes with the punished behavior and decreases the occurrence of the aversive stimulus are reinforced and acquire a higher probability of expression.

Along these lines, we recently developed a model that provides both positive and negative incentives capable of turning animals away from the pursuit of drugs. We compared the effect of the punishment of cocaine seeking after differential access to cocaine and when behavioral reallocation was facilitated or not facilitated by an alternative reinforcer. Rats self-administered cocaine under a seeking–taking chain schedule in which pressing the seeking lever in the first link gave access to another taking lever. A single press on this lever in the taking link delivered i.v. cocaine after which the seeking–taking chain recycled. After training, animals were allowed a period of differential exposure to the drug, with either 1 or 6 h of daily access to cocaine self-administration. Rats were then re-baselined on the seeking–taking task and subjected to intermittent punishment of the seeking responses. In both groups, some animals could nose-poke for sucrose independently of the cocaine seeking–taking task. After 12 days of 6-h cocaine availability, animals escalated their early cocaine loading and showed enhanced resistance to punishment compared to animals with 12 days of 1 h of cocaine availability. However, the introduction of an alternative reinforcer, for example, sucrose, abolished these differences between the two groups. Interestingly, even in this situation when reallocation was facilitated by an alternative sucrose reinforcer, a subpopulation of rats remained completely resistant to punishment of drug seeking, that is, the rats continued to seek cocaine compulsively. Thus, whereas the availability of an alternative reinforcer enhances the tendency to abstain from drug seeking in face of adverse consequences, some
rats after intoxication persistently use drug despite both positive and negative incentives for abstinence.

Similarly, most of the data obtained from punishment-induced suppression of drug use have identified a sub population of animals that are resistant to punishment (39, 44) or prone to relapse (126, 151). In some cases, an overlap of the population resistant to punishment and a preexisting alteration in decision-making processes, for example, impulsivity, has been reported (45, 151). Thus, considering individual differences in preclinical models is particularly important with regard to the human condition, since not every individual that uses drugs becomes addicted. Instead a transition from casual use to compulsive cocaine seeking and taking develops only in highly vulnerable individuals after prolonged periods of cocaine self-administration (see Fig. 1) (47).

This observation has contributed to a shift in the understanding of drug addiction from an exclusively drug-related to a more integrated point of view (152). In fact, Olievenstein (quoted in (153)) emphasized the fact that drug addiction reflects the unfortunate encounter between a predisposed individual and the drug in a facilitating context or circumstance. Therefore, animal models that demonstrate individual differences in the predisposition to develop compulsive-like behavior directed at obtaining drugs, may represent valuable tools and offer important insights into the understanding of the neurobiological basis of the vulnerability to shift from controlled to compulsive drug use.

There are two main strategies when developing preclinical models of drug addiction. The first category refers to models developed to understand the psychobiological, neurological, cellular, and molecular processes involved in a particular aspect of the pathology. Therefore, these models specifically address one aspect of the pathology, including a diagnostic criterion, such as escalation of intake, resistance to punishment, high motivation for the drug, or habitual instrumental performance, vulnerability to relapse, cognitive flexibility, a neurobiological mechanism such as behavioral sensitization (50, 75, 154, 155) or hedonic allostasis (156, 157). The basic underlying construct is that a specific manipulation of the population exposed to an addictive drug, either by extending the period of drug availability or manipulating the amount of drug available, or both, will trigger a specific behavioral, neural, or molecular response in all the population tested that will provide information about the psychological, neural, or molecular substrates of the aspect of addiction under investigation. The animal models of habitual or compulsive drug seeking we have discussed so far are good illustrations of this strategy. A good example of such animal models is the escalation model developed by Ahmed and Koob (37, 57). This was the first model to address pathological drug self-administration in an animal...
Habit Formation and Compulsion

model. It is based on the idea that differential exposure to the drug may trigger differential behavioral responses. And indeed, whereas rats exposed to drug self-administration for 1 h a day (short-term access, or ShA) maintain a stable pattern of drug self-administration for months, animals exposed to 6 h a day (long-term access, or LgA) lose this control and escalate their intake day after day (see Chap. 10). Comparing the two populations has provided interesting insights into the hedonic status of animals failing to regulate drug self-administration. They have provided critical insights into our understanding of the neurobiological substrates of drug addiction and need to be developed even further. However, these models cannot address other aspects of drug addiction, such as interindividual differences in the vulnerability to develop the pathology and their behavioral and biological correlates. They perhaps also have limitations as preclinical tools in terms of being able to test potential therapeutic strategies in humans since they do not capture the multisymptomatic nature of drug addiction. Indeed, although it might be considered that integrating all the information obtained using the various animal models that have revealed different aspects of the pathology may provide an integrative overview of drug addiction, it is unlikely that such an approach is ideal, since drug addiction is not a sum of symptoms but a neurobiological disorder that can be diagnosed by different symptoms, each of which reflects a subcomponent of a disorder of the brain.

Thus, the second category of animal models of drug addiction takes into account both interindividual differences and the complementary strategy of meeting diagnostic criteria of the pathology in humans. According to the DSM-IV, for an individual to be diagnosed as addicted, three out of seven diagnostic criteria of drug abuse over the last 12 months must be met. In the last 5 years, a new type of preclinical model based on this approach has provided interesting insights into the behavioral and neurobiological substrates of the vulnerability to addiction-like behavior in the rat.

In this model, the DSM-IV diagnostic criteria 3, 6, and 7 (Table 1), namely (i) inability to refrain from drug seeking, (ii) high motivation for the drug, and (iii) maintained drug use despite negative consequences, have been operationalized by, respectively, (i) drug seeking during periods when the drug is not available and signaled as such, (ii) break points during progressive ratio schedules of reinforcement, and (iii) and persistence of self-administration despite punishment by contingent electric footshocks (Fig. 9). For each of these three addiction-like criteria, the animals in an experimental group are ranked according to their score. If a rat’s score is included in the 40% highest percentile of the distribution, this rat is considered positive for that addiction-like criterion and is given an arbitrary criterion score of 1. Then
the arbitrary criterion scores for each of the three addiction-like criteria are added, and consequently four different groups are identified depending on the number of positive scores: 0 criteria, 1 criterion, 2 criteria, and 3 criteria rats.

The core of the model is based on the comparison of 3 criteria and 0 criteria rats. 3 criteria rats show very high scores for each of the three addiction-like criteria and are therefore considered as “addicted,” whereas 0 criteria rats are considered resistant to addiction. The 3 criteria rats represent ~20% of the population exposed to cocaine (Fig. 9), an incidence similar to that observed in human drug-using populations (47). Although 3 criteria rats do not differ from 0 criteria rats at early stages of cocaine self-administration and, more importantly, self-administer the...
same amount of cocaine throughout the experiment (44), their behavior directed toward the drug eventually diverges: 3 criteria rats progressively develop a higher motivation for the drug, are unable to refrain from drug seeking and are resistant to punishment (44–46). They also show escalation of self-administration when given long periods of access to the drug and therefore fulfill a fourth addiction criterion, namely inability to control drug intake (44). The 3 criteria rats also show a high vulnerability to relapse in response to contingent infusions of the drug or contingent presentations of a drug-associated stimulus (46). Thus, even though selected on the three addiction-like criteria, after chronic exposure to cocaine, 3 criteria rats display other important complementary features of addiction as defined in the DSM-IV: escalation and vulnerability to relapse. These observations provide the model with both construct and predictive validities. Moreover, since addiction-like behavior emerges in 3 criteria rats only after extended exposure to the drug, these results highlight the importance of the interaction between a vulnerable phenotype and chronicity of drug use in the development of compulsive drug self-administration (Fig. 1).

In investigating behavioral markers of the vulnerability to develop addiction-like behavior, we recently developed an “addiction severity scale” in rats (45, 46). This variable is computed from the sum of the reduced centered normal distributions, that is, the mean of the population is subtracted from the score of each individual and the result is divided by the standard deviation so that the distribution is characterized by a mean of 0 and a standard deviation of 1, for each of the three addiction-like criteria. We suggest that this reflects the addiction severity index in humans (49, 158–162). This “addiction severity scale” allows for simple dimensional studies such as correlation and regression, and is therefore a useful tool to address predictive factors of compulsive drug use.

Although differential drug exposure is not necessary for the development of addiction-like behavior, we have identified that the early pattern of cocaine self-administration (measured by inter-infusion intervals), and sensitivity to the incentive properties of cocaine (measured as sensitivity to cocaine-induced reinstatement (163)), predict the subsequent development and severity of addiction-like behavior (Fig. 10). Thus, 3 criteria rats develop two important features of cocaine addiction (164, 165) soon after the initiation of cocaine self-administration, namely a “binge-like” pattern of self-administration and, using the drug-induced reinstatement procedure, what we have interpreted as increased drug-induced “craving.”

Interestingly, we have also established that addiction-like behavior is predicted by the behavioral characteristic of impulsivity.
in rats (45) (Fig. 11) but not the locomotor response to novelty, an animal model of sensation seeking (167) related to the vulnerability to acquire drug self-administration (166). Thus, highly impulsive rats, identified on the basis of their level of premature responses during long intertrial intervals in the five-choice serial reaction-time task (168, 169), show much higher scores than low impulsive littermates in the rat addiction severity scale after chronic cocaine self-administration. This difference is attributable to the development of compulsive behavior in high impulsive rats, since these animals maintain cocaine self-administration despite punishment by contingent electric footshocks, whereas the rest of the population suppresses responding (Fig. 11) (45). However, high impulsive and low impulsive animals do not differ in their locomotor response to a new environment, nor in their propensity to acquire cocaine self-administration, a behavioral feature that is instead predicted by the high locomotor response to novelty, which is therefore an independent behavioral trait (45, 166) (Fig. 11).

This evidence suggests that the vulnerability to initiate drug use is independent of the vulnerability to shift from controlled to compulsive drug taking, and therefore provides new insights into behavioral and psychological factors that facilitate different stages in a drug user’s history. In particular, the demonstration that the high impulsive characteristic predicts the shift to compulsive drug
Habit Formation and Compulsion

369

Taking behavior is of major interest since a shift from impulse control failure to compulsivity has been suggested to play a major role in the development of drug addiction in humans (Fig. 1) (156, 157).

At the neurobiological level, this suggests that the lower D2 dopamine receptor binding in the nucleus accumbens that characterizes highly impulsive rats (170) may in some way facilitate the progressive devolution of control over behavior to the dorsal striatum, which, together with a preexisting failure in top-down executive control, may facilitate the progression toward compulsivity and drug addiction.

6. Conclusions

The last decade has seen a major development in the theoretical and experimental approaches to drug addiction. Whereas before the end of the twentieth century animal models had focused mainly on the reinforcing properties of addictive drugs and the reward pathway in the brain (171), there has been a growing...
realization that more realistic models of human pathology were required, as illustrated by a request for application from NIDA in 1995 stating:

NIDA is encouraging research to develop innovative preclinical methods and models that would identify treatment agents for the entire spectrum of cocaine and opiate abuse, from pre-addiction through abstinence, relapse, and recovery. Several preclinical models of drug abuse, such as the animal self-administration and animal drug discrimination models, have been useful laboratory methodologies. However, animal models to study multiple phases of cocaine or opiate abuse have not been well developed. This has hampered the identification and development of potential new medications to treat abuse of these substances. Development of additional animal models could further help in identifying and developing medications to treat cocaine and opioid abuse.

Beginning in 2004, we have developed animal models that address the transition to habitual and then compulsive drug seeking or drug taking behavior, and these models have already provided new insights into the psychological and neurobiological substrates of the different stages of drug addiction.

Thus, the acquisition of drug self-administration depends upon the ventral striatum and its inputs from the dopaminergic neurons of the ventral midbrain, since this system is widely held to represent the primary neurobiological substrate for the reinforcing effects of addictive drugs (27, 172). However, dopamine-dependent processes in the dorsolateral striatum underlie well-established, or habitual, cocaine seeking (58–60). These preclinical observations have been complemented by observations of the specific activation of the dorsal striatum in response to cocaine-associated stimuli (173, 174) in imaging studies with human addicts and a profound decrease in myelin-associated transcripts in the dorsal striatum of human cocaine addicts (175). However, the apparent ventral to dorsal striatum shift in the locus of control over cue-controlled instrumental drug seeking behavior is not an all-or-nothing mechanism. It indicates that the ventral striatum, which is initially involved in controlled drug seeking behavior, becomes “subordinate” to the dorsolateral striatum in terms of the control of drug seeking responses. Indeed, we have also established that dopamine-dependent interactions between the ventral and the dorsolateral striatum are necessary for the establishment of habitual drug seeking behavior (58). Thus, when cocaine seeking becomes habitual, Pavlovian incentive influences converging from the BLA and the OFC onto the core of the nucleus accumbens (AcbC) are channeled to the DLS via the ascending dopamine-dependent intrastriatal circuitry, thereby enhancing the development of incentive habits (56). These observations embody a neurobiological model of drug seeking habits developed in the early 2000s (50, 54, 55) which has encouraged a broadening of
the focus of investigations from the nucleus accumbens to the entire striatum, especially the dorsal striatum. This is illustrated by a Pubmed query with dorsal, striatum, and addiction as key words. Of the 114 entries obtained in early 2009, only nine refer to publications from before 2000, even though only few have so far used animal models of habitual drug seeking behavior.

The same development can be seen in animal models of compulsive drug seeking and drug taking: Wolffgramm and colleagues (176) first reported loss of control over alcohol intake in 1995, but only after 1998 and the development of the animal model of escalation of drug intake (37) has the compulsive aspect of drug addiction been integrated into more elaborated animal models (39, 40, 44–46). However, at this early stage, the neurobiological substrates of compulsive drug seeking and taking have not been clearly established although the body of evidence supports the notion that addictive drugs alter prefrontal executive function (177–180). The development of compulsive drug use can therefore be viewed as a loss of prefrontal executive control over incentive habits that underlie drug seeking and taking. Protracted exposure to addictive drugs may diminish the influence of top-down executive control by the PFC, thereby facilitating the impact of Pavlovian motivational influences on instrumental drug seeking responses. Additionally, by subverting orbitofrontal-dependent decision-making processes (140, 141, 181–183), drugs of abuse may bias individual choices toward drugs and diminish sensitivity to negative feedback, thereby promoting compulsive drug seeking.

A major issue for further research is thus to elucidate the psychological and neurobiological mechanisms involved in the development of compulsive drug seeking and taking behaviors, teasing apart the relative involvement of prefrontal cortical and striatal processes. Further research is also needed to broaden the theoretical and empirical data presented here to classes of drugs other than psychostimulants, for which our understanding is far more advanced than for opiates or alcohol. Indeed, based on recent data obtained in our laboratory that, unlike cocaine, escalation of heroin intake is not predicted by impulsivity 184, we predict that the psychological vulnerability to stimulant and opiate addiction might differ markedly.

Acknowledgments

This research was supported by grants from the Medical Research Council and Wellcome Trust and was conducted within the Behavioral and Clinical Neuroscience Institute in the University of Cambridge.
References

15. Goldberg SR (1973) Comparable behavior maintained under fixed-ratio and second-order schedules of food presentation, cocaine injection or d-amphetamine injection in the squirrel monkey. J Pharmacol Exp Ther 186:18–30


377
Habit Formation and Compulsion


dependence diagnoses from Addiction Severity Index composite scores. J Subst Abuse Treat 31:17–24


Impulsivity
Andrea Bari, Trevor W. Robbins, and Jeffrey W. Dalley

Abstract
Impulsivity is a multifaceted behavioural trait commonly linked to drug abuse and addiction involving rash or risky behaviour and a strong tendency towards spur-of-the-moment, poorly judged decisions and actions. At its core, impulsivity arises through an inability to adequately suppress or inhibit inappropriate behaviour and by a general intolerance to delayed gratification, a tendency also widely found in abstinent drug addicts. Despite intensive research, however, it remains unclear whether impulsivity arises from neural abnormalities produced by the chronic exposure of individuals to drugs such as alcohol and cocaine (‘state impulsivity’) potentially via interactions with medial temporal lobe and frontal cortical structures (e.g., amygdala, hippocampus, anterior cingulate cortex, orbitofrontal cortex) or whether instead ‘trait impulsivity’ and brain disorders linked to it – for example, attention deficit/hyperactivity disorder (ADHD) – predispose to drug use and addiction. This chapter considers both possibilities from a neural systems and psychological perspective drawing on evidence from animal models and clinical research.

Key words: Impulsivity, Drug addiction, Dopamine, Delay discounting, 5-CSRTT, Stop task

1. Introduction

It has been recognised for some time that certain personality traits, including impulsiveness, risk-taking and sensation-seeking strongly influence the trajectory from controlled drug use to repeated use and addiction (1–3). The psychological construct of impulsivity itself is often characterised as a failure to adequately inhibit behaviour and by a predisposition toward rapid, unplanned actions without due consideration of the consequences of such behaviour (4, 5). It can take many forms (e.g., intolerance to delayed gratification or an inability to stop an already initiated motor response) and has long been associated with addiction and substance use disorders (e.g., (2)).
The origin of impulsivity in regular drug users is thought to arise from two, potentially interacting mechanisms: either as a consequence of chronic drug intake causing long-term plasticity and neurotoxic effects on the brain, or as a pre-existing trait that predisposes individuals to compulsive drug taking tendencies (2, 6–9). In practice, these mechanisms are difficult to disentangle in human drug addicts because cognitive and intellectual abilities prior to drug use are not normally known. In addition, it is virtually impossible to control for variations in drug exposure in addicts, including the drug itself and consumption rate (10). Animal models thus provide a valuable means of investigating the neurobiology and causal influences of impulsivity underlying the development of drug addiction.

A prominent hypothesis of the role of impulsivity in initial drug use and addiction posits that the early stages of drug taking are driven by positive reinforcing mechanisms and an impulsive urge and preoccupation to use drugs despite presumably being aware of the future negative consequences of such reckless behaviour. These early drug-related experiences often elicit such feelings as excitement and anticipation, leading up to drug use and perhaps feelings of guilt and remorse after the drug taking act itself (11, 12). Following protracted drug exposure, a shift from impulsivity to compulsivity is hypothesised to occur whereby the control of drug seeking is thought to be determined by negative reinforcement processes (11). This devolution in control to compulsive drug seeking is hypothesised to be motivated by a desire of the addict to alleviate unpleasant or aversive emotional/affective states associated with the withdrawal state (13). However, until only very recently (see (14)) empirical support for this hypothesis has been lacking.

This chapter reviews the diverse conceptualization and operational classification of impulsivity, which increasingly is considered as a complex and multidimensional psychobiological trait (5, 15, 16). We describe the various tasks used to assess impulsivity in animals and discuss the importance and value of animal models in understanding the causal influence of impulsivity in behaviour linked to drug use and addiction.

2. Defining Impulsivity

Impulsivity is considered a multifactorial psychological construct, which is not underpinned by a single neurobiological mechanism. Indeed, several researchers have proposed different taxonomies for subdividing this complex behavioural phenotype, (e.g., (5, 17–20)). Generally, impulsivity has been defined as the inability to withhold or stop a response in the face of negative consequences;
Impulsivity preference for a small immediate reward versus a larger but delayed one; acting without forethought or before all necessary information is available; novelty/sensation-seeking and an increased propensity to engage in risky behaviours. These definitions clearly cover a broad range of behaviours that rarely correlate with each other and sometimes are even conceptually incompatible. Of course, not all impulsive behaviours are disadvantageous. Dickman (21) pointed out the existence of functional impulsivity, which can have adaptive value in certain situations. For example, impulsive individuals show superior performance compared with non-impulsive individuals in situations when there is short time available to make a decision or when facing very-easy-to-solve problems (22, 23).

The core subcomponents of impulsivity are considered to include impaired planning and decision-making, risk-taking, motor hyperactivity and general inattentiveness (17, 20, 24). Others definitions focus on impaired behavioural inhibition (19, 25) and the inability to withhold from responding or to tolerate delayed gratification as evident, for example, in children with attention deficit/hyperactivity disorder (ADHD) (18, 26). As well as being impulsive, individuals diagnosed with the combined subtype of ADHD are also hyperactive and show poor attention (27). ADHD sufferers, as well as individuals diagnosed with oppositional defiant disorder and conduct disorder are characterised by poor behavioural control and disinhibited behaviour and, not surprisingly, are at higher risk of developing drug dependence compared to the control population (28). According to some authors, individuals who show strong extroversion – characterised by high novelty-seeking and low harm avoidance – are also more susceptible to drug use than low extroversion individuals (29, 30). The personality trait of novelty/sensation-seeking is considered by several theories to be linked to the impulsivity construct and is regarded as a combination of reward sensitivity and insensitivity to negative outcomes, thus overlapping to some extent with a general definition of impulsivity (31).

Moeller et al. (4) defined impulsivity as ‘a predisposition towards rapid, unplanned reactions to internal or external stimuli without regard to the negative consequences of these reactions to themselves or others’, thus separating impulsivity from sensation-seeking and poor judgement.

However defined, impulsivity is generally regarded as a consequence of impaired executive functioning and, more specifically, of dysfunctional inhibitory processes. Inhibition itself is a higher-order supervisory cognitive function serving to reduce interference from irrelevant distracting stimuli, the suppression of unwanted memories, actions or unpleasant emotions, and the avoidance of concurrent activation of incompatible responses (32). Harnishfeger (33) distinguished cognitive from
**behavioural** forms of inhibition; the first mediating the control of cognitive contents and attentional processes, the second relating to overt behaviour such as response inhibition and delayed gratification. More recently, Aron (32) described the division of ‘inhibition’ into automatic and active/willed subtypes. In higher mammals, the ability to exert inhibitory control over automatic reflexes and conditioned responses has been suggested to have evolved to allow slower cognitive processes to guide behaviour in certain circumstances (6, 34).

Impulsivity can be measured in a number of ways in humans, for example, by self-report questionnaires or by observing behaviour in natural settings (35–39). It can also be assessed using laboratory tasks that measure distinct operational definitions of impulsivity in humans and other animals (5, 16, 40–44). Three main types of behavioural tasks are broadly used to measure impulsivity (1) extinction and reversal learning paradigms, where impulsivity is operationally defined as the inability to inhibit a response previously rewarded but now punished; (2) delay and probability discounting (broadly defined as decision-making paradigms) where the subject has to choose actions that are more rewarding in the long run, thus delaying gratification; and (3) response inhibition paradigms, where impulsivity is defined as the inability to stop an ongoing action or to refrain from initiating a response within a predetermined interval of time. The first class of tests measures an aspect of behaviour more akin to compulsivity and perseveration, because often the action becomes habitual and no longer controlled by the outcome. The last two categories of behavioural tasks have been the most exploited in the literature on drug addiction (9) and will be reviewed in the subsequent sections.

### 3. Assessing Impulsivity in Rodents

In order to assess impulsivity, several analogous tasks have been devised for use in humans and rodents. The main advantages of behavioural tasks over questionnaires are that they can be scaled for use in a number of animal species, they provide objective measures suitable for repeated testing and they are more sensitive to the ‘state’ of the subject at the moment of testing than many self-report personality inventories. The possibility to measure impulsivity in laboratory animals is especially important when exploring the causal relationship between impulsivity and drug addiction. In humans, it is often difficult to elucidate whether impulsive traits predate substance abuse or are simply a consequence of chronic drug exposure because the majority of studies are correlational and/or retrospective. Behavioural tasks used to assess
Impulsivity in rodents, relevant to addiction research, can be divided broadly into those measuring impulsive choice (or impulsive decision-making) and those measuring response inhibition (or impulsive action) (8, 9, 16). The main difference between these two forms of impulsivity is that impulsive choice paradigms embed a ‘waiting’ component after a particular action has been elicited; this action is usually a choice between two or more alternatives and is based on expected value and on subjective discounting parameters. In response inhibition paradigms, the waiting component, if present, occurs before the response has been produced and the outcome is all-or-nothing depending on the successful inhibition of a prepotent motor response. Impulsive choice temporally precedes response inhibition, which occurs at a later stage of processing, sometimes even after the response output itself, as in the stop-signal reaction time (SSRT) task.

3.1. Delay Discounting Paradigms

Impulsive decision-making is commonly assessed using delay-discounting (DD) paradigms. In these tasks, impulsive choice is defined as preference for a small, immediate reward over a larger but delayed reward (45–47). Different laboratories have used different versions of this paradigm, sometimes substituting the delays with probabilities; here we will refer to the classic variant with increasing delays to a large magnitude incentive.

Typically, the animal is required to make an operant response (e.g., lever press or nose-poke) for one of two available choices in order to receive a reward. The reward is usually food, water or drug, and is delivered immediately, in a small quantity, upon responding on one of the two choices, or after a delay, but in a larger quantity, if the alternative choice is selected (see (48) for details). By varying the delay to, and/or the magnitude of, the larger reinforcer, it is possible to calculate the so-called indifference point, that is, the point at which the two different rewards (small/immediate and large/delayed) are chosen with equal frequency. Several indifference points – obtained independently by manipulating reward size and delay – are then plotted to obtain a discount function (49). This function can be described by a hyperbolic curve in both humans and rodents, although nonhuman animals are generally believed to behave more impulsively than humans (i.e., they exhibit steeper discounting functions) (50).

The DD procedure is particularly suited to model motivational states in drug addiction. Indeed, drug abuse can be conceptualised as a preference for immediate, small and short-lasting rewards (e.g., euphoric effects of a particular drug) versus long-term larger benefits such as social acceptance, a rewarding career and good health. Delay aversion on the DD task predicts substance use and relapse in both animal (51–54) and human (55, 56) subjects. It also provides a useful measure of the effects of stimulant drugs on DD, which can sometimes depend on the presence of a
cue stimulus during the delay period associated with the larger reinforcer, on the nature of the reward used and on baseline (i.e., pre-drug) levels of impulsivity (8, 57).

These effects depend not only on the class of abused drug and precise level of exposure but also on important cross-species differences. For example, alcohol has been shown to increase impulsivity on a DD task in rats (47, 58) but not in humans (59, 60), whereas repeated administration of cocaine (61–63), methamphetamine (64) and nicotine (65) all increase impulsivity in rats on this task (see Table 1). By contrast, acute administration of methylphenidate (71, 77), methamphetamine (64) and the non-stimulant drug atomoxetine (90) increases delay tolerance in rats (i.e., decrease impulsivity). Other research has found that the selective dopamine (DA) reuptake inhibitor GBR-12909 reduces impulsivity on this task, while DA receptor antagonists increase it (71, 96, 97). Thus, in general, it seems that acutely increasing catecholaminergic brain activity decreases impulsive choice, especially in impulsive subjects (96, 98, 99). Chronic psychostimulant administration, on the other hand, increases impulsive choice in the DD paradigm, (e.g., (61)), possibly as a result of functional neuroadaptive changes in the mesolimbic DA system (100, 101). By contrast, global serotonin (5-HT) and acute tryptophan depletion (ATD) have no significant effects on impulsive choice in the DD paradigm (102, 103), even though acute administration of the 5-HT1A receptor agonist 8-OH-DPAT causes impulsive responding (104) in the same task.

3.2. Stop Signal Reaction Time and Go/No-Go Tasks

The most widespread tests of response inhibition are the go/no-go and SSRT paradigms. These tasks have been successfully adapted for use in animals and have high face and predictive validity. In the go/no-go paradigm, subjects are required to respond to a cue – the go signal – and not to respond to a different infrequent stimulus – the no-go signal. Impaired go/no-go performance has been reported in children with ADHD (105) and in individuals using different classes of drugs, including cocaine (106, 107), alcohol (108), tobacco (109), but not MDMA (the principal component of ecstasy) or cannabis (110). In animals, acute (66), but not chronic (61) administration of cocaine impairs behavioural inhibition on the go/no-go task.

The SSRT task is a sophisticated variant of the go/no-go task where subjects are required to cancel an already initiated motor response following the presentation of an unexpected stop-signal (usually auditory or visual) (25). The action to be inhibited is made prepotent by its high frequency and fast execution. Stop trials (usually 20–25% of total trials) are randomly interspersed among go trials in order to make the stop-signal unpredictable for the subject. By varying the timing of the stop-signal, it is possible to measure the duration of the inhibitory process itself.
Table 1
Summary of the effects of some widely abused drugs on distinct forms of impulsivity in animals. The effects of drugs used clinically in ADHD and other disorders are also shown

<table>
<thead>
<tr>
<th>Substance</th>
<th>Delay discounting</th>
<th>Go/no-go</th>
<th>SSRTT</th>
<th>5-CSRTTT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cocaine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>?</td>
<td>↑ (66)</td>
<td>?</td>
<td>↑ (67)</td>
</tr>
<tr>
<td>rep/chronic/self-admin</td>
<td>↑ (61, 63, 68)</td>
<td>⇔ (61)</td>
<td>↓ (69)</td>
<td>⇔ (70)</td>
</tr>
<tr>
<td><strong>Amphetamine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>↓↑ (45, 57, 71)</td>
<td>↑ (72)</td>
<td>↑ (73)</td>
<td>↓ (74)</td>
</tr>
<tr>
<td>rep/chronic/self-admin</td>
<td>⇔ (75)</td>
<td>?</td>
<td>?</td>
<td>⇔ (76)</td>
</tr>
<tr>
<td><strong>Heroin/morphine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>↑ (77, 78)</td>
<td>?</td>
<td>⇔ (79)</td>
<td>↑ (79)</td>
</tr>
<tr>
<td>rep/chronic/self-admin</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>⇔ (70)</td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>↑ (80)</td>
<td>?</td>
<td>↑ (73)</td>
<td>↑ (81)</td>
</tr>
<tr>
<td>Alc. pref. bred animals</td>
<td>↑ (82, 83)</td>
<td>↑ (84)</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td><strong>Nicotine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>?</td>
<td>?</td>
<td>↑ (A. Bari and T.W. Robbins, unpublished findings)</td>
<td>↑ (67, 74)</td>
</tr>
<tr>
<td>rep/chronic/self-admin</td>
<td>⇔ (86)</td>
<td>?</td>
<td>?</td>
<td>↑ (87)</td>
</tr>
<tr>
<td><strong>Therapeutic drugs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>↓ (71)</td>
<td>?</td>
<td>↓ (88)</td>
<td>↓ (74)</td>
</tr>
<tr>
<td>Atroventaxetene</td>
<td>↓ (90)</td>
<td>?</td>
<td>↓ (90)</td>
<td>↓ (89)</td>
</tr>
<tr>
<td>Modafinil</td>
<td>?</td>
<td>?</td>
<td>↓ (88)</td>
<td>↑ (91)</td>
</tr>
<tr>
<td>SSRIs</td>
<td>↓ (92) ⇔ (45)</td>
<td>?</td>
<td>⇔ (93)</td>
<td>?</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>↑ (92, 94)</td>
<td>↑ (95)</td>
<td>↑ (A. Bari and T.W. Robbins, unpublished findings)</td>
<td>↑ (81)</td>
</tr>
</tbody>
</table>

Abbreviations: ↓ decreased impulsivity; ↑ increased impulsivity; ⇔ no effect; ? no studies available; rep = repeated treatment; alc. pref. = alcohol preferring.
(the SSRT) and to derive an inhibition function \(^{(25, 40)}\). Previous research has demonstrated that the SSRT is consistently longer and more variable in ADHD individuals \(^{(111, 112)}\) as well as in drug abusers \(^{(113–115)}\).

The SSRT is decreased in rats by drugs commonly used for the treatment of ADHD, although for some drugs – especially the psychostimulants – the effect is dependent on baseline performance \(^{(73, 88, 116)}\). Stopping efficiency is also improved by non-stimulant drugs such as atomoxetine, an effect that has been observed in both rats and humans. However, unlike psychostimulant drugs, this effect does not appear to depend on baseline performance \(^{(90, 93, 117)}\). The exact mechanism of atomoxetine's beneficial action is still unclear but may involve increased noradrenaline (NA) function in the prefrontal cortex (PFC) since GBR-12909, a selective DA reuptake inhibitor, tends to speed go responses but not stop responses; whereas guanfacine, a selective alpha-2 adrenoceptor agonist, which diminishes ascending noradrenergic activity \(^{(118, 119)}\), slows SSRT and impairs stop-signal accuracy in the rat \(^{(93)}\). Moreover, the speeding effect of methylphenidate on the SSRT in slow-stopping animals is not blocked by the DA receptor antagonist \textit{cis}-flupenthixol \(^{(88)}\), suggesting a non-dopaminergic mechanism of action. Finally, in ADHD children, increasing dopaminergic transmission by l-dopa administration does not appear to influence SSRT, while the tricyclic antidepressant desipramine – which inhibits the reuptake of NA – decreases SSRT \(^{(120)}\). Thus, noradrenergic neurotransmission appears to be important for the inhibition of an already initiated response \(^{(41)}\), whereas DA appears to selectively modulate the go response, potentially at the level of the striatum \(^{(121, 122)}\). Conversely, 5-HT seems to have a marginal role, if any, in this form of behavioural inhibition because 5-HT global depletion, acute tryptophan depletion and citalopram administration do not alter SSRT in humans and rats \(^{(93, 117, 123, 124)}\).

### 3.3. Five-Choice Serial Reaction Time Task (5-CSRTT)

The 5-CSRTTT is an automated operant behavioural task used widely for the assessment of sustained attention and impulsivity in rodents \(^{(125, 126)}\). The basic task is modelled on the continuous performance task used to study human attentional processes \(^{(127, 128)}\). The rodent version of the task requires animals to detect brief flashes of light presented pseudo-randomly in one of five holes and to make a nose-poke response in the correct spatial location in order to receive a food reward. Rats are trained to monitor a horizontal array of apertures and to withhold from responding for a fixed or variable inter-trial interval (ITI) until the onset of the stimulus (see \(^{(129)}\) for details). Generally, the accuracy of stimulus discrimination provides an index of attentional capacity, while premature responses – made before the presentation of the stimulus – are regarded as a form of impulsive behaviour and hence a failure in impulse control \(^{(125)}\).
Acutely administered stimulant drugs invariably increase impulsive behaviour on the 5-CSRTT (125), an effect likely mediated by increased DA activity in the nucleus accumbens (NAC) (130, 131). On the other hand, atomoxetine, which produces no appreciable effects on subcortical DA (132), decreases impulsivity on the 5-CSRTT (89, 90, 133). The selective depletion of brain 5-HT by intracerebroventricular infusions of 5,7-dihydroxytryptamine (5,7-DHT) in adult rats produces long-lasting hyperactivity as well as increased impulsivity on this task (134). Attentional impairments on the 5-CSRTT mainly result from selective lesions of the cortical cholinergic system and, under certain conditions, following disruption of the ascending noradrenergic system (126, 135–139).

Recent research has highlighted a strong link between impulsivity on the 5-CSRTT and different stages of the drug addiction cycle. Thus, animals selected for high levels of premature responses (high impulsive rats; HI) display higher rates of cocaine and nicotine self-administration compared with low-impulsive rats (LI) (52, 140). Moreover, as we will discuss in the next section, high impulsivity on the 5-CSRTT predicts the transition to compulsive drug seeking and taking (14).

Scientific research on drug addiction has focused traditionally on motivational processes that lead to, and are altered by, pathological drug use. For example, repeated drug use has been hypothesised to increase the incentive motivational properties of drugs and drug cues and stimuli associated with drug use (141). This effect may bias decision-making processes toward drug procurement and the immediate euphoria-producing effects of drugs at the expense of future negative outcomes. Indeed, chronic exposure to stimulant drugs is associated with morphological abnormalities in a number of brain areas involved in behavioural inhibition, impairing the ability of the subject to refrain from using drugs and discounting negative future consequences of chronic drug abuse (6, 31).

More recently, an important role for impulsivity and other behavioural traits in drug addiction has been recognised (8, 31, 142). It has been found, for example, that subjects displaying high levels of exploratory behaviour and sensation/novelty-seeking are more likely to initiate drug use (24, 143, 144). Thus, animals showing increased novelty-induced locomotor activity (i.e., the ‘high responder rat or HR’) show a greater propensity to self-administer psychomotor stimulant drugs such as amphetamine and cocaine (143, 145), a characteristic postulated to be mediated by increased activity of the brain dopaminergic systems (146).
Other studies have shown that individuals with personality traits related to impulsivity begin drug use earlier and show a generally higher rate of drug abuse than the general population (147–151). However, the precise link between impulsivity and the initiation of drug use is still a matter of considerable debate and uncertainty. It is possible that people start to use drugs because they value the immediate rewarding or pleasurable effects of the drug more than future larger goals, such as stable personal relationships and a rewarding career. Such individuals tend to be insensitive to delayed gratification (55, 56, 152). Other forms of impulsivity – such as impaired inhibition in the stop task – also correlate with drug use (113–115), an association mediated in part by the failure of such individuals to inhibit behaviour directed towards drug-associated environmental cues (31). An alternative explanation is that individuals with personality traits related to extroversion (153) tend to be exposed to more diverse environments compared with introverted individuals, thus increasing their probability of coming into contact with other drug users (154). Once the drug is available in the immediately surrounding environment, factors such as peer pressure, underlying impairments in behavioural inhibition, disregard for future negative consequences, reward dependence and low harm avoidance all hypothetically increase the probability of experimentation with drugs. Accordingly, de Wit and Richards (31) describe the addiction process as being determined by heightened reward sensitivity and decreased behavioural inhibition. These characteristics are thought to combine together to increase the likelihood of initiating substance use and relapse after abstinence. According to Dawe and colleagues (155), individuals with high reward sensitivity would experience greater pleasure by using the substance, while poor behavioural inhibition would result in the continuation of drug use despite negative consequences. A third causal pathway in the development of drug addiction relates to ‘stress reduction’ (156, 157), consistent with the ‘self-medication’ hypothesis of addiction (158). Thus, poor behavioural inhibition and the excessive pursuit of rewarding activities that are often socially unacceptable and/or illegal can lead to undesirable consequences (e.g., social isolation, loss of employment), which may drive drug use as a means to alleviate the distress caused by such events.

A number of studies in laboratory animals have supported a causal link between impulsivity and drug addiction, specifically by influencing the different stages of the drug addiction cycle, (e.g., (8)). For example, underlying deficits in delay discounting have been shown to influence the acquisition or initiation of both cocaine (51, 53) and ethanol self-administration (54). The maintenance phase of drug use, which is often accompanied by drug binging and escalation (159), is influenced by spontaneously high levels of impulsivity on the 5-CSRTT. Thus, rats selected for
high impulsivity on the 5-CSRTT show a robust escalation of cocaine (140) and nicotine (52) self-administration. Therefore, ‘trait-like’ impulsivity on the 5-CSRTT appears to predict the escalation, but not the initiation of drug intake, which instead is predicted by a high locomotor response to novelty (i.e., the HR phenotype).

High impulsivity on the 5-CSRTT also predicts the transition or switch to compulsive drug seeking as shown using a rat model based on three key diagnostic criteria of drug abuse from the DSM-IV, namely, increased motivation to take the drug (criterion 6), an inability to inhibit drug seeking (criterion 3), and continued drug use despite negative or adverse consequences (criterion 7) (14). The degree of impulsivity, as measured by the 5-CSRTT, was found to correlate with the propensity of the rats to compulsively self-administer cocaine as measured by the resistance of HI rats to punishment-induced suppression of responding for cocaine. Thus, ‘trait’ impulsivity appears to play a causal role in facilitating the transition from initial drug exposure to habitual and ultimately compulsive form of drug taking (see Fig. 1).

Finally, there is recent evidence that ‘trait’ impulsivity in rats can influence the propensity for relapse to drug seeking and taking under certain conditions (160, 161). Thus, drugs that reduce impulsivity (e.g., atomoxetine) may also lessen the likelihood for relapse or reinstatement of drug taking after a period of abstinence.

**Fig. 1. Hypothetical inter-relationship between impulsivity and substance abuse.** Pre-existing impulsive traits are postulated to facilitate drug use (light grey pathway) in part by short and long-lasting interactive effects of chronic drug exposure on behavioural inhibition and reward sensitivity (dark grey pathway). Trait impulsivity in this schema includes both clinical forms – caused by psychiatric disorders such as ADHD or acquired brain damage – and non-clinical, stable personality subtypes. State impulsivity is hypothesised to be triggered by initial drug use, stressful life events and, in the more advanced stage of drug use, by drug-related stimuli leading to craving and chronic relapse to drug taking activities. Both trait and state forms of impulsivity are assumed to lead to maladaptive decision-making which, in turn, encourage further drug use to alleviate the distress caused by increasingly negative life events.
The high co-morbidity between clinical impulsivity and substance use/dependence is postulated to reflect an overlapping pattern of neural and neurochemical abnormalities in limbic fronto-striatal brain networks leading to a characteristic impairment in cognitive control over behaviour (6, 162). Such impairments in control may be determined in part by genetic influences in the case of antecedent or trait impulsivity (1), or via harmful interactive effects on PFC functioning of chronic exposure to stimulant and opiate drugs (see Fig. 1). In the latter case, chronic exposure to drugs is hypothesised to result in a form of cognitive impulsivity (or ‘state’ impulsivity) produced by (i) frontal cortical dysfunction leading to inhibitory control deficits and (ii) an augmentation of the incentive motivational properties of stimuli associated with drug use, putatively via impaired subcortical processing of stimuli at the level of the amygdala and NAC (6).

In the schema shown in Fig. 1, both ‘trait’ and ‘state’ forms of impulsivity are hypothesised to facilitate the transition from first drug use to repeated use and addiction by increasing the control over behaviour by stimulus–reward associations and by decreasing fronto-cortical functioning. The net result is a failure to adequately evaluate the consequences of risky or inappropriate drug taking behaviour, thereby facilitating continued drug use leading in turn to a further exacerbation and impairment in inhibitory control mechanisms mediated by the PFC and related structures (see Fig. 2).

The core pathologies of ADHD and drug addiction are hypothesised to involve frontal cortical brain regions (dorsolateral PFC, anterior cingulate cortex, orbitofrontal cortex OFC) and basal ganglia structures, including especially the NAC and caudate nucleus (collectively, the striatum). In human addicts, the presentation of drug-related stimuli produces a reliable activation of limbic brain structures (164, 165) whilst cocaine abusers exhibit marked morphological abnormalities in OFC (166) and are impaired on tasks dependent on OFC function such as probabilistic reversal learning and gambling tasks (167, 168). They are also impaired on go/no-go and stop task paradigms (106, 107, 114). Several studies confirm these findings with evidence of structural and metabolic abnormalities in the PFC and striatum following extended drug exposure both in humans and other animals (e.g., (101, 169)). Indeed, our own research has revealed lasting impairments in inhibitory response control, attention, and motivational variables in rats exposed contingently to intravenous methamphetamine, MDMA and heroin (70, 76, 170), consistent with recent reports showing enduring effects of cocaine on working memory and sustained attention in rats (171, 172), as well as tasks sensitive to OFC function (173–175).
Impulsivity

The main neural loci mediating different forms of impulsivity in rodents, including delay discounting impulsivity, SSRT and impulsive responding on the 5-CSRTT, have been extensively investigated in recent years (see (8, 16, 176, 177) for recent reviews on this topic). Key findings include the demonstrations that impulsive choice on the DD paradigm is increased by selective lesions of the core, but not shell sub-region of the NAC (178, 179) and by lesions of the OFC ((180, 181), but see (182)). Other forms of impulsivity, including impulsive actions, depend on the functional integrity of the medial PFC, especially the anterior cingulate cortex and infralimbic cortex (183) as well as the NAC and areas of the medial striatum considered homologous to the caudate in humans (116, 176, 184). Taken together these findings suggest a level of convergence in the neural systems that underlie distinct forms of impulsivity that may reflect more general psychological processes such as ‘waiting’ or ‘stopping’ impulsivity (123). Indeed, rats selected for spontaneously high levels of impulsivity on the 5-CSRTT also show delay intolerance on the DD paradigm but are unimpaired on other forms of impulsivity, including the SSRT task and Pavlovian conditioned approach (185).

Previously, we reported using dedicated small animal positron emission tomography (PET) that hyper-impulsive rats on

Fig. 2. Simplified schema showing how chronic drug exposure progressively impairs the inhibitory influence of the PFC and evokes a shift in dopamine function from ventral (i.e., NAC) to more dorsal areas of the striatum, especially those domains implicated in habitual forms of behaviour. In brief, chronic drug abuse is believed to impair the inhibitory influence of the PFC on striatal and mesostriatal structures (light grey curved arrows) resulting in behaviour that is progressively controlled by immediate, reward-related impulses. Hyperactivity of the mesolimbic dopaminergic system is thought to enhance stimulus-reward associations (6) (light grey straight arrows) leading to habitual drug taking – mediated by more dorsal regions of the striatum (163) – as well as increased susceptibility for relapse even after long periods of drug abstinence.
the 5-CSRTT have a reduced availability of dopamine D2/3 receptors in the ventral striatum (140). They also maintain significantly higher rates of intravenous cocaine and nicotine self-administration (52, 140). The magnitude of the change in dopamine D2/3 receptors was inversely related to the severity of impulsivity suggestive of a possible causal link between dopamine D2/3 receptors and impulsivity, consistent with previous findings of delay aversion in rats with selective lesions of the NAC (178). These and other data (51) indicate that impulsivity – as defined by the inability to wait or bridge delays to future reinforcement – may be causally involved in drug abuse vulnerability rather than the other way around. Thus, the reduced availability of dopamine D2/3 receptors in abstinent cocaine addicts (186) may in part be a pre-existing abnormality that confers risk for drug bingeing which in turn accelerates the transition from controlled drug use to habitual and ultimately compulsive patterns of drug taking (161).

6. Concluding Remarks

In this chapter, we have highlighted recent significant advances in our understanding of the role of impulsivity in the drug addiction cycle together with putative neural substrates. However, it is clear that much further work is needed to elucidate (i) the precise modifications caused by drugs of abuse on neuronal circuits involved in behavioural inhibition and reward sensitivity and (ii) the causal role and brain mechanisms of impulsivity in substance use disorders. Animal models provide a valuable source of convergent information because they allow drug exposure to be precisely controlled whilst, in addition, providing a metric of cognitive and behavioural performance prior to drug experience. Studies in human addicts are often confounded by interpretative issues relating to variable drug histories and pre-morbid intellectual capabilities.

In closing, it is worth noting that many of the drugs used to calm hyperactive and impulsive children are themselves widely abused by humans (187). A major challenge for future research will be to determine whether prescribed stimulant treatment of ADHD (e.g., with Ritalin®) offers protection against future problem drug use or instead exacerbates and speeds up the transition to compulsive drug use in susceptible individuals. It will also be important to compare these effects with newer non-stimulant drugs such as atomoxetine, which have proven clinical efficacy in ADHD (188, 189). Finally, a major objective for future research should be to continue to develop sophisticated animal models with high construct and face validity to investigate further the
neural and psychological mechanisms underlying the switch from impulsivity to compulsive drug seeking and taking and how these changes interact with vulnerable personality traits and chronic drug exposure.

Acknowledgements

The work reviewed in this chapter was funded by grants from the MRC (G0401068, G0600196, G0701500, G0802729), the Wellcome Trust (076274/z/04/z) and by a consortium joint award from the MRC and Wellcome Trust (G0001354) within the Cambridge University Behavioural and Clinical Neuroscience Institute. AB was supported by a studentship from the MRC.

References


Impulsivity


Chapter 15

Binge Drug Taking

Herbert E. Covington III and Klaus A. Miczek

Abstract

The act of bingeing represents the culmination of a potentially abusive behavioral routine, and underscores the beginning of an addiction cycle. Experimental binges provide a valid model for examining aspects of the gradual progression from drug use to abuse, particularly when attempting to identify environmental and genetic factors that may prompt intermittent behavioral routines to become dysregulated, as exemplified during cycles of bingeing. Here we explore binge behavior in animals with a particular focus on data obtained from self-administration studies that utilize conditions that allow for both unrestricted and prolonged access to cocaine, opiates, alcohol or food. Many behavioral and neural effects of bingeing are shared between these substances, indicating that the repetitive nature of binge behavior for many types of drug abuse may be related to the dysregulation of common neural circuits. Interestingly, intense bingeing occurs with the emergence of two potential changes in behavior; an increase in the rate or an increase in the persistence of behavioral responding. These two changes indicate that control over bingeing may be determined by a number of processes, including sensitization, tolerance, and withdrawal.

Key words: Cocaine, Heroin, Alcohol, Food, Binge, Drug abuse, Compulsive behavior

1. Defining Binge Behavior

Many metaphorical descriptions have been used to depict the transition from normative to uncontrollable “dysregulated” behavioral routines. For instance, recreational users who are suddenly captivated by an irresistible urge to take their drug of choice without being able to limit intake could be described as crossing over the Rubicon, or alternatively, spiraling out of control (1). Yet the question still remains as to whether the transition from use to abuse is gradual or sudden. Unfortunately, the precise environmental conditions and the duration of exposure to the reinforcing stimuli that trigger dependence vary dramatically across populations of
drug users. Why study binge behavior? Binges represent an important transition stage in the addiction cycle, and are indicated by an uncontrollable and repetitive behavior. It remains unclear whether or not a first “binge” signifies the bona fide transition to substance dependence, or if such an occurrence streamlines this process. Undoubtedly, the act of bingeing dramatically impacts on behavioral patterns typically expressed from day to day. The operational definition of a binge can thus be summarized as high rates of a reinforced behavior (i.e., responding at a rate that is equal to or above the average rate of responding emanated during conditions of limited access) over abnormally long intervals of time (i.e., indicated by a dysregulation of circadian controlled behavioral routines, such as food and water intake, sleeping, grooming) without inhibitory feedback from potentially adverse consequences such as significant changes in body weight, motor activity and affect (2).

Several theories have contributed substantially to the experimental analyses of addiction, which are discussed more fully throughout this textbook that may provide explanations for the emergence of binge patterns of behavior. The incentive-sensitization theory of addiction (see Chap. 7) postulates that repeated administrations of a drug, such as cocaine, promotes an increase in the incentive-salience (i.e., wanting) of the drug through adaptations in the mesocorticolimbic dopamine system (3). The process of sensitization can be readily examined by observing an increasingly stronger behavior, most often locomotor activity, with repeated exposures to a psychomotor stimulant (4, 5). Sensitization to a psychomotor stimulant persists for a long time, and these animals tend to self-administer larger amounts of a drug of abuse as compared to nonsensitized animals under various access conditions (6–11). The transition to drug abuse has also been postulated to occur as an accumulation of opponent-processes that emerge in response to a reward-induced (e.g., cocaine) state (12, 13). Considerable evidence supports the proposal that hedonic allostatics (14) contributes to escalated cocaine taking and underlies the negative affect present during withdrawal after a binge (see Chap. 10). Another theoretical model proposes that instrumental (i.e., goal directed) responding for an unconditioned reward (i.e., cocaine) gradually switches to a Pavlovian (i.e., stimulus-response) habit (see Chap. 13) (15, 16). Experimental work has highlighted the significance of certain limbic brain areas throughout phases of conditioning for cocaine reinforcement. A shift from ventral (planned or action-outcome responding) to dorsal (habitual responding) striatal mechanisms is therefore also likely to contribute to routine bingeing (17). The possibility for each of the above mentioned processes to overlap, occur concomitantly, or take effect at different times during the development and persistence of binge behavior is reasonable (18).
In addition to drugs of abuse, many natural reinforcers that aid in the maintenance of healthy and productive lifestyles can, at least in some individuals, promote binge-like behavioral routines despite severely negative consequences. While a balanced diet is necessary for survival, excessive overeating without control over food intake (i.e., binge eating disorder) is tightly correlated with obesity and a perpetuation of health problems (19). Sexually compulsive behavior (e.g., sex binging) is characterized by extended periods of time engaged in sexual encounters or activities that are socially or occupationally disruptive (20). As with binge eating disorders, compulsive sexual behavior tends to occur intermittently and is separated by intervals of sex-avoidance (21). Both binge eating and compulsive sexual behavior can emerge in adolescence or in adulthood, and predictors for their onset typically include early life stressors (i.e., social stressors including sexual abuse) as well as low scores on rating scales of self-esteem (20, 22). Similarly, a diagnosis for compulsive buying, or “binge shopping,” is determined by an irresistible urge to shop in the absence of conscious control over spending behaviors (2). Binge shopping occurs through an uncontrollable urge to excessively accumulate personal items with intentions of enhancing one’s image reflecting potential deficits in self-esteem.

Behavioral similarities that occur across various compulsive disorders suggest the existence of an underlying neural mechanism controlling binge-like behavior. Particular attention has been placed on the mesocorticolimbic dopamine system, as activity within this circuitry is critical for promoting the natural rewarding properties of stimuli such as food and sex, and has also been demonstrated as being essential in the expression of behavioral responses to drugs of abuse (23–27). In addition, while impulsivity may exist in several distinct forms, impulsive acts typically precede all compulsive drives (28). Mood and anxiety disorders also accompany each of these compulsive disorders at significantly higher frequencies than their rate of occurrence in the overall population (29, 30).

2. Experimental Models of Bingeing

Significant advances have been made through the establishment of experimental models that aid in the exploration of the neural and behavioral consequences of bingeing for a variety of substances. Importantly, progress in this area has benefited greatly from exploiting the use of interval and ratio-based schedules of reinforcement (31), which allow for conditions of varying access to a particular reward (32). Reinforcers of binge-like behavior vary considerably regarding their ability to disrupt behavioral
performance. Those that significantly disrupt ongoing behavior (e.g., alcohol, food) appear to benefit from protocols that provide limited access to reinforcement (i.e. access over the course of a few hours each day or on intermittent days of the week). In contrast, reinforced behavioral responses that can be maintained continuously over prolonged periods of time such as with psychomotor stimulants can be closely examined by employing fixed ratio (FR) schedules of reinforcement. Given the heavy burden of compulsive disorders as defined by personal, social, and economic indices, it is critical that we further our understanding regarding specific patterns of bingeing behavior.

Currently, two questions are in particular need of resolution. First, an exploration of the environmental conditions that promote the transition from controlled to uncontrollable behaviors must be undertaken. Second, it will be important to investigate the neural changes that occur during this transition, which ultimately enable and sustain binge behaviors. Insight gained from experimental strategies, which address these issues would arguably reduce occurrences of compulsive disorders through the development of novel intervention strategies and offer better forms of treatment. Unfortunately, at least three obstacles hinder experimental progress in the area of drug abuse pharmacology and therapeutic intervention. First, the general public and funding agencies have historically been skeptical of recognizing compulsive disorders as illnesses. Therefore many, if not most, of the individuals suffering from compulsive behavioral syndromes are not appropriately treated, at least in the initial stage. Second, human studies that focus on compulsive disorders primarily rely on self-reports or rating scales, often rendering it difficult to dissociate co-morbid influences on compulsive habits of interest, including the interplay between environmental and genetic factors contributing to these disorders. Lastly, it remains challenging to experimentally study binge behavior in animal models that incorporate natural reinforcers such as sex or food, and it is similarly challenging to examine experimentally binge behaviors such as shopping or gambling. A cardinal diagnostic criterion of compulsive disorders is the intense feeling of guilt, remorse, and regret following a binge (2), which also cannot be systematically measured in animal models. Most scientific advances contributing to our understanding of compulsion are derived from animal models of feeding and drug abuse. However, treatment strategies for illnesses such as pathological gambling have been successfully employed by extrapolating from mechanisms that have been characterized for other addictions (33).

There are distinct forms of continuous access, or experimen-tal binges, in preclinical models. One type of binge involves the experimenter injecting drugs to the animal independent, or non-contingent, of its ongoing behavior, such as repeated injections of
cocaine three to five times in hourly intervals (34). By contrast, a second type of binge consists of self-administration models of continuous access that allows the animal to control their rate of consumption (35). There are clear and unique characteristics in the degree to which contingency between an active response and drug delivery can affect brain function and behavior. Animals receiving infusions of cocaine noncontingently of a specific behavioral response have higher mortality rates than those that self-administer cocaine at similar dose-ranges and rates of administration during conditions of extended access (36). Systematic manipulations of self-administration protocols for food, psychomotor stimulants, opiates, or alcohol can be studied under well-controlled access conditions that allow for prolonged intervals of intake. We describe in the next sections of this chapter how many of these substances can reliably reinforce a behavior during conditions of long access. The primary focus in the current discussion will pertain to uncontrollable taking of drugs and other commodities and how certain individual, pharmacological, and environmental factors can facilitate binge patterns of self-administration. Even still, it will be important for future studies to examine the neuronal and behavioral differences between individuals that are and are not capable of voluntarily ceasing their behavioral response during prolonged periods of intake, at the cost of many other physiological needs.

3. Cocaine Binge

It is estimated that approximately 16% of the general population has tried cocaine at least once (37); however, those that abuse cocaine only represent a small proportion of total users (38). Estimates report that <1% of the total population of the USA abuses cocaine (39), as defined by criteria established by the DSM-IVR (2). Remarkably, this small but significant sub-population of cocaine users is expected to consume more than 90% of the available drug supply each year during binges (40). The development of cocaine dependence by the user is not a subtle transition; the gradual loss of control over cocaine that occurs with intermittent usage is revealed during an initial prolonged binge. Thereafter, subsequent cocaine binges typically last for as long as the drug remains available to the user (i.e., lasting between 4 h and 6 days) (41, 42). Withdrawal symptoms after a cocaine binge primarily include changes in affect. These symptoms emerge and persist until the next binge is initiated, which is typically days or weeks later. Thus, like other forms of drug abuse, cocaine dependence is recognized as a relapsing disorder accompanied by a concurrent escalation of intake. Identification of specific risk factors that
promote the transition from intermittent cocaine use to escalated binge taking continues to challenge researchers and clinicians. The difficulty in identifying risk factors is likely due to the small number of recreational users that actually binge, and the long delay prior to initiating binge behavior, a process that often takes years to develop (41, 43, 44).

Experimental intravenous self-administration models that allow for limited daily access to cocaine (e.g., ca. 15 mg/kg/day or 2 h of access) reveal a pattern and rate of behavioral responding that is well controlled and remains stable for long periods of time in rodents and primates (45–47). These access conditions have permitted the systematic assessment of rates of responding under the control of various schedules of cocaine reinforcement, the role of neural modulators on cocaine’s reinforcing effects and genetic contributions to the propensity to take cocaine (for reviews see 48–51). Most self-administration studies that examine the neural and behavioral impact of cocaine have used limited access conditions, because shortly after the introduction of intravenous self-administration techniques it was observed that prolonged access to cocaine resulted in lethality (52). When access to cocaine is continuous, cocaine responding is initially highest during the active phase of the sleep/wake cycle. Within only a few days circadian-controlled patterns of self-administration behavior deteriorate, during which time cocaine usurps complete control over behavior (53, 54). The dysregulated pattern of self-administration that emerges after a few days of bingeing consists of a combination of intense intervals of responding separated by intermittent pauses in cocaine-taking behavior. By comparison, heroin self-administration can be maintained for much longer durations before behavior becomes dysregulated, or mortality ensues (55). Despite the difficulties in studying cocaine self-administration under extended access conditions, long access conditions reveal a pattern of self-administration behavior that resembles the high levels of intake during cocaine dependence in human populations (35, 56–61).

Given our current understanding of patterns of intravenous cocaine self-administration that occur over extended access, experiments have been successfully designed to examine the neural and behavioral effects of prolonged access without producing lethal outcomes (62–65). When provided with unlimited access to cocaine for 12–16 h at dose ranges between 0.25–1.5 mg/kg/infusion, rats will respond continuously under the control of cocaine reinforcements at very stable and predictable rates (60, 66). The effect of 12 continuous hours of cocaine self-administration produces alterations in the concentration of biogenic amines in the nucleus accumbens quite differently in comparison to more limited access conditions (e.g., 3 h access) (58, 67, 68). Throughout 12 h of continuous access, extracellular
concentrations of dopamine and serotonin increase significantly more than those observed at baseline before the bingeing behavior commenced (68). Within an hour after termination of a 12-h binge, however, serotonin and dopamine levels fall significantly below baseline concentrations and remain suppressed for at least 6 subsequent hours (58, 67). The changes in dopamine and serotonin concentrations observed in rats at the cessation of continuous cocaine access correspond very closely to the onset and duration of affective disturbances (e.g., anhedonic, anxiogenic and depressive-like symptoms) that occur during withdrawal from binges in the clinical population (43). Likewise, intracranial self-stimulation (ICSS) thresholds correlate with changes in dopamine and serotonin levels in the nucleus accumbens during and after prolonged access to cocaine (35). Specifically, ICSS thresholds are lowered during cocaine taking, an effect that is interpreted to reflect increased sensitivity to rewards. In contrast, ICSS thresholds are significantly increased shortly following a 12-h cocaine binge, and this increase in set-point persists up to 72 h. These deficits in ICSS thresholds are interpreted to reveal an anhedonic state, which occurs clinically after a cocaine binge (69). Interestingly, the duration of cocaine access and the amount of cocaine intake during conditions of extended access correlate tightly with changes in ICSS thresholds (14, 35). When studies in rats allow for continuous access to cocaine, an increased emission of ultrasonic vocalizations in response to tactile stimulation (e.g., a 15 psi air puff) shortly after a 16 h binge also indicates heightened levels of anxiety (70). Deficits in ICSS thresholds and hypersensitive startle-induced vocalizations after a binge can both be restored to baseline following renewed access to cocaine or the dopamine agonist bromocriptine (69, 71). These data support the hypothesis that affective withdrawal symptoms may further promote drug seeking behavior and continue the addiction cycle (1).

In addition to studying the effects of cocaine bingeing on the brain and behavior, it is equally important to understand how environmental influences can promote such an intense behavior. Extending access conditions slightly beyond the regulated phase of cocaine taking (e.g., beyond 16 h following the start of a binge) promotes a shift to irregular patterns of responding (60). Such dysregulated responding occurs later during the cocaine binge and is the result of a “burst and pause” pattern of responding, as rats will cease to self-administer cocaine over long intervals of time such as at the onset of their light phase (54). The typical pattern of circadian-controlled responding for cocaine during a 12-h
binge can be disrupted by a prior history of stress or drug challenges in such a way that responding for cocaine remains continuous over the later hours of a binge (see Fig. 1) (73). Thus, several questions regarding the types of environmental influences that can promote high levels of intake during binges can be addressed. In addition, the concept of “controllability” over cocaine taking during a binge can be examined in two separate ways. First, control over cocaine intake for long periods of time can be defined as an ability to regulate intake by adjusting to changes in the dose that is received in a predictable manner. These adjustments are operationally defined by a correspondence between a specific dose of cocaine and the following post infusion interval (PII) (e.g., 0.2 mg/kg/infusion is followed by a 2 min PII, and a 0.4 mg/kg/infusion by a 4 min PII). Second, control over cocaine intake can
be defined as an ability to limit intake within a defined interval of time. Using well-established models of binge-like behavior (63, 74), it becomes possible to observe whether certain factors lead to dysregulated responding or a persistence of responding as compared to non-manipulated control conditions. Thus, an advantage of prolonged binge protocols is that they allow for an examination of individuals exposed to different environmental, social, and/or genetic factors on subsequent patterns of intake during a binge.

Behaviorally sensitized animals will self-administer significantly more cocaine during conditions of extended access (75). In particular, 24-h binges reveal that prior injections of cocaine, morphine or exposures to episodes of social defeat stress increase the persistence of responding for cocaine, corresponding to increases in the cumulative intake of cocaine as compared to nonsensitized controls (see Fig. 2). The persistence of cocaine responding can be compared across various treatment groups by performing survival analyses of the percentages of animals responding under each condition over consecutive hours of the binge (see Fig. 3). Vulnerability to cocaine addiction varies significantly across

![Graph showing cumulative cocaine intake over time for different conditions.](image-url)

Fig. 2. Behavioral sensitization increases the persistence of responding for cocaine during conditions of continuous access. The pattern of cocaine self-administration during a 24-h binge in stress-sensitized (filled circles, n=8), cocaine-sensitized (filled triangles, n=8), morphine-sensitized (filled squares, n=8), and control rats (open circles, n=24) is shown in the top insert. The last 8 h of the 24-h binge in groups of sensitized and control rats are highlighted in the lower graph. Total cocaine intake at hour 24 is significantly different between stress or cocaine-sensitized and control rats (Reprinted from (75). With permission).
different populations of drug users. These data support the hypothesis that stressors, similar to a history of drug administrations, can potentially facilitate binge behavior (76). Since the introduction of “stress” to the field of physiology and medicine, this concept has been linked to the pathogenesis of several CNS disorders (77, 78). Empirical evidence supports the hypothesis that certain types of environmental stressors enhance the incidence of psychiatric illness (for reviews see 76, 79, 80). However, stress-induced increases in cocaine bingeing are sensitive to parameters of stress exposure including the intensity, duration, intermittency, and controllability of the stressor (64). Brief intermittent social stress episodes appear to be critical for the emergence of social stress-induced binge cocaine taking in rodents (75, 81). Brief episodes of social defeat stress sensitize behavioral, physiological and neural responses to subsequent stressors and stimulant challenges. Conversely, repeated aggressive acts that are also inherently stressful do not induce behavioral sensitization or promote an increase in cocaine bingeing (see Fig. 4) (82).

Episodic social defeat stress, like intermittent psychomotor stimulant administration, depends on NMDA receptor activation in the VTA during the development of augmented behavioral responses (83, 84). Brief exposures to stress appear to stimulate

![Graph showing percent responding throughout binge](image)
neural responses that undergo adaptations in dopamine cell bodies and that intensify drug taking behavior over the later hours of a 24-h binge. Blockade of NMDA receptors in the VTA during episodic social defeat protects against the behavioral tendency to prolong cocaine self-administration during later hours of continuous (24 h) cocaine access (see Fig. 5). The impact of brief episodes of social stress on persistent cocaine binging is also long lasting, as it persists for at least 2 months after the last social defeat event, a further indication that enduring neuronal plasticity in brain reward processes is induced by some stressors (73).

3.2. Controllability: Stability of the Rate of Cocaine Intake During a Binge

In addition to the process of behavioral sensitization, a history of extended access to self-administered cocaine (i.e., 6-h sessions/day, which leads to a significant escalation of intake over consecutive sessions (56)) also increases cocaine intake during a 24-h variable-dose binge (see Fig. 6, top) (85). The process of escalation entails an increase cocaine intake during a 24-h binge, particularly during the first half of the binge (see Fig. 6, top). In addition, the persistence of cocaine intake over the later hours of a 24-h binge
is intensified, primarily, by the earlier experience of intermittent social defeat stress (see Fig. 6, bottom), or intermittent administration of psychomotor stimulants (75).

Experimental binge assays that use multiple doses of cocaine within a 24-h interval of unlimited access have begun to elucidate features of dysregulation in the rates of cocaine taking. Initially, experiments were designed to examine the effects of social defeat stress on an animal’s ability to adjust to varying doses of cocaine reinforcement, while levels of cocaine intake increase during unlimited access to the drug (60). This goal was accomplished by a systematic analysis of the post-infusion interval across varying unit doses of self-administered cocaine (0.2, 0.4 or 0.8 mg/kg/infusion, in irregular sequence) conducted over a 24-h continuous access binge. Previous observations suggest that rats begin to lose control over the rate of intake only after extended hours (>10 h) of cocaine self-administration (60, 75). Therefore, the effect of behavioral sensitization could arguably either strengthen or weaken...
the response contingency for cumulative cocaine infusions at the time during which persistence of cocaine taking is expected to occur. An analysis of post-infusion intervals reveals that social defeat stress decreases the length of the intervals that follows doses of cocaine infused over the entire binge (see Fig. 5a and b). Second, it is clear that sensitized rats continue to adjust their rate of responding to varying unit doses of cocaine, as indicated by a lack of variation in the post-infusion interval for each dose delivered over the course of a 24-h binge (see Fig. 5a). In support of the hypothesis that prolonged cocaine bingeing develops as
animals gain control over their rate of cocaine self-administration, rats have been observed to significantly reduce their overall variation in responding over repeated 16-h access binges (see Fig. 7). Specifically, variations in responding that are prominent after 10 or more hours of cocaine self-administration are largest during an initial binge, as compared to subsequent binges. Thus, continuous binge intake appears well controlled, as defined by the ability to adjust to varying doses.

An animal’s ability to continue responding in a reliable and stable manner for cocaine over long periods of time occurs in the presence of intense stereotyped behavioral routines (see Figs. 1 and 8) (86). Tolerance to the rate disruptive effects of cocaine-induced stereotyped behavior may contribute to an ability to self-regulate higher amounts of cocaine taking, although this has not been experimentally verified.

Individual variations in cocaine intake can predict vulnerability to cocaine taking behavior during prolonged binges (87). Adaptations in dopaminergic signaling in response to extended access to cocaine (14, 15, 64) strengthen subsequent responding for cocaine by increasing response rates, stabilizing the ability to adjust to changes in unit doses of the drug and increase the persistence of responding. Intermittent social stress episodes that

![Graph showing cumulative cocaine intake and variability in response over repeated binges](Image)
facilitate prolonged bingeing correspond with decreased functional activity of cells in the infralimbic and prelimbic prefrontal cortex and increased functional activity of the amygdala (73); changes that are consistently observed in cocaine addicts (88). The prefrontal cortex and amygdala are critical for the attentional, goal-directed, repetitive, and hedonic components of cocaine self-administration behavior (15). Future experiments that systematically dissect the roles of particular forms of neuronal plasticity on binge-like forms of cocaine taking are bound to provide much needed translational information for the initiation, termination, persistence, and reoccurrence of cocaine abuse (89, 90).
4. Opiate Binge

Estimates report that over 500,000 Americans try heroin each year, and in 2007, approximately half of that number were dependent on, or abusing, the drug (39). Heroin abuse is sustainable for years and is characterized by stable and regular patterns of intake (91). This style of opiate use deviates from the cyclical patterns of behavior typically associated with other drugs of abuse, as in the case of cocaine bingeing (92). The use of opiates, such as heroin, results in severe physical dependence within a rather short period of time. Therefore, maintenance administrations of opiate agonists are often necessary to relieve withdrawal symptoms during periods of abstinence (93). Transitioning from initial use to heroin abuse is characterized by significant increases in daily intake, yet these increases usually occur at seemingly predictable intervals in order to reduce the opportunity for withdrawal symptoms to occur. As a result of intense withdrawal, opiate bingeing is likely to occur more frequently than other forms of bingeing (e.g., food, sex, cocaine), leading to notably high rates of morbidity and mortality (91).

In 1962, James R. Weeks first implemented the use of a swivel to allow for minimally restrictive intravenous access to morphine in rats in order to examine the effects of opiate self-administration over extended periods. By incorporating the use of an operant behavior (e.g., lever pressing) with intra-jugular catheter infusions, Weeks demonstrated how morphine can be reliably self-administered over long periods of time. Predictive, external and face validity of continuous access to opiates was thus established, as morphine was found to maintain stable levels of operant responding during 25–30 h of unlimited access across an extended range of doses. Small increases in the FR requirement increased behavioral responding during continuous access, but did not alter the rate of drug intake. Rates of intake are, however, controlled by the unit dose of morphine infused (i.e., higher doses promoted fewer infusions). The pattern of intake clearly reveals the rapid reinforcing and sedative effect of opiates since each infusion was immediately followed by a long pause in responding. In fact, Weeks found that patterns of responding were well maintained with a 10 mg/kg morphine dose that results in only two infusions/hour. The opioid receptor antagonist nalorphine systematically increased rates of responding confirming a critical role for opioid receptors in morphine’s reinforcing effects. Classical withdrawal signs (e.g., tremors, diarrhea, increased respiration, circling, and rearing (94, 95)) appear within 24 h following the discontinuation of a morphine binge documenting that physical dependence can be maintained by conditions of extended access (it is important to reemphasize here that physical dependence was
initially established in this pioneering study via experimenter-administered morphine prior to self-administration).

It is difficult to model uncontrollable opiate use during a single binge since most animals will only self-administer during their active phase, due to a circadian cessation of drug taking at the onset of the inactive phase. Continuous access to opiates over numerous days reveals a gradual increase in daily intake; an effect that holds true for humans, nonhuman primates, and rodents (53, 55, 96, 97). Experimental approaches that model the transition to heroin dependence have revealed both escalated intake and persistent patterns of responding over repeated opiate binges; however, this effect takes an extended period of time to arise as compared to behavioral changes observed after only a few cocaine binges (55). Koob and colleagues (98) provide a clear example of how prolonged heroin access (23 h/day) influences patterns of self-administration over repeated days. During the first week of access, intake is relatively stable, with responses occurring primarily during the dark (i.e., active) phase. An escalation of heroin intake is observed in the first 4 days of access as indicated by increased numbers of self-infusions within the first hours of each binge. Within 3 weeks, a diurnal pattern of responding clearly emerges. Specifically, the nocturnal peak and the diurnal nadir during each photo cycle increases over consecutive days of access. Within 3 weeks, average daily intake curves are significantly elevated. Thus, it appears that during unlimited access to heroin, like cocaine bingeing, a shift in an individual’s control over intake occurs in two separate ways: (1) by inducing an “escalation” in the rate of intake at the beginning of access and (2) by progressively increasing the “persistence” of responding over long periods of time.

In response to opiate administration, both tolerance and sensitization occur concomitantly. Tolerance to the depressant and analgesic effects of opiates after repeated administration depends on excitatory amino acid receptors (99, 100). At the same time, excitatory amino-acid-dependent behavioral sensitization becomes evident (100). Excitatory amino acids appear critical for the induction of tolerance and sensitization to opiates and psychomotor stimulants, however, they do not appear to play a role in their long-term expression (101, 102). This implies that immediate responses to opiates promote lasting changes in down-stream neural mechanisms. Both tolerance and sensitization processes may contribute to opiate dependence, opiate bingeing and a propensity for relapse to drug taking after long periods of abstinence (103). Significant decreases in the expression of dopamine transporters and opiate receptors, in the ventral tegmental area, and hypothalamic nuclei, respectively, are likely to contribute to the dysregulation of normal behavioral routines and progressive increases in opiate intake (104, 105). The emergence of increased
wakefulness over successive days of opiate intake, and corresponding changes in the pattern of food intake, imply that the types of neural mechanisms that control circadian processes (i.e., behaviors following the sleep/wake cycle) become disrupted after repeated opiate administration, particularly after high levels of intake that occur during opiate binges (96, 106).

5. Alcohol Binge

Half of the population of the USA occasionally drinks alcohol, and about 20% binge drink (i.e., more than 5 drinks on one occasion) each month (39). Binge drinking most often occurs at weekly intervals, and only 7% of the total population consists of binge drinkers that do so more than five times in a given month. Increasingly more adolescents take part in alcohol binging, further emphasizing the need to understand the neurobiological factors that contribute to alcoholism and interventions for treatment (39, 107, 108).

Humans, nonhuman primates, and rodents present an extensive range of individual preferences for alcohol within and across their respective populations, at least when self-administering orally (109–111). Individual preferences for alcoholic solutions have helped identify trait characteristics that may relate to genetic, environmental or developmental predispositions for bingeing behavior (112–114). Irregular patterns of alcohol intake are commonly reported when using animal models that provide continuous access to alcohol, and it is therefore difficult to achieve the high blood concentrations observed clinically after binging (115). Only a small portion of monkeys (Macaca fascicularis) allowed continuous access to alcohol are inherently susceptible to developing patterns of intake that lead to high blood concentrations, an effect that occurs predominately in males (116). These monkeys steadily increase their daily alcohol intake within the first 2 weeks of access, until approximately 4 g/kg are consumed during each session. As with humans, binge drinking behavior in monkeys is limited almost exclusively to the active phase of the light/dark cycle, with minor changes in the pattern of daily intake for up to 6 months (116).

Despite taste preferences, voluntary alcohol drinking can be intensified in rodents and monkeys by manipulating the initial exposure to alcohol using alcohol fading schedules, or forced exposures to an alcohol-rich vapor (117–119). Alcohol fading procedures typically begin by allowing access to a sweetened drinking solution, to which an alcohol concentration is gradually added, and alcohol content is progressively increased over repeated trials. Eventually, the sweet is faded out of the solution in a gradual
manner over consecutive days. This method can achieve dependence on alcohol, and levels of intake increase significantly over time, with blood concentrations reaching ca. 150 mg/100 mL during daily binges (120). Binge-like drinking in rodents persisted for weeks after inducing dependence, indicative of lasting changes in the brain that sustained binge patterns of alcohol intake. Fading procedures can identify environmental factors that promote high levels of intake, as rhesus monkeys (Macaca mulatta) with a history of consuming sweetened alcohol solutions in a social environment at later time points drank high levels of unsweetened alcohol when isolated from their social group (121). However, the impact of environmental and social stressors on binge drinking is not obvious, as mostly decreases in alcohol intake have been reported in response to acute and chronic stressors (122–124). In light of these and epidemiological data, there remains substantial evidence to support an interaction between the effects of stress and alcohol intake (63, 125).

Exposure to ethanol vapors also readily induces alcohol dependence, and facilitates escalated or binge-like patterns of intake in rodents (126, 127). Intermittent exposure to alcohol vapor facilitates an escalation of voluntary intake more effectively than continuous vapor exposure (128). Intermittent exposures to alcohol vapor also progressively sensitized withdrawal behaviors in mice, indicating that a sensitization process, in addition to tolerance, contributes to increased binge drinking (129). In fact, administration of alcohol, like opiates, clearly induces both tolerance and sensitization and both of these processes depend on NMDA receptor activation (130–133). Unfortunately, it is difficult to identify with precision whether or not the consumption of alcohol during binges after experimental manipulations results from increased rates of intake within a given time or if intake is prolonged over an access period, although both patterns of responding are likely to occur (116). It is clear, however, that prolonged intervals of alcohol deprivation after daily limited access can substantially increase responding for alcohol when access is renewed, a behavioral phenomenon shared with other types of experimental bingeing that are typically dependent on an oral route of administration (134).

### 6. Binge Eating

Binge eating disorder is the most common eating disorder affecting approximately 5% of the general population (i.e., this disorder is more highly represented in the population than both anorexia nervosa and bulimia nervosa combined) (30). Patterns of behavior that characterize binge eating are typical of other abusive
behaviors that involve bingeing. For example, binge eaters display self-imposed restrictions over food intake, or over intake of a specific type of food, for sustained intervals of time. These periods of caloric deprivation are subsequently followed by prolonged episodes of high calorie binge intake (135). During these binges, significantly higher amounts of food are consumed in comparison to what constitutes a normal meal, and the binge is most often followed by feelings of remorse and guilt (2).

Experimental attempts to model binge eating behavior in animal models were initiated in the mid-twentieth century. Immediately following the advent of operant conditioning procedures, and with the development of experimental conditions that promote increased or decreased rates of responding for fluids and palatable foods, it became possible to explore protocols that could be used for inducing a substantial increase in food intake (136–138). Earlier work on compulsive overeating identified how social stressors (e.g., social isolation, maternal separation, etc.) and environmental stressors (e.g., food deprivation, footshock, tailpinch, etc.) can immediately and persistently escalate eating behavior in rats (139–141) and monkeys (142). Given the strong correlation between stress and overeating in clinical populations, results from these types of experiments have provided much insight into the neural mechanisms mediating stress-induced consumatory behaviors. For instance, acute stressors that induce a strong and rapid increase in food intake depend upon dopaminergic and opiate receptor activation (139, 143). Chronic mild stressors also lead to elevations in feeding, and an interaction between stress and diet is correlated with increased brain expression of brain-derived neurotrophic factor (BDNF), arginine vasopressin (AVP) and the cocaine-amphetamine regulator of transcription (CART) in male, as well as leptin in female, rats (144).

Experimental conditions that restrict food availability and limit access to food at specific times can greatly increase food consumption during intervals of access, particularly in rodents (32, 145). Limited access conditions have proven to be useful in examining numerous consequences of binge eating due to the face validity of this procedure, since the human condition is also characterized by cycles of food restriction and overeating (135). Binge eating by rats during limited access can be accomplished without food restriction, but simply by permitting access to fatty and sweet foods within predetermined intervals (e.g., for 1–2 h several times a week). Intermittent access to a high calorie diet escalates over the first few access sessions, and this form of bingeing behavior remains stable for extended periods of time. Escalated food consumption during binges can be inhibited by the administration of GABA-B agonists and opioid antagonists in lower dose ranges that do not affect normal feeding behavior (146, 147).
Intermittent access to a sugar solution has been employed to study how high sugar intake induces behavioral and neural adaptations that are similar to those induced by drugs of abuse. Intermittent exposure to sugar can induce behavioral cross-sensitization to psychomotor stimulants and produce a withdrawal syndrome during intervals when sugar access is discontinued (148, 149). Typically, food intake is associated with an initial rise in the release of dopamine into the nucleus accumbens, possibly indicative to its hedonic effects; this effect dissipates with renewed access (150). With repeated sugar binges, however, accumbal dopamine release continues to be reliably elevated (151). Likewise, withdrawal from a sugar binge corresponds to a decline in dopamine concentrations with a concomitant increase in acetylcholine levels in the nucleus accumbens. This result is similar to the effects of withdrawal from opiates and psychomotor stimulants (149). Increases in dopamine D1 receptor expression, decreases in dopamine D2 receptor expression and decreases in the expression of enkephalin in the nucleus accumbens have been reported after intermittent access to sweetened solutions, which has also been reported after psychomotor stimulant and opiate administration (149, 152, 153). Allostatic shifts in the modulatory effect of opioids, dopamine and acetylcholine within the nucleus accumbens in response to eating behavior is potentially related to a negative affective state that facilitates later bingeing (154). Thus, models of addiction to sweet substances have led to the proposal of neural “triggers” that may underlie the emergence of binge-eating patterns of behavior (154).

7. Summary

1. Studies under very long access conditions have revealed that animals will binge on various substances that are abused by humans. Experimental binges approximate isomorphic and mechanistic models of abusive behavioral routines, both of which are considered important criteria for translating data from animals to human psychiatric disorders (155).

2. Intense bingeing can occur through two distinct changes in behavior: an increase in the rate of bingeing, or by an increased persistence of binge behavior. Increased rates and persistence in drug intake under continuous access conditions have highlighted a role for specific environmental and genetic factors that promote vulnerable phenotypes for binge-like behavior.

3. Overlap in behavioral routines and neural mechanisms that are necessary for promoting binge behavior for various commodities indicates that a switch from drug “use” to “abuse”
may be related to the dysregulation of neural circuits that share common elements.

4. Future experiments promise to add substantial insight into the mechanisms via which therapeutic interventions reduce the rate or duration of binge behavior.

Acknowledgments

The experimental work from the authors’ laboratory was supported by R01 DA002632 (KAM, PI). The authors also wish to thank Ian Maze and Amy Arguello for their valuable comments on this chapter.

References

and nonautomatic processes. Psychol Rev 97:147–168
43. Gawin FH, Kleber HD (1986) Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations Arch Gen Psychiatry 43:107–113
72. Morgan D, Brebner K, Lynch WJ, Roberts DC (2002) Increases in the reinforcing...
efficacy of cocaine after particular histories of reinforcement. Behav Pharmacol 13: 389–396


84. Vezina P, Queen AL (2000) Induction of locomotor sensitization by amphetamine requires the activation of NMDA receptors in the rat ventral tegmental area. Psychopharmacology (Berl) 151:184–191

85. Quadros I, Miczek KA. Two modes of intense cocaine binging: increased persistence after social defeat stress and increased rate of intake due to extended access conditions in rats. Submitted 2009.


99. Trujillo KA, Akil H (1991) Inhibition of morphine tolerance and dependence by the


131. Broadbent J, Weitemier AZ (1999) Dizocilpine (MK-801) prevents the development of sensitization to ethanol in DBA/2J mice. Alcohol Alcohol 34:283–288


Chapter 16

Withdrawal

Alasdair M. Barr, Heidi N. Boyda, and Ric M. Procyshyn

Abstract

Drugs of abuse generate diverse behavioral and physiological effects. One feature common to many abused drugs is the phenomenon of “withdrawal,” which results from abrupt termination of drug administration. The initial phases of drug withdrawal, often referred to as the “crash” phase in humans, are characterized by dramatic psychological changes which may or may not be accompanied by somatic symptoms. The psychological symptoms, including disruptions in affect and cognition, are most commonly observed following psychostimulant withdrawal. This transient state strongly resembles the symptoms of major depressive disorder (MDD) in humans. The majority of rodent paradigms of psychostimulant withdrawal accurately model this human condition, and sophisticated preclinical protocols have been used to quantify psychological changes, such as anhedonia. In the present review, we describe past and current progress in modeling psychostimulant withdrawal in rodents, as well as potential limitations of this model.

Key words: Amphetamines, Anhedonia, Behavior, Cocaine, Psychostimulant, Rodent, Withdrawal

1. Introduction

As discussed elsewhere in this book in detail, most drugs of abuse have acute physiological effects in both humans and animals. Many of these effects represent a strong deviation from the body’s natural state of equilibrium, and therefore endogenous homeostatic mechanisms are recruited throughout not only the body, but also the central nervous system, in order to counter and reduce the ongoing effects of the drug (1). In general, the greater the concentration and the longer the duration of drug exposure, the more pronounced the homeostatic counteradaptations (2). At some point during drug taking, either for voluntary or involuntary reasons, drug intake ceases. From that point on, the physiological effects of the drug rapidly decrease as the remaining drug
is metabolized and cleared from the body. By contrast, the drug counteradaptations typically do not diminish nearly as rapidly. The body is therefore left in a state where potent homeostatic mechanisms remain essentially unopposed; this represents one aspect of the complex phenomenon described as “drug withdrawal.” The dramatic “somatic” withdrawal symptoms that are frequently associated with termination of central depressants, such as heroin and alcohol, come most vividly to mind when considering the effects of drug withdrawal (3, 4). These include sweating, muscle aches and cramping, nausea, vomiting and diarrhea for opiates, and tremors, seizures, and even death for alcohol. Eventually the counteradaptations subside, but this may require considerable time.

In a similar manner to the overt physiological effects of abused drugs, the acute psychological and pleasurable effects of drugs of abuse are also countered, presumably as a consequence of homeostatic physiological adaptations. During the period of drugs withdrawal, adaptations to prolonged drug exposure contribute to a cluster of psychological symptoms (5, 6). For over three decades, these psychological effects of drug withdrawal have been described within the theoretical framework of an opponent-process theory of motivation (7) (see Fig. 1). Consistent with this theory, the previously pleasurable effects of different drugs of abuse are inevitably followed during withdrawal by emotional states opposite in effect, and of a longer duration, as the body seeks to restore its “hedonic equilibrium.” This dysphoric state, whose psychological, behavioral, and physiological indices represent the operational definition of the term “withdrawal” as used in this synopsis, are most pronounced after abstinence from classical psychoactive drugs, which include both depressants and stimulants. However, in the case of depressant drugs, the marked somatic symptoms of withdrawal represent a potent confound in measuring the more

![Fig. 1. The opponent-process theory of motivation, as envisioned by Solomon and Corbit (1974). According to the model, emotional stimuli or experiences evoke one emotional state (a), which then triggers an opponent state (b), which serves to bring the emotional state back to a neutral or baseline point. (a) Initial presentation of stimulus. The first stimulus present elicits a large emotional change (a). The (b) state persists beyond the stimulus presentation, leading to an emotional change that is opposite to the (a) state. (b) Stimulus presentation after repeated exposures. With experience, the (b) process increases in strength and duration such that it is more effective at countering the emotional changes associated with the (a) state. This leads to a larger peak of the opponent emotional change after the stimulus is removed.](image-url)
subtle psychological effects, particularly when studying withdrawal effects in animal models (5). This can be due to interference of physical symptoms on dependent measures that are utilized to assess underlying psychological states, or because the physical symptoms themselves are distressing.

For this reason, the most informative work on the psychological effects of drug withdrawal has been performed with animal models of exposure to psychostimulant drugs. This class of drugs represents a range of different compounds, which include milder central nervous system stimulants (typically legal) such as caffeine and nicotine, through to potent stimulants (typically illegal) such as cocaine and methamphetamine. While exposure to higher doses of stimulant drugs, particularly nicotine, can result in some somatic symptoms during withdrawal (8, 9), these effects are much less obvious and severe than with the depressants. Thus, there are far fewer confounding physical symptoms to interfere with measurement of underlying psychological states in psychostimulant withdrawal. Thus, rodent paradigms of psychostimulant withdrawal have allowed the detailed investigation of the complex behavioral and affective sequelae associated with the termination of drug intake.

2. Neurobiology of Psychostimulant Drugs

2.1. Molecular Mechanisms of Psychostimulants

Uncovering the neural substrates and molecular mechanisms of psychostimulant drugs provides insight into the physiological counteradaptations that accompany drug withdrawal. Psychostimulants activate the central nervous system directly, with the more potent drugs increasing the activity of both peripheral and central monoaminergic and cholinergic systems. A subclass of psychostimulant drugs acts on the central nervous system mainly through non-exocytic mechanisms. The mechanism of action of amphetamines, a commonly used class of psychostimulant drug, has been described in extensive detail recently in several excellent reviews (10, 11). The principal mechanisms underlying amphetamines’ physiological properties are outlined in Fig. 2. The large majority of studies have focused on the capacity of amphetamines to modulate dopamine release, due to this neurotransmitter’s crucial role in addiction, and therefore we have a much more detailed understanding of how amphetamines act on dopamine substrates than we do for other monoamines, such as serotonin and norepinephrine.

Earlier studies characterized several biochemical pathways that modified dopamine levels in the brain. Reports in the 1970s and early 1980s observed that treatment with amphetamines enhanced dopamine synthesis by increasing tyrosine hydroxylase activity (12, 13). This effect, though, may be biphasic, with lower
to medium levels of drug increasing enzyme activity, but higher levels of amphetamines inhibiting dopamine synthesis. The physiological mechanisms that account for increased tyrosine hydroxylase activity are largely unknown. Amphetamines also increase dopamine levels by inhibiting monoamine oxidases (MAO), the enzymes that are present on the outer mitochondrial membrane that are responsible for amine catabolism in presynaptic terminals. Amphetamines are competitive inhibitors of MAO. Evidence indicates that some amphetamines exhibit a differential affinity for MAO-A versus MAO-B (14).

Recent examination of the mechanism of action of amphetamines has focused on two main molecular substrates on dopamine neuronal terminals: the plasmalemmal dopamine transporter (DAT) and the vesicular monoamine transporter-2 (VMAT-2). The plasmalemmal DAT (15) has been studied most extensively in its relation to the physiological effects of amphetamines,
although the results of studies examining the norepinephrine (NET) and serotonin (SERT) transporters are generally consistent with the DAT studies. Considerable evidence suggests that amphetamines are a substrate of the Na\(^+/\)Cl\(^-\)-dependent DAT. At a molecular level, based on the exchange-diffusion model (11), amphetamines compete with synaptic dopamine at the extracellular site on the DAT. As concentrations of dopamine are much greater inside the cell than outside, and the DAT can transport dopamine in a bidirectional manner, the binding of amphetamines to the extracellular side of the transporter has the net effect of causing cytosolic dopamine to be reverse-transported outside of the cell. At higher concentrations of amphetamines, these lipophilic drugs diffuse directly across the plasmalemmal membrane and increase cytosolic concentrations of dopamine, meaning that this process may not even require binding to the extracellular site of the DAT. The activity of the DAT in the exchange diffusion model is highly dependent on a number of cell signaling pathways, as this molecular mechanism is closely regulated. Key signaling pathways include phosphatidylinositol 3-kinase (PI3K) and calmodulin-dependent protein kinase II (16, 17).

The DAT is able to cause non-vesicular release of dopamine through a second mechanism in addition to the slow exchange process, which has been characterized in stably expressed heterologous cell systems. This process involves a conformational change of the DAT into a “channel-like mode” that enables brief bursts of a dopamine efflux at a concentration resembling vesicular exocytotic release (18). Importantly, the DAT can also be internalized as part of an endocytotic recycling pathway. This property removes it from the plasmalemmal membrane and thus obviates its capacity to reduce synaptic levels of dopamine. In vitro studies in heterologous cell systems note that amphetamine drugs increase DAT internalization (19). This process remains an area of ongoing study, but there is mounting evidence that amphetamines increase the formation of DAT complexes, which may affect the rate at which the transporter can be recycled back to the cell surface (20).

The second major molecular substrate of amphetamines is the VMAT-2, which is an integral membrane protein that transports monoamines from the intracellular cytosol into synaptic vesicles, using a vacuolar-type H\(^+\) pumping ATPase (21). The ATPase produces a pH gradient across the vesicle membrane, with a mildly acidic vesicular internal environment of approximately pH 5.5 (22). According to the “weak base hypothesis” (11), amphetamines cause synaptic vesicles to release dopamine and other monoamines into the surrounding presynaptic cytosol by reversing the proton gradient between the inside and outside of the vesicle. Amphetamines are weak bases, with a p\(K_a\) of 9.9 (23). It is hypothesized that when these basic amphetamines enter the vesicle
in sufficient concentrations, the internal pH is disrupted. This decreases the capacity of the vesicle to store monoamines. Although this theory continues to remain a leading hypothesis for the mechanism of action of amphetamines, there is recent evidence that this effect can only occur at drug concentrations well above those when monoamine uptake by the VMAT-2 is evident (24). Thus, at lower concentrations, amphetamines may compete with the substrate of the VMAT-2 and inhibit, in a dose-dependent manner, the accumulation of the monoamines in the synaptic vesicles (25). Alternately, the role of VMAT-2 in the amphetamines’ action may involve its redistribution from the synaptic vesicle to an as-yet-unidentified cellular location (11). Converging evidence from experiments with rat striatal preparations note that multiple, high doses of amphetamines, designed to mimic human “binge”-like behavior, cause decreased vesicular uptake of dopamine. These studies noted that, while levels of the VMAT-2 were decreased in preparations that included only the vesicles, the concentration of VMAT-2 in entire homogenates remained unaltered (26, 27). These intriguing findings suggest that there was a redistribution of the VMAT-2 to an alternate, unknown, cellular location.

The effects of amphetamines and most other psychostimulants in the brain, therefore, are largely a result of the stimulation of monoamines. Based on local distribution of monoamine terminals, levels of monoamines increase in a regionally dependent manner. For example, monoamine release is greatest in regions of the densest monoamine terminal innervation, such as dopamine release in the striatum. Preclinical studies in nonhuman primates with procedures such as cerebral microdialysis have enabled us to measure directly extrasynaptic levels of monoamines; these studies indicate that monoamines can be substantially elevated after even single acute drug doses. For instance, a single dose of 1 mg/kg of methamphetamine in adult male rhesus monkeys elevated dopamine levels in the striatum by 1350% compared to baseline within 30 min, and remained close to 500% above baseline 2.5 h later (28). Given the ubiquitous functions and distribution of monoamines in the brain, it is likely that many other neurotransmitters and neuropeptides are altered in a secondary manner following the dramatic elevations in monoamines caused by psychostimulants.

Although it is not a focus for many preclinical studies, psychostimulants also have potent physiological effects on peripheral systems. It is therefore unclear whether peripheral changes contribute to the psychological effects of stimulant drugs and influence drug withdrawal. As an example, amphetamines stimulate epinephrine and norepinephrine release by the medulla of the adrenal glands, and can simulate the effects of activation of the sympathetic division of the central nervous system during a stress response (29). Thus, psychostimulants can acutely cause an acceleration of heart
and lung action through vasoconstriction and bronchodilation respectively, while muscle activity is primed via transient hyperglycemia and dilation of blood vessels in skeletal muscle. Nonessential physiological activity, such as stomach and intestinal function, is inhibited by many stimulants. Levels of cortisol (corticosterone in rodents) and adrenocorticotropic hormone, the major peripheral stress hormones, are increased by over 200% following administration of amphetamines (30) and remain elevated for hours after the final exposure to the drug. Chronic over-stimulation of the stress response by psychostimulant use may exceed the capacity of the organism to maintain normal health – a term referred to as “allostatic” load (31) – which can result in a range of medical problems, including severe cardiovascular complications related to chronic hypertension and cardiovascular disease. Additional medical problems in human psychostimulant users include respiratory symptoms, infectious disease, dental sequelae, and malnourishment. It is worth remembering that these medical comorbidities are rarely considered in rodent models of drug administration and withdrawal, yet they detract significantly from the quality of life for many stimulant drug abusers, and may be of greater concern for addicts than the less life-threatening psychological effects of drug withdrawal.

In order to model the effects of psychostimulant withdrawal in animals, it is first necessary to understand the phenomenon in humans. Fortunately, the behavioral and psychological effects of drug exposure and withdrawal have been well described for multiple psychostimulant drugs, including amphetamines and cocaine (in its different forms).

Understanding the initial effects of psychostimulant drugs is important, as the opponent-process theory predicts that the effects of drug withdrawal will be opposite to the initial changes. The type and severity of the acute psychological effects of most psychostimulants depend on a number of variables, including both the method of administration, as well as the amount of drug used. The most rapid onset of psychological effects occurs after either smoking or intravenous injection of the drug. Oral ingestion or snorting of the drug produces a delayed onset of psychological effects and reduces bioavailability of amphetamines, due to first-pass metabolism. Controlled laboratory studies of acute, low-to-moderate doses of psychostimulants show that they cause cognitive changes that include increased arousal, enhanced concentration, and improved attention. In addition to cognitive
changes, affective changes include decreased appetite and increased libido, as well as elevated mood and increased confidence (32). With higher acute doses, most psychostimulants may induce intense euphoria, often referred to as a drug “high.” However, when high doses of psychostimulants are administered for extended periods, they may cause dysphoria; symptoms include restlessness and anxiety, and are commonly associated with tremors and dyskinesia. With regards to “binge” drug use, which typically lasts over multiple days, the euphoric effects of the drug usually diminish while dysphoria and compulsive behavior increase throughout the course of the binge – an effect known as “tweaking”; this phenomenon is consistent with the predictions of the opponent-process theory. Chronic, binge users of psychostimulant drugs can exhibit highly focused and repetitive behaviors, known as “punding” (33), such as the stereotyped handling and sorting of objects. Importantly, substantial attention has been paid to the capacity of high and sustained doses of psychostimulants to cause psychological abnormalities that resemble the symptoms of psychosis (34). Psychotic symptoms frequently include sleeplessness, paranoia, hallucinations, and delusions (formication and grandiosity) and have been associated with irritability and unprovoked aggression. These latter physical and psychological symptoms that occur in chronic stimulant users represent a challenge to preclinical models of drug withdrawal at a number of levels. Firstly, they indicate that, with chronic use, the negative effects of drug exposure may be evident even before drug termination, and therefore the timing of withdrawal effects can be critical in animals. Secondly, the capacity of residual psychological symptoms such as psychosis, which can last well after drug termination and through the peak periods of drug withdrawal, may represent a subtle and largely unnoticeable confound in preclinical models, if homologous effects occur in animals.

One of the predictions of the opponent-process theory is that, with repeated drug exposure, counteradaptations occur that oppose the primary rewarding effects of the drug. Most experimental work is consistent with this hypothesis in that withdrawal from psychostimulant drugs produces a cluster of distinct psychological effects that contrast acute reward. A seminal paper by Gawin and Kleber (35) described, in detail, the abstinence symptomatology of chronic cocaine users, and determined that the course of the post-binge withdrawal syndrome was categorized into three distinct phases. The first of these, referred to as the “crash”, was characterized by “extreme dysphoria…full anhedonia…irritability, anxiety (and) a subjective sense of confusion,” as well as “temporary suicidal ideation” in 43% of the sample patient group. The initial crash phase lasted for up to 4 days, after which subjects entered the “withdrawal” phase, which was represented by milder dysphoria, substantial anhedonia, and anergia, with
increased anxiety and irritability. This “withdrawal” phase continued for up to 10 weeks, whereupon subjects gradually entered the “extinction” phase, which reflected a return to prior functioning. More recent studies have provided relatively little support for the multiphase sequence that Gawin and Kleber originally described, rather noting a gradual, linear decrease in the severity of withdrawal symptoms. However, the types of symptoms that Gawin and Kleber initially reported have remained consistent. When viewed in totality, these combined symptoms bear a striking similarity to those of major depressive disorder (MDD). This interesting similarity creates a more structured framework for describing the effects of psychostimulant withdrawal, and may also help us to understand better the neurobiological basis of drug withdrawal. The strong similarities between both disorders have resulted in the widespread assessment of psychostimulant withdrawal symptoms with psychiatric diagnostic tools that are most commonly used to measure MDD, such as the Hamilton Rating Scale for Depression and Beck Depression Inventory (6).

Depressive disorder is diagnosed based upon the presence of a number of symptoms, as defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (see Table 1). Symptoms

Table 1

<table>
<thead>
<tr>
<th>Major depression</th>
<th>Psychostimulant withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioral (DSM-IV criteria)</td>
<td></td>
</tr>
<tr>
<td>Depressed mood and/or irritability</td>
<td>Severely depressed mood and/or irritability</td>
</tr>
<tr>
<td>Diminished interest/pleasure in daily activities</td>
<td>Loss of interest/pleasure in daily activities</td>
</tr>
<tr>
<td>Large increase/decrease in appetite</td>
<td>Increase in appetite</td>
</tr>
<tr>
<td>Insomnia or excessive sleepiness</td>
<td>Excessive sleepiness</td>
</tr>
<tr>
<td>Psychomotor agitation or retardation</td>
<td>Psychomotor retardation</td>
</tr>
<tr>
<td>Fatigue or loss of energy</td>
<td>Fatigue and/or loss of energy</td>
</tr>
<tr>
<td>Diminished ability to think or concentrate</td>
<td>Poor ability to concentrate/confusion</td>
</tr>
<tr>
<td>Feelings of worthlessness and/or guilt</td>
<td></td>
</tr>
<tr>
<td>Recurrent thoughts of death or suicide</td>
<td>Significant suicidal ideation</td>
</tr>
<tr>
<td>Behavioral (non-diagnostic)</td>
<td></td>
</tr>
<tr>
<td>Feelings of restlessness</td>
<td>Restlessness</td>
</tr>
<tr>
<td>Comorbid anxiety</td>
<td>High levels of anxiety</td>
</tr>
<tr>
<td>Carbohydrate craving</td>
<td>Increased craving for carbohydrates</td>
</tr>
<tr>
<td>Elevated drug self-administration</td>
<td>Greater drug seeking and taking behaviors</td>
</tr>
</tbody>
</table>
must include the presence of at least one of two core symptoms, which are severely depressed mood and anhedonia – defined as a loss of interest or pleasure in normally rewarding activities. Both depressed mood and anhedonia are prominent and reliably documented during withdrawal from cocaine and amphetamines. Furthermore, nearly all of the remaining seven diagnostic symptoms are widely noted during psychostimulant withdrawal. Appetite changes during psychostimulant withdrawal are frequently reported as a pronounced hyperphagia, which is opposite to the acute anorectic effects of most psychostimulants (36–38). Also, in contrast to the acute effects of psychostimulants on wakefulness, drug withdrawal is associated with hypersomnia (35, 39, 40). Psychomotor retardation typically occurs during psychostimulant withdrawal, opposite to the acute psychomotor agitation during initial exposure to the drug. Similarly, unlike the increased energy that is experienced when first taking psychostimulants, contrary feelings of fatigue are the norm during withdrawal (41, 42). Cognitive impairments reported during psychostimulant abstinence include decreased concentration and confusion (43, 44). Importantly, increased rates of suicidal thoughts and ideations have been noted during psychostimulant withdrawal (45–47). Indeed, to our knowledge, the only DSM-IV symptom of MDD that has not been reported during withdrawal is “feelings of worthlessness or excessive or inappropriate guilt.” We are unsure whether this is due to their absence, or simply because such feelings have not been evaluated.

There are also additional behavioral and affective symptoms shared by both depression and psychostimulant withdrawal that are not diagnostic symptoms of MDD. People with MDD and those in the later stages of psychostimulant withdrawal display increased cravings for drugs of abuse, possibly as a way to self-medicate the dysphoria (for review, see (48)). Both disorders are associated with increased irritability (37, 49), as well as feelings of internal restlessness (35, 50). Additionally, strong cravings for carbohydrates are common during psychostimulant withdrawal and in MDD (47, 51, 52).

4. Effects of Psychostimulant Withdrawal in Animals

It has long been known that drugs that deplete monoamine stores in the brain can induce depressive-like symptoms. The use of monoamine-depleting drugs represents one of the earliest forms of animal models of depression. After the discovery that administration of the antihypertensive drug reserpine (Serpasil) induced the symptoms of clinical depression in approximately 15% of human patients, a large number of experiments were
Withdrawal conducted to establish the nature of this phenomenon. As a consequence, the reserpine animal model of depression was established, in which behavioral alterations such as sedation and ptosis, as well as physiological changes such as hypothermia and hyperalgesia, could be induced by injecting rodents with reserpine. However, as noted above, the presence of various confounding somatic symptoms, as well as the relatively poor pharmacological validity of the model, meant that the reserpine model fell out of favor. During the 1980s, important work on the psychological effects of psychostimulant withdrawal indicated that psychostimulants could be used to develop a better model to understand depressive-like symptoms. Much of the preclinical work that has examined both the physiological and psychological effects of psychostimulant withdrawal has therefore been conducted with this goal in mind. Thus, based on the human similarities of psychostimulant withdrawal to MDD, and the application of psychostimulant withdrawal in rodents as a model of depression, the principle focus of many studies has been to examine behavioral and psychological changes that are largely comparable to the symptoms of MDD in humans. For these changes to be relevant, they must exhibit behavioral changes in the animal model that are homologous, or at least analogous, to the symptoms of the disorder: i.e., have “construct validity” (53). Of interest, behavioral changes during a state of psychostimulant withdrawal are not limited to mammals, and have been characterized in species as diverse as zebrafish (54) and planarians (55). However, as the vast majority of studies of psychostimulant withdrawal have focused on rodents, and mainly rats, we limit the current discussion to these species only.

There are a number of clear benefits to studying psychostimulant withdrawal in rodents as opposed to humans. Firstly, it is possible to engage in more invasive techniques to understand better the underlying neurobiology of the condition; while this is not a focus of the present review, it is worth noting that many excellent studies have advanced our understanding of the physiological changes that take place during psychostimulant withdrawal. Secondly, we are able to obtain a “pure” evaluation of the effects of withdrawal from a specific drug, under known conditions of drug administration. Human studies of drug withdrawal are complicated by numerous factors, which include poly-substance abuse and lack of knowledge about drug dosing, not to mention high rates of physical and psychiatric comorbidity. The use of preclinical models provides the opportunity for a relatively unambiguous view of the withdrawal effects under specified and controlled conditions.

4.1. Dysphoria

While there are two core symptoms of MDD, depressed mood and anhedonia, it has so far proven to be a difficult task for
behavioral neuroscientists to model the former in preclinical rodent studies of psychostimulant withdrawal. Undoubtedly, this is because the symptom in humans is determined largely by self-report, and unlike most of the other symptoms of MDD, it is difficult to infer from objective assessment or behavioral observation. Clearly, rodents are theoretically unable to tell us how they “feel,” and so we must depend on indirect measures. However, several interesting studies have reported that rodents express higher rates of ultrasonic distress vocalizations during cocaine withdrawal (56, 57); these types of vocalizations are typically emitted when rodents are exposed to highly stressful situations. At least, these vocalizations are consistent with increased fear and anxiety, and could also reflect other negative affective states. It is worth noting that the evaluation of depressed mood in patients does not have to come exclusively from self-reported feelings. In the DSM-IV, it is noted that not all patients with MDD are able or willing to state explicitly any feelings of dysphoria, and in such a case, “the presence of depressed mood can be inferred from the person’s facial expression and demeanor.” Analogously, it has been reported that rats in psychostimulant withdrawal ((58) and personal observations) may exhibit an altered appearance, such as displaying decreased grooming behavior and a huddled stature, that are suggestive of self-neglect and negative affect.

Nevertheless, a number of creative and thoughtful studies have reported findings that are strongly suggestive of, and consistent with, the presence of internal psychological states that resemble depressed mood or dysphoria. It has been shown that rats which were exposed to multiple doses of amphetamine and then tested during drug withdrawal displayed altered behavior in the forced swim test (59). This procedure, also known as the Porsolt test and the behavioral despair test, is a widely used screen that was initially developed to measure the behavioral effects of compounds with antidepressant properties, in which antidepressant drugs increase the time spent active (inversely, decreasing the amount of time spent immobile) in an inescapable container filled with water. The model has a high pharmacological validity, and when tested with the appropriate control for general locomotion, discriminates well against other classes of drugs. More recently, and perhaps controversially, the test has also been used in rodents to detect the presence of depressive-like symptomatology. Although the face validity of the paradigm as a technique to measure depressed mood is questionable, a number of experimental manipulations in rodents that generate depressive-like states in humans also increase immobility in these tasks (60, 61). Rats tested during amphetamine withdrawal displayed decreased time in active behaviors and more time immobile, which the authors interpreted as “depressive-like” behavior. Similar effects in the same series of studies were also found for mice, which
Withdrawal exhibited increased immobility in the tail suspension test. Increased immobility in the forced swim test was also observed following a sub-chronic regimen of thrice-daily cocaine treatment in rats (62). A separate group has reported increased immobility by rats in the forced swim test when tested 9–12 weeks after treatment with the psychostimulant drug 3,4-methylenedioxymethamphetamine (MDMA, “Ecstasy”) (63, 64), although this time frame would appear longer than the effects of withdrawal that are typically observed in rodents.

Using a sophisticated drug discrimination paradigm in which rats were trained to respond on specific levers to identify distinct interoceptive states, rats in amphetamine withdrawal would generalize the condition to treatment with a low dose of the typical antipsychotic drug haloperidol (65). Low doses of haloperidol in normal controls has been described as an extremely dysphoric and unpleasant experience (66), implying that the rodents were able to generalize between this state and that of amphetamine withdrawal. While dysphoria and depressed mood are not equivalent, they are both common to psychostimulant withdrawal in humans. Additional evidence of dysphoria in rodents during drug withdrawal comes from studies that have used the conditioned place preference/avoidance procedure. Several studies have reported that rats will quickly learn the association between an environment paired with cocaine, so that they will freely spend more time in that environment. These same animals will rapidly avoid an environment paired with cocaine withdrawal (67, 68), implying a negative affective state.

We have previously reported that animals tested during withdrawal from a binge-like regimen of \( d \)-amphetamine display altered behavior in a successive negative contrast paradigm (69) (see Fig. 3). In this protocol, rats are trained reliably to anticipate a reward of a consistent value; in this particular instance, rats were freely able to consume a 32% sucrose solution for a limited time. If this reward is unexpectedly replaced by one of lesser value (a 4% sucrose solution), animals will normally consume lower levels of the new reward than subjects that have only ever been exposed to this lesser reward will. This phenomenon of successive negative contrast has been widely demonstrated across different species, including rodents, primates, and humans. Multiple explanations have been provided to account for the expression of successive negative contrast, many of which are based on the generation of negative affective states in the animals. Disappointment and frustration are prominent amongst these affect-based theories, which posit that the animal develops such negative feelings as it fails to find the reward that it had predicted to receive and instead finds one of a lesser value. In our study, the rats that were “down-shifted” during amphetamine withdrawal exhibited a greater degree of negative contrast, and for a longer duration, than those
not in withdrawal, possibly reflecting a greater negative state (70). These data are also consistent with anhedonia (see below), with a decrease in the incentive salience of the lesser reward compared to its salience in unshifted animals, as downshifted animals not only consume less of the reward but also decrease their approach speed toward it.

### 4.2. Anhedonia

The second core symptom of MDD, anhedonia, is characterized by a decrease or absence of pleasure or interest in normally rewarding activities. As may be expected, it has proven to be significantly easier to model this symptom in rodents, as it is relatively straightforward to train them to respond for a wide variety of both natural and artificial rewards, and also to quantify the interest in such rewards. We have previously reported our findings that amphetamine withdrawal in rats was associated with reduced interest in a sexually receptive conspecific (71), as well as reduced motivation to obtain a sweet sucrose solution (72).

In the former study, \( d \)-amphetamine withdrawal was evaluated by using male rats that were well-trained and exhibited high levels of sexual activity before the drug treatment conditions were implemented, thereby maximizing our opportunity to see reductions in motivated responding. Bi-level chambers were used during training...
in which male rats had once weekly access to females; these chambers effectively create a perimeter “racetrack” area around which the female rat can pace sexual behavior, allowing for more naturalistic mating behavior. An additional advantage to this design is that, with training, male rats engage in anticipatory searches between the levels prior to the introduction of the female, providing a reliable index of sexual interest before copulation begins. Thus, we were able to measure both appetitive and consummatory components of sexual behavior (see Fig. 4). Following extended training, the male rats were tested for sexual behavior during amphetamine withdrawal. Twelve hours after the administration of a 4-day escalating-dose schedule of \(d\)-amphetamine, anticipatory locomotor activity, measured as the side changes in the bi-level chamber, in the 5-min period prior to the presentation of an estrous female was reduced, reflecting a decrease in appetitive search behaviors. Furthermore, post ejaculatory intervals were significantly longer in \(d\)-amphetamine-treated rats, demonstrating a reduction in an additional component of motivated sexual behavior. These effects were replicated within the same

![Graphs showing side changes and post-ejaculatory intervals](image)

**Fig. 4.** Effect of withdrawal from a 4-day escalating regimen of \(d\)-amphetamine or vehicle on male sexual behavior. Tests occurred every 5 days with the parentheses indicating a test conducted 12 h after withdrawal from the drug regimen. (a) Anticipatory approaches to the female across sessions. (b) Post-ejaculatory intervals across testing. Stars indicate a significant difference between groups (*\(p<0.05\), **\(p<0.005\)).
experiment after the animals were exposed to the same drug schedule a second time, and similar results were obtained. We also noted strong trends toward fewer mounts and intromissions in d-amphetamine-treated rats during the period of drug withdrawal. The results of this study indicated that withdrawal from an escalating dose schedule of d-amphetamine decreases certain appetitive components of sexual behavior in sexually experienced male rats, but leaves their copulatory behaviors fundamentally unaltered. This could be interpreted as evidence for anhedonia, as interest is decreased, but the physical capacity to consummate the “reward” is unaltered.

In our second study, we were interested in evaluating how d-amphetamine withdrawal affected the motivation to obtain a rewarding sucrose solution. To quantify this, rats were trained to lever press for a sucrose solution under a progressive ratio schedule of reinforcement. Based on the requirements of the progressive ratio schedule, animals increase the number of lever presses for each subsequent reward (0.5 mL of a 4% sucrose solution), until a point was reached at which they failed to obtain the next reward within the time available: a measure known as the “breaking point” (73–76). After rats had been trained to respond stably for this reward, they were subjected to the 4-day escalating dose schedule of d-amphetamine, and then tested at different time points during drug withdrawal. Rats that were in amphetamine withdrawal exhibited lower breaking points than control animals for at least 72 h after drug termination when responding for the 4% sucrose solution under the progressive ratio schedule (see Fig. 5) (72, 77). This is strong evidence that amphetamine

![Fig. 5](image-url)

Fig. 5. The effect of withdrawal from a 4-day escalating regimen of d-amphetamine or vehicle on responding for a 4% sucrose solution under a progressive ratio schedule of reinforcement. Values represent the break points (±S.E.M.) or final ratio achieved and cumulative total responses of both groups during baseline sessions (B) and after drug administration (1–11 days). Stars indicate a significant difference between groups (**p<0.001).
withdrawal induces anhedonia, as the rats displayed reduced motivation to obtain the natural reward. After conducting a more detailed analysis of the data, we noted that both drug-treated and control animals met the required number of responses in approximately the same amount of time at lower ratios of responding. By contrast, the rats in d-amphetamine withdrawal took significantly longer than controls to complete the requirements at higher response ratios, when the amount of effort necessary to obtain a fixed reward was substantially greater. It is not likely that this is due to psychomotor fatigue, as rats in drug withdrawal can exhibit extremely high rates of responding for rewarding electrical brain stimulation (see below) at a much greater rate than the current study. We therefore interpret these data as evidence that motivation to obtain the reward increasingly declines as the demands of the task become greater, or alternately as the ratio of the reward to the effort cost decreases. Thus, anhedonia is manifested more clearly under conditions where greater motivation is required. In support of this hypothesis, we noted that animals in d-amphetamine withdrawal did not differ in their consumption of the sucrose solution when it was freely available.

Despite these data, the effects of psychostimulant withdrawal on the consumption of a sucrose solution may be more complex that they initially appear. We have previously analyzed the microstructural licking behavior of rats when they were able to freely consume a 4% sucrose solution for 10 min, which is a well-established technique used to measure hedonic response to palatable stimuli (69). In rats that were tested during withdrawal from the escalating-dose schedule of d-amphetamine (Barr, Genn, and Phillips, unpublished observations), there was a significant effect on microstructural licking parameters. Analysis of the results revealed that, in the earlier stages of fluid consumption, the pattern of the licking behavior by rats in psychostimulant withdrawal resembled that observed with a more concentrated sucrose solution in non-withdrawn animals. This implies that psychostimulant withdrawal in rodents may cause a transient initial increase in palatability for a sucrose solution. Data from the progressive ratio experiment, therefore, indicate that withdrawal is associated with a reduced motivation to respond for a sucrose solution while free consumption remains unaffected, even as the palatability of the solution may be increased. These findings reveal the complexity of assessing measures of reward in rodents during drug withdrawal, and highlight the need for specificity in behavioral testing. Despite these interpretational difficulties, we believe that the progressive ratio task may be particularly suited to measuring the decreased motivation associated with psychostimulant withdrawal (78) as an important component of anhedonia. In addiction, although we chose to use a sucrose solution as the natural reward, a large number of alternate rewards could be utilized.
Other researchers have used different operant techniques to demonstrate decreased motivation during psychostimulant withdrawal. Rats that responded under a fixed-ratio schedule for a sweetened drinking solution during cocaine withdrawal exhibited lower rates of lever pressing, which was reversed by re-administration of cocaine (79). In studies of the earlier stages of cocaine withdrawal, rats decreased responding for cocaine during the first interval of a second-order schedule (80, 81): this indicates decreased drug seeking behavior and reflects a reduced interest in responding for the reward. As a caveat, though, testing anhedonia with drugs of abuse is a potentially confounding task. Unlike most natural rewards, people with depressed mood and anhedonia often engage in higher rates of drug seeking behavior than normals. The reason for this continues to be debated, but it has been proposed that the capacity of many drugs of abuse to induce supra-physiological stimulation of endogenous reward pathways may represent a unique opportunity to feel pleasure, and therefore a way to self-medicate. Thus, other groups have noted that rodents demonstrate increased drug seeking behavior during withdrawal (80, 82). The disparities between studies are likely due to subtle but important methodological differences, such as responding directly for drugs versus responding under a second-order schedule. As noted above, we have generally found greater effects of withdrawal on appetitive versus consummatory behaviors, and responding for conditioned rewarding stimuli may well be more sensitive to the effects of withdrawal than directly responding for primary rewards. Exploratory and general investigative activity is also commonly disrupted during psychostimulant withdrawal, often reflected as a decreased interest in novel objects in the environment (83–86).

Anhedonia has also been appraised during psychostimulant withdrawal using a number of alternate techniques, including motivation for nonnatural rewards. Most commonly in this latter category, preclinical rodent studies have measured anhedonia during psychostimulant withdrawal with the use of reinforcing electrical brain stimulation. This includes direct stimulation via implanted chronic electrodes of the lateral hypothalamus, ventral tegmental area, and substantia nigra through the use of intracranial self-stimulation (ICSS) protocols. The ICSS procedure allows experimenters to quantify accurately the current intensity or frequency of the electrical stimulus necessary to maintain operant responding in rodents, and provides a reliable and sensitive measure of the state of the animal’s reward system. Increases in the frequency or current of the electrical stimulus required to maintain responding, depending on the specific protocol that is being used, are interpreted as diminished hedonic capacity (87), whereas decreases in current magnitude or frequency represent enhanced function of the endogenous reward system (88) (see Chap. 1 by
Withdrawal Markou for more detail). These changes can be measured under multiple different response protocols: one of the most common is identifying the currents that are required to maintain half-maximal and threshold levels of responding. Acute exposure to most drugs of abuse, including psychostimulants, causes a lowering of current parameters needed to maintain responding, consistent with activation of brain reward centers (89–92). The administration of higher doses of drugs of abuse and their subsequent termination can create a state of withdrawal, in which significant elevations in current parameters are needed to maintain responding, indicating a loss of reward function and therefore anhedonia. The size and duration of ICSS deficits during psychostimulant withdrawal are directly in proportion to the amount of drug that has been consumed (2, 93, 94), as is noted in humans, and effects of comparable magnitude are found with both amphetamines and cocaine. A similar degree of anhedonia is evident in rats that self-administer (95), receive passive injections (96), or subcutaneously absorb drug from implanted minipumps (93). The duration of the effects of psychostimulant withdrawal typically ranges from 2 to 6 days (97), although some indices may be present for up to 3 weeks (98). Indeed, one study that examined the effect of amphetamine withdrawal on ICSS from electrodes placed in the substantia nigra (99) observed much longer term effects. Using our 4-day, escalating-dose binge-like schedule of \textit{d}-amphetamine treatment, we examined the effects of withdrawal on ICSS responding using an ascending-series rate/intensity paradigm that has been widely employed to measure hedonic changes in rodents (100). Chronic stimulating electrodes were placed in the lateral hypothalamus, and rats were trained to press a lever for electrical stimulation. Following termination of drug treatment, rats exhibited reduced levels of ICSS responding for up to 60 h, manifested by a significant increase in currents that were required to maintain both half-maximal and threshold levels of responding for ICSS. At present, it is not clear why there is such a large variation in the duration of the anhedonic effects of psychostimulant withdrawal when using ICSS procedures. Some of this is likely due to methodological differences in how the animals respond, such as discrete versus non-discrete trials, as well as the location of the stimulating electrode. Methodological details for the ICSS paradigm are provided in Chap. 1.

4.3. Changes in Appetite and Sleep

Simple homeostatic behaviors, such as eating and sleeping, occur naturally in rodents and can be reliably measured. These behaviors are, therefore, also amenable to quantification during the withdrawal from psychostimulant drugs. Alterations in body weight and food consumption are common in people who abuse psychostimulants, and are typically manifested as a decrease in appetite and body weight. Rodents that are treated with psychostimulant
drugs at medium to higher doses initially exhibit anorectic effects, similar to humans, as they show decreased interest in and consumption of food (101). However, a rebound effect has been described after the termination of amphetamine treatment: animals displayed hyperphagia and consumed greater amounts of food (102). This is generally comparable to the withdrawal phase, although drug doses were not as high in this study as has been used in many other studies of psychostimulant withdrawal. As noted above, we have also previously found that withdrawal from a binge-like dose of \( d \)-amphetamine was associated with changes in microstructural licking by rats for a sucrose solution, consistent with an increase in the palatability of the solution. During psychostimulant withdrawal, rodents also display a clear preference for foods with a lower protein but higher fat and carbohydrate content (103). To our knowledge, this effect has not been tested and described in humans during psychostimulant withdrawal, but it is entirely consistent with the literature of MDD in which patients display a strong preference for foods rich in carbohydrates and fat, such as chocolate (52, 104). Given that rodents in psychostimulant withdrawal may exhibit a decreased motivation to obtain food reinforcement when the demand requirements for it is increased, careful consideration is needed in designing studies to model symptoms related to hypo/hyperphagia.

Sleep alterations are a common effect of psychostimulants, and obviously amphetamines continue to be used widely as a pharmacological method of promoting wakefulness. After exposure to psychostimulants and initial insomnia, rodents can exhibit a rebound effect during drug withdrawal, in which hypersomnia is evident (105). An additional effect of this hypersomnia is to cause a change in diurnal feeding activity. During cocaine withdrawal, rats shifted food consumption that occurs primarily in the dark/active phase of the circadian cycle to a more fragmented pattern of activity, which included a proportionally greater amount of time spent feeding during the light/resting phase (106). Sleep itself may also be altered during psychostimulant withdrawal. As noted in humans, withdrawal can potently disrupt sleep architecture. In a similar manner, rats displayed a post-drug increase in REM sleep density after withdrawal from chronic cocaine (107).

4.4. Psychomotor Retardation and Fatigue

Both psychomotor retardation and fatigue are one of the more consistently reported symptoms of psychostimulant withdrawal in humans. While the clear majority of rodent studies of drug withdrawal have also reported decreased locomotor activity (58, 108–114), this effect is not universal (83, 115). In those studies where decreases in activity are reported, there may be additional movement-related anomalies, such as mild catalepsy (116–118). A number of different possible explanations may account for the lack of consistency with regards to deceased locomotor activity.
Dose and type of drug will be an influencing factor (119), but the insightful work of White and colleagues suggests that decreases in locomotor activity may also be highly time-dependent, with greatest effects occurring around 20 h after the final drug treatment (120). Therefore, studies that examine locomotor activity during a relatively brief span that does not include this period may minimize their opportunity to observe decreases in activity. Additionally, the effects of psychostimulant withdrawal on locomotor activity tend to be more noticeable when baseline activity is higher, as this provides a greater magnitude of activity reduction. Thus, larger decreases in locomotor activity are reported during the dark/active phase (121–123), as well as during exposure to a novel environment (124). The assessment of “fatigue” in rodents is a far more complicated task. In humans who experience psychostimulant withdrawal, fatigue is typically assessed by self-report (35). As this is not possible in rodents, the presence of fatigue can, at best, be inferred from external observation, such as general posture and activity, although confounding factors such as reduced motivation complicate this assessment. We have previously suggested (5) that tasks that offer animals a choice between low effort/low reward and high effort/high reward types of responding or other variants of effort-related choice behavioral tasks may represent a fruitful option for investigating rodent homologues of fatigue (125).

4.5. Cognitive Changes

With an abundance of sophisticated animal models of cognition available, some of the best preclinical research on psychostimulant withdrawal has been conducted in this broad domain. As noted above, cognitive impairment has been reported in humans during psychostimulant withdrawal, most commonly as deficits in both memory and attention (44, 126, 127). In rodent studies, the effects of psychostimulant withdrawal are evident in cognitive systems ranging from pre-attentive cognitive processing through to higher-order cognitive functions.

A number of studies have measured the effects of withdrawal from psychostimulant drugs on prepulse inhibition of the acoustic startle reflex. This is a pre-attentional cognitive process that regulates information flow, and involves a complex neural circuitry sharing many of the same brain nuclei as those responsible for behavioral responses to psychostimulant drugs (128). Reduced prepulse inhibition has been associated with psychotic disorders, such as schizophrenia and psychostimulant-induced psychosis (129). Therefore, there is a strong a priori rationale to examine the effects of psychostimulant withdrawal on prepulse inhibition. The effects of cocaine and amphetamine withdrawal on prepulse inhibition have been studied in detail by Feldon and colleagues in a series of papers (130–136). In the earliest study, withdrawal from repeated daily low doses of either cocaine or amphetamine had no
effect on prepulse inhibition (130), nor was there an effect on prepulse inhibition when the authors increased the dosing regimen to an escalating dose schedule, with a maximum dose per injection of 5 mg/kg (132, 134–136). Similarly, there was no effect on prepulse inhibition during withdrawal when amphetamine was infused continuously via an osmotic minipump (131). However, when the escalating dose was increased to a maximum of either 8 mg/kg (132) or 10 mg/kg (132, 134) per injection, long-lasting decreases in prepulse inhibition were observed when first measured, starting 4 days after drug termination, suggesting that a threshold dose may be needed to see withdrawal effects on this parameter. It is important to note, however, that most behavioral effects of psychostimulant withdrawal are greatest in the first 3 days of withdrawal. The effects on prepulse inhibition, therefore, may have occurred even with the lower doses of drug that were not tested in this time window. Interestingly, withdrawal from lower doses of cocaine and amphetamine that had no effect on prepulse inhibition did alter latent inhibition of the active avoidance response, which is another form of attentional processing.

Higher order attentional processing in rats during psychostimulant withdrawal has been determined using a five-choice serial reaction time (5-CSRT) task. This task measures a number of different components of “attention,” such as selective attention, vigilance, and executive control. In animals that were trained to self-administer either d-amphetamine or methamphetamine, withdrawal from the drug produced significant deficits on a number of individual parameters (137). These mainly included a reduction in the number of correct responses, an increase in omissions, and a general slowing of responding to the visual targets; in the case of d-amphetamine these deficits were apparent for at least 4 days, while effects of methamphetamine withdrawal were still significant 2 weeks later. Impulsivity during cocaine withdrawal has also been reported using this task (138), and rats in amphetamine withdrawal exhibited altered performance in the differential reinforcement of low rates of responding operant conditioning task (DRL-30) (139), whereby animals displayed a behavioral profile consistent with increased impulsivity. Memory impairment during psychostimulant withdrawal has not been widely studied: one report noted that rats in withdrawal from a moderate dose escalating-dose schedule of d-amphetamine exhibited normal acquisition of a water maze task and performance in a probe trial (140), while reversal learning was enhanced in these animals. There is clearly plenty of opportunity to examine alternate subdomains of cognition during psychostimulant withdrawal, including executive function and working memory.

4.6. Anxiety

Although not a defining symptom of MDD, increased anxiety is common in the disorder, as well as during psychostimulant
withdrawal in humans. Not only does it represent an important symptom for study, it also serves as a potential confound in numerous behavioral procedures when testing constructs, such as memory in maze tasks. A wide range of preclinical protocols has been used to note consistent and large anxiogenic effects of psychostimulant withdrawal from both cocaine and amphet-amines. Specific tasks include fear-potentiated startle (141), ultrasonic vocalization (56), defensive burying (142), suppression of punished responding (143), the elevated plus maze (62, 144, 145), and conditioned avoidance (110) (although see (146)).

5. Conclusion

In summary, an extensive body of evidence describes a cluster of symptoms of psychostimulant withdrawal in humans that, at least at the phenomenological level, closely resembles MDD, particularly the atypical subtype. Using this as a framework, numerous preclinical rodent studies of psychostimulant withdrawal and behavior have focused on those symptoms that are most readily modeled in animals: perhaps the best understood of these is anhedonia. As the face and construct validity of animal models of psychostimulant withdrawal continue to evolve, we are able to develop ever more accurate models with which to understand the underlying neurobiology of withdrawal as a first stage in developing novel treatments for this important component of addiction. There are numerous challenges ahead in this field, including head-to-head comparison of different psychostimulants and other drugs of abuse, as well as greater standardization between research laboratories of drug dosing, route of administration, and timing of withdrawal effects. Nevertheless, important progress has been made in this endeavor, providing critical insight into one of the critical stages of drug addiction in humans.

References


gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156(2–3): 117–154


Relapse

Suzanne Erb and Franca Placenza

Abstract

The most insidious aspect of drug addiction in humans is a high and recurrent propensity to relapse. Over the past several decades, the reinstatement procedure has received widespread use as an animal model of drug relapse, to study the basic mechanisms underlying drug-seeking responses in laboratory animals. The objectives of this chapter are twofold. The first is to describe the primary paradigms and procedures that have been developed to study reinstatement of drug-related behaviors in the laboratory. The second is to define and characterize the three major triggers of reinstatement to drug seeking that constitute the foundation of this work. These triggers include priming injections of a previously self-administered drug, reexposure to drug-associated cues, and exposure to stress. The role of these triggers in reinstatement will be characterized within the context of an overview of key behavioral findings in the literature and their theoretical implications.

Key words: Reinstatement, Extinction, Self-administration, Conditioned place preference, Conditioning

1. Introduction

Drug addiction can be conceptualized as a chronic relapsing disorder, characterized by recurrent bouts of drug use with intervening periods of withdrawal and abstinence. Relapse may in fact represent the single-most predictive outcome of a diagnosis of addiction (1) and, as such, it poses one of the greatest challenges for treatment (2, 3). The problem of relapse is made more difficult by the fact that the time separating periods of drug use can vary on the order of hours and days to months and even years, and that different motivational influences may govern the likelihood of a full relapse episode depending on time since the last exposure to the drug (4–6). Furthermore, work carried out at a basic research level would suggest that the different triggers of relapse (e.g., a “taste” of the drug, reexposure to drug-associated
cues, or exposure to stress) act via largely dissociable neuroanatomical systems to induce relapse (e.g., (7–13)) and may in fact be more or less likely to precipitate a relapse episode depending on the amount of time that follows termination of drug use (5, 14–18). From a treatment perspective, therefore, the problem of relapse is complex and requires careful consideration of multiple factors and influences.

Over the past several decades, a major research objective in the drug addiction field has been to understand the motivational processes and neurobiological underpinnings contributing to drug craving and relapse to drug use (see (5, 6, 12, 19–21) for recent reviews). In this regard, some of the major advances that have been made have come about as a result of studies carried out in the laboratory, using animal models of relapse. This work has focused in large part on identifying the triggers of relapse, and determining the conditions under which those triggers are most likely to precipitate a full relapse episode. In addition, a major emphasis has been on characterizing the neurobiological systems contributing to drug craving and relapse.

We will begin here by describing the major reinstatement procedures that have been developed over the past several decades to address mechanistic and phenomenological questions of relapse to drug seeking in the laboratory. Subsequently, an overview of some of the key behavioral findings and their theoretical implications will be provided, with an emphasis on studies aimed at characterizing the major triggers of reinstatement. These triggers include administration of a priming injection of a drug of experience, reexposure to drug-associated cues, and exposure to environmental or pharmacological stressors. Although, as mentioned, a major focus of drug reinstatement studies has been identifying the neurobiological systems involved, a review of these studies is beyond the scope of this chapter. The interested reader is, therefore, referred to several recent reviews on the topic (6, 7, 12, 22, 23).

Animal models of drug relapse share common features that qualify them as so-called reinstatement procedures. Thus, the term reinstatement is typically used to define relapse behavior in laboratory animals. Technically, reinstatement is defined as the resumption of a previously reinforced response when the unconditioned reinforcer or reward is presented non-contingently after a period of extinction training. During extinction training, the previously reinforced response gradually declines because reinforcement is withheld. Importantly, testing for reinstatement occurs under extinction conditions, in that responses are without scheduled consequence (24).

2. The Reinstatement Model
In addition to drug-seeking behaviors, reinstatement procedures have been used to study appetitive responses conditioned to food or sucrose (e.g., (25–28)), and aversive responses associated, primarily, with the conditioning of fear (e.g., (29–32)). Although the noncontingent presentation of the stimulus that originally maintained the behavior is what has traditionally defined reinstatement, these procedures have been adapted to study the ability of alternate stimuli, presented at test, to induce reinstatement. In fact, a major focus of the relevant drug literature in the past 10 years has been on environmental and pharmacological stressors as triggers for reinstatement (5, 33, 34). For example, acute exposure to intermittent footshock stress after a period of extinction training serves as a powerful stimulus for provoking the reinstatement of drug seeking in rats with a history of cocaine, heroin, nicotine, or alcohol self-administration (35–38).

From a historical perspective, the most conventional reinstatement procedures are based on the drug self-administration paradigm, in which animals are trained to perform an operant response for drug reinforcement (e.g., (24, 35, 39, 40)). More recently, alternate reinstatement procedures have been developed that are based on the conditioned place preference (CPP) paradigm, a widely used measure of the rewarding properties of drugs of abuse (e.g., (41–46)). In the next sections, these paradigms and their procedural variations for studying reinstatement will be described.

A generalized schematic of the reinstatement procedure based on the drug self-administration paradigm is presented in Fig. 1. In reinstatement studies of this kind, animals are trained to perform an operant response, such as a lever press or nose poke, to obtain intravenous infusions of a psychostimulant (e.g., amphetamine, cocaine, nicotine) or opiate (e.g., heroin, morphine), or oral access to alcohol. Following training, extinction of the drug-reinforced behavior is accomplished by no longer reinforcing the behavior; that is, the operant response is either without consequence or results in an infusion of saline. Once the behavior is extinguished (i.e., responding is reduced to a low baseline level), animals are tested for the reinstatement of drug seeking induced by a specific triggering stimulus or event (e.g., priming injection of the drug, presentation of a drug-associated stimulus, or exposure to stress). Thus, the reinstatement of drug seeking is defined, operationally, as an increase above baseline in the number of occurrences of the previously reinforced behavior (e.g., number of lever presses on the previously reinforced lever) in response to the presentation of a triggering stimulus, after a period of extinction (24).

Since 1971, when the first report of drug-induced reinstatement of drug seeking appeared in the literature (40), three variations of the general procedure have been developed and used to
study reinstatement of drug seeking in laboratory rats and monkeys. The differences that define these procedures relate primarily to whether drug self-administration, extinction, and testing sessions occur within the same day or on different days (47). Depending on the nature of the question being asked, some of these procedures are more suitable than others (see below), but reliable and consistent results have been obtained using all three procedures.

In one procedure, known as the “between-sessions” procedure (47), the various phases of the experiment occur sequentially, such that self-administration, extinction, and testing sessions are given on different days (e.g., (36, 40, 48)). Thus, animals are initially trained to self-administer a drug during consecutive daily sessions and, once stable self-administration is acquired (typically less than 20% variation from the mean number of infusions over several sessions), extinction conditions are introduced in subsequent daily sessions. After a predetermined extinction criterion is reached (e.g., 10 or fewer responses in a 3 h session or less than 20% responding on the last relative to first day of extinction), tests for reinstatement are given in the next daily sessions.

In an alternate procedure, the “within-sessions” procedure, self-administration training, extinction, and test sessions all occur within the same day (e.g., (39)). As in the case of the between-sessions
procedure, animals are initially trained to self-administer a drug during daily sessions. In this case, however, once animals have acquired stable self-administration, subsequent daily sessions involve drug self-administration followed immediately by extinction training (to a predetermined extinction criterion) and then a test for reinstatement.

In the third procedure, the “between-within-sessions” procedure, once animals have acquired stable self-administration, sessions of extinction training and testing for reinstatement occur within the same day. Thus, drug self-administration occurs during daily sessions that are separate from extinction and testing; however, extinction and testing occur on the same day (e.g., (15, 49)). In studies using this procedure, animals are typically given multiple 1-hour daily extinction sessions separated by 5–30 min, in order to achieve stable, low levels of responding; these extinction sessions are then followed by a test for reinstatement. With the addition of a withdrawal period between the last day of self-administration training and the first day of extinction and testing for reinstatement, the “between-within” procedure has proved especially useful for studying the effects that different periods of drug withdrawal can have on extinction responding and reinstatement of drug seeking (15).

In the past decade, several laboratories have adapted the CPP paradigm to study the reinstatement of extinguished preferences for previously drug-paired environments (41–46). CPP training is a Pavlovian conditioning procedure that involves discrete pairings of a drug, such as amphetamine, cocaine, or morphine, with a distinct environmental context, and pairings of saline with a different environmental context. At test, the animal is allowed to freely explore both contexts in a drug-free state. Evidence of a CPP is demonstrated by the animal spending more time in the drug- than saline-paired context, or relatively more time in the drug-paired context after than before the conditioning phase.

A generalized schematic of the CPP reinstatement procedure is presented in Fig. 1b. Two variations of the procedure have been developed that differ with respect to the way in which extinction is carried out. In one procedure, extinction is accomplished by giving repeated saline injections in both contexts, such that exposure to the previously drug-paired context is no longer rewarded (41–44). In the second procedure, animals are given repeated CPP tests on consecutive days. In this case, each test constitutes an extinction trial, since testing occurs in the absence of drug. In both procedures, testing for reinstatement is conducted by presenting the animals with a triggering stimulus, such as a noncontingent priming injection of the drug or exposure to an acute stressor, prior to providing the animal with free access to both the drug- and saline-paired context. Thus, reinstatement is defined
operationally, as a proportionately greater amount of time spent in the previously drug-paired context, relative to saline-paired context, after a period of extinction.

3. The Triggers of Reinstatement

3.1. Drug Priming

One of the basic tenets of the organization Alcoholics Anonymous is that consumption of a single alcoholic beverage will greatly increase the probability of a full relapse. In fact, the idea that a small amount of drug can prime desire for more drug has been supported by several experimental findings obtained in psycho-stimulant and opiate-dependent subjects, and alcoholics (e.g., (50–56)). For example, in one study carried out with cocaine-experienced users, intravenous doses of the drug, relative to a placebo, were associated with marked increases in subjective ratings of cocaine craving (51). In another study, employing functional MRI, subjective ratings of cocaine-induced craving were closely correlated with focal signal increases in the nucleus accumbens, a key structure of the brain reward circuitry (50).

Studies of drug-priming-induced craving in experienced users parallel studies of drug-priming-induced reinstatement of drug seeking in rats and monkeys, in which noncontingent priming injections of a previously self-administered drug have been established as highly effective stimuli for inducing the reinstatement of drug seeking. In an original series of studies by Stretch and Gerber (40, 57, 58), noncontingent intravenous or intramuscular injections of amphetamine or cocaine produced a reliable reinstatement of responding in monkeys that had been trained to self-administer those drugs and had subsequently undergone extinction of the drug-taking behavior. In subsequent experiments using several variations of the reinstatement procedure, these effects were replicated in rats with histories of cocaine, heroin, and morphine self-administration (39, 59, 60). More recently, the drug priming effect was further extended to rats with histories of nicotine (61, 62), alcohol (37, 63), cannabinoid (64, 65), and MDMA (ecstasy) (66) self-administration. In addition, the drug priming effect was found to generalize to the CPP paradigm and, more specifically, the reinstatement of morphine- (43, 45, 67, 68) and cocaine- (44, 69) induced CPPs.

In addition to the drug of experience, drugs from the same pharmacological class as the self-administered drug can effectively induce reinstatement. For example, in monkeys trained to self-administer cocaine, priming injections of amphetamine induce the reinstatement of cocaine seeking (58, 70). Likewise, in rats, priming injections of amphetamine restate cocaine seeking (39), and priming injections of morphine restate heroin seeking (60). Drugs from
different pharmacological classes than the self-administered drug, but affecting similar neurotransmitter systems, are also effective in inducing reinstatement of drug seeking. Such “crossover” priming effects have been demonstrated between opiates and psychostimulants (39, 60, 70, 71), nicotine and alcohol (72, 73), cannabinoids and psychostimulants (74), and cannabinoids and opiates (64, 65).

One explanation of the drug priming effect is that a non-contingent injection of the drug reinstates extinguished drug-seeking responses by activating the brain systems underlying its discriminative stimulus effects (40, 75). By this account, during testing for reinstatement, the priming injection elicits certain effects of the self-administered drug that signal to the animal the availability of response-contingent drug reinforcement. There are, however, several discrepancies between the outcomes of drug discrimination and drug priming studies that question the validity of this explanation. For example, some drugs shown to substitute for another drug in a drug discrimination procedure (e.g., D1-like receptor agonists for cocaine) do not induce reinstatement of responding for that drug (71). Conversely, some pharmacological manipulations that prime the reinstatement of drug seeking e.g., intra-VTA injections of morphine prime the reinstatement of heroin seeking (76) do not substitute for the drug in a drug discrimination procedure (35, 77). These findings suggest that the neurobiological systems underlying the discriminative stimulus and the priming effects of drugs on reinstatement are largely distinct. From this perspective, it is of interest that a neurobiological dissociation has also been reported between the discriminative stimulus and rewarding effects of drugs (77, 78), and that the priming effects of drugs have been attributed, at least in part, to the same brain systems that mediate their rewarding effects (79).

Indeed, findings from crossover priming studies with psychostimulants and opiates have led to the interpretation that activation of the mesocorticollimbic dopamine system, largely responsible for the rewarding effects of drugs of abuse, mediates reinstatement by both classes of drugs. For example, amphetamine injected into the nucleus accumbens reinstates heroin seeking (80) and injections of morphine into the ventral tegmental area reinstate cocaine seeking (76). Although these and similar findings are suggestive of a relationship between drug-priming-induced reinstatement and drug reward, other findings have failed to confirm a relationship. There are in fact several examples in the literature of pharmacological manipulations that either facilitate or interfere with drug self-administration, while having minimal or no effect on drug-priming-induced reinstatement of drug seeking. Likewise, there are examples of pharmacological manipulations that alter the effectiveness of drug-priming-induced reinstatement while having little or no effect on drug self-administration (see (23, 47)).
The mesocorticolimbic dopamine system is involved not only in the acute rewarding properties of drugs, but is also thought to underlie their incentive motivational and goal-directed effects (79, 81). As such, the reinstating effects of drug priming may also be associated with the ability of drugs to produce an incentive motivational state that leads to drug-seeking behavior (79, 81). According to this idea, priming injections of a drug enhance the incentive salience (i.e., “attractiveness”) of previously drug-related cues, and thus enhance behaviors that facilitate approach to those cues. Such cues may include a visual or auditory stimulus paired with an operant response (e.g., lever press) or, in the case of studies based on the CPP procedure, contextual cues. In support of this view, it has been shown that the removal of drug-associated cues during tests for reinstatement attenuates the effectiveness with which priming injections induce reinstatement of drug seeking (40).

Clearly, events other than reexposure to drugs can provoke relapse to drug seeking. Indeed, after a period of abstinence, drug seeking is antecedent to drug taking. One such event that has been the focus of considerable study is exposure to cues that have become associated with the experience of drug taking. For example, reexposure to the people, places, or paraphernalia associated with drug use can elicit strong drug craving, and it has been argued that such cue-elicited craving is fundamental to the cycle of relapse in addiction (82). Given the crucial role that drug-associated cues are purported to play in drug craving and relapse to drug use, it is perhaps not surprising that reinstatement procedures have been used extensively to study the basic mechanisms mediating their effects.

In an initial laboratory demonstration of cue-induced reinstatement, Davis and Smith (1976) (59) trained rats to self-administer morphine under conditions in which a lever press response led to a drug infusion and the simultaneous sounding of a buzzer. Subsequently, animals underwent extinction training in the absence of the buzzer cue; that is, during extinction, lever presses led neither to drug infusions nor presentations of the buzzer. Subsequently, animals underwent extinction training in the absence of the buzzer cue; that is, during extinction, lever presses led neither to drug infusions nor presentations of the buzzer. At the time of testing for reinstatement, animals were given a noncontingent presentation of the buzzer prior to access to the previously drug-reinforced lever. Under these conditions, reinstatement of responding was observed. In subsequent studies, the findings of Davis and Smith (59) were replicated and extended to animals trained to self-administer other drugs, including cocaine, heroin, ethanol, and nicotine, in combination with other types of discrete stimuli, such as a light cue or tone (39, 83–86).

Although the noncontingent presentation of a discrete drug-associated cue reliably reinstates drug seeking, it is generally not
as effective in doing so as is, for example, a contingent presentation of a cue \((39, 87)\) or a priming injection of the self-administered drug \((e.g., (39))\). This observation has led researchers to study factors that might influence the effectiveness with which a discrete cue induces reinstatement of drug seeking. The objective of this work, in part, has been to optimize the procedures for studying cue-induced reinstatement. For example, it has been shown that a compound drug-associated cue \((e.g., \text{light plus tone})\) is much more effective than a singular cue \((\text{light or tone})\) in inducing the reinstatement of drug seeking. In one study, response-contingent cocaine infusions led to varied presentations of a light, tone, or light plus tone compound stimulus \((88)\). Following extinction training in the absence of the drug and cues, noncontingent presentation of the compound stimulus reinstated drug seeking, whereas presentations of either stimulus alone led to a much weaker effect on reinstatement, or was without effect. As mentioned previously, another factor that influences the effectiveness with which a drug cue induces reinstatement of drug seeking is its contingency of presentation at test. More specifically, it has been reported that presenting the discrete cue at test contingent on the operant response facilitates the reinstatement of drug seeking, relative to presenting the cue in a noncontingent fashion \((39, 87)\).

In addition to discrete cues, discriminative cues that predict drug availability have been found to reinstate drug seeking. The first demonstration of this was obtained using a runway model of relapse, in which rats were initially trained to traverse a straight-arm maze for intravenous infusions of heroin in the goal box \((89)\). On subsequent trials, the subjects learned to discriminate between two odors. One odor, the so-called \(S^+\), was accompanied by intravenous infusions of heroin in the goalbox and the other, the so-called \(S^-\), was accompanied by a saline infusion in the goalbox. Thus the \(S^+\) signaled the availability of heroin, whereas the \(S^-\) signaled its absence. Discrimination training was considered to have occurred once the latency to reach the goalbox was less on \(S^+\) than \(S^-\) trials, at which time extinction conditions were introduced. During extinction training, entries into the goalbox were without scheduled consequence. On test day, animals were given varied presentations of the \(S^+\) or \(S^-\) before runway trials. Presentations of the \(S^+\), relative to the \(S^-\), reduced latencies to reach the goalbox; that is, the \(S^+\) reinstated the extinguished runway behavior.

More recently, the effect of discriminative cues on the reinstatement of drug seeking has been studied using the drug self-administration procedure \((90–93)\). In these studies, rats are trained to perform an operant response \((e.g., \text{lever press})\) for intravenous infusions of a drug or saline, or for oral access to alcohol or water, during multiple daily sessions. Throughout sessions in which drug or alcohol is available, a discrete visual or auditory
stimulus (i.e., $S^+$) is presented; throughout sessions in which responding results in saline infusions or access to water, an alternate visual or auditory stimulus (i.e., $S^-$) is presented. Following discrimination training and subsequent extinction sessions, in which responding is without scheduled consequence, noncontingent presentations of the $S^+$, but not $S^-$, are found to reinstate drug seeking.

Several studies have been carried out to characterize the phenomenology of reinstatement induced by discriminative cues. In one such study, rats that were trained to discriminate between the availability of intravenous infusions of cocaine versus the availability of saline exhibited strong reinstatement of cocaine seeking by presentations of the $S^+$, throughout a month-long period of repeated testing, and following an additional 3-month drug-free period (92). These results suggest that cocaine-associated discriminative cues are highly resistant to extinction and are persistent in their motivational effects on drug-seeking responses. In another study in which rats were trained to discriminate between stimuli associated with the availability of alcohol versus water, reinstatement of alcohol seeking was induced by an olfactory, but not an auditory, discriminative stimulus. These results suggest that, at least in the case of alcohol-trained animals, the efficacy of discriminative stimuli in inducing reinstatement may depend on their modality of presentation (94).

Using an adapted so-called renewal procedure (32), Crombag and Shaham (2002) (95) demonstrated that the contextual cues associated with drug taking can also contribute to the reinstatement of extinguished drug-seeking responses. The renewal effect refers to the recovery of a conditioned response when testing occurs in a different context than that in which the response was extinguished. In the study by Crombag and Shaham (95), rats were trained to self-administer a heroin–cocaine speedball mixture in a context containing a set of distinctive visual, tactile, auditory, and olfactory stimuli. They subsequently underwent extinction training in an alternate context, containing a different set of distinctive visual, tactile, auditory, and olfactory stimuli. At the time of testing, animals that were returned to the context in which the original training had occurred exhibited renewed responding on the previously drug-reinforced lever.

Theoretical explanations for how drug-associated stimuli, be they discrete drug-associated cues, discriminative stimuli signaling the availability of drug, or contextual cues associated with the drug-taking environment, can come to acquire control over drug-seeking behaviors have been the focus of numerous seminal papers in the drug addiction field. Explanations have focused largely on Pavlovian learning processes, the idea of occasion setting, and the idea that drug-associated cues, through associative learning, can come to acquire incentive motivational properties that facilitate
Relapse

There are many correlational studies implicating life stress as an important factor contributing to increased rates of drug and alcohol use, and as a trigger for relapse in individuals with substance abuse disorders (55, 100–104). A relationship between stress and relapse in drug addicts has also been studied under controlled laboratory conditions. For example, the induction of psychological stress, using a guided imagery procedure involving recall of personalized stress situations, was found to increase subjective reports of cocaine and alcohol craving in recently abstinent cocaine-dependent subjects seeking treatment (105, 106). Moreover, using these procedures, stress-induced cocaine craving reportedly predicted the incidence of cocaine relapse following inpatient treatment (see (105)).

Since 1995, reinstatement procedures have been used extensively to explore the relationship between stress and relapse to drug seeking in laboratory animals. To date, much of this work has used intermittent exposure to mild, electric footshocks as the stress manipulation. In an initial study carried out in animals trained to self-administer heroin, Shaham and Stewart (1995) (35) demonstrated that 10 min of exposure to brief intermittent electric footshocks served as a powerful stimulus for inducing the reinstatement of extinguished drug-seeking behavior. Furthermore, this effect of footshock on reinstatement persisted after an additional 4–6 week drug-free period and additional extinction training. In subsequent experiments, the effect of footshock on the reinstatement of heroin seeking was replicated and extended to animals with histories of cocaine, alcohol, and nicotine self-administration, as well as self-administration of a cocaine–heroin (“speedball”) mixture (see (6, 7, 23, 37, 38, 47)). The effect of footshock on the reinstatement of drug seeking was also found to generalize to the CPP paradigm, where it has been found to effectively induce reinstatement of both morphine and cocaine CPPs after drug-free periods of more than 1 month (42, 46, 107).

In addition to generalizing to the CPP procedure, the effects of footshock on reinstatement of drug seeking have been found to generalize to certain other types of stressors. One such highly effective stressor is 21 h of food deprivation, which induces the reinstatement of cocaine and heroin seeking at levels comparable to that induced by footshock stress (108–110). Likewise, various pharmacological stressors have been found to induce the reinstatement of drug seeking. For example, i.c.v. injections of the stress-related neuropeptide, corticotropin-releasing factor (CRF),

3.3. Stress
reinstate heroin (111), cocaine (49), and alcohol (112) seeking, and injections of CRF into the bed nucleus of the stria terminalis (BNST), a brain region critically involved in the effects of stress on reinstatement, reinstate cocaine seeking (113). Likewise, systemic injections of the corticosterone synthesis inhibitor metyrapone, reinstate heroin seeking, presumably by acting acutely to reduce negative feedback in the hypothalamus and pituitary (111). Finally, central injections of the alpha-2 antagonist, yohimbine, reinstate responding in animals trained to self-administer alcohol (114), methamphetamine (115), or cocaine (116).

As occurs in the reinstatement of drug seeking, the effects of footshock on the reinstatement of extinguished CPPs also generalize to other stressors. In one study, 15 min of exposure to restraint, 15 min of exposure to tail pinch, or exposure to social defeat were all equally effective stressors for reinstating extinguished morphine CPPs in mice (117). In other studies, conditioned stimuli previously paired with footshock reinstated extinguished CPPs induced by cocaine in rats (118, 119). Interestingly, conditioned stimuli previously paired with footshock are ineffective in inducing the reinstatement of heroin or cocaine self-administration. It has been suggested that the discrepancy in the effects of the conditioned stressor on reinstatement of CPP versus self-administration may be due to the conditioned stressor inducing conditioned freezing, a response elicited unconditionally by footshock stress and incompatible with the performance of an operant behavior (34).

Other studies aimed at further characterizing the effects of stress on reinstatement have focused on how the schedule of drug access during the training phase may influence the subsequent magnitude of reinstatement testing. For example, in one study, rats given 11 h, relative to 1 h, of daily access to intravenous heroin showed a gradual escalation in heroin intake during the self-administration phase. Moreover, this escalation in drug intake was associated with a slower rate of extinction and a greater level of responding to footshock during tests for reinstatement, suggesting an association between an escalation in heroin intake and persistent increase in motivation to seek heroin following a drug-free period (120). In a similar study carried out in cocaine-trained rats, it was found that 6, relative to 2, hours of daily cocaine self-administration was associated with an escalation in drug intake over a 14-day training period, and this escalation in drug intake corresponded to an increased magnitude of reinstatement by footshock stress or i.c.v. injections of CRF (121).

From a theoretical perspective, it is of interest that the phenomenon of stress-induced reinstatement is quite specific to the reinstatement of drug-reinforced behaviors. For example, footshock does not reinstate extinguished responding previously maintained by food pellets (122), sucrose pellets, or sucrose
solution (7, 108). Footshock, however, does reinstate responding for brain stimulation reward. In animals trained to lever press for electrical stimulation to the septal region, and subsequently subject to extinction of the operant response, 5 and 15 min of exposure to footshock stress reinstated responding to a level comparable to that observed in animals with a history of drug self-administration (108). Based on the findings that footshock reinstates responding for drug and brain stimulation, but not natural nondrug reinforcers such as food and sucrose, it has been suggested that the stressor facilitates pathways compatible with general approach behaviors, but not with motivational behaviors that have evolutionary significance, such as behaviors maintained by food and sex (see (7) for a discussion).

The effect of stress on reinstatement appears to be highly sensitive to manipulations of context. In one study, it was revealed that footshock stress was only effective in inducing the reinstatement of drug seeking if it was administered in the environment in which animals had previously self-administered the drug and subsequently undergone extinction; that is, footshock did not reinstate drug seeking if exposure to the stressor occurred in a novel context, suggesting an important interaction between the stressor and drug environment in its effects on reinstatement (108). In subsequent related studies, exposure to i.c.v. injections of CRF or footshock stress were effective in inducing the reinstatement of cocaine seeking when a significant time delay was imposed between the presentation of the stressor and testing for reinstatement, but only so long as the stressor and delay period were experienced in the same context as that in which animals had self-administered the drug and undergone extinction (i.e., in the self-administration chamber) (49, 123). For example, animals that were injected with CRF and placed in the self-administration chamber for 2 h prior to the start of testing, exhibited robust reinstatement of responding. In contrast, animals that were injected with CRF and returned to their home cage for the 2 h delay period failed to exhibit reinstatement (49). These findings were explained within a contextual conditioning account of reinstatement, based on that originally put forward by Bouton and colleagues (32, 124–126). Specifically, it was argued that experiencing the stressor in the context of the self-administration chamber served to elicit a conditioned excitatory response developed to that context during training that overcame the presumably inhibitory processes maintaining extinction responding. On the other hand, when the acute effects of the stressor were experienced outside the self-administration chamber (in the home cage), the peptide failed to elicit the conditioned excitatory response; as a result, the processes governing extinction were maintained (49). This explanation of stress-induced reinstatement is also consistent with the idea that stress acts during
extinction, as it does during reinstatement testing, to disinhibit responding that is under inhibitory control (127).

4. Conclusions

The most insidious aspect of drug addiction in humans is the chronic relapse to drug use. Thus, successful treatment is incumbent on achieving an understanding of the complex mechanisms underlying the phenomenon. In the past several decades, the reinstatement model has proved a powerful tool for studying the basic underpinnings of long-term relapse to drug use. Indeed, the triggers of reinstatement to drug seeking that have been the focus of this chapter are those that drug addicts report as the primary triggers of drug craving and relapse in their own experience, and data generated using variations of the reinstatement procedure have led to considerable progress in the identification and characterization of the processes underlying the behaviors that govern relapse (6).

As mentioned in the Introduction, a major recent focus of studies on the reinstatement of drug seeking has been on identifying the neural substrates of reinstatement to drug seeking by reexposure to drugs, drug cues, and stressors ((6, 7, 12, 22, 23) for recent reviews). This work, together with the more phenomenological work emphasized in this chapter, allows several general conclusions to be drawn about the basic substrates of drug relapse. First, the collective work suggests that the reinstating effects of the different triggers of reinstatement are largely independent of drug class and experimental procedure; that is, the different triggers of reinstatement tend to be associated with similar outcomes, regardless of the animal’s drug history or the type of reinstatement procedure employed (i.e., self-administration versus CPP). Second, the behavioral effects of the different triggers of reinstatement are mediated by neural and corresponding motivational processes that are overlapping, but not identical (7). This observation has important clinical implications for the development of appropriate pharmacological and/or behavioral strategies for treatment, because it underscores the complexity of the systems involved, and suggests that a single approach to treatment is unlikely to be effective under all conditions. Finally, the collective work speaks to the idea that drugs of abuse can produce profound and long-lasting changes in the function of the central nervous system, and that these changes can be reflected in a long-term vulnerability to relapse.
References


## Index

### A

Acetylcholine (ACh) ................................. 214  
   muscarinic ACh receptor ........................ 214  
   nicotinic ACh receptors (nAChRs) .......... 34–36, 108–114, 119  
ADE. See Alcohol deprivation effect  
Adrenocorticotropic hormone (ACTH) ........ 118–119, 209–210, 216, 218, 219, 437  
Alcohol .................................................. 133–158, 329–330, 420–421  
Alcohol deprivation effect (ADE) ................ 138, 144, 151–152, 156–157  
Allostasis .................................................. 279–280  
Amphetamine ............................................. 294–299  
Amygdale  
   basolateral amygdala ...................... 182, 185, 316, 319, 326, 327, 330, 331, 352  
   central amygdala ............................ 25, 34, 36, 315  
Anhedonia ........................................... 29, 30, 91, 348–442, 444–449, 453  
Anorexia nervosa ............................... 208, 209, 211, 214–215, 218, 219, 222, 421  
Autoshaping ........................................... 239–240, 252, 257–258  

### B

Baclofen ................................................. 111, 113, 258  
Barbiturate ............................................. 41–42, 69, 242  
BDNF. See Brain-derived neurotrophic factor  
Bed nucleus of the stria terminalis (BNST) ........ 25, 35, 36, 471–472  
Behavioral economics .............................. 73–75, 147–148, 243–244, 256, 259, 270, 280  
Benzodiazepine ....................................... 41–43, 69, 242, 385  
Binge drinking ......................................... 150–151, 156, 420, 421  
BNST. See Bed nucleus of the stria terminalis  
Brain-derived neurotrophic factor (BDNF) ........ 422  
Bulimia nervosa ..................................... 208, 209, 211, 214–215, 222, 421  

### C

Calcium-calmodulin (CaM)-dependent protein kinase II (CaMKII) ................................. 193–201  
CaMKII. See Calcium-calmodulin (CaM)-dependent protein kinase II  
cAMP. See Cyclic AMP  
Cannabinoid  
   cannabinoid receptor ........................... 21, 26, 39–40, 44, 112, 114, 246, 330, 466, 467  
   cannabinoid 1 (CB1) receptor .......... 39, 40, 112, 214–215, 221, 246  
Cannabis ................................................. 350, 384  
CART. See Cocaine-amphetamine regulator of transcription  
CCK. See Cholecystokinin  
Cholecystokinin (CCK) ............................. 211–213, 215  
Cocaine-amphetamine regulator of transcription (CART) .................................. 209, 211, 212, 214, 422  
Compulsion ............................................ 103, 207, 337–371, 406  
Conditioned place aversion (CPA) .............. 174. See also Place conditioning  
Conditioned place preference (CPP) ............ 4, 11, 112, 140, 146–147, 151, 155–156, 170, 173–186, 249, 253, 322–324, 443, 463–466, 468, 471, 472, 474. See also Place conditioning  
Conditioned reinforcement .......................... 65–66, 181, 326–327, 350  
Conditioned suppression ........................... 69–70, 281, 359–361  
Conditioning  
   classical ............................................ 26, 64, 91, 92, 320  
   contextual ......................................... 140, 156, 256, 473  
   operant .............................................. 92, 93, 139, 142, 144, 248, 422, 452  
Context  
   contextual cue .................................... 139, 140, 145, 184, 329, 468, 470  
   contextual reinstatement ......................... 140, 324  
   environmental ................................... 66, 140, 152, 294, 297, 299, 301–303, 305, 465  

CORT. See Corticosterone
Cortex
  cingulate ........................................... 119, 390, 391
  infralimbic ......................................... 344, 391, 417
  medial prefrontal cortex (mPFC) .............. 119, 182
  orbitofrontal cortex (OFC) ...................... 316, 319, 320,
  352, 353, 355, 370, 390, 391
  prefrontal cortex (PFC) ..................... 25, 26, 28, 30–31, 35,
  191–194, 199, 303, 320, 371, 386, 390, 391, 417
Corticosterone (CORT) .............................. 118–119, 216, 255,
  257–258, 437, 472
Corticotronin releasing factor (CRF) .......... 86, 114, 119,
  271–273, 471–473
Corticotronin-releasing hormone (CRH) ..... 210, 211, 214,
  216–220
Cortisol .................................................. 216, 220, 225–256, 437
CPA. See Conditioned place aversion
CPP. See Conditioned place preference
Craving ................................................. 152–157, 283–284, 311–332
CRF. See Corticotronin releasing factor
CRH. See Corticotronin-releasing hormone
CS. See Conditioned stimulus
Cyclic AMP (cAMP) ..................................... 193, 200–201
  response element binding protein ........... 193

D
DA. See Dopamine
Delta FosB .................................................. 193
Dependence ........................................ 87–91, 102, 154–155, 284–285
Depression .............................................. 28, 37, 58, 70, 91, 207, 439–441
Dopamine (DA). 15, 58, 110, 150, 191, 220, 248, 302, 319,
  346, 384, 404, 433, 467
  D1 DA receptor ........................................ 110, 200–201, 251, 324,
  325, 329, 423
  D2 DA receptor ........................................ 15, 31, 110, 248, 251,
  329, 330, 369, 391–392, 423
  D3 DA receptor ........................................ 110, 391–392
Drug discrimination .................................. 59, 370, 443, 467
Drug seeking ....................................... 66–68, 103, 114, 134, 139, 140,
  152–154, 168, 180–186, 192, 240–241, 247,
  249, 253, 254, 256, 280, 283–284, 293–294,
  312, 315–321, 323–324, 326–329, 331,
  338–344, 349–371, 380, 387, 389, 392–393,
  409, 448, 462–474
Drug taking .......................................... 58, 59, 67–68, 89, 93, 182, 184,
  192, 197, 243, 247, 257, 270, 283, 293–306,
  313, 315, 317, 321, 326–328, 337–338,
  403–424, 431, 466, 468, 470
D+Tetraydrocannabinol (D+THC) .... 21, 28, 39–40, 257
Dysphoria ................................................. 41, 134, 432, 438–444
E
Enriched environment .................................. 251–254, 259
Estrogen .................................................... 215, 221, 246
Ethanol ........................................ 22, 69, 133, 172, 240, 274, 345, 468
Extinction ............................................. 42, 68–69, 72, 92–95, 103, 108–109, 114, 118,
  139, 140, 151, 152, 181–186, 239, 247–250, 252,
  254, 280, 283–284, 320–321, 324, 325, 344–348,
F
Feeding .................................................. 142, 207–211, 213–217, 219–222,
  239–240, 254, 342–343, 406, 422, 450
Fentanyl .................................................. 38–39, 91–92, 246, 254–255, 275, 276
Food restriction ....................................... 85, 106, 120, 142, 208, 215,
  218, 220, 222, 254, 363, 422

G
GABA. See Gamma aminobutyric acid
Gambling .............................................. 268, 390, 406
Gamma aminobutyric acid (GABA) .............. 111, 113,
  114, 258, 302, 330, 422
Genetic ................................................. 39, 71, 107, 113, 134–136, 141, 147,
  148, 151–152, 157, 179, 211–215, 220–221, 238,
  250, 251, 258, 259, 390, 406, 408, 411, 420, 423
Ghrelin .................................................... 210, 211, 214
Glucocorticoid ....................................... 210, 216–218, 220
Glutamate ............................................. 26, 35–36, 110–111, 114, 119, 193, 198–199,
  201, 239, 257, 330, 344
AMPA glutamate receptor ......................... 193, 198–201
NMDA glutamate receptor ......................... 29, 34, 111, 119,
  179, 182, 198, 257–258, 412–414, 421

H
Habit ...................................................... 37, 84, 88, 90, 92–97, 314, 315,
  320, 337–347, 401, 406
Heroin ................................................... 20, 57, 83, 183, 243, 272, 294,
  317, 351, 385, 408, 432, 463
Hippocampus ........................................... 25, 120, 191–194, 216–217, 316
Homeostasis ............................................ 216
6-Hydroxydopamine (6-OHDA) .................. 112, 113, 192
Hypothalamus ......................................... 25, 28, 30–31, 43, 119,
  209–212, 216, 220, 286, 448, 449, 472

I
ICSS. See Intracranial self-stimulation
ICV. See Intracerebroventricular
Impulsivity ........................................ 207, 245, 247–248, 258, 320, 340,
  355, 364, 367–369, 371, 379–393, 405, 452
Incentive motivation .................................. 25, 89, 96, 139, 145–146,
  155, 316, 318, 319, 321, 322, 327, 330, 331, 353,
  387, 390, 468, 470–471
Incentive salience ................................. 315, 319, 355, 404, 444, 468
Incubation ............................................. 152–153, 283, 327–329
Inhibition ............................................. 10–11, 110, 116–118, 169, 193,
  195–197, 215, 216, 222, 248, 359, 361, 381–389,
  392, 451–452
Intra-cerebroventricular (ICV) .............. 36, 90–92, 213, 244, 387
Intracranial self-stimulation (ICSS) .......................... 3–44, 91, 120, 409, 448, 449
Intravenous self-administration (IVSA) .............. 14, 85, 89, 101–110, 112–123, 408

K

L
Leptin ................................................................ 210–214, 216–218, 220, 221, 422
Locus coeruleus .................................................. 25, 31

M
MAO. See Monoamine oxidase
Maternal separation ............................................. 149, 254, 422
MDMA. See 3,4-Methylene dioxy- N-methylamphetamine
Medial forebrain bundle (MFB) ......................... 25, 40, 43
Mesolimbic dopamine ....................................... 25, 58, 112, 253, 320, 323–326, 391
Methamphetamine ........................................... 26, 33, 57, 58, 246, 274–275, 279–281, 283, 284, 323, 324, 328, 330, 384, 390, 433, 436, 452, 472
3,4-Methylene dioxy- N-methylamphetamine (MDMA) ... 22, 41–43, 294, 384, 390, 443, 466
MFB. See Medial forebrain bundle
MK-801 .......................................................... 257
See also Nonhuman primate
Monoamine oxidase (MAO) ...................................... 115–118, 434

N
NA. See Noradrenaline
NAcc. See Nucleus accumbens
Naloxone ............................................... 20, 21, 37, 38, 87, 88, 90, 94, 112, 182, 329
Naltrexone ............................................... 20, 38, 88, 112, 153, 182, 317–318, 329
NE. See Norepinephrine
Neuroendocrine ........................................... 209, 212, 221
Neuropeptide Y (NPY) ................................... 210–212, 214–217, 220

O
Obesity .......................................................... 63, 208, 209, 211–215, 217, 220–222, 330, 405
Obssessive compulsive disorder (OCD) .............. 222, 356
6-OHDA. See 6-Hydroxydopamine
Opioid receptor
δ opioid receptor ............................................. 38, 214
κ opioid receptor ............................................. 38
μ (mu) opioid receptor .................................. 38, 84, 113, 250, 302–304
Opponent process ............................................ 317–318, 404, 432, 437, 438

P
Pain ................................................................ 38, 63, 70, 84, 88, 90–92, 254, 281
Paraventricular nucleus (PVN) ......................... 119, 210–211, 216–217, 220
PCP. See Phencyclidine
Pedunculopontine tegmental nucleus (PPTg) ......... 112–113
Peptide .......................................................... 209–214, 216–217, 473
Phencyclidine (PCP) ........................................ 28, 29, 41, 42, 184, 240, 246
PKA. See Protein kinase A
Place conditioning ............................................ 59, 66, 151, 155, 167–186
Polydipsia ....................................................... 102–103, 105, 138, 363
Polydrug .......................................................... 277, 294, 297–301
PPTg. See Pedunculopontine tegmental nucleus
Prenatal ........................................................... 255, 257, 259
Progesterone ..................................................... 246
Protein kinase A (PKA) ..................................... 193, 200–201

Animal Models of Drug Addiction
Index
PVN. See Paraventricular nucleus

Reward

Schedule of reinforcement

Tolerance

An i mAl mo d e l s o f dr u g Ad d i c t i o n

Stimulus

Learning

Stress

Smoking

Social isolation

Sensitization

Streptomycin

Subcutaneous

Sensitization

Streptomycin

Subcutaneous