INDIVIDUAL VARIATION IN THE MOTIVATIONAL PROPERTIES OF REWARD CUES

by

Benjamin Thomas Saunders

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in the University of Michigan
2013

Doctoral Committee:

Professor Terry E. Robinson, Chair
Assistant Professor Brandon J. Aragona
Professor Kent C. Berridge
Associate Professor Geoffrey G. Murphy

In memory of Lovie Mae Marino

Acknowledgments

First and foremost, I would like to thank my graduate advisor, Terry Robinson, for his world-class mentoring, and serving as a constant inspiration for the kind of investigator I hope to be one day. I also owe many thanks to the members of my committee – Kent Berridge, Brandon Aragona, and Geoff Murphy – for their time and critical input on this thesis. In particular, Kent Berridge has been an invaluable source of input on papers I have written here at Michigan. Brandon Aragona has also been a wonderful mentor-friend to me, and I really appreciate his support.

In my time in the Robinson lab, I was surrounded by many smart, thoughtful, and dedicated people, who not only provided absolutely essential training and technical support, making this research possible, they also became wonderful friends. In particular, I want to thank Lindsay Yager for being the best lab partner ever, Paul Meyer and Vedran Lovic for countless discussions about science and life, and Liz Cogan for keeping the lab "fancy". Thanks are also due to previous members of the Robinson lab for their training and friendship earlier on in my graduate career, including Jake Jedynak, Ken Wakabayashi, Kristi Pickup, and Courtney Cameron. Shelly Flagel deserves special thanks for her considerable input on my projects and writing. Many talented undergraduates were also critically involved in my research along the way, and it is no exaggeration to say that this dissertation would not have been possible without them. Specifically, I thank Elizabeth O'Donnell, Jacqueline Antonishek, Viktoria Lesya, Taylor Swabash, Adam Dziuba, and Ethan Kolderman for their help. Elizabeth O'Donnell in particular

was critically involved in several experiments, and is now generating her own exciting data. I can't thank her enough for sticking with me for so long.

Outside of lab my time at Michigan was filled with more great friends. My topnotch cohort – Jeremiah Bertz, Caitlin Orsini, Jocelyn Richard, and Eila Roberts – has in particular been a source of inspiration and amazing support. Additionally, Alex DiFeliceantonio, Matt Howe, Jeff Pettibone, Kyle Pitchers, Chris Fitzpatrick, Elyse Aurbach, Howard Gritton, Katy Goldey, Anne Berry, Steve Mahler, Seth Wescott, Eric Jackson, Alaina Case, Johnny Saldate, Shanna Harkey, Arif Hamid, Giovanna Paolone, Kirsten Porter-Stransky, Daniel Castro, Caitlin Vander Wheele, Aneesha Badrinarayan, Robert Schmidt, and Morgan Gustison. You all made this a great place to work. A special thanks to Jocelyn, for being great in so many ways. A few people from beyond Michigan also deserve acknowledgment here. Anne Foreman, for helping inspire me to go into research many years ago, and Jarrod Curry, for keeping me grounded in the real world and up to date on music and X-Men knowledge. Finally, my parents deserve probably the most credit here, for providing a lifetime of unconditional love and support for anything I ever wanted to do. As does my brother, Nick, for being my all time greatest best friend.

Table of Contents

List of Figures

Figure

Abstract

Reward-associated stimuli are critically involved in the organization of behavior. Such cues, if they are attributed with incentive motivational properties, can act as *incentive stimuli*, with the power to evoke complex emotional and motivational states that drive reward-seeking behavior. Incentive stimuli can motivate behavior via multiple psychological processes, including attracting approach, reinforcing actions themselves, and/or spurring on new or ongoing behavior, and these processes rely on a distributed set of overlapping, but partially distinct brain systems. Critically, not only do cues guide adaptive reward seeking, they also contribute to compulsive disorders, such as addiction and binge eating.

Importantly, there is substantial variation in the tendency of different individuals to assign motivational value to cues. Some rats (sign trackers), for example, find a discrete Pavlovian food-predictive cue attractive and desirable, in that it motivates approach behavior, and these animals will work avidly to obtain the cue. Other rats (goal trackers), alternatively, learn the cue's association with food, but do not find the cue itself attractive or desirable, instead approaching the location of food delivery. Thus, reward cues only acquire incentive stimulus properties in some individuals. This may have broad implications for understanding both natural variations in normal reward seeking, as well the factors that contribute to differences in susceptibility to psychopathologies such as addiction. The primary goals, here, are to better understand the neural mechanisms responsible for this variation, and to establish the extent to which variation in food-cue responsivity predicts variation in behavior motivated by drug cues.

In the experiments in chapter 2, I examined the role of dopamine signaling within the nucleus accumbens in the expression of different types of Pavlovian conditioned responses. I found that dopamine signaling within the accumbens core is necessary for the expression of sign-tracking, but not goal-tracking, approach behavior. The results suggest that dopamine in the accumbens mediates the incentive motivational, rather than learned, value of reward cues.

In chapter 3, I examined whether the individual variation in responsivity to discrete food cues exhibited by sign and goal trackers predicts the degree to which a discrete cocaine cue motivates behavior. I found that a discrete cocaine cue was more important for maintaining cocaine self administration, and served as a conditioned reinforcer to reinstate cocaine seeking behavior to a greater degree in sign trackers than it did for goal trackers. This suggests that some individuals may be more "reactive" to discrete cues associated with both food and drugs.

In chapter 4, I utilized a novel procedure to examine individual variation in the ability of a discrete cocaine cue to spur cocaine-seeking behavior in the face of adverse consequences. I found that a discrete cocaine cue, evoked much greater cocaine-seeking behavior in sign trackers than goal trackers, even in the face of negative costs associated with the seeking response. Additionally, I found that blockade of dopamine signaling within the accumbens core prevented this cue-evoked cocaine seeking, and amplification of dopamine transmission was sufficient to enhance seeking responses. This suggests that there is considerable individual variation in the ability of discrete drug cues to evoke motivational states that drive behavior, and dopamine signaling in the accumbens core is necessary and sufficient for this process.

With experiments in chapter 5, I examined whether there were similar individual differences in the motivational properties of cocaine itself. I found that sign trackers were willing to work much harder to obtain infusions of cocaine, in the absence of discrete drug cues, compared to

goal trackers. Additionally, exposure to a small amount cocaine (a "prime") elicited robust reinstatement behavior in sign trackers compared to goal trackers. This suggests that the interoceptive cues associated with drugs also spur different levels of motivation in different individuals.

In chapter 6, I investigated potential individual variation in the ability of drug-associated contextual cues to motivate behavior. I found that a cocaine context instigated much more robust conditioned hyperactivity, and reinstated greater cocaine-seeking behavior in goal trackers, relative to sign trackers. Furthermore, dopamine signaling within the accumbens core was necessary for this reinstatement effect. Taken together with my other results, this indicates that sign trackers preferentially assign motivational significance to discrete reward information, while goal trackers do to contextual information.

Thus, the individual variation expressed by sign and goal trackers reflects broad underlying variability in reward-cue processing. Given that these animals exhibit sensitivity to different types of reward-associated information, there may be multiple "vulnerability" states underlying a variety of maladaptive patterns of reward-seeking behavior. Further exploration of the mechanisms underlying variation in responsivity to reward cues will provide a better understanding of the organization of adaptive reward seeking, as well as the development of many psychopathologies.

Chapter 1

Introduction

To survive, animals must navigate a complex, ever-changing environment, and stimuli associated with different behavioral outcomes help organisms do this, in part by coordinating approach towards desirable stimuli and avoidance of potentially harmful ones (Hebb, 1955; Ikemoto, 2010; Moltz, 1965; Schneirla, 1959). Thus, from worms to humans, environmental cues play an important role in guiding individuals to successfully seek out what is critical for survival, by signaling the current or future availability, location, quality, and/or quantity of rewards. The sensory systems of different animal species have evolved specifically to enable efficient processing of particular types of reward cues important for their survival. Color vision, for example, is thought to have evolved in many species, including insects and primates, due to selection pressures favoring the ability to visualize colorful flowers and fruits, which facilitates successful foraging. Thus, environmental cues serve a phylogenetically ancient purpose: to increase the probability of acquiring rewarding stimuli and avoiding aversive stimuli.

The role of cues in reward seeking

Reward "cues", as I will discuss them here are broadly defined, and can exist in any single sensory modality, or combinations of different modalities. I will roughly classify reward cues into three categories. First, cues can be discrete, localizable stimuli. Often in rodent and human experiments, this type of cue is also transiently present in the environment, in that its appearance

is explicitly associated in time with some event. Second, cues can be static, contextual stimuli (e.g., places where rewards are acquired and/or consumed). Third, internal states produced by the experience of rewards themselves can serve as cues. It is important to point out, however, that while these broad definitions work well for laboratory studies, in a natural world setting free from experimental constraints, there is some degree of overlap. As one example, discrete, localizable cues may also be static in the environment. Additionally, in experimental settings, cues are often relatively simple, unimodal stimuli, such as lights, tones, images, or a test chamber, but in reality, reward cues are generally complex compound stimuli.

In now classic studies, Pavlov (1927) demonstrated that if a previously neutral stimulus (conditional stimulus, CS) reliably predicts the delivery of a reward (unconditional stimulus, US), over time the CS will come to elicit a conditional response (CR). Pavlov found that in hungry dogs if the ticking of a metronome was paired with food delivery, the sound of the metronome itself (the CS) came to elicit salivation (the CR). Given that the dogs initially salivated unconditionally when presented with the US, Pavlov referred to the CS-elicited CR as a *conditioned reflex* (Pavlov, 1927). For many years after these experiments, researchers described Pavlovian conditioned behavior largely in terms of stimulus – response (S-R) *habits* (Berridge, 2001). That is, as a consequence of learning, a Pavlovian CS comes to evoke a rigid, inflexible behavioral response. Researchers have now long known, however, that beyond eliciting simple, reflexive CRs, CSs may also be attributed with *incentive motivational properties* ("incentive salience"), becoming *incentive stimuli*, and thus acquire the ability to activate complex emotional and motivational states (Berridge, 2001; Bindra, 1978; Bolles, 1972; Cardinal et al., 2002a; Konorski, 1967; Rescorla, 1988; Toates, 1986; Trowill et al., 1969; Young, 1959; Young, 1966). Incentive salience refers specifically to the acquired perceptual and motivational properties of a

stimulus that render it attention grabbing and "wanted"[1] (Berridge and Robinson, 1998). Thus, Pavlovian CSs not only have predictive or associative value – they signal information about rewards – they can also acquire powerful motivational properties of their own, acting as incentive stimuli. Importantly, the motivational properties of a reward or reward cue are not simply a fixed characteristic of the stimulus itself, but are modulated by the physiological state of the individual (Cabanac, 1979; Toates, 1986; Young, 1959). For example, when one is hungry, the incentive value of rewards and their cues is potentiated, when sated, their value is relatively diminished. Various circumstances, therefore, such as hunger, thirst, or even drug-induced states can modulate the motivational value of learned reward cues. The complexity of these psychological responses to rewards and cues – well beyond simple S-R habits – has the effect of greatly increasing the flexibility and diversity of an individual's behavioral repertoire, allowing for adaptive reward seeking (Toates, 1986).

Barry Everitt and colleagues (e.g., Cardinal et al., 2002a; Everitt et al., 2001; Milton and Everitt, 2010) have developed a useful conceptualization of incentive stimuli that emphasizes three fundamental properties. An incentive stimulus 1) is attractive and attention grabbing, drawing individuals into close proximity with it. 2) It is itself desirable, in the sense that it can reinforce actions to obtain it. 3) Its presence can evoke a conditioned motivational state capable of both instigating seeking behavior, and invigorating ongoing behavior. Collectively, these properties define an incentive stimulus but, importantly, they are psychologically dissociable, and rely on overlapping but distinct neural systems (Cardinal et al., 2002a; see below). Taken together, if a reward-associated cue acquires these properties it is, in effect, transformed from a

[1] Here I will follow the convention of Berridge and Robinson (1993, 2003). "Wanting", with quotation marks, refers to implicit motivational drives (incentive salience) for which conscious awareness is not necessary. This is distinct from wanting, without quotation marks, which refers to explicit conscious desires. In the preclinical studies described here, I will refer to motivational drives as states of "wanting" for rewards and associated stimuli.

predictive but motivationally "cold" CS into a "hot" incentive stimulus, which can exert motivational control over behavior (Cardinal et al., 2002a; Meyer et al., 2012a).

Conditioned Approach

An important feature of an incentive stimulus is its ability to grab one's attention and attract, which has the effect of drawing individuals into close physical proximity with it, and thus usually with the reward itself. Experimentally, this phenomenon is measured as Pavlovian conditioned approach behavior. It was demonstrated years ago that if a localizable Pavlovian cue reliably predicts the presentation of a reward, some animals will learn to approach the CS itself, even though no response is necessary to obtain the reward (Brown and Jenkins, 1968). This CS-directed approach behavior was called "sign-tracking" (Hearst and Jenkins, 1974), the word "sign" referring to the cue, and often includes vigorous engagement with the cue that mimics the consummatory response associated with the type of reward delivered (Davey and Cleland, 1982; Jenkins and Moore, 1973; Pavlov, 1932). Originally, the term "autoshaping" was used to describe the procedure that produces this type of Pavlovian CR (Brown and Jenkins, 1968), but this is actually a misnomer, because with the Pavlovian procedure no responses are ever reinforced (i.e., shaped). Indeed, the development of conditioned approach is not due to accidental reinforcement or "superstitious" behavior (Skinner, 1948). This was neatly demonstrated in Pavlovian conditioning studies in which a negative contingency was implemented, whereby contact with the CS resulted in omission of the reward. Under these conditions, animals continue to approach and sometimes even contact the CS, despite no longer receiving reward (Killeen, 2003; Lajoie and Bindra, 1976; Schwartz and Williams, 1972; Timberlake and Lucas, 1985; Williams and Williams, 1969). Many species of animals, including birds, fish, rats, mice, monkeys, and humans, are known to exhibit sign-tracking behavior

(Breland and Breland, 1961; Brown and Jenkins, 1968; Burns and Domjan, 1996; Cole and Adamo, 2005; Gamzu and Schwam, 1974; Hearst and Jenkins, 1974; Nilsson et al., 2008; Pithers, 1985; Tomie et al., 2012; Wilcove and Miller, 1974; Williams and Williams, 1969).

Conditioned Reinforcement

In addition to being attractive, incentive stimuli can also become desirable, in the sense that they will reinforce actions that lead to their obtainment. In experimental terms, incentive stimuli act as conditioned or secondary reinforcers. Conditioned reinforcers can serve as a bridge between delays in the receipt of primary reinforcers, capable of maintaining responding for long periods of time in the absence of the primary reward, and they can support the learning of new and complex behavioral chains (Fantino, 1977; Hall, 1951; Hull, 1943; Kelleher and Gollub, 1962; Mackintosh, 1974). This has the effect of greatly increasing the persistence and complexity of behavior. Cues are thought to result in conditioned reinforcement via two mechanisms: by triggering a general motivational state, independent of particular outcomes, and/or by evoking a representation of a specific rewarding outcome that reinforces further behavior (Burke et al., 2007, 2008; Parkinson et al., 2005).

The conditioned reinforcing properties of reward cues can become quite powerful. For example, they maintain behavior in the absence of rewards (Di Ciano and Everitt, 2004), they are resistant to extinction (Arroyo et al., 1998; Di Ciano and Everitt, 2005; Panlilio et al., 2005), and continue to reinforce responding even after US devaluation (Davis and Smith, 1976). The ability of cues to act as conditioned reinforcers is clearly illustrated by many reward self-administration studies utilizing traditional extinction-reinstatement procedures to model relapse behavior (Nair et al., 2009; Shaham et al., 2003). In these studies, animals are trained to self administer a reward in the presence of an explicitly associated cue (often a light or tone), after which instrumental

responding is extinguished in the absence of the cue. The cue's ability to reinstate and maintain reward-seeking behavior is examined in a reinstatement test, wherein responses again produce the reward-paired cue, but under extinction conditions (that is, they do not receive the primary reward). Using this procedure, many studies have demonstrated that cues associated with a variety of rewards reinstate reward-seeking behavior (de Wit and Stewart, 1981; Kruzich et al., 2001; Milton and Everitt, 2010; Nair et al., 2009; Shaham et al., 2003). I should note that these studies typically refer to this effect as "cue-*induced* reinstatement", but in fact the way these procedures are typically applied the cue does not "induce" an action, but the action produces the cue, and therefore it is presumably the conditioned reinforcing properties of the cue that primarily increase responding.

Conditioned Motivation

Finally, incentive stimuli can arouse or evoke a conditioned motivational state that spurs reward-seeking behavior (Bindra, 1968; Cardinal et al., 2002a; Milton and Everitt, 2010). This is an important mechanism by which cues produce craving[2], which may not only instigate new actions to procure the reward, but also invigorate ongoing actions. The ability of a Pavlovian cue to invigorate instrumental behavior has traditionally been examined using Pavlovian-to-instrumental transfer (PIT) procedures (Estes, 1943; Estes, 1948; Holmes et al., 2010; Lovibond, 1983; Ostlund and Maidment, 2012; Rescorla and Solomon, 1967). Typically, individuals first receive Pavlovian training, where a cue is paired with reward delivery independent of any action. This is followed by an instrumental training phase, where the individual learns to make a response (e.g., lever press) for a reward. Subsequent *noncontingent* presentations of the Pavlovian cue (under extinction conditions) increase the rate, or "vigor", of instrumental

[2] As with wanting and "wanting", I will distinguish craving, without quotation marks, which reflects a conscious subjective state of desire, from "craving", which is an inferred implicit conditioned motivational state.

responding for reward. Similar to conditioned reinforcement, two varieties of this transfer effect have been described. First, PIT can occur in an outcome-*independent* manner (Dickinson and Dawson, 1987), where a cue enhances instrumental responding for any appetitive outcome, even those the cue was never paired with. For example, Balleine (1994) demonstrated that rats trained to self administer water responded at a higher rate when presented with either a water-associated cue, or a food-associated cue. Importantly, this general ability of cue to invigorate instrumental behavior is tied to internal motivational states, such that transfer is greatest when the individual is highly motivated for the associated rewards. If rats have been stated on food, for example, a food-associated cue does not increase responding for water (Balleine, 1994). Second, an outcome-*specific* form of transfer can occur (Colwill and Rescorla, 1988; Kruse et al., 1983), where a cue biases instrumental actions to favor the one that produces the same outcome that was paired with that cue. This form of transfer appears to be somewhat less dependent on internal motivational states (Corbit et al., 2007). Therefore, Pavlovian cues can directly modulate instrumental actions via multiple, dissociable processes.

As described above, most reinstatement studies typically use procedures in which the conditioned reinforcing properties of cues primarily control behavior (that is, the cue is presented contingent upon an action). But noncontingent presentation of rewards and reward cues can also produce a conditional motivational state that invigorates or reinstates extinguished reward seeking. Skinner (1938) demonstrated long ago that, following extinction, non-contingent presentation of a food pellet to rats reinstated responding. Similarly, Rescorla and Skucy (1969) found that giving rats noncontingent food retarded the rate of extinction of food-seeking, and this occurred even if continued responding delayed the next availability of food. Many of these reward "priming" studies have now demonstrated that exposure to even small amounts of a

variety of rewards can renew extinguished or long abstinent instrumental behavior (de Wit, 1996; Jaffe et al., 1989; Konorski, 1967; Skinner, 1938).

Reward cues can promote maladaptive behavior

Cues, while serving these vital roles in directing adaptive reward-seeking behavior, under certain conditions, may also serve as powerful temptations that can promote maladaptive patterns of behavior (Nesse and Berridge, 1997). This is best illustrated in many types of human psychopathology, where cues can instigate pathological reward-seeking in disorders such as compulsive eating and gambling, hypersexuality, and addiction.

For a large part of the second half of the twentieth century, the predominantly held psychological explanation for why addicts continue to self administer drugs despite many adverse consequences was because doing so alleviated an aversive state associated with withdrawal symptoms (Koob and Le Moal, 2001; Lindesmith, 1968; Solomon and Corbit, 1974). This was partly due to the popularity of drive-reduction theories at the time (Hull, 1943), but also because the majority of early drug self-administration studies utilized opiates, which produce physical dependence that leads to marked withdrawal symptoms upon abstinence. Many studies (e.g., Deneau et al., 1969; Stewart et al., 1984; Woods and Schuster, 1971) eventually demonstrated, however, that opiate use – and that of other drugs – develops and progresses in the absence of physical dependence and withdrawal symptoms. Furthermore, studies began to demonstrate that relapse of drug seeking could be instigated, through presentation of drug-associated cues or contexts, or by "priming" individuals with small amounts of drug, even in users who had been long abstinent (de Wit and Stewart, 1981; Hodgson et al., 1979; Stretch and Gerber, 1973). Based on studies like this, consensus began to shift to the view that drug use, similar to consumption of biologically relevant rewards such as food and water (Bindra, 1978), is

usually – though not always – mediated by the positive incentive motivational properties of drugs and associated cues, rather than an internal drive to reduce a negative withdrawal state. In regard to drug use this conceptual change was summarized by Stewart et al. (1984), who noted, "Need and drive views of motivation are gradually being replaced by a view … that ascribes a primary role to incentive stimuli as the generators of motivational states and elicitors of actions". It is, "the drug itself, or the presentation of a stimulus previously paired with the drug, that acts to create a motivational state that facilitates drug-seeking behavior" (p. 251, 256), a view that currently has broad support (Milton and Everitt, 2010; Robinson and Berridge, 1993). Indeed, we now know that if drug cues act as incentive stimuli, they may become especially critical for the development and persistence of addiction, in part because they facilitate three "routes to relapse" (see Figure 4 in Milton and Everitt, 2010). They may 1) bias attention, eliciting approach to drug-associated places and paraphernalia; 2) reinforce actions that lead to obtaining drugs; and 3) spur intense drug seeking by evoking a conditioned motivational state (e.g., implicit or explicit "craving"). These incentive motivational properties of drug cues, though dissociable, in addicts may work in concert to promote relapse, such that maintaining abstinence becomes overwhelmingly difficult. Indeed, several theories of addiction have emphasized the importance of drug cues (Di Chiara, 1998; Robinson and Berridge, 1993; Stewart et al., 1984; Tomie, 1996).

Until recently, there was no clear evidence that drug cues would support conditioned approach behavior directed towards the drug cue itself (i.e., sign tracking) in non-human animals, as is readily demonstrated with food cues (see above). Indeed, as recently as 2005, Everitt and Robbins (2005) speculated with regard to Pavlovian drug cue approach, "it may…be that the behavioral influence of CSs associated with drugs and natural reinforcers differ fundamentally in this regard" (p. 1482). Nevertheless, several studies using rats have now

demonstrated that drugs delivered in a variety of fashions do in fact support conditioned approach behavior (Cunningham and Patel, 2007; Krank et al., 2008; Tomie et al., 2008; Uslaner et al., 2006). Tomie (1996) was amongst the first to suggest that the ability of drug cues to instigate approach and engagement is important in the development of maladaptive drug use, given that many drug-associated stimuli (e.g., drinking containers, needles, and pipes, etc) are embedded within drug-delivery apparatuses. If these cues become attractive and facilitate approach and engagement, the likelihood of continued drug use will be high.

The ability of drug cues to act as conditioned reinforcers is another important mechanism contributing to persistent drug-seeking behavior. A variety of studies have demonstrated that, as with food cues, cues associated with drugs will maintain drug-seeking behavior for long intervals between drug delivery events, and support complex drug-seeking behavioral sequences (Arroyo et al., 1998; Di Ciano and Everitt, 2003, 2004; Di Ciano and Everitt, 2005; Everitt and Robbins, 2005; Goldberg and Tang, 1977; Katz, 1979; Kelleher, 1966; Kelleher and Goldberg, 1977; Schindler et al., 2002). Di Ciano and Everitt (2004) found, for example, that a visual cue associated with cocaine can actually reinforce the learning of a novel instrumental action, and maintain responding in the absence of the drug across two months of intermittent testing. Consistent with this, other studies demonstrated that self administration of drugs is more robust when a cue is associated with drug delivery, compared to when drug delivery is unsignaled (Caggiula et al., 2009; Caggiula et al., 2001; Panlilio et al., 1996; Schenk and Partridge, 2001). Many further studies using the traditional extinction-reinstatement procedure showed that animals will reinstate extinguished drug-seeking behaviors in order to receive presentations of a drug-associated cue alone (e.g., de Wit and Stewart, 1981; Shaham et al., 2003)

The presence of drug-associated cues can elicit conditioned motivational states that instigate drug-seeking behavior (Milton and Everitt, 2010). This is often the mechanism by which drug craving develops, and is thus an important way that drug cues promote relapse behavior. Additionally, exposure to small amounts of drug itself, and therefore, the interoceptive cues associated with the drug experience, can instigate craving and relapse (de Wit and Stewart, 1981; Hodgson et al., 1979; Jaffe et al., 1989). As mentioned above, the ability of cues to produce conditioned motivation is often measured in tests of Pavlovian-instrumental transfer, where the presence of a Pavlovian CS invigorates current instrumental behavior. However, until recently there was no clear experimental evidence that a drug-associated cue can produce a PIT effect. In an important study addressing this issue, LeBlanc et al. (2012) demonstrated drug cue PIT, showing that presentations of a cocaine-associated CS acutely increased the rate of ongoing self-administration behavior in rats. Interestingly, they found that the presence of a Pavlovian cocaine CS invigorated behavior during both the "seeking" and "taking" phases of the self-administration behavioral chain, which are analogous to the approach/preparation and consumption phases of drug taking. Though this topic requires more investigation, the results of LeBlanc et al. (2012) suggest that Pavlovian drug cues directly invigorate behavior at multiple points along the progression of drug use.

Neural mechanisms of Pavlovian reward cue processing

Considerable research suggests that the neural systems recruited by motivationally significant events are similar across many different classes of rewards, such as food, sex, and drugs (Cardinal et al., 2002a; Childress et al., 2008; Haber and Knutson, 2010; Ikemoto, 2010; Ikemoto and Panksepp, 1999; Kalivas and Volkow, 2005; Kelley, 2004a; Kelley, 2004b; Kelley and Berridge, 2002; Kelley et al., 2005; Kenny, 2011; Kuhn and Gallinat, 2011; Nair et al., 2009;

Volkow and Wise, 2005). These reward circuits comprise a wide, distributed network, including mesocorticolimbic dopamine pathways, which I will discuss in detail below. Though each may have specific functional roles in different reward-related processes, several brain regions, including the ventral tegmental area (VTA), dorsal and ventral striatum, ventral pallidum, thalamus, habenula, amygdala, and prefrontal/anterior cingulate/orbitofrontal cortex (PFC/ACC/OFC) are all known to be "engaged" by reward-associated cues (Cardinal et al., 2002a; Kalivas and Volkow, 2005; Kelley et al., 2005; Koob and Volkow, 2010; Schiltz et al., 2007). Together, these regions constitute a motivational system (Figure 1.1), comprised of cortico-striato-pallido-thalamic loops with extensive reciprocal interregional connectivity (Belin and Everitt, 2008; Belin et al., 2009; Haber et al., 2000; Haber and Knutson, 2010; Kalivas and Volkow, 2005; Zahm, 2000, 2006). Specifically, dopamine neurons in the substantia nigra and VTA project to subcortical targets in the ventral pallidum, nucleus accumbens core and shell, dorsal striatum, and hippocampus, and also to frontal-cortical areas such as the PFC and the "cortical-like" amygdala (Beckstead et al., 1979; Fields et al., 2007; Ikemoto, 2007; Swanson, 1982). The VTA receives GABAergic inputs from the nucleus accumbens, ventral pallidum, and habenula, and glutamatergic inputs from hippocampus and PFC, all of which regulate dopamine signaling (Carr and Sesack, 2000; Geisler and Zahm, 2005; Kalivas, 1993; Watabe-Uchida et al., 2012). The nucleus accumbens in particular sits at an important junction within this system, receiving the densest dopamine projections from VTA, as well as having reciprocal connections with the ventral pallidum, amygdala, hippocampus, and PFC/ACC/OFC (Berendse et al., 1992; Brog et al., 1993; Fields et al., 2007; Heimer et al., 1991; Hurley et al., 1991; Ikemoto, 2007; Kelley and Domesick, 1982; Kelley et al., 1982; Mogenson, 1987; Nauta et al., 1978; Zahm, 2000). Within the thalamus, the mediodorsal nucleus acts as an indirect relay between these

cortical and subcortical structures, as it receives input from the ventral pallidum, and sends projections to frontal cortical areas (Groenewegen, 1988; Ongur and Price, 2000; Ray and Price, 1992).

The ability of cues to act as incentive stimuli is dependent on the functional integrity of this motivational circuit, though the specific cells and systems required for each psychological process are somewhat dissociable (Cardinal et al., 2002a; Milton and Everitt, 2010). *Conditioned approach*: Pavlovian conditioned approach is dependent on signaling within nucleus accumbens, central amygdala, ACC, and OFC (Blaiss and Janak, 2009; Chudasama and Robbins, 2003; Parkinson et al., 1999; Parkinson et al., 2000a; Parkinson et al., 2000b). *Conditioned reinforcement*: The conditioned reinforcing properties of reward cues are dependent on the ventral striatum (likely nucleus accumbens), OFC, and basolateral amygdala (Burke et al., 2007, 2008; McDannald et al., 2011; Parkinson et al., 2001). *Conditioned motivation*: The neural systems supporting the ability of reward cues to produce a conditioned motivational state have been most clearly examined using PIT procedures. These studies suggest that the general and outcome-specific versions of PIT have somewhat dissociable neural substrates. For example, while both forms require an intact VTA, general PIT is dependent on the nucleus accumbens core, central amygdala, and dorsolateral striatum, while specific PIT requires the nucleus accumbens shell, basolateral amygdala, OFC, mediodorsal thalamus, and dorsomedial striatum (Corbit and Janak, 2007a; Corbit and Balleine, 2005, 2011; Corbit et al., 2007; Corbit et al., 2001; Hall et al., 2001; Holland and Gallagher, 2003; Murschall and Hauber, 2006; Ostlund and Balleine, 2007, 2008).

Cue processing within dopamine systems

Within the larger, distributed reward circuits described above, signaling in dopamine neurons projecting from the VTA to ventral striatal regions such as the nucleus accumbens is thought to be central to motivated behavior. Considerable debate exists, however, about dopamine's exact role, or roles, in reward (Beeler et al., 2012; Berke and Hyman, 2000; Berridge, 2007; Berridge and Robinson, 1998; Bromberg-Martin et al., 2010; Di Chiara, 1998; Ikemoto, 2010; Robinson et al., 2005; Salamone and Correa, 2012; Salamone et al., 2007; Saunders and Richard, 2011; Schultz, 2007; Wise, 2004). One view is that phasic signaling of dopamine neurons provides a "prediction-error" signal necessary for learning stimulus-reward associations (Bayer and Glimcher, 2005; Montague et al., 1996; Schultz et al., 1997). This hypothesis stems from electrophysiological recordings of dopamine neurons in the VTA and substantia nigra, as well as electrochemical measurements of actual dopamine release within the nucleus accumbens. These studies show that a phasic dopamine response that initially occurs to an unexpected reward (US), over the course of training, transfers in time to the CS that predicts reward delivery (Cohen et al., 2012; Day et al., 2007; Pan et al., 2005; Schultz, 1998; Schultz et al., 1997; Waelti et al., 2001). Additionally, these studies suggest that dopamine signaling also modifies cached predictive associations. For example, if a reward is bigger than expected based on the CS's current learned predictive value, dopamine neurons fire more, if it is smaller than expected, they fire less (i.e., a negative prediction error), leading to new learning (Pan et al., 2005; Schultz et al., 1997; Waelti et al., 2001).

Alternatively, others have argued that mesolimbic dopamine is not necessary for learning stimulus-reward associations per se, but for conferring learned reward cues with incentive salience, transforming them into "wanted", motivationally potent incentive stimuli (Berridge, 2007; Berridge, 2012; Berridge and Robinson, 1998). An important prediction from the incentive

salience hypothesis of dopamine is that changes in dopamine signaling can modify the motivational value of learned CSs 'on-the-fly', without the need to re-experience CS-US pairing (Zhang et al., 2012; Zhang et al., 2009). This is in contrast to learning-based accounts (Daw et al., 2005; Schultz et al., 1997; Sutton, 1988), which state that dopamine prediction errors update the learned value of a CS incrementally, on a trial-by-trial basis. It has been difficult to separate the potential contribution dopamine makes to learning from its contribution to incentive salience, because reward cues often acquire these properties together. However, recent studies have exploited individual variation in the tendency to attribute cues with motivational value, as discussed below, to dissociate these properties of reward cues (Berridge and Robinson, 2003; Robinson and Flagel, 2009). This will be addressed in the experiments in chapter 2.

Individual differences in responsivity to reward-associated stimuli

In the sections above, I reviewed how if reward-associated stimuli acquire incentive motivational properties they can guide behavior, by eliciting conditioned approach, serving as conditioned reinforcers, and by instigating conditioned motivational states. Importantly, however, accumulating evidence suggests individuals vary dramatically in the degree to which food-associated cues acquire these motivational properties.

It turns out there is considerable individual variation in the extent that CS-US pairing leads to the development of a strong sign-tracking (ST) conditioned approach CR (Tomie et al., 2000). Zener (1937) first described such variation in dogs, for which a bell was paired with the delivery of food. These studies were nearly identical to those done by Pavlov, but in his case Zener released the dogs from their harnesses, allowing them to move freely. Zener found that the type of CR the CS came to elicit varied across dogs. Some dogs exhibited "small but definite movement of approach toward the conditioned stimulus…followed by a backing up later to a

position to eat"; similar to what was later called sign-tracking behavior. Other dogs, however, exhibited "an initial glance at the bell" followed by "a constant fixation…to the food pan" (p. 391). Studies after this described similar individual variation in approach behavior, but Boakes (1977) was the first to systematically describe goal location-directed conditioned approach in the context of autoshaping experiments, which he termed "goal-tracking (GT)". I will use this ST/GT terminology here in respect of historical precedence.

This individual variation in conditioned approach behavior has recently been explored in a series of studies in rats utilizing a simple Pavlovian conditioning procedure, in which the extension of a lever (the CS) is paired with delivery of a food pellet (the US) into an adjacent food hopper. Under these conditions, where a discrete localizable cue that can also be manipulated is presented (versus, for example, a tone), some rats come to preferentially approach and engage the lever-CS itself (sign-trackers; "STs"), as described above. However, upon lever-CS presentation other rats (goal-trackers; "GTs"), initially glance at the lever-CS, but then go immediately to the food hopper (Figure 1.2), and make head and mouth movements in the hopper while awaiting food delivery (Mahler and Berridge, 2009). Yet other rats are ambivalent, alternating responses (Flagel et al., 2007; Meyer et al., 2012a). Both STs and GTs appear to learn their respective CRs at the same rate, indicating that the food cue is an equally effective CS – it evokes a reliable approach CR in both – the conditioned approach response is just directed to different locations in the environment (Robinson and Flagel, 2009). Critically, the different approach behaviors of STs and GTs are not a reflection of general differential learning capabilities, as both groups learn a variety of tasks equally well (Morrow et al., 2011; Robinson and Flagel, 2009; Saunders and Robinson, 2010). Rather, we have suggested that variation in the topography of the CR reflects underlying variation in the propensity to attribute incentive

salience to discrete Pavlovian CSs (Flagel et al., 2009; Flagel et al., 2007; Meyer et al., 2012a; Robinson and Flagel, 2009). Thus, only for STs does the discrete cue acquire those incentive stimulus properties that make it attractive.

Consistent with this interpretation, there is also considerable individual variation in the extent to which reward-associated cues acquire conditioned reinforcing properties. For example, for STs, the same food cue that was attractive during Pavlovian training is also an effective conditioned reinforcer (i.e., these rats will learn a new instrumental response for presentations of just the cue). For GTs, however, who did not approach the lever but instead the food hopper, the lever is a comparatively less effective conditioned reinforcer (Lomanowska et al., 2011; Meyer et al., 2012a; Robinson and Flagel, 2009). Furthermore, Yager and Robinson (2010) found that a food-associated cue was more effective in reinstating food-seeking behavior after extinction in STs than in GTs. These studies provide additional support for the hypothesis that STs and GTs differ in their propensity to attribute incentive salience toward some types of reward-associated cues (Meyer et al., 2012a). While several studies have characterized individual variation in the propensity to attribute incentive salience to reward cues by assessing their ability to motivate approach behavior, and act as conditioned reinforcers, few have assessed variation in the ability of cues to evoke a conditioned motivational state, as measured specifically with PIT procedures. To our knowledge, only one study has examined individual variation in PIT. Barker et al. (2012) recently found that mice vary in the degree that a food-associated CS invigorates food-seeking behavior.

In summary, many studies, using a variety of procedures, indicate that Pavlovian stimuli, in addition to informing an individual about upcoming rewards, can acquire powerful incentive properties. While there is little disagreement about this general concept, it is important to point

out that it is often assumed, either explicitly or implicitly, that a CS will also necessarily function as an incentive stimulus – that is, CSs generally also act as incentive stimuli. The individual differences in reward-cue responsivity described above, however, demonstrate that this assumption is not valid. For both STs and GTs a discrete localizable Pavlovian cue serves as an effective CS, evoking CRs, but it is attributed with incentive salience to a much greater degree in STs than GTs. Thus, a discrete food cue acquires the ability to instigate conditioned approach towards it, to act as a powerful conditioned reinforcer, and to arouse a conditioned motivational state to a greater extent in STs than GTs. This leads to the following conclusion: the conditional, predictive relationship between a CS and a US is not sufficient to confer motivational properties to the CS (Robinson and Flagel, 2009). For that to occur, learned cues must be attributed with incentive salience (Berridge, 2007; Zhang et al., 2012; Zhang et al., 2009).

As described above, drug-associated cues are critically involved in the development of maladaptive reward-seeking behavior, as evidenced by disorders such as drug addiction. Importantly, there are large differences in the vulnerability to develop addiction-related disorders. For example, only a small subset of the general population ever becomes addicted to drugs, even though the vast majority of people use a potentially addictive substance at some point in their lives (Anthony et al., 1994). Based on the studies demonstrating individual variation in responsivity to food-related cues described above, I suggest that a possible source of variation in susceptibility to maladaptive drug use may be differences in the ability of drug cues to gain motivational control over behavior. Specifically, individuals for whom drug cues acquire exaggerated incentive salience will find them difficult to resist, and will therefore be more vulnerable to developing the persistent and compulsive patterns of drug seeking characteristic of addiction.

Summary of the present experiments

With the experiments described in this dissertation I aim to better understand individual variation in reward cue responsivity, with the goal of achieving two aims. 1) Better understand the neural systems that underlie variation in Pavlovian conditioned approach responses. 2) Determine whether individual variation in the tendency to attribute incentive motivational value to a discrete food cue, as measured by conditioned approach behavior, predicts variation in responsivity to a variety of drug-associated cues.

Chapter 2: Dopaminergic regulation of individual variation in Pavlovian conditioned approach

Previous work has demonstrated that the acquisition of a sign-tracking CR is dependent on dopamine signaling, but the acquisition of a goal-tracking CR is not (Flagel et al., 2011b). It remained unclear from this study, however, the role of dopamine in the *performance* of already acquired sign- and goal-tracking behavior. In this chapter (Saunders and Robinson, 2012), I investigated this by administering flupenthixol, a nonselective dopamine receptor antagonist, directly into the nucleus accumbens core of rats after they had learned stable sign- and goal-tracking behavior. I found that flupenthixol dose-dependently attenuated a sign-tracking CR, but had little to no effect on a goal-tracking CR. Additionally, after administration of flupenthixol into the accumbens, sign-tracking behavior was fully impaired on the very first trial, before new learning via updated prediction-errors could occur. Consistent with the incentive salience hypothesis of dopamine function, this suggests that fluctuations in mesolimbic dopamine signaling can dynamically modify the motivational value of reward cues, without the need to re-experience the CS-US association (see Berridge, 2012; Zhang et al., 2012; Zhang et al., 2009).

Chapter 3: Individual variation in the ability of a discrete cocaine cue to maintain self administration and serve as a conditioned reinforcer to reinstate extinguished drug seeking

Discrete drug cues are important for the maintenance of self-administration behavior (Schenk and Partridge, 2001), and can reinstate extinguished drug seeking by reinforcing drug-seeking and -taking actions (i.e., by acting as conditioned reinforcers) (Shaham et al., 2003). In this chapter (Saunders and Robinson, 2010), I investigated whether a discrete cocaine cue was differentially important for maintaining self-administration behavior and reinstating extinguished drug seeking in STs and GTs. I trained STs and GTs to self administer cocaine in the presence of a discrete visual cue. Once rats reached stable behavior, I removed the cue, though left cocaine available. This resulted in a large reduction in the rate of self administration of STs, but not GTs. Furthermore, I trained a separate group of STs and GTs to self administer, then extinguished them. On a reinstatement test day, they then had the opportunity to respond, under extinction conditions, to receive the cocaine-associated cue. I found that under these conditions, STs showed much more robust reinstatement compared to GTs. Thus, discrete drug cues acquire greater control over drug seeking, and greater conditioned reinforcing power, in individuals for whom a discrete food cue also acquires these properties.

Chapter 4: Individual variation in the ability of a discrete cocaine cue to instigate reinstatement in the face of adverse consequences: role of accumbens dopamine

In addition to acting as conditioned reinforcers, drug cues can also promote reinstatement by evoking conditioned motivational states that spur drug-seeking actions (Milton and Everitt, 2010). This is an important mechanism by which craving is generated in human addicts, but most preclinical studies utilize a procedure (described above) that only allows for a clear assessment of the conditioned reinforcing properties of the cue. In this chapter, I utilized a novel procedure akin to PIT to investigate differences in the ability of a Pavlovian cocaine cue to instigate an instrumental action in STs and GTs. I trained rats to self administer cocaine in the presence of a

discrete visual cue, but then instead of extinction, I imposed escalating negative consequences (footshock) to drug seeking, such that rats discontinued cocaine use on their own. I then presented rats with a reinstatement test session during which the cocaine cue was presented to them noncontingently. STs reinstated much more in response to the discrete noncontingent cue, relative to GTs. Additionally, I found that antagonism of dopamine transmission in the nucleus accumbens core blocked this cue-evoked drug seeking, while potentiation of dopamine release, via administration of amphetamine in the core, potentiated reinstatement. This suggests that there are considerable individual differences in the ability of a Pavlovian cocaine cue to evoke a conditioned motivational state that instigates instrumental drug-seeking actions, and dopamine signaling within the accumbens core is causally involved in the generation and/or maintenance of this conditioned motivational state.

Chapter 5: Individual variation in the motivational properties of cocaine and cocaine-primed reinstatement

In addition to external stimuli, the internal state produced by the interoceptive cues associated with drugs themselves is important for the maintenance and reinstatement of drug seeking behavior (Stewart et al., 1984), because they evoke conditioned motivational states that instigate or invigorate behavior. In this chapter (Saunders and Robinson, 2011b), I investigated ST/GT variation in the motivational properties of cocaine itself. I trained STs and GTs to self administer cocaine in the absence of any explicitly-paired discrete cues. Once stable behavior was achieved, I shifted the response requirement to a progressive ratio schedule, which required rats to make more and more responses for every cocaine infusion. I found that STs continued responding for cocaine for much longer than GTs, reaching significantly higher "breakpoints" on the progressive ratio schedule. Following extinction training, I then tested the ability of cocaine

itself to reinstate cocaine seeking. Rats received an i.p. injection of cocaine before a final extinction session. STs, under these conditions, reinstated much more in response to the cocaine "prime" relative to GTs. These results suggest that there are also large individual differences in the ability of drugs themselves to evoke conditioned motivational states to maintain and reinstate drug seeking.

Chapter 6: Individual variation in cocaine context conditioning: role of accumbens core dopamine

Contextual stimuli, like discrete and interoceptive cues, are also important for the regulation of reward-seeking behavior. For example, returning rats to a drug-associated context, after they were extinguished in an alternate context, results in robust reinstatement of drug seeking (Fuchs et al., 2005). In this chapter, I investigated the extent to which there is individual variation in the ability of drug-related contextual information to motivate behavior. First, I examined conditioned hyperactivity in response to a cocaine context. STs and GTs received cocaine injections either paired with a unique test chamber, or unpaired in their home cage. On the test day, I injected all rats with saline, and found that GTs showed greater locomotor behavior when placed in the cocaine-associated context, relative to STs. In a second experiment, I trained STs and GTs to self administer cocaine in the absence of any explicitly-paired discrete cues. Rats were then extinguished in either the cocaine-training context, or a novel context. For the reinstatement session, all rats returned to the cocaine context. I found that under these conditions, GTs reinstated much more than STs. In a follow-up experiment, I redid the context reinstatement study, but administered flupenthixol into the nucleus accumbens core just prior to the reinstatement test. This manipulation blocked the context-induced reinstatement in GTs. These studies suggest that GTs preferentially assign motivational value to contextual reward-related

information, and dopamine signaling in the accumbens core is necessary for the expression of that motivational value. Taken together with my other studies, these results indicate that STs are not necessarily more vulnerable to addiction-like traits, as we have suggested, but that STs and GTs have different vulnerabilities, given that their brains process motivationally relevant information differently.

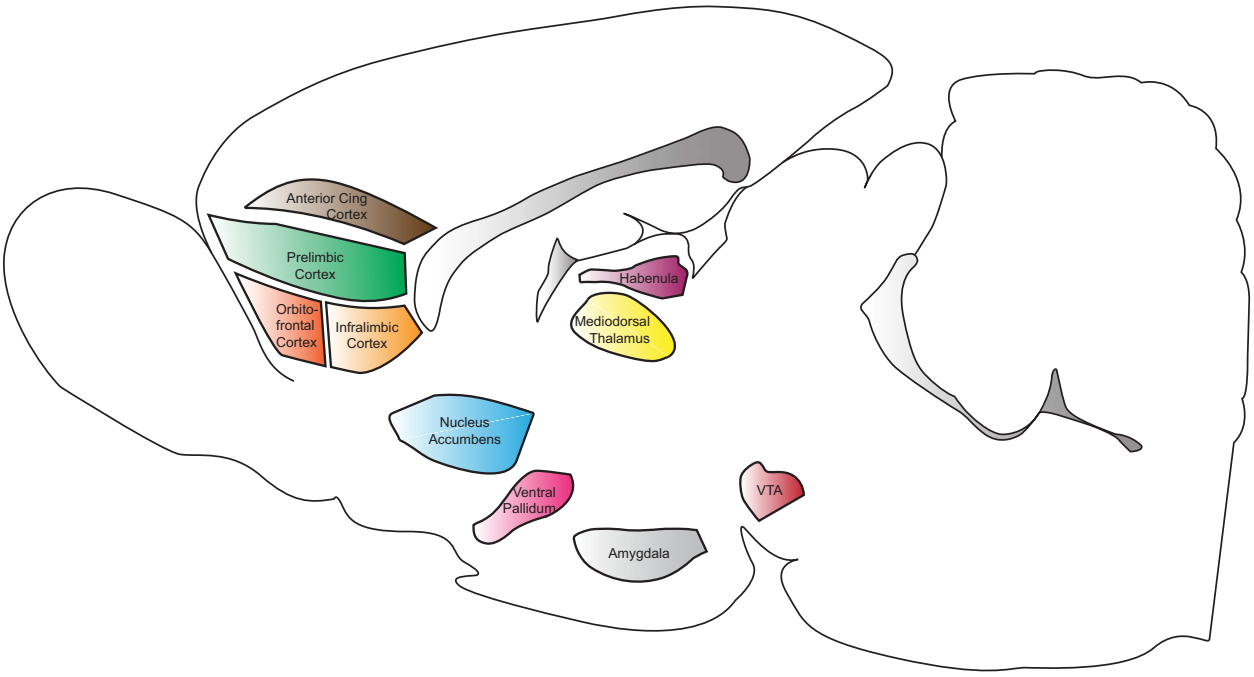


Figure 1.1. Major brain systems involved in processing reward-associated stimuli. This sagittal view of a rat brain shows some of the major regions that are "engaged" by experience with rewards and associated stimuli.

Figure 1.2. Representative pictures of a rat engaged in a ST CR (left), directed at the discrete CS (lever), and a rat engaged in a GT CR (right), directed at the food hopper, during the CS period (note that the lever is extended in both pictures).

Chapter 2

The role of accumbens core dopamine in the expression of Pavlovian conditioned responses

Introduction

There is general agreement that dopamine signaling within mesolimbic brain circuitry contributes to reward, but its exact role is less clear. One view is that phasic dopamine activity provides a prediction-error signal necessary for learning stimulus-reward associations (Bayer and Glimcher, 2005; Montague et al., 1996; Schultz et al., 1997). In contrast, others have argued that dopamine is not necessary for learning, but for attributing incentive salience to reward cues (Berridge, 2012; Berridge and Robinson, 1998; Zhang et al., 2012). It is difficult to parse these psychological functions, because the predictive and incentive values of reward-associated stimuli are strongly correlated and often change together. However, individuals vary in the extent to which they attribute reward cues with motivational properties, and this variation can be exploited to dissociate these components of reward (Berridge and Robinson, 2003; Flagel et al., 2009; Robinson and Flagel, 2009).

When a Pavlovian conditional stimulus (CS) predicts delivery of a food reward (US), only in some animals (sign-trackers, STs; Hearst and Jenkins, 1974) does the cue become attractive, eliciting approach towards it, and become desired, in that animals will work to obtain it (Robinson and Flagel, 2009). For others (goal-trackers, GTs; Boakes, 1977) the cue itself is not attractive, and is less desirable, but nevertheless, it comes to reliably evoke conditioned approach towards the location of impending food delivery (Flagel et al., 2011b; Lomanowska et al., 2011;

Robinson and Flagel, 2009). Thus, the cue is an equally predictive and effective CS for both STs and GTs, and it comes to evoke a conditioned response (CR) in both, but only in STs is the predictive CS attributed with incentive salience, rendering it an attractive and desirable "incentive stimulus" (Flagel et al., 2009).

Flagel et al. (2011b) took advantage of this variation to examine the role of dopamine in stimulus-reward learning. They reported that learning a ST CR, but not a GT CR, was associated with transfer of a phasic dopamine signal from the US to CS, and systemic injection of a dopamine antagonist prevented learning of a ST CR, but not a GT CR (see also Danna and Elmer, 2010). They suggested, therefore, that dopamine plays a selective role in attributing incentive salience to reward cues during learning. Flagel et al. (2011b) also reported that the performance of *both* sign and goal-tracking was impaired by dopamine antagonism. However, it is difficult to interpret this result, because the effects occurred at doses that also produced nonspecific reductions in motor activity. The purpose of this study was to further explore the role of dopamine in the performance of these two forms of Pavlovian conditioned approach, after they were acquired, using intracerebral drug administration to obviate nonspecific effects of dopamine antagonism on behavior. We focused on the core of the nucleus accumbens (NAcC), because of the considerable evidence this region is critical in mediating the learning and performance of motivated behaviors (Cardinal et al., 2002a; Ikemoto and Panksepp, 1999), and because it shows dopamine prediction-error signals (Day et al., 2007).

Materials and Methods

Subjects

Male Sprague Dawley rats (N=53) (Harlan, IN) weighing 275-325 grams at surgery were individually housed in a temperature and humidity controlled colony room kept on a 12-hr

light/12-hr dark cycle (lights on at 0800 hr). Water and food were available ad libitum (i.e., rats were not food deprived at any time). After arrival rats were given one week to acclimate to the colony room before testing began. During this period they were repeatedly handled by the experimenters. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus

Behavioral testing was conducted in standard (30.5 x 24.1 x 21 cm) test chambers (Med Associates Inc., St. Albans, VT, USA) located inside sound-attenuating cabinets. A ventilating fan masked background noise. For Pavlovian training each chamber had a food cup located in the center of one wall, 3 cm above a stainless steel grid floor. Head entries into the food cup were recorded by breaks of an infrared photobeam located inside. A retractable lever that could be illuminated from behind was located 2.5 cm to the left or right of the food cup, approximately 6 cm above the floor. The location of the lever with respect to the food cup was counterbalanced across rats. On the wall opposite the food cup, a red house light remained illuminated throughout all experimental sessions. Lever deflections and beam breaks were recorded using Med Associates software.

Surgery

Rats were anesthetized with ketamine hydrochloride (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The skull of each rat was leveled and chronic guide cannulae (22 gauge stainless steel; Plastics One) were inserted bilaterally 2 mm above the target site in the NAcC (relative to Bregma: anterior +1.8 mm; lateral +1.6 mm; ventral -5.0 mm). Guide cannulae were secured with skull screws and acrylic cement, and wire stylets (28 gauge, Plastics One) were inserted to prevent occlusion.

After surgery, rats received antibiotic and carprofen (5 mg/kg) for pain. Rats were allowed to recover from surgery for at least 7 days before testing began.

Microinjections

Dopamine receptor blockade was achieved with microinjections of the relatively nonspecific dopamine receptor antagonist, flupenthixol (Sigma, St. Louis, MO). We chose a nonspecific antagonist in order to block all actions of endogenous dopamine within the NAcC, to assess the general (i.e., not specific to a particular receptor) function of dopamine in the expression of different forms of Pavlovian conditioned approach behavior. Flupenthixol was administered in four doses: 0, 5, 10, and 20 µg in 0.9% sterile saline. Drug doses were based on previous studies (e.g., Di Ciano et al., 2001). Intracerebral microinjections were made through 28 gauge injector cannulae (Plastics One) lowered to the injection site in the NAcC (ventral -7.0 mm relative to skull), 2 mm below the ventral tip of the guide cannulae. During infusions, rats were gently held by the experimenter. All infusions were administered bilaterally in a volume of 0.5 µl/side, delivered over 90 s using a syringe pump (Harvard Apparatus, Holliston, MA) connected to microinjection cannulae via PE-20 tubing. After infusions, the injectors were left in place for 60 s to allow for drug diffusion before being withdrawn and replaced with wire stylets. All infusions were separated by at least 1 additional day of behavioral testing without treatment.

Procedure

Pavlovian training: Pavlovian training procedures were the similar to those described previously (Flagel et al., 2007; Saunders and Robinson, 2010). For two days prior to the start of training, 10 banana-flavored pellets (45 mg, BioServe, #F0059; Frenchtown, NJ) were placed in the home cages to familiarize the rats with this food. Approximately one week after surgery, rats were placed in the test chambers, with the lever retracted, and trained to retrieve pellets from the

food cup by presenting 25 45-mg banana pellets on a variable time (VT) 30-sec schedule. All rats retrieved the pellets and therefore the next day they began Pavlovian training. Each trial consisted of insertion (and simultaneous illumination) of the lever (CS) into the chamber for 8 s, after which time the lever was retracted and a single food pellet (US) was immediately delivered into the adjacent food cup. Each training session consisted of 25 trials in which CS-US pairings occurred on a variable time (VT) 90-s schedule (the time between CS presentations varied randomly between 30 and 150 s). Lever deflections, food cup entries during the 8-s CS period, latency to the first lever deflection, latency to first food cup entry during the CS period, and food cup entries during the inter-trial interval were measured.

Quantification of behavior using an index of Pavlovian conditioned approach (PCA): For some analyses rats were classed into three groups: (1) Those who preferentially interacted with the lever ("sign-trackers", STs), (2) those who preferentially interacted with the food cup during lever presentation ("goal-trackers", GTs), and (3) those who had no clear preference for the lever or food cup ("intermediates", INs). The extent to which behavior was lever (CS) or food-cup directed was quantified using a composite index (Lovic et al., 2011; Meyer et al., 2012b; Saunders and Robinson, 2011b) that incorporated three measures of Pavlovian conditioned approach: (1) the probability of either deflecting the lever or entering the food cup during each CS period [P(lever) - P(food cup)]; (2) the response bias for contacting the lever or the food cup during each CS period [(#lever deflections - #food-cup entries) / (#lever deflections + #food-cup entries)]; and (3) the latency to contact the lever or the food cup during the CS period [(lever deflection latency - food-cup entry latency) / 8]. Thus, the Pavlovian conditioned approach index (PCA Index) score consisted of [(Probability difference score + Responses bias score + Latency difference score) / 3]. This formula produces values on a scale ranging from -1.0 to +1.0, where

scores approaching -1.0 represent a strong food cup-directed bias and scores approaching +1.0 represent strong lever-directed bias. The average PCA Index score for days 4 and 5 of training was used to class rats. Rats were designated STs if they obtained an average index score of +0.5 or greater (which means they directed their behavior towards the lever at least twice as often as to the food cup), and as GTs if they obtained a score of -0.5 or less. The remaining rats within the -0.49/+0.49 range were classed as INs.

Experiment 1: The effect of flupenthixol on two forms of Pavlovian conditioned approach behavior: 10 min between drug treatment and testing

Training and microinjection tests: Rats initially underwent Pavlovian training for 8 consecutive days, with no drug pretreatment, as described above. After the 8th day of training, by which time conditioned responding had stabilized, rats were given a vehicle microinjection before the next training session. Each rat was subsequently given an injection of each of three doses of flupenthixol (5, 10, and 20 μg) in a counterbalanced order, followed by a second vehicle injection before the final session. After all microinjections, rats were placed in holding boxes for approximately 10 minutes before being moved to the testing chambers for the start of the session. The days rats received microinjections were separated by 1-2 days of additional Pavlovian training without pretreatment to ensure conditioned responding was maintained between treatments.

Video coding of orienting behavior: A subset of STs (n=8) was video recorded during vehicle and flupenthixol administration sessions. Video was scored offline to quantify approach/contact and orientation to the CS. An orientation response was scored if the rat made a head and/or body movement in the direction of the lever during the period it was extended, even if it did not

approach or contact the CS. A contact was scored if the rat approached and touched the lever with its nose, mouth, and/or forepaw, even if contact failed to produce a deflection of the lever.

Experiment 2: The effect of flupenthixol on lever (CS) directed Pavlovian conditioned approach behavior: 35 min between drug treatment and testing

Training and microinjection tests: A separate group of STs (n=11) were tested in order to investigate the time course of flupenthixol effects found in Experiment 1 (see below). These rats received Pavlovian training exactly as in Experiment 1, for 10 sessions, then received vehicle and flupenthixol (20 μg) injections, in a counterbalanced order, before separate test sessions. Following microinjections, rats were placed in holding chambers for 35 minutes and then moved to the testing chambers for the start of the session.

Extinction: After the last test with a drug injection all rats were trained for three additional days, to once again stabilize performance, and then all underwent extinction training over the next four consecutive days. For these four sessions, no food pellets were delivered upon lever retraction, but conditions were otherwise the same as during Pavlovian training. Rats received a vehicle microinjection before the first extinction session.

Histology

After the completion of all behavioral testing, rats were anesthetized with an overdose of sodium pentobarbital and their brains were removed and flash frozen in isopentane chilled to approximately -30 C by a mixture of isopropyl alcohol and dry ice. Frozen brains were sectioned on a cryostat at a thickness of 60 μm, mounted on slides, air-dried, and stained with cresyl violet. Microinjection sites were verified by light microscopy and plotted onto drawings from a rat brain atlas (Paxinos and Watson, 2007).

Statistical Analyses

Linear mixed-models (LMM) analyses of variance (ANOVA) were used for all repeated-measures data. The best-fitting model of repeated measures covariance was determined by the lowest Akaike information criterion score (Verbeke, 2009). Depending on the model selected, the degrees of freedom were adjusted to a non-integer value, however, according to journal instructions, we report the unadjusted degrees of freedom alongside the LMM results. Significant interactions were followed by main effects and planned comparisons. Bonferroni corrections were used for planned comparisons between vehicle and each drug dose where appropriate. Paired t tests were used to compare mean order scores of IN rats and to compare mean orientation and contact behavior from video coded data. Statistical significance was set at $p < 0.05$.

Also note that in Experiment 1 we found that the drug did not start to have much effect until about half way through the session, and so we conducted Experiment 2 to determine if this was because of a delayed onset of drug action. We determined that, indeed, there was a delay before the drug exerted its full effect. Therefore, the data shown for Experiment 1 (Figures 2.2, 2.4, 2.5, and 2.7) are from trials 13-25, because this is when we determined the drug exerted its full behavioral effects (see Figure 2.6A). The full session data were also analyzed for all measures described, and similar effects were found. For the sake of brevity, we chose not to present the full session data, but only data from trials 13-25 for Figures 2.2, 2.4, 2.5, and 2.7. Although both analyses resulted produced a similar pattern of results, the effect is most clearly illustrated in the later trials, not surprisingly, because only in these trials was the drug exerting its full effect.

Results

Experiment 1: Individual variation in Pavlovian conditioned approach behavior

Figure 2.1 (top) illustrates the degree of individual variation in conditioned responding in the rats used in Experiment 1, by plotting the distribution of individual PCA Index scores (n=42). There was wide variation in the type and prevalence of different forms of Pavlovian conditioned approach behavior. The type of response made on each single trial was categorized as: (1) Contact with the food cup only (CUP ONLY), (2) contact with the lever only (LEVER ONLY), (3) contact with the food cup first followed by a lever contact (CUP FIRST; i.e., both responses occurred within the same 8-s CS period), (4) contact with the lever first followed by a food cup contact (LEVER FIRST), and (5) no food cup or lever contacts (NONE). The percentage of trials with each type of response was averaged over training sessions 4-8, which corresponded to the emergence of stable PCA behavior (see below), and for illustrative purposes we grouped rats into 4 subgroups based on their PCA Index scores. As shown in Figure 2.1 (bottom), the distribution of response types varied markedly as a function of PCA Index score. Rats with Index scores in the most negative range (-1.0 to -0.5) made over 90% CUP ONLY and CUP FIRST trials (Figure 2.1, left pie graph). Rats with scores between -0.49 and 0 were also biased toward the food cup on a majority of trials, showing 59% CUP ONLY and CUP FIRST trials, but also exhibited substantial lever-directed behavior, with 30% LEVER ONLY and LEVER FIRST trials (Figure 2.1, second pie graph). Within the 0 to +0.49 score range, rats were biased towards the lever on a majority of trials, with 74% LEVER FIRST and LEVER ONLY trials, but still had a sizable proportion of food cup trials, with 20% CUP ONLY and CUP FIRST trials (Figure 2.1, third pie graph). Finally, rats with scores from +0.5 to +1.0 made over 90% LEVER ONLY and LEVER FIRST responses (Figure 2.1, right pie graph). Note that rats with scores on the extreme ends of the Index distribution almost exclusively made ONLY responses, whereas rats with intermediate scores had large numbers of BOTH trials (i.e., CUP FIRST or LEVER FIRST).

Flupenthixol selectively impairs the expression of lever (CS) directed behavior

The effect of flupenthixol on two forms of Pavlovian conditioned approach behavior was analyzed in two different ways. In the first analysis rats were not subdivided into groups based on their behavior, but the analysis was based on the type of conditioned response (CR) observed on every individual trial, in each individual rat tested in Experiment 1 (i.e., independent of a rat's PCA Index score). A lever-directed CR was defined as a trial on which a rat made either a LEVER ONLY or a LEVER FIRST response (Figure 2.2, panels A-C). A food cup-directed CR was defined as a trial on which a rat made either a CUP ONLY or CUP FIRST response (Figure 2.2, panels D-F). As noted in the Methods section (*Statistical Analyses*), the data presented in Figure 2.2 correspond to trials 13-25 of experimental sessions, to capture the period when the drug exerted its effect (see below).

Probability: A two-way ANOVA comparing the effect of flupenthixol (vehicle, 5, 10, or 20 μg) as a function of CR type (lever versus food cup-directed responses) showed that flupenthixol reduced the probability of responding during the CS period (effect of treatment, $F_{(3,41)} = 17.444$, $p < 0.001$), but this effect varied depending on whether the CR was directed towards the lever or towards the food cup (CR type X treatment interaction, $F_{(3,41)} = 5.036$, $p = 0.005$; Figure 2.2A and 2.2D). One-way ANOVAs revealed that flupenthixol dose-dependently reduced the probability of a lever-directed CR (effect of treatment, $F_{(3,41)} = 9.378$, $p < 0.001$; Figure 2A), but had no significant effect on the probability of a food cup-directed CR (no effect of treatment, $F_{(3,41)} = 0.330$, $p = 0.803$; Figure 2.2D).

Contacts: Similarly, flupenthixol reduced the total amount of responding, indicated by the total number of contacts made during CS presentation (effect of treatment, $F_{(3,41)} = 19.450$, $p < 0.001$), but the magnitude of the effect again varied by type of CR (CR type X treatment

interaction, $F_{(3,41)} = 3.988$, $p = 0.015$; Figure 2.2B and 2.2E). Flupenthixol produced a reduction in number of lever deflections at all doses tested (effect of treatment, $F_{(3,41)} = 9.675$, $p < 0.001$; Figure 2.2B). There was a significant effect of flupenthixol on the number of head entries into the food cup during the CS period (effect of treatment, $F_{(3,41)} = 4.305$, $p = 0.010$), but this effect was more modest, as indicated by the significant interaction (above) and that the effect was significant only after treatment with the highest dose of flupenthixol (Figure 2.2E).

Latency: Finally, the latency to make a CR upon CS presentation was increased by flupenthixol (effect of treatment, $F_{(3,41)} = 19.540$, $p < 0.001$), but only for lever-directed responses (CR type X treatment interaction, $F_{(3,41)} = 4.953$, $p = 0.005$; Figure 2.2C and 2.2F). One-way ANOVAs revealed that the latency to contact the lever was increased (effect of treatment, $F_{(3,41)} = 13.779$, $p < 0.001$; Figure 2.2C), but the latency to make a head entry into the food cup was unaffected (no effect of treatment, $F_{(3,41)} = 1.871$, $p < 0.151$; Figure 2.2F).

Flupenthixol influences behavior to a greater extent in sign-trackers than goal-trackers

We next analyzed the data by classing rats as STs or GTs based on their PCA Index score, as described above and elsewhere (Saunders and Robinson, 2011b). GTs were defined as rats with PCA scores between -1.0 and -0.5, who made over 90 % CUP ONLY and CUP FIRST trials during training (Figure 2.1, left pie graph). STs were defined as rats with PCA scores between +0.5 and +1.0, who made over 90 % LEVER ONLY and LEVER FIRST trials during training (Figure 2.1, right pie graph). Intermediates (INs) had PCA scores between -0.49 and +0.49 (Figure 2.1, middle pie graphs). Figure 2.3 shows the time course of learning Pavlovian conditioned approach responses in animals grouped in this fashion, across the initial 8 days of training. Similar to previous reports (Flagel et al., 2007), with training, rats classed as STs developed a high probability of rapidly approaching and vigorously engaging the lever (Figure

2.3A-C), rarely contacting the food cup. In contrast, rats classed as GTs learned to rapidly approach and enter the food cup upon CS onset, and rarely contacted the lever itself (Figure 2.3D-F). The approach behavior of INs vacillated between lever and food cup, as they showed a similar likelihood of contacting the lever or entering the food cup during lever extension, and did so with similar latencies. Thus, these data clearly illustrate that, as a function of CS-US pairing, both STs and GTs acquired a conditioned response (they both learned), as we have reported previously (Robinson and Flagel, 2009; Saunders and Robinson, 2010; Yager and Robinson, 2010), they just directed their conditioned approach response to different places.

Figure 2.4 shows the effects of flupenthixol on STs and GTs. INs were excluded from this analysis because we wanted to directly compare two groups that differed markedly in the extent to which they attributed incentive salience to the CS. Additionally, the data presented in Figure 2.4 correspond to trials 13-25 of experimental sessions (see above).

Probability: A two-way ANOVA showed that flupenthixol significantly altered the probability of approach behavior (effect of treatment, $F_{(3,28)} = 25.834$, $p < 0.001$; Figure 2.4A) but the magnitude of this effect was greater in STs than GTs (group X treatment interaction, $F_{(3,28)} = 8.384$, $p < 0.001$). Independent one-way ANOVAs revealed that flupenthixol reduced the probability of approach in STs (effect of treatment, $F_{(3,28)} = 37.936$, $p < 0.001$; Figure 2.4A) at doses of 10 and 20 µg. The effect in GTs (effect of treatment, $F_{(3,28)} = 3.174$, $p = 0.041$; Figure 2.4A) was significant only at the highest dose tested ($p = 0.04$).

Contacts: Flupenthixol administration also influenced how avidly animals responded, as indicated by number of contacts (effect of treatment, $F_{(3,28)} = 31.451$, $p < 0.001$; Figure 2.4B, data expressed as % of vehicle contacts), but only in STs (group X treatment interaction, $F_{(3,28)} = 14.677$, $p < 0.001$). Independent one-way ANOVAs showed that flupenthixol decreased the

number of times STs engaged the lever (effect of treatment, $F_{(3,28)} = 43.470$, $p < 0.001$; Figure 2.4B), and did so at all doses tested (ps < 0.001). In contrast, flupenthixol had no significant effect on the number of head entries into the food cup in GTs (no effect of treatment, $F_{(3,28)} = 1.716$, $p = 0.183$; Figure 2.4B).

Latency: Finally, flupenthixol administration influenced the rapidity of approach, measured as the latency from CS onset to the first lever deflection or head entry (effect of treatment, $F_{(3,30.012)} = 21.966$, $p < 0.001$; Figure 2.4C), but again, this effect varied as a function of group (group X treatment interaction, $F_{(3,28)} = 9.751$, $p < 0.001$). Independent one-way ANOVAs showed that flupenthixol increased the latency of approach to the CS in STs (effect of treatment, $F_{(3,28)} = 28.122$, $p < 0.001$; Figure 2.4C), and did so at all doses tested (ps < 0.001). However, although the effect of flupenthixol on latency in GTs was statistically significant based on the one-way ANOVA (effect of treatment, $F_{(3,28)} = 3.048$, $p = 0.044$; Figure 2.4C), none of the paired comparisons revealed a statistically significant effect of any given dose.

Figure 2.4D shows the number of food cup head entries rats made during the inter-trial interval (ITI), the period between CS presentations, which serves as an indirect measure of the effect of flupenthixol on general motor activity. GTs made more non-CS food cup entries than STs (effect of group, $F_{(3,28)} = 5.698$, $p = 0.024$). However, by the end of training their rate of food cup entries during the CS period (mean = 0.376 entries/s, SEM = 0.055) was much higher than during non-CS periods (mean = 0.072 entries/s, SEM = 0.010) (see also Meyer et al., 2012b), indicating that GTs discriminated between CS and non-CS periods. Importantly, there was no effect of flupenthixol administration on the number of inter-trial interval food cup entries made by either group (no effect of treatment, $F_{(3,28)} = 0.132$, $p = 0.94$), indicating that flupenthixol did not impair general motor activity at the doses we used.

Effect of flupenthixol on the topography of the CR in STs, GTs and INs

Figure 2.5A shows a more detailed analysis of the effects of just the highest dose of flupenthixol (20 µg) on different types of CRs in STs, GTs and INs (as defined above and shown in Figure 2.1). For the sake of simplicity, CUP FIRST and CUP ONLY trials were grouped together (CUP trials) and LEVER FIRST and LEVER ONLY trials were grouped together (LEVER trials). Figure 2.5A shows that in all groups flupenthixol reduced the proportion of LEVER trials, but not CUP trials, and increased NONE trials. *Goal-trackers*: As expected, after vehicle GTs made mostly CUP trials (78% CUP, 16% LEVER, and 6% NONE trials). Following 20 µg flupenthixol, CUP trials were modestly reduced to 70% of trials, while LEVER trials were halved to 8% and NONE trials increased to 22% (Figure 2.5A, left pie graphs). *Intermediates*: After vehicle INs exhibited roughly equal proportions of CUP (42%) and LEVER (41%) trials, with 17% NONE trials. Administration of 20 µg flupenthixol selectively reduced LEVER trials to 19% and increased NONE trials to 41%, while leaving CUP trials unaffected, at 40% (Figure 2.5A, middle pie graphs). *Sign-trackers*: When treated with vehicle STs made LEVER responses 97% of the time, with 0% CUP, and 3% NONE trials. Flupenthixol administration altered this response pattern by reducing the proportion of LEVER trials by roughly half, to 43%, and increasing NONE trials to 42%. Interestingly, CUP trials in STs increased modestly, to 15% (Figure 2.5A, right). In summary, the effect of flupenthixol was to primarily decrease LEVER trials and increase NONE trials, with modest effects on CUP trials, regardless of the phenotype.

We next sought to further characterize the effect of flupenthixol on IN rats, which exhibit substantial numbers of both LEVER and CUP responses during a single CS period (i.e., BOTH responses). Thus, we analyzed trials in which rats both contacted the CS and entered the food cup during a single CS period (BOTH trials) and calculated an order score [(# LEVER FIRST

trials - # CUP FIRST trials)/total # BOTH trials] ranging from -1.0 to +1.0, to determine which response occurred first. A positive order score represents a tendency to contact the lever first followed by the food cup on BOTH trials whereas a negative score indicates a bias towards contacting the food cup first. Figure 2.5B shows the average order scores for INs following vehicle and flupenthixol administration (for simplicity, flupenthixol doses were collapsed). After vehicle, INs tended to approach the lever first on BOTH trials. After flupenthixol administration, this order bias reversed and INs showed a tendency to approach the food cup first. A paired t test revealed that this change in order score was significant (paired t test, $t_{(9)} = 3.315$, $p = 0.009$).

Experiment 2: Time course of flupenthixol effects on STs

We next examined the time course of flupenthixol's effect on the amount of lever-directed behavior specifically in STs, on a trial-by-trial basis, during the test session when they were treated with the highest dose of flupenthixol (20 μg). In Experiment 1, after flupenthixol administration, there was no effect on lever contacts during the first few trials (relative to vehicle), but over the test session there was a gradual decrease in responding, as indicated by a significant trial X treatment interaction ($F_{(24, 312)} = 1.643$, $p = 0.031$; Figure 2.6A). This interaction is clearly illustrated by comparing responding on the first two trials to the last two trials of the test session (Figure 2.6B). Two possible mechanisms could explain this time course. (1) Delayed drug effects: It is possible that flupenthixol had not yet reached the full extent of its action in vivo within 10-min post-injection. (2) Pavlovian 'extinction mimicry': Administration of dopamine antagonists to rats during an instrumental food-seeking paradigm causes an progressive decrease in responding over time, even when food remains available (Wise et al., 1978), which has been interpreted as an extinction-like effect (but see Phillips et al., 1981; Salamone et al., 2012). In order to test whether the delayed suppression of sign-tracking seen in

Experiment 1 was due to delayed drug action or to an extinction-like effect we conducted a second experiment in a separate group of STs (n=11). In this experiment we imposed a 35-min delay after vehicle and flupenthixol (20 μg) microinjections before beginning testing. This delay was chosen because it was approximately the amount of time before flupenthixol produced a marked reduction in sign-tracking behavior in Experiment 1 (see Figure 2.6A).

As Figure 2.6C and 2.6D show, following a 35-min post-injection delay, the number of lever contacts was maximally suppressed on the very first trial. Comparison of lever contacts during the first and last two trials clearly shows the immediacy of the flupenthixol effect (Figure 2.6D). Over the course of the session there was a gradual decrease in the number of lever contacts after both vehicle and flupenthixol treatment, but the rate of this decrease was the same under both conditions (no trial X treatment interaction, $F_{(24,240)} = 0.748$, $p = 0.799$). Note that rats were not food deprived in these experiments, and so the gradual decline in the responding per trial across the test session, even after vehicle, may be because the rats become increasingly sated as the session progressed. In conclusion, these data suggest that the delayed onset of flupenthixol's effect evident in Experiment 1 was not due to an extinction-like effect, but to a delay in peak drug effect, which is why data from trials 13-25 were selected for analysis in Experiment 1.

Extinction of ST behavior

In order to more directly contrast the effects of flupenthixol with Pavlovian extinction, the STs in Experiment 2 were given four sessions of PCA extinction training, during which no food pellets were delivered following lever retraction. Figure 2.6E shows the time course of lever deflections, trial-by-trial, during these extinction sessions, relative to the vehicle test session, when pellets were delivered. In contrast to flupenthixol administration, when there was a 35-min delay in testing (Figure 2.6C), on day 1 of extinction, sign-tracking behavior was initially

identical to vehicle, and decreased gradually across the session. Examination of the first and last two trials clearly shows that the pattern of behavior on day 1 of extinction differed from that seen following flupenthixol, when testing was delayed by 35 min (compare Figure 2.6F and 2.6D). Days 2 and 4 of extinction are also shown in Figure 6E. While within-session extinction was clear by day 2, sign tracking during the first trials of the session remained similar to vehicle levels even on day 4. Thus, the effect of flupenthixol, when testing was delayed by 35 min, had a very different temporal profile than the effect of extinction training, further indicating that flupenthixol did not produce an extinction-like effect (see also Phillips et al., 1981; Salamone et al., 2012).

Flupenthixol does not affect an orienting CR in STs

To further examine the possibility that flupenthixol decreased sign-tracking behavior because dopamine transmission in the NAcC is necessary to maintain the learned association between lever extension and reward delivery, we looked at the effect of flupenthixol on another CR that develops in response to presentation of a CS: a conditioned orienting response. We operationally defined this as a head and/or body movement in the direction of the lever, even if it did not bring the rat into close proximity to it (which would be classed as an approach response; see below). Importantly, we have found that with lever(CS)-US pairing, *both* STs and GTs acquire a conditioned orienting response, and they do not differ in their rate of learning this CR, even though only STs develop a high probability of approaching the lever (L.M. Yager, unpublished observations). To examine the role of dopamine in the expression of the orienting CR in STs, a subset of STs (n=8) from Experiment 1 were video recorded during the vehicle and 20 μg flupenthixol test sessions. This video was scored offline to quantify the occurrence of conditioned orienting behavior. As shown in Figure 2.7, after training, the probability of a

conditioned orienting response on each trial was very high (over 90%), and this was not influenced by treatment with the high dose of flupenthixol (paired t test relative to vehicle, $t_{(7)}$ = 0.168, p = 0.436; left bars). This is important, because it establishes that after flupenthixol administration the learned CS-US association is still intact in STs, even though they do not approach the CS. As before, for data presented in Figure 2.7 we restricted our analysis to trials 13-25 of experimental sessions.

As an aside, the conditioned orienting response described here should not be confused with the conditioned orienting response described by Holland and his colleagues in a series of papers (e.g., Han et al., 1997; Holland, 1977). That CR consists of rearing in close proximity to a visual stimulus, which by our criteria would be classed as an approach response.

We also used this video to determine if the effect of flupenthixol on the expression of a ST CR could be because more effort was required to physically deflect the lever (recorded as a ST CR) than to break a photobeam (recorded as a GT CR). To do this we scored the occurrence of CS-evoked approach responses, independent of whether the lever was deflected. Thus, in this analysis approach was defined as merely coming into close proximity to the lever, touching it, but not necessarily deflecting it. This approach response is therefore directly comparable to approach to the food cup, and therefore there is no difference in the "effort" required for a ST versus a GT response. Figure 2.7 shows that in STs flupenthixol decreased the probability of approaching the lever regardless of whether an approach response was scored by simple proximity to it (paired t test relative to vehicle, $t_{(7)}$ = 3.910, p = 0.003; middle bars), or by physically deflecting the lever (paired t test relative to vehicle, $t_{(7)}$ = 4.437, p = 0.002; right bars). Thus, when controlling for potential differences in effort between ST and GT CRs, dopamine antagonism still had a selective effect in reducing sign-tracking. This suggests that our results

cannot be explained by the view that dopamine is involved in effort-related processes necessary for motivated behavior to occur (Robbins and Everitt, 1992; Salamone et al., 2007).

Histological verification of cannulae placements

Figure 2.8 illustrates the location of microinjection tips within the NAcC for rats used in Experiment 1. Placements for rats in Experiment 2 were similar (data not shown).

Discussion

Flagel et al. (2011b) reported that dopamine plays a very selective role in stimulus-reward learning – it is necessary to attribute incentive salience to cues predictive of reward, but not to learn a CS-US association (see also Danna and Elmer, 2010; Shiner et al., 2012). We extend that notion here, and report that dopamine in the NAcC also plays a selective role in the *performance* of Pavlovian CRs already acquired. Dopamine blockade within the NAcC markedly degraded the expression of Pavlovian conditioned approach behavior directed towards the CS itself (sign-tracking), but not conditioned approach behavior directed towards the food cup (goal-tracking, see also Chang et al., 2012). These findings have a number of implications for thinking about the role of mesolimbic dopamine in reward.

Mesolimbic dopamine as a prediction-error signal necessary for learning.

Electrophysiological recordings from dopamine neurons in the ventral tegmental area and substantia nigra, and direct measures of release events within the NAcC, have shown that a phasic dopamine response transfers from an unexpected reward (US) to the CS that predicts reward delivery, over the course of training (Cohen et al., 2012; Day et al., 2007; Pan et al., 2005; Schultz et al., 1997). These studies led to the hypothesis that phasic dopamine transmission provides a prediction-error signal, coding the discrepancy between actual and

predicted events, that is required for learning stimulus-reward associations (Bayer and Glimcher, 2005; Montague et al., 1996; Schultz et al., 1997). Therefore, blocking dopamine transmission within NAcC could be functionally equivalent to reward omission, which produces a pause in dopamine neuron firing (i.e., a negative prediction error; Cohen et al., 2012; McClure et al., 2003; Pan et al., 2005; Schultz et al., 1997), leading to new learning. If, under dopamine blockade, the predictive value of the CS was negatively adjusted, trial by trial, this could produce a gradual reduction in sign-tracking behavior, similar to that seen in instrumental responding (Wise et al., 1978).

The results suggest, however, that the effect of flupenthixol on sign-tracking behavior was not due to updating a prediction-error signal (i.e., new learning). With an optimal delay between flupenthixol administration and testing, the expression of sign-tracking behavior was maximally suppressed on the *very first trial.* This indicates that dopamine antagonism altered the value of the CS in the absence of new learning (i.e., without new prediction-error computations; see also Shiner et al., 2012). This is in contrast to the gradual, multi-session-long decay in sign-tracking observed when actual extinction conditions were in effect (see also Phillips et al., 1981). These results are complementary to previous studies in which dopaminergic activity was *increased* by drugs or sensitization, also producing an immediate increase in responding (Smith et al., 2011; for review see Berridge, 2012; Tindell et al., 2005; Tindell et al., 2009; Wyvell and Berridge, 2001). Of course, a learning-based interpretation also cannot account for why the GT and conditioned orienting CRs were not similarly decreased. However, as put by Berridge (2012, p. 1139), "advocates of dopamine-learning theories may reply that only some forms of reward learning require dopamine". But he goes on to say, "so what particular forms of learning would those advocates suggest need dopamine?" "Pavlovian reward learning was the original source of

the dopamine prediction-error hypothesis" … "if not for Pavlovian reward learning, then for what learning is dopamine needed?"

Mesolimbic dopamine as an incentive salience signal. It has been argued that the primary role of mesolimbic dopamine in reward is to attribute incentive salience to rewards and their associated cues, making them attractive and desirable, and capable of exerting motivational control over behavior (Berridge, 2007; Berridge, 2012; Berridge and Robinson, 1998). That is, dopamine in the accumbens is involved in transforming a predictive, but "cold" informational CS, into a "hot" motivating *incentive stimulus*. This concept was formalized in a recent computational model of incentive salience (Berridge, 2012; Zhang et al., 2012; Zhang et al., 2009). In contrast to traditional "model-free" forms of stimulus-reward learning (see Daw et al., 2005; Sutton, 1988; Sutton and Barto, 1998), which require the cached learned value of a Pavlovian CS be updated incrementally, via new dopamine prediction errors (Schultz et al., 1997), the incentive salience model predicts that dopamine's role is specifically in transforming the motivational value of learned CSs 'on-the-fly', without the need to re-experience CS-US pairing, as observed here.

In thinking about the differential effect of dopamine antagonism on the learning and expression of a ST CR vs. a GT CR it is important to consider the distinction between a conditional stimulus (CS) and an incentive stimulus, a distinction long emphasized by learning theorists (Berridge, 2001; Bindra, 1978; Dickinson and Balleine, 1994, 2002; Konorski, 1967; Toates, 1986). Our recent studies on individual variation in the attribution of incentive salience to reward cues indicate that a perfectly effective CS may or may not also function as an incentive stimulus. Only in STs is the CS transformed into a powerfully attractive and desirable "motivational magnet" (Flagel et al., 2009; Flagel et al., 2011b; Flagel et al., 2007; Meyer et al.,

2012b; Robinson and Flagel, 2009; Saunders and Robinson, 2010; Yager and Robinson, 2010), and our results suggest that it is this transformation that requires dopamine in the NAcC. We suggest, therefore, that dopamine antagonism attenuates the learning (Flagel et al., 2011b) and performance (present results) specifically of a ST CR because it degrades the motivational properties of the CS, which are required for the CS to become attractive, but without necessarily compromising the CS-US association (Berridge, 2012; Berridge and Robinson, 1998).

Of course, dopamine neurons in the midbrain project to many other forebrain regions, including the NAcc shell, dorsal striatum, amygdala, prefrontal cortex, and hippocampus, and they are not homogeneous in their pattern of activity (Bromberg-Martin et al., 2010; Fields et al., 2007; Lammel et al., 2011; Witten et al., 2011), raising the possibility that dopamine signaling within other regions are necessary for learning stimulus-reward associations. However, recording and release studies cannot establish whether dopamine activity in any structure is *necessary* for any particular function – by their nature such studies are only correlative. It is important to keep in mind, therefore, that the systemic administration of flupenthixol, which would block dopamine receptors in all brain regions, failed to prevent the learning of a GT CR (Flagel et al., 2011b). This suggests that dopamine in no brain region is necessary for learning all CS-US associations. The data reported here are consistent with this notion (although, of course, here we can only draw strong conclusions about the NAcC\).

The fact that dopamine antagonism had little effect on the performance (and learning, Flagel et al., 2011b) of a GT CR suggests, of course, that for GTs the CS did not function as an incentive stimulus. At first glance this may seem inconsistent with recent reports that systemic dopamine antagonism (Wassum et al., 2011) or inactivation of the nucleus accumbens via GABA receptor agonists (Blaiss and Janak, 2009) can decrease goal-directed CRs. However, an

important procedural difference may explain the discrepancy. These studies assessed goal approach in response to an *auditory* CS, and when a tone is used as the CS all rats develop a GT CR (i.e., rats do not approach a tone CS; Cleland and Davey, 1983). As mentioned above, when a discrete localizable cue is used as the CS it serves as a more effective conditioned reinforcer in STs compared to GTs (Robinson and Flagel, 2009), but, if an auditory CS is used we have found it is an effective conditioned reinforcer in both STs and GTs, suggesting a tone cue is attributed with motivational properties in both STs and GTs (P.J. Meyer, unpublished observations). Thus, for any given individual, whether a cue acquires motivational properties may vary depending on sensory modality, and perhaps even the extent to which it can be engaged and manipulated (Chang et al., 2012; Holland, 1977). This also suggests that the form of the CR alone may not always indicate whether a CS is attributed with motivational properties, or whether performance of the CR is dopamine-dependent. Sometimes a CR directed to the location of reward delivery may reflect activation of a Pavlovian conditioned motivational state (and be dopamine-dependent) and other times not. Additional tests are required to determine the psychological and neurobiological processes underlying what may otherwise appear to be exactly the same behavior.

Nevertheless, here, expression of a goal-tracking CR did not require dopamine in the NAcC, and so we should consider what psychological process might underlie goal-tracking under these conditions. We can only speculate, but one possibility is that it is governed by a cognitive reward expectancy process (Bindra, 1978; Dickinson and Balleine, 1994; Toates, 1986). If presentation of the CS evokes an explicit cognitive representation of the outcome (US), this could result in goal-directed approach to the food cup to await delivery of the expected reward. Goal-directed instrumental behavior governed by explicit cognitive expectations ("instrumental incentives")

does not require dopamine (Dickinson et al., 2000; Lex and Hauber; Wassum et al., 2011; Yin et al., 2006), but instead may depend on endogenous opioid signaling (Wassum et al., 2009), which has also been implicated in some aspects of goal-tracking behavior (Difeliceantonio and Berridge, 2012; Mahler and Berridge, 2009). Therefore, the goal-directed approach seen here may be akin to behavior governed by an act-outcome association, which is thought to be dependent more on corticostriatal than mesolimbic circuits (Balleine and Dickinson, 1998; Daw et al., 2005), and may be related to so-called 'model-based' forms of learning (Daw et al., 2005; Dayan and Balleine, 2002; Glascher et al., 2010; Tolman, 1948). Consistent with this notion, cue-induced c-fos mRNA expression in corticostriatal regions was correlated in GTs, but not STs (Flagel et al., 2011a), suggesting the possibility of greater "top-down" cortical regulation of behavior in GTs.

Finally, we should note that the distinction between STs and GTs is not absolute, and although each shows a strong propensity to make a specific CR on any given trial (for as yet unknown reasons) most rats will still make the non-preferred CR on some trials. Of course, this vacillation is most pronounced in INs. This raises the interesting possibility that the psychological process that governs behavior may shift dynamically on a trial-by-trial basis, from one that is dopamine-dependent to one that is not, a hypothesis that can be tested.

In conclusion, we suggest that the role of dopamine in the NAcC in stimulus-reward learning is to attribute incentive salience to reward cues, transforming predictive CSs into powerful incentives, which can motivate not only normal behavior, but are also more likely to instigate maladaptive behavior (Robinson and Berridge, 1993).

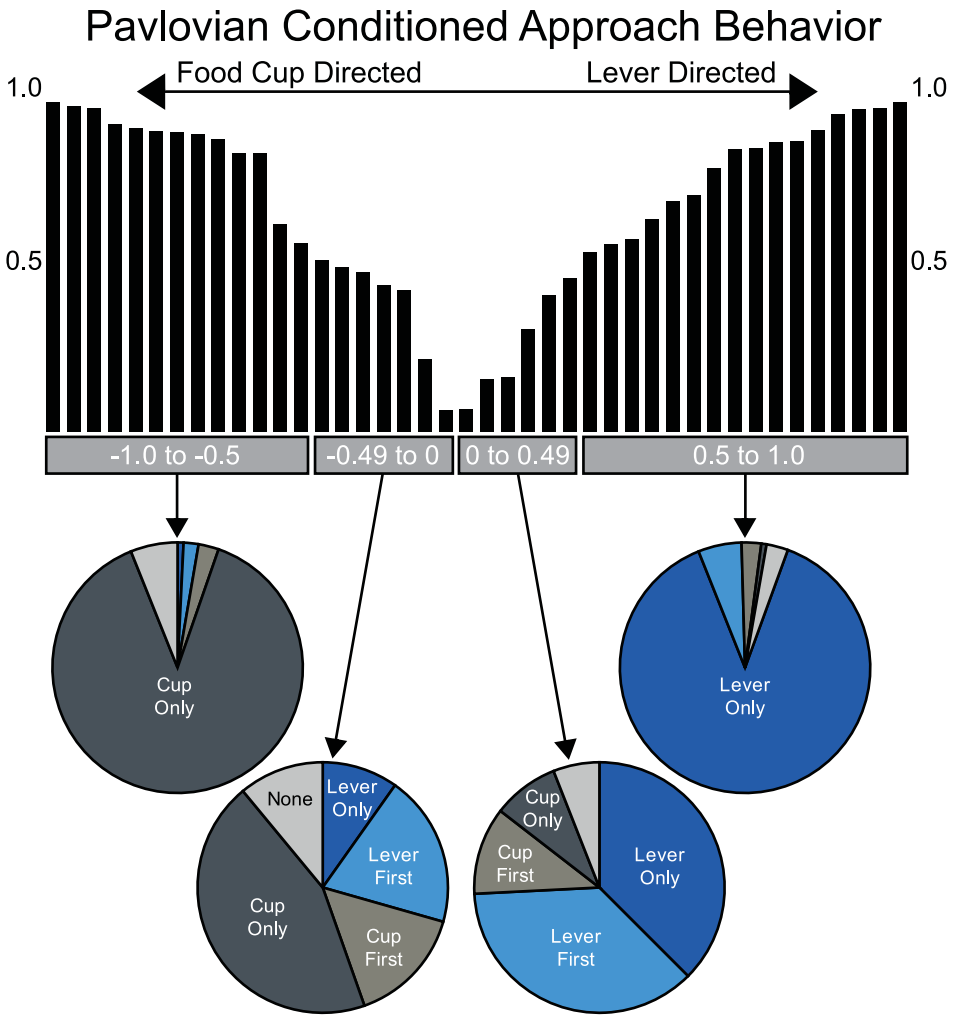


Figure 2.1. Individual variation in Pavlovian conditioned approach behavior. The top section shows PCA Index scores for individual rats (n=42) used in Experiment 1. Moving from left to right, scores range from -1.0 (food-cup biased) to +1.0 (lever biased). The bottom section illustrates the proportion of different response types for the rats with PCA Index scores within four different ranges. Five response types were possible on a given trial: (1) Contact with the food cup only (CUP ONLY), (2) contact with the lever only (LEVER ONLY), (3) contact with the food cup first followed by a lever contact (CUP FIRST; i.e., both responses occurred within the same 8-s CS period), (4) contact with the lever first followed by a food cup contact (LEVER FIRST), and (5) no food cup or lever contact (NONE).

Figure 2.2. Effects of flupenthixol on two types of Pavlovian conditioned approach. Lever-directed (**A**-**C**) and food cup-directed (**D**-**F**) CRs were quantified for all rats tested in Experiment 1 (n = 42). Data presented correspond to trials 13-25 of experimental sessions. **A**, The probability of lever-directed CR occurrence. The probability of lever-directed CRs was significantly reduced after flupenthixol doses 10 μg ($p = 0.001$) and 20 μg ($p < 0.001$). **B**, Number of lever-directed CRs. The number of lever-directed CRs was reduced following each dose of flupenthixol (ps < 0.004). **C**, Latency to make a lever-directed CR. The latency of lever-directed CRs was significantly longer after 5 μg ($p = 0.021$) as well as 10 and 20 μg (ps < 0.001) doses of flupenthixol. **D**, Probability of food cup-directed CR occurrence. **E**, Number of food cup-directed CRs. The highest flupenthixol dose (20 μg) produced a small but significant reduction in the number of food cup-directed CRs ($p = 0.022$). **F**, Latency to make a food cup-directed CR. *p < 0.05, **p < 0.01 (relative to vehicle). Error bars indicate SEM.

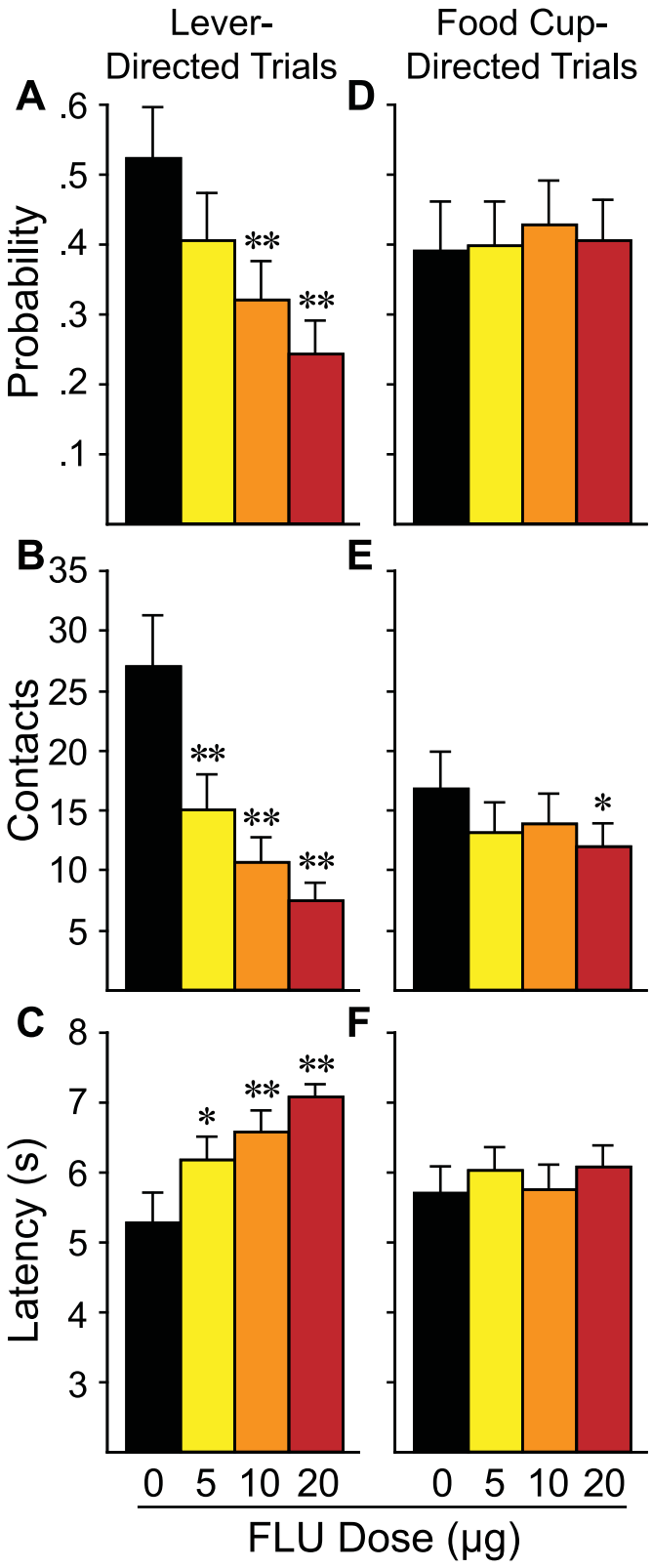
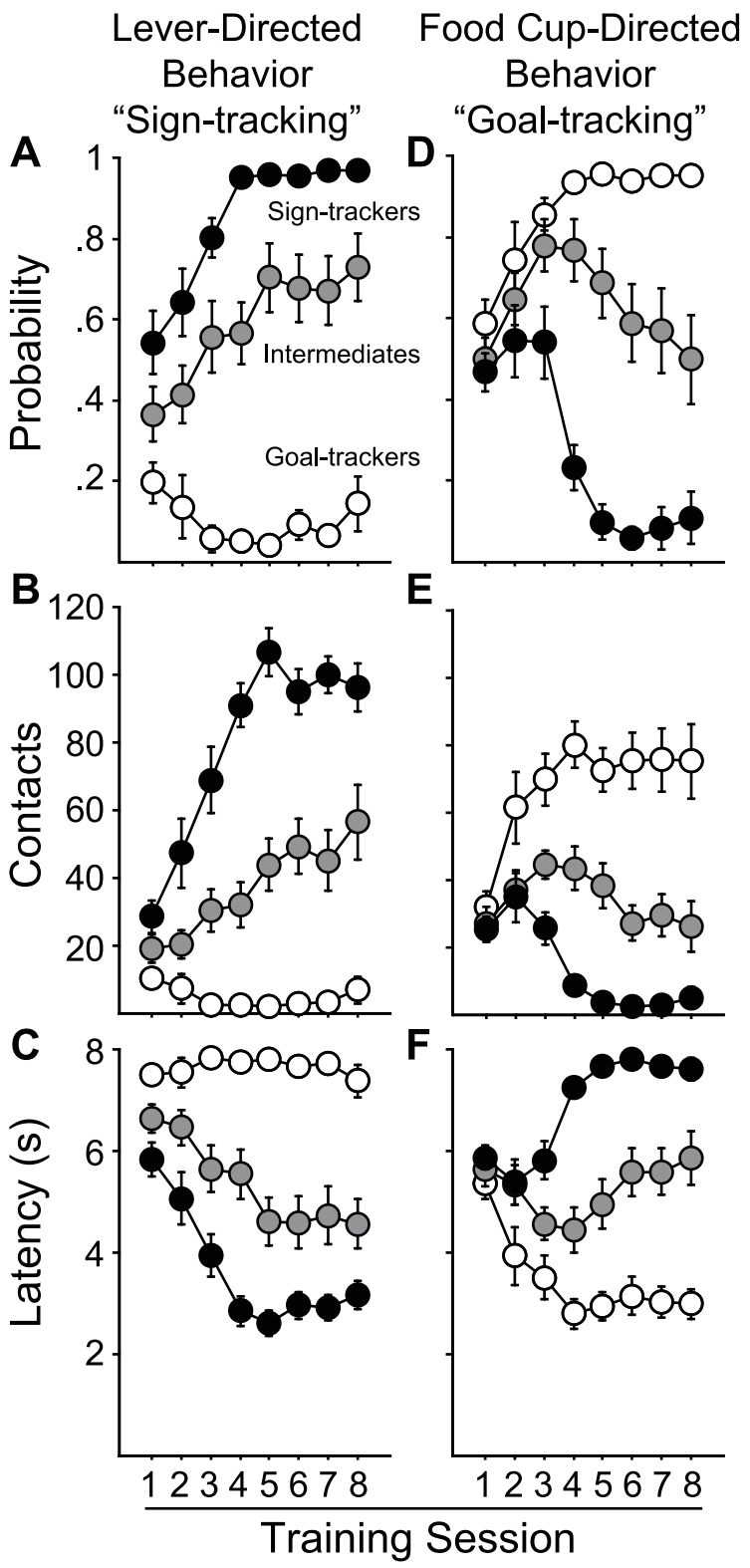

Lever-
Directed Trials
Food Cup-
Directed Trials
A
D
Probability
.6
.5
.4
.3
.2
.1
**
**
B
E
Contacts
35
30
25
20
15
10
5
**
**
**
*
C
F
Latency (s)
8
7
6
5
4
3
*
**
**
0 5 10 20
0 5 10 20
FLU Dose (µg)

Figure 2.3. Pavlovian conditioned approach training. Lever-directed behavior ("sign-tracking", **A**-**C**) and food cup-directed behavior ("goal-tracking", **D**-**F**) across 8 days of training for rats classed as sign-trackers (STs, n=16), goal-trackers (GTs, n=13), or intermediates (INs, n=13). Error bars indicate SEM.


Lever-Directed Behavior "Sign-tracking"
Food Cup-Directed Behavior "Goal-tracking"
A
D
B
E
C
F
Probability
Contacts
Latency (s)
Sign-trackers
Intermediates
Goal-trackers
1
.8
.6
.4
.2
120
100
80
60
40
20
8
6
4
2
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8
Training Session

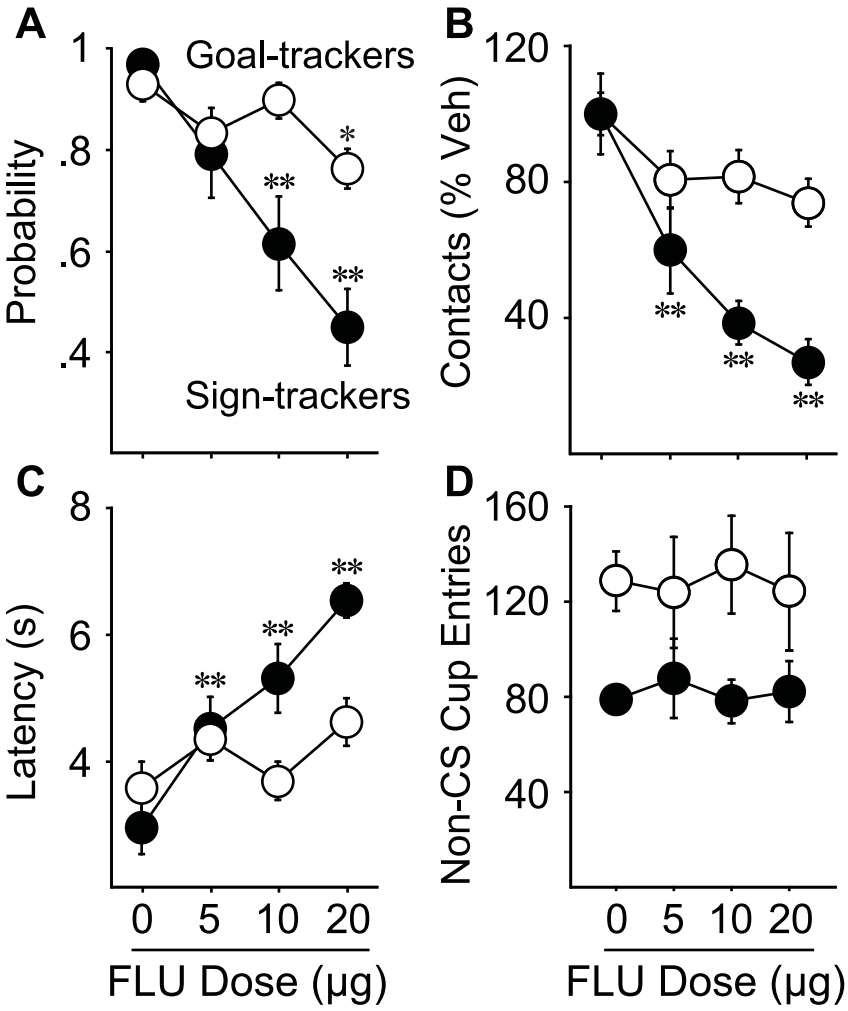


Figure 2.4. Effects of flupenthixol on STs (n=16) and GTs (n=13). Data presented correspond to trials 13-25 of experimental sessions. **A**, Probability of making an ST or GT CR. **B**, Number of ST or GT CRs (Statistical analysis was done on the raw data, but in **B** the data are expressed as a % of vehicle, in order to directly illustrate the relative size of the effect of flupenthixol that would otherwise be obscured by group baseline differences in total responding). **C**, Latency to make an ST or GT CR. **D**, Number of non-CS food cup entries. *$p < 0.05$, **$p < 0.01$ (relative to vehicle). Error bars indicate SEM.

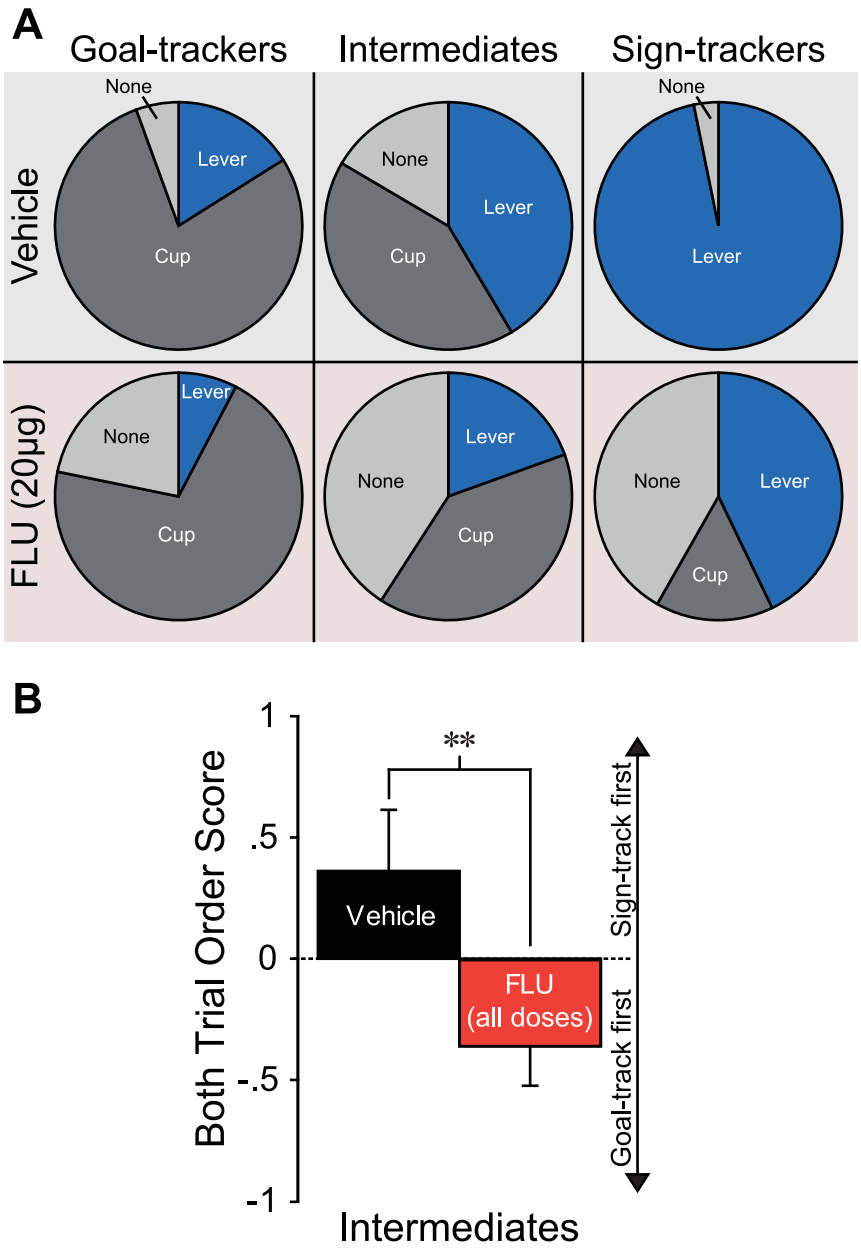
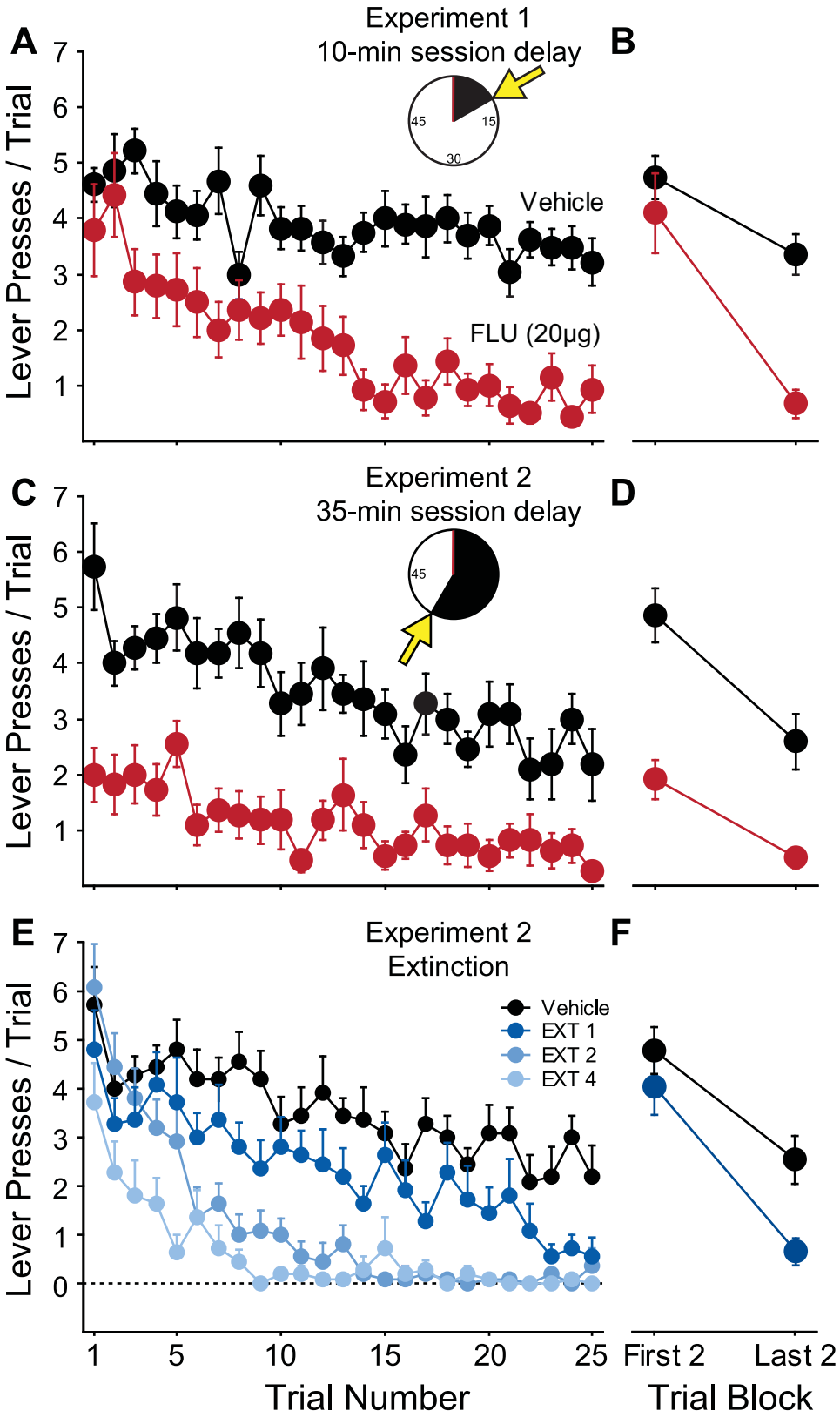


Figure 2.5. Effects of flupenthixol on response topography and order of responding. Data presented correspond to trials 13-25 of experimental sessions. **A**, Proportion of CUP trials (CUP FIRST + CUP ONLY trials), LEVER trials (LEVER FIRST + LEVER ONLY trials), and NONE trials for STs, GTs, and INs following vehicle (top circles) or 20 µg flupenthixol (bottom circles). Flupenthixol specifically reduced LEVER trials, but not CUP trials, and increased NONE trials, in all groups. **B**, Effect of flupenthixol on the order of responding in INs. An order score was calculated as follows: [(# LEVER FIRST trials - # CUP FIRST trials)/total # BOTH trials]. Following vehicle administration, INs had a positive order score, indicating a bias towards making a lever-directed CR first. After flupenthixol administration, this score reversed, indicating a bias towards making a food cup-directed CR first. **$p < 0.01$ (relative to vehicle). Error bars indicate SEM.

Figure 2.6. Time course of flupenthixol and extinction effects on lever-directed CRs. *Experiment 1* (**A**-**B**): 10-min delay between drug administration and testing. **A**, The number of lever-directed CRs among STs (n=16) across the 25-trial test session and **B**, on the first and last two trials, after vehicle and 20 µg flupenthixol administration. *Experiment 2* (**C**-**F**): 35-min delay between drug administration and testing. **C**, Number of lever-directed CRs among STs (n=11) across the 25-trial test session and **D**, on the first and last two trials, after vehicle and 20 µg flupenthixol administration. **E**, Number of lever-directed CRs across the 25-trial test session for vehicle and extinction days 1, 2, and 4. **F**, Average number of lever-directed CRs on the first and last two trials of the vehicle session and extinction day 1. Error bars indicate SEM.


A
Experiment 1
10-min session delay
45
15
30
Vehicle
FLU (20µg)
Lever Presses / Trial
B
C
Experiment 2
35-min session delay
45
D
E
Experiment 2
Extinction
Vehicle
EXT 1
EXT 2
EXT 4
F
Trial Number
First 2
Last 2
Trial Block

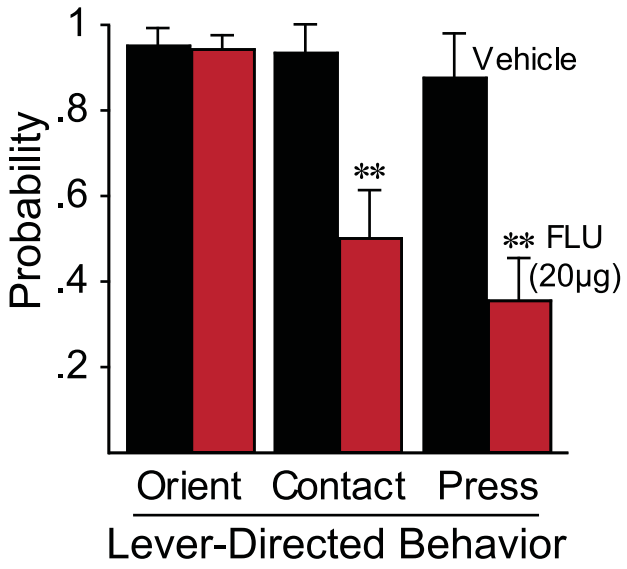


Figure 2.7. Lever orientation and approach behavior. Data presented correspond to trials 13-25 of experimental sessions. *Left bars*, Video-scored probability of making a conditioned orienting response for a subset of STs (n=8) in Experiment 1 following vehicle and 20 µg flupenthixol. *Middle bars*, Video-scored probability of approaching the lever following vehicle and 20 µg flupenthixol. *Right bars*, Probability of making a computer-scored lever deflection following vehicle and 20 µg flupenthixol. **$p < 0.01$ (relative to vehicle). Error bars indicate SEM.

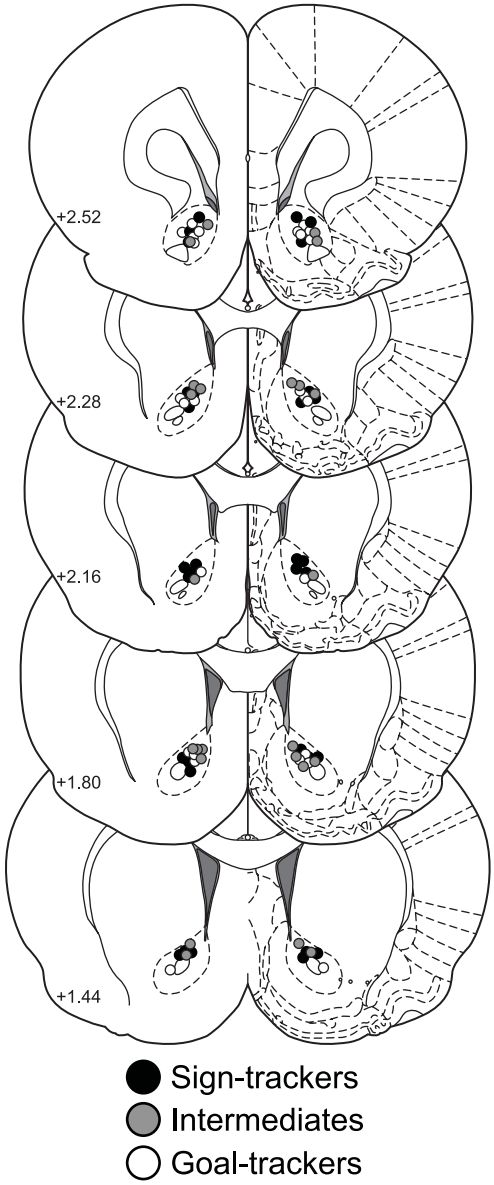


Figure 2.8. Location of microinjection tips within the NAcC relative to Bregma for STs, GTs, and INs used in Experiment 1.

Chapter 3

A cocaine cue acts as an incentive stimulus in some, but not others: implications for addiction

Introduction

Cues associated with rewards can themselves act as powerful incentives, arousing desire and instigating actions. For example, food-related cues can motivate eating even when one is sated (Schachter, 1968; Weingarten, 1983), and in addicts, drug cues grab the attention more powerfully than other stimuli (Field and Cox, 2008) and can produce craving and/or relapse (Ehrman et al., 1992; O'Brien et al., 1998). Even drug cues outside of conscious awareness activate brain motive circuits (Childress et al., 2008). The importance of drug-associated cues in motivating behavior has been confirmed using animal models of addiction. Drug cues are attractive and "wanted" – they are approached (Uslaner et al., 2006) and animals will work to get them (Davis and Smith, 1976; Di Ciano and Everitt, 2004; Goddard and Leri, 2006); drug cues help maintain drug-seeking and -taking behavior – their omission greatly decreases seeking and self-administration (Arroyo et al., 1998; Caggiula et al., 2001; Panlilio et al., 1996; Schenk and Partridge, 2001; Schindler et al., 2002); and drug cues are powerful instigators of reinstatement/relapse – promoting renewed drug seeking after extinction or prolonged abstinence (de Wit and Stewart, 1981; Shaham et al., 2003). For reward-associated cues to motivate, however, they must acquire the ability to act as *incentive stimuli* – i.e., be attributed with incentive salience (Berridge, 2001; Bindra, 1978; Bolles, 1972; Cardinal et al., 2002a; Stewart et al., 1984).

How do cues acquire the ability to motivate? It is often assumed that cues (conditional stimuli, CS) become incentive stimuli merely as a function of their conditional relationship with a reward (unconditional stimulus, US), but in fact, the ability of a CS to predict a US and evoke a conditional response (CR) is not sufficient to confer incentive value to the CS (Flagel et al., 2009; Robinson and Flagel, 2009; Tomie et al., 2008; Zener, 1937). When a localizable cue (a lever-CS) is associated with the receipt of food reward, only in some rats does the lever-CS itself become attractive, eliciting approach and engagement with it, and in these animals the lever-CS is also an effective conditional reinforcer (that is, animals will work to get it). But in other rats the lever-CS does not attract. These animals are fully capable of learning a Pavlovian CR, but the CR is not directed towards the CS, but rather, towards the place food will be delivered, and in these rats the lever-CS is relatively ineffective as a conditional reinforcer (Robinson and Flagel, 2009). Animals for which a localizable cue acts as an attractive incentive stimulus are called “sign-trackers” (STs - they approach the cue or "sign"; Hearst and Jenkins, 1974) and those for whom the cue does not have incentive properties are called “goal-trackers” (GTs - they learn to approach the location of reward delivery, or “goal”; Boakes, 1977). Note that the cue is predictive and acts as a fully effective CS, supporting Pavlovian learning, in both STs and GTs, and their respective CRs are learned at a comparable rate. But the cue itself serves as an attractive incentive stimulus only for STs (Flagel et al., 2009; Robinson and Flagel, 2009).

Thus, only in susceptible individuals are localizable food cues attributed with incentive salience, acquiring the ability to attract and to reinforce new learning. It is important to know if there are similar individual differences in the propensity to attribute incentive salience to drug cues, because if drug cues act as incentives - “as a persistent goad to response generation” (Stewart et al., 1984) in some individuals but not others - it is presumably the former who will

have difficulty resisting them and thus be susceptible to continued drug use and addiction. We asked, therefore, whether individual differences in the propensity to attribute incentive salience to a food cue, determined prior to any drug experience, predicts individual differences in the ability of a cocaine cue to motivate drug-seeking and drug-taking behavior.

Material and Methods

Pavlovian training. Male Sprague Dawley rats were initially trained using a Pavlovian conditioning procedure described previously (Flagel et al., 2007). Briefly, an illuminated retractable lever (the conditioned stimulus, CS) located to the left or right of a food magazine was inserted into the chamber for 8 s. As the lever was retracted, a single 45-mg banana-flavored food pellet (the unconditioned stimulus, US) was delivered into the magazine. This CS-US pairing occurred on a *response-independent* random-interval 90-s schedule 25 times per session for three sessions. The animals were then classified as sign-trackers or goal-trackers as described previously (Flagel et al., 2007). Lever deflections, magazine entries, latency to the first lever deflection, magazine entries during the intertrial interval, and latency to magazine entry during CS presentation were measured. The average number of lever presses across training sessions was used to distinguish sign-tracking (lever-directed) and goal-tracking (food tray-directed) behavior (1). Using this indicator, animals were divided into two groups, sign-trackers (ST, top 33% lever presses) and goal-trackers (GT, bottom 33% lever presses). The intermediate group (middle 33% lever presses) was excluded from further study.

Surgery. After Pavlovian training animals were prepared with intravenous catheters as described previously (Crombag et al., 2000) under ketamine hydrochloride (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia. Following surgery catheters were flushed daily with 0.2 ml sterile saline containing gentamicin (5 mg/ml). During self-administration testing catheters were

flushed with this solution before and after each session. Once a week, catheter patency was tested by injection of 0.1 ml sodium thiopental (20 mg/ml in sterile water, i.v.). Only rats that become ataxic within 5 s were considered to have patent catheters and included in the analysis.

Self-administration. Self-administration sessions began one week after surgery in chambers outfitted with two nose ports, but no levers or food magazine. A nose poke into the active port resulted in an intravenous injection of cocaine HCl (0.5 mg/kg/infusion in 25 µl delivered in 1.6 s) on a fixed ratio (FR) 1 schedule. After an infusion there was 20-s timeout period, during which time the active nose poke port light was illuminated. This light served as the CS signaling cocaine delivery. To guarantee that all animals received exactly the same number of drug injections, and therefore the same number of CS-US pairings, all animals were initially allowed to take 5 infusions (i.e., the length of the session was determined by how long it took to take 5 injections). This infusion criterion (IC) was then increased to 10, 15, 25, 40, and finally 80 infusions. Animals were tested at each IC for two consecutive sessions. At IC 40, the dose was lowered to 0.2 mg/kg, to produce increased response rates necessary to result in sessions lasting approximately 1-2 hrs. This lower dose was used for the remainder of testing, and was chosen because doses in this range have been found to be sensitive to the presence or absence of a CS (4). Animals who failed to acquire self administration (ST = 2, GT = 2), or for whom catheter patency was not maintained (ST = 3, GT = 5), were removed from the analysis.

Experiment 1: Cue removal and resistance to extinction

After STs (final $n = 14$) and GTs (final $n = 16$) achieved an IC of 80, and showed stable levels of self-administration behavior, a cue removal test was conducted. For these two days all conditions were the same, and an active nose poke resulted in an infusion of cocaine, and the animals were allowed to take 80 injections – but the cue light (CS) was not illuminated. After

two days of cue removal the CS was reintroduced and daily testing continued for four more days. The influence of cue removal on self-administration behavior was assessed by quantifying the rate of self-administration (injections/min).

To examine resistance to extinction in STs and GTs, animals were then tested under cued extinction conditions, during which time a nose poke resulted in the illumination of the CS, but no cocaine injection. Extinction sessions were fixed at 1 hr and responding was measured until both groups no longer showed a downward trend in responding for four consecutive sessions.

Experiment 2: Individual differences in cue-induced reinstatement.

An independent group of rats received Pavlovian training as described above to identify STs (final $n = 13$) and GTs (final $n = 12$). These rats were then trained to self-administer cocaine, exactly as described above. Following the acquisition of self-administration all animals underwent extinction training for 7 daily 1-hr sessions. During these sessions, rats were not attached to the infusion pump swivel, and nose pokes had no programmed consequences (that is, animals received neither the drug nor the CS). Following extinction training (during which neither cues nor drug were available) all rats were left undisturbed in their home cages for a 30-day "incubation" period (Grimm et al., 2001), after which they were returned to the self-administration chambers for a single cue-induced reinstatement test. During this test session animals were attached to the infusion pump swivel and a nose poke into the active port resulted in illumination of the nose port light (the CS) for 5 s, but no drug infusion.

Statistical Analysis. Linear mixed-effects models were used to analyze all repeated measures data (Verbeke, 2009). Two-way ANOVAs were used to compare responding on active and inactive nose pokes during reinstatement testing. Bonferroni corrected post hoc comparisons were conducted where appropriate. Statistical significance was set at $P < 0.05$.

Results

Group differences in the propensity to approach and engage a food-related cue

A summary of the experimental design is shown in Figure 3.1. In adult male rats presentation of a lever-CS for 8 s was followed immediately by the unconditional delivery of a food pellet on each of three days of training. As described previously (Flagel et al., 2007), the one third of the rats showing the greatest number of lever deflections during the CS period were designated sign-trackers (STs) and the one third showing the fewest goal-trackers (GTs). The remaining rats were not used in any of these experiments. Rats designated as STs or GTs exhibited very different behavior on the last day of training (Figure 3.2). During the CS period STs had a high probability of rapidly approaching (Figure 3.2a, c) and vigorously engaging the lever-CS (Figure 3.2b). In contrast, during the CS period rats designated GTs seldom approached the lever-CS, but instead rapidly approached and engaged the food cup (Figure 3.2 d-f). Thus, as in our previous studies (23), the lever was a predictive CS in both STs and GTs, and was effective in evoking a CR in both, but the food cue was attractive in STs but not GTs.

No group differences in the acquisition of cocaine self-administration

Following Pavlovian training all rats were equipped with an intravenous (i.v.) catheter and trained to make an instrumental response (nose poke) for an i.v. injection of cocaine. In this situation the injection of cocaine (the US) was paired with illumination of the nose port, and therefore this light served as the cocaine-paired cue (drug CS). The training procedure was designed to eliminate any potential group differences in the number of CS (light) - US (cocaine) pairings and to mitigate possible differences in acquisition of self-administration behavior. Thus, rats were allowed to take a fixed number of injections each day (i.e., session length was determined by how long it took to reach the criterion number of injections), and the number

allowed increased over days of training (Figure 3.1). With this procedure group differences in acquisition would be evident by differences in the rate of self-administration, but there were no group differences for this measure at any infusion criterion (Figure 3.3a; $F_{(1,73)} = 1.108$, $p = .296$). Both groups also made the same number of active ($F_{(1,83)} = .107$, $p = .744$) and inactive nose pokes ($F_{(1,43)} = 2.429$, $p = .126$), and discriminated between the active and inactive nose ports (Figure 3.3b).

Removal of the cocaine cue decreases self-administration in STs but not GTs

By the end of training at the final infusion criterion (80 injections of 0.2 mg/kg per test session) all rats showed stable levels of cocaine self-administration (first 3 sessions shown in Figure 3.4A). During these baseline sessions each cocaine injection was accompanied by illumination of the nose poke port. On sessions 4 and 5 a nose poke into the active port still produced an i.v. injection of cocaine, but the light did not illuminate. Removal of the drug cue sharply decreased the rate of self-administration in STs by approximately half, and the magnitude of this effect was significantly correlated with how vigorously animals engaged the lever-CS on the first day of Pavlovian training ($r = .545$, $p = .022$). In GTs cue removal produced a small decrease in self-administration, but this was not statistically significant. Figure 3.4b shows the data averaged over the 3 baseline sessions and the 2 cue removal sessions (effect of group, $F_{(1,36)} = 4.954$, $p = .03$; effect of phase, $F_{(1,50)} = 19.332$, $p < .0001$; group x phase interaction, $F_{(1,50)} = 4.668$, $p = .01$; posthoc tests showed no effect of cue removal in GTs, $p > .05$, but a large effect in STs, $p < .001$). Reintroduction of the cocaine cue reinstated baseline levels of self-administration in STs within a few days (sessions 6-9 in Figure 3.4a). All rats continued to discriminate between the active and inactive nose poke during cue removal (Active

M = 224.88, SEM = 13.57; Inactive M = 24.43, SEM = 6.95), and there were no group differences in the total number of active or inactive responses (data not shown).

STs showed resistance to extinction relative to GTs

After the resumption of baseline levels of self-administration (session 9 in Figure 3.4) all animals underwent 28 days of extinction training, during which time nose pokes no longer produced cocaine, but they *did result in illumination of the light CS*. Over this period of time both STs and GTs slowly decreased responding for the CS (Figure 3.5, effect of session block, $F_{(6,125)} = 15.459$, $p < .0001$), and there was a small but significant group difference in responses during this cued extinction phase (effect of group, $F_{(1,321)} = 4.806$, $p = .029$; interaction non-significant). Inspection of these data indicates that STs made more responses for the CS than GTs, primarily during the first four sessions (Figure 3.5b-inset, one-tailed t test, $t_{(54)} = -2.02$, $p = 0.024$).

A cocaine-associated cue produces more robust reinstatement in STs than GTs

An independent group of rats underwent Pavlovian training to identify STs and GTs, exactly as above (data not shown - they are similar to those in Figure 3.2). The rats were then trained to self-administer cocaine exactly as described above (data not shown – they exhibited the same pattern of acquisition as illustrated in Figure 3.3). After stable responding all animals underwent 7 days of extinction training, except in this experiment nose pokes had no consequence (that is, nose pokes produced neither cocaine nor the CS). Under these conditions there were no group differences in responding during extinction (data not shown). After extinction all animals were tested for cue-induced reinstatement of responding similar to previous studies (30). In this test nose pokes again resulted in presentation of the cocaine CS (illumination of the nose-poke port), but no cocaine was delivered.

Sign trackers showed more robust reinstatement of cocaine seeking than GTs (Figure 3.6, the group x time [block] interaction was significant, $F_{(5,45)} = 2.871$, $p = .025$). This was because STs increased their number of active nose pokes over the session ($F_{(1,61)} = 5.867$, $p = .018$), but GTs did not (Figure 3.6a). A comparison of active and inactive port responses (Figure 3.6b, the dashed line indicates the average inactive port responses) indicated that both groups made more active nose pokes than inactive nose pokes, but STs reinstated responding to a significantly greater degree than GTs, as indicated by a significant group by port interaction ($F_{(1,46)} = 6.489$, $p = .014$; effect of group, $F_{(1,46)} = 5.539$, $p = .023$; effect of port, $F_{(1,46)} = 93.463$, $p < .0001$).

Discussion

If cues associated with rewards are attributed with incentive salience they acquire the ability to act as incentive stimuli. Incentive stimuli have three fundamental properties: (1) they attract, eliciting approach towards them; (2) they are "wanted", in the sense that animals will work to get them; and (3) they can spur ongoing instrumental actions to obtain the associated reward, as in the Pavlovian to instrumental transfer effect (Berridge, 2001; Cardinal et al., 2002a). We have reported, however, that if a localizable cue is associated with presentation of a food reward at a different location the cue acquires incentive motivational properties in some individuals, but not others - even though all individuals learn the CS-US association as indicated by the development and vigor of their respective CRs (Flagel et al., 2009; Robinson and Flagel, 2009). This suggests that the ability of a cue to act as an incentive stimulus is dissociable from its ability to act as a conditional stimulus (CS) that evokes a conditional response (CR; Robinson and Flagel, 2009). In the present study we asked whether rats that have a propensity to attribute incentive salience to a food cue are the same ones for whom a cocaine cue would come to motivate cocaine self-administration behavior - maintain drug taking and instigate reinstatement after extinction. They

are. The cocaine cue was much more effective in motivating drug-taking behavior and instigating reinstatement in STs (who approached the food cue) than in GTs (who did not).

It is well known that drug cues are important for maintaining and reinstating drug-seeking behavior, presumably because they spur incentive motivation for drugs (Arroyo et al., 1998; Davis and Smith, 1976; Schenk and Partridge, 2001; Shaham et al., 2003). In most drug self-administration studies drug delivery is paired with presentation of a cue for good reason. This is because omitting the cue greatly attenuates self-administration behavior (Caggiula et al., 2001; Chaudhri et al., 2005; Kippin et al., 2006; Panlilio et al., 1996; Schenk and Partridge, 2001). Indeed, cues are capable of maintaining responding for long periods of time even in the absence of the drug, on second-order schedules of reinforcement, for example (Everitt and Robbins, 2000; Schindler et al., 2002). Of course, there is a large literature on both human and non-human animals concerning the importance of drug cues in instigating reinstatement/relapse (Shaham et al., 2003). In addicts, many drugs cues (e.g., people, places and paraphernalia) evoke craving and/or relapse (Ehrman et al., 1992; O'Brien et al., 1998), and their attention is biased towards such cues (Field and Cox, 2008). In rats, even stimuli associated with a single session of cocaine self-administration can motivate renewed drug-seeking a year later (Ciccocioppo et al., 2004). In such studies there is, however, considerable individual variation in the effectiveness of cues in motivating behavior. The data reported here suggest this variation is not just due to experimental error, but much of it may be due to real individual variation in the propensity for cues to acquire incentive motivational properties. In the present study we considered only the effects of discrete localizable stimuli (a lever CS and a light CS), but many other manipulations influence self-administration behavior and reinstatement/relapse - including contextual stimuli, stress, and a "prime" with the drug itself (Crombag et al., 2008; de Wit, 1996; Shaham et al., 2003). It

remains to be determined whether variation in the propensity to attribute incentive salience to a discrete localizable cue also predicts the ability of these other classes of stimuli to produce desire and instigate actions. Furthermore, in the present experiment the cocaine cue was located at the same place where a response produced an injection of cocaine, a situation that may produce especially "compulsive" behavior (Tomie et al., 2008). Further studies will be required to explore the influence of different arrangements between the CS and manipulandum, and whether similar individual differences are evident if a cue is paired with cocaine in a response-independent manner (Uslaner et al., 2006).

It is important to emphasize that the differences between STs and GTs reported here are not a function of differential exposure to either the drug itself, the number of CS-US pairings, or learning *per se*. We used a training procedure that held these variables constant across animals. Rather than using sessions of a fixed duration we allowed each animal to take the same number of injections each day, insuring that all animals would be exposed to the same amount of drug and the same number of CS-drug pairings. Indeed, there were no group differences in learning cocaine self-administration behavior. Furthermore, the difference between STs and GTs in the Pavlovian task is not attributable to differences in learning, as both groups learn their respective CRs at a comparable rate (Robinson and Flagel, 2009).

What might account for the large difference between STs and GTs in their propensity to attribute incentive value to either the food cue or the cocaine cue? Presumably this is due to variation in the operation of brain systems that attribute incentive salience to reward cues, or brain systems that moderate that process. Of course, there is a wealth of evidence implicating ascending mesotelencephalic dopamine (DA) systems in Pavlovian conditioned motivational processes (Berridge and Robinson, 1998; Cardinal et al., 2002a; Di Chiara, 1998), which

prompts questions about differences in DA function between STs and GTs. Unfortunately, there has been very little research on this question, although the little that has been done suggests such differences exist (Tomie et al., 2000; Tomie et al., 2008). For example, a recent report (Flagel et al., 2007) found higher levels of nucleus accumbens dopamine D1 receptor mRNA and lower levels of D2 mRNA in STs compared to GTs, and another found STs are more prone to cocaine sensitization (Flagel et al., 2008).

Recent studies on two selectively-bred lines of rats also suggest a relationship between the propensity to attribute incentive salience to reward-related cues and DA (Flagel et al., 2010). Rats selectively-bred for up to 18 generations for a high or low locomotor response to a novel environment (bHRs and bLRs, respectively) differ dramatically in their propensity to attribute incentive salience to reward cues. Essentially all bHR animals approach a cue associated with food or with cocaine (they are STs) and no bLRs do so (they are GTs). Furthermore, a food cue is a more effective conditional reinforcer in bHR/STs than bLR/GTs. There are a number of differences in the DA systems of these animals. The bHR/ST rats are more sensitive to the psychomotor activating effects of the DA D2/3 agonist quinpirole, and this behavioral supersensitivity is associated with a much greater proportion of DA $D2^{high}$ receptors in the dorsal striatum in these animals (despite lower D2 mRNA and no difference in total D2 receptor binding). Also, studies using fast-scan cyclic voltammetry revealed that bHR/ST animals have more frequent spontaneous DA "release events" in the accumbens core. Thus, the available data suggest that animals prone to attribute incentive salience to reward cues (STs) have a more active DA system than those who do not (GTs) (Flagel et al., 2010). In addition, with Pavlovian training there is a transfer of the phasic DA signal (assessed with fast-scan cyclic voltammetry in the accumbens core) from a food US to a lever-CS in STs, but this does not occur in GTs (Flagel

et al., 2011b). It appears that during Pavlovian conditioning the transfer of a phasic DA signal from the US to the CS is not due to a gain in predictive strength, but requires that the CS be attributed with incentive salience - as it is in STs but not GTs. This may be why acquisition of a ST CR is DA-dependent whereas learning a GT CR is not (Flagel et al., 2011b). Taken together, these data suggest that STs and GTs differ in dopaminergic signaling in ways that may be related to differences in their tendency to attribute incentive salience to stimuli.

The extent to which the ST/GT and HR/LR traits are genetically (or epigenetically) related remains to be determined (Flagel et al., 2010). In outbred animals these two traits are not correlated (Flagel et al., 2009). Nevertheless, there are other traits that may contribute to addiction vulnerability and are associated with the tendency to show a ST or GT phenotype. Relative to GTs, both bHR/ST animals (Flagel et al., 2010) and outbred STs have difficulty withholding an action in order to receive a reward, as assessed using a differential reinforcement of low rates (DRL) task, and STs show more premature ("impulsive") responses on a 2-choice serial reaction time task (Lovic et al., 2011). These studies suggest that STs tend to show "impulsive actions" relative to GTs. Of course, individuals that combine both a propensity to make "impulsive actions" and to be motivationally aroused by drug cues may be especially susceptible to addiction.

Many of the behaviors characteristic of STs are similar to those seen after a lesion of the subthalamic nucleus (STN). Animals with a STN lesion more readily acquire cocaine self-administration, more readily approach ("sign-track") cues associated with both food and cocaine reward, and show impulsive actions on a DRL task (Uslaner et al., 2008; Uslaner and Robinson, 2006; Uslaner et al., 2005). These symptoms may be due to an increased tendency to attribute incentive salience to reward cues in animals with a STN lesion and, therefore, the STN may be

part of a neural system that normally moderates the degree to which incentive salience is attributed to reward cues (Uslaner et al., 2008). It should be adaptive to attribute incentive value to cues that predict the location of natural rewards, because approaching such cues would often lead one to the reward itself. It may also be important, however, to moderate this process, because the excessive attribution of incentive salience to cues may be maladaptive and contribute to psychopathologies, including over-eating (Schachter, 1968; Weingarten, 1983) and addiction (Kapur, 2003; Robinson and Berridge, 1993). Thus, the differences between STs and GTs may be due to intrinsic differences in DA systems that actively attribute incentive salience to reward cues, to differences in systems that oppose this process, such as the STN, or both.

In conclusion, we report that it is possible to predict, *prior to rats having any experience with cocaine*, which individuals will most likely show cue-induced reinstatement of cocaine-seeking following extinction, and for which ones a cue will motivate drug-taking behavior. It appears rats that have a propensity to attribute incentive salience to a food cue also attribute incentive salience to a cocaine cue. The neural mechanism(s) responsible for these differences are unknown, although preliminary data implicates DA systems. Of course, individuals for whom drug cues become attractive will have more difficulty resisting them than individuals for whom cues are mere predictors of reward. Thus, cues may continually goad some individuals to action (Robinson and Berridge, 1993; Stewart et al., 1984), and this trait may render these individuals most susceptible to addiction. It will be important to determine the psychological and neurobiological mechanisms responsible for variation in the propensity to attribute incentive salience to reward cues, as this may not only increase susceptibility to addiction, but to other compulsive behavioral disorders as well, including eating disorders and compulsive gambling.

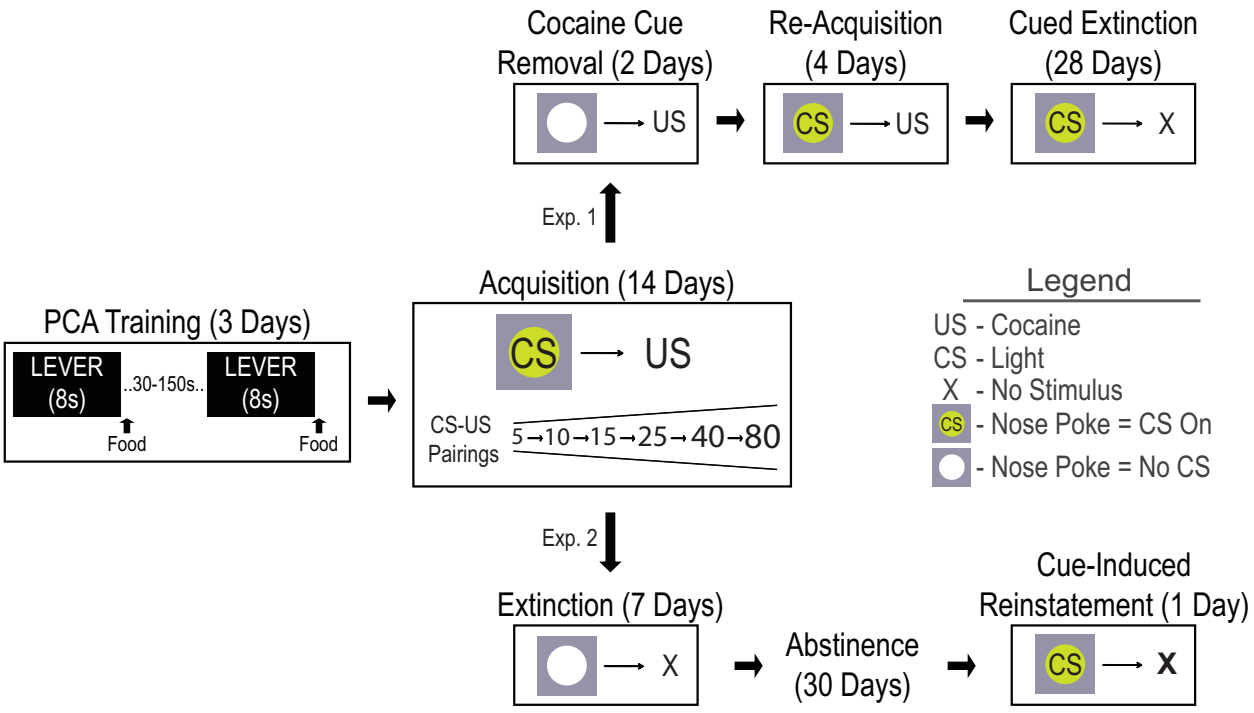


Figure 3.1. Schematic illustrating the experimental design. All animals received identical Pavlovian conditioned approach (PCA) and cocaine self-administration acquisition training. Following acquisition, Experiment 1 (Exp. 1), and Experiment 2 (Exp. 2) diverged as shown. During self administration, the conditional stimulus (CS) was a nose poke port cue light and the unconditional stimulus (US) was an intravenous infusion of cocaine. Depending on the experimental phase, an active nose poke produced the CS and US (Acquisition/Re-Acquisition), the CS but no US (Cued Extinction), no CS but the US (Cue Removal), or nothing (X – Extinction).

Figure 3.2. Behavior directed towards the lever-CS ("sign-tracking") or the food cup (the location of US delivery; "goal-tracking") during the 8 s CS period on the final day of Pavlovian training. The topography of the conditional response (CR) is different in rats designated sign-trackers (STs, *n*=14) vs. goal-trackers (GTs, *n*=16). The mean ± SEM for (**a**) probability of approaching the lever [# trials with a lever contact/#trials per session] (CS) during the 8 s CS period, (**b**) number of lever contacts (**c**) latency to the first lever contact after CS presentation, (**d**) probability of approach to the food tray during the 8 s CS period, (**e**) number of food tray contacts during the CS period, and (**f**) latency to the first food tray entry after CS presentation. *****, indicates significant group differences, $p < 0.001$.

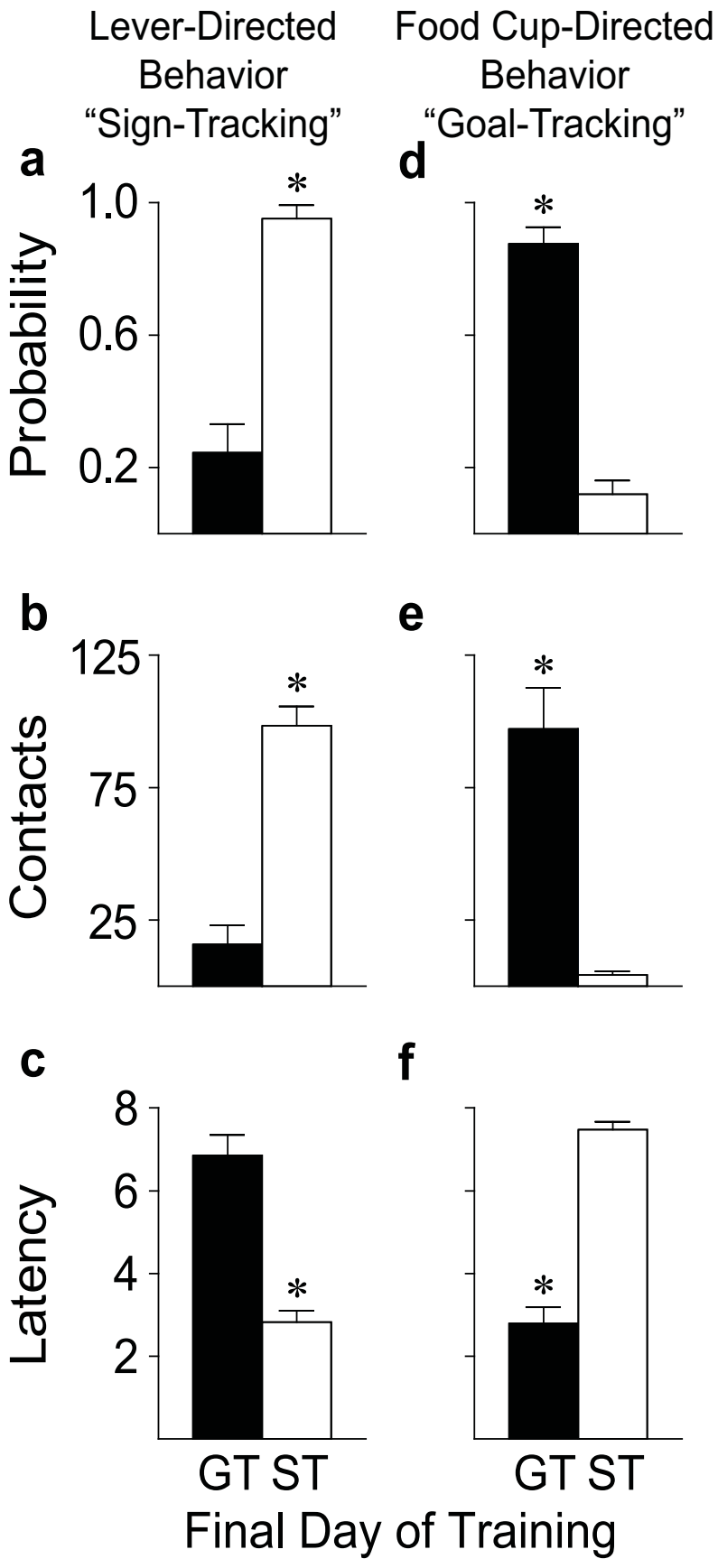
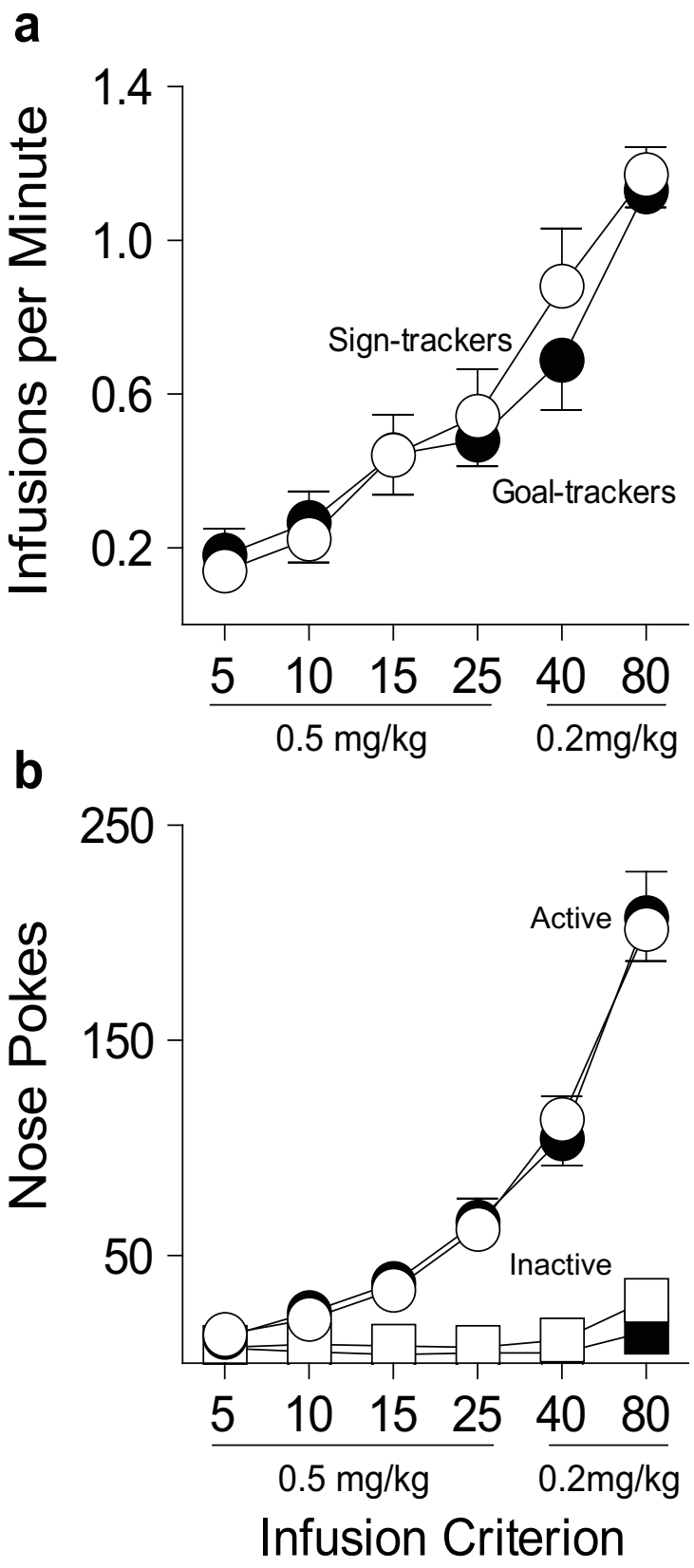

Lever-Directed Behavior "Sign-Tracking"
Food Cup-Directed Behavior "Goal-Tracking"
a
d
b
e
c
f
Probability
1.0
0.6
0.2
Contacts
125
75
25
Latency
8
6
4
2
*
GT ST
GT ST
Final Day of Training

Figure 3.3. Acquisition of cocaine self-administration behavior in sign-trackers (*n*=14) and goal-trackers (*n*=16). (**a**) The mean ± SEM number of cocaine infusions per minute for infusion criteria 5, 10, 15, and 25 (0.5 mg/kg/inf) and 40 and 80 (0.2 mg/kg/inf). (**b**) The mean ± SEM number of active and inactive nose poke responses at each infusion criterion.


a
Infusions per Minute
1.4
1.0
0.6
0.2
Sign-trackers
Goal-trackers
5
10
15
25
40
80
0.5 mg/kg
0.2mg/kg
b
Nose Pokes
250
150
50
Active
Inactive
5
10
15
25
40
80
0.5 mg/kg
0.2mg/kg
Infusion Criterion

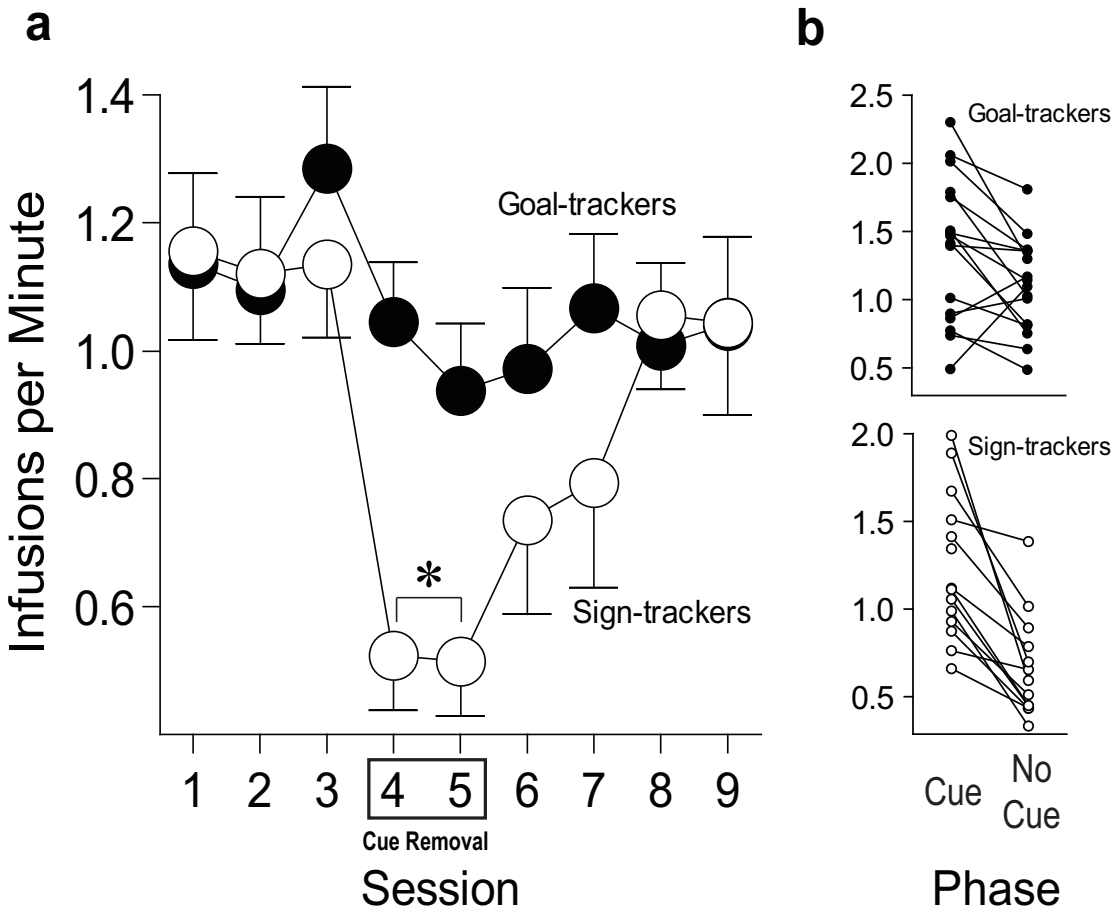


Figure 3.4. Effects of removal of a cocaine-associated cue on self-administration behavior in sign-trackers (*n*=14) and goal-trackers (*n*=16). (**a**) The mean ± SEM number of cocaine infusions (0.2 mg/kg/infusion) per minute in sign-trackers and goal-trackers in the presence of the cocaine cue (sessions 1-3, 6-9) and when the light cue was not presented along with the injection of cocaine (sessions 4-5). (**b**) The rate of cocaine self-administration (infusions/min) for individual animals averaged over the baseline period (cocaine cue present, sessions 1-3) and the cue removal sessions (sessions 4-5). *, indicates a significant group difference, $p < 0.05$.

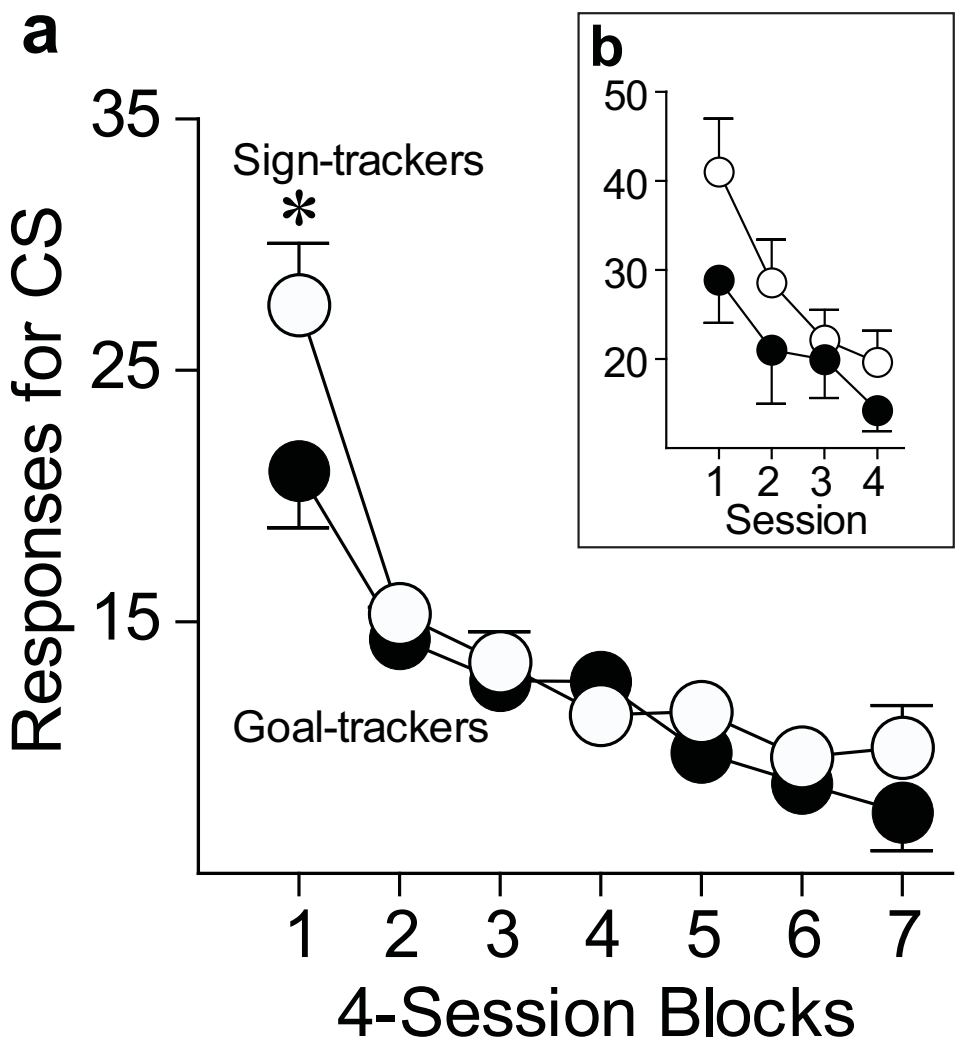


Figure 3.5. Extinction of responding for cocaine in sign-trackers (*n*=14) and goal-trackers (*n*=16), when a response continued to produce the cocaine-associated CS. (**a**) The mean ± SEM number of active responses for the CS, expressed in 4-session blocks. (**b**) The mean ± SEM number of active responses for the CS during the first 4 extinction sessions (effect of group, $F_{(1,85)} = 4.57$, $p = 0.035$). *, indicates a significant group difference, $p < 0.05$.

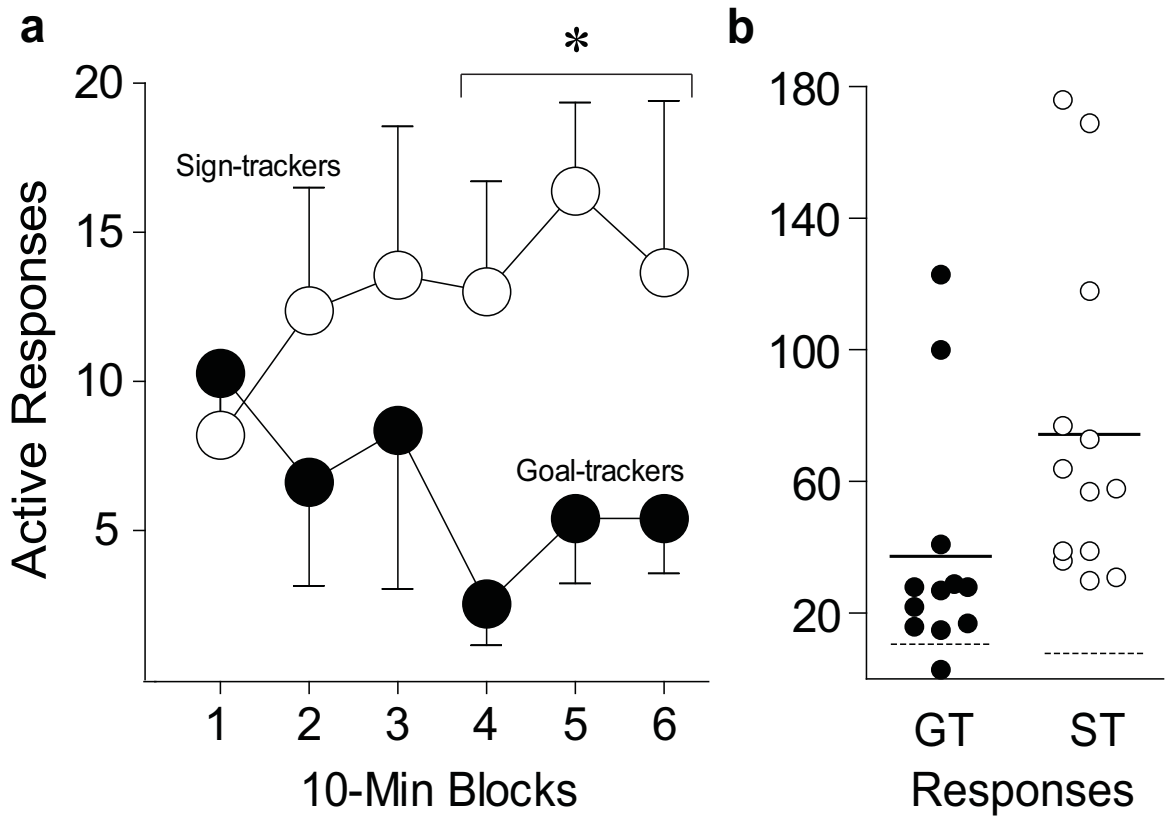


Figure 3.6. Cue-induced reinstatement of responding in sign-trackers (*n*=12) and goal-trackers (*n*=13). Following extinction, all animals were given a single, 60-min cue reinstatement test session, in which active responses resulted in presentation of the cocaine cue. (**a**) Mean ± SEM number of active port nose pokes in 10-min blocks over the 60-min session. (**b**) The total number of active port responses in individual animals (the solid horizontal line indicates the group mean and the dashed line the average number of responses at the inactive port). *, indicates a significant group difference, $p < 0.05$.

Chapter 4

Cue-evoked cocaine "craving": role of dopamine in the accumbens core

Introduction

For addicts, cues associated with drug use can acquire powerful control over motivated behavior, and can contribute to high rates of relapse, via three overlapping but dissociable processes (Milton and Everitt, 2010). First, they can become attractive and attention grabbing, eliciting approach toward themselves, which may draw an addict into close proximity with drugs and/or drug delivery equipment. Second, they become desirable in their own right, acting as conditioned reinforcers. This has the effect of increasing the persistence and flexibility of drug-seeking behaviors for long periods of time between drug availability. Third, and perhaps most important for relapse, they can evoke conditioned motivational states ("wanting" or "craving") capable of spurring new drug-seeking actions or invigorating ongoing ones, even after long periods of abstinence (Milton and Everitt, 2010; Robinson and Berridge, 1993; Robinson and Berridge, 2001).

In a series of preclinical studies we have found, however, that there is considerable individual variation in the extent to which reward cues, including drugs cues, acquire Pavlovian motivational properties, and thus the ability to act as incentive stimuli. When a Pavlovian conditional stimulus (CS), predicts the delivery of food reward (unconditional stimulus, US), only some rats (sign-trackers, STs; Hearst and Jenkins, 1974) come to find the cue attractive and

desirable, in that they will approach it and will avidly work to obtain it. For others (goal-trackers, GTs; Boakes, 1977), the cue itself is less attractive, and it is less desired, but it nevertheless elicits conditioned approach directed at the location of food delivery (Flagel et al., 2007; Robinson and Flagel, 2009). Thus, the cue is an equally effective CS for both STs and GTs, in that it produces a conditioned response (CR) in both, but only for STs is it attributed with incentive salience, rendering it a motivationally potent incentive stimulus. Importantly, we have found that individual variation in the propensity to attribute incentive salience to a food cue, as measured by conditioned approach behavior, predicts the extent to which discrete *drug cues* motivate behavior. For example, for STs, a cocaine-associated cue is more attractive, reliably eliciting conditioned approach towards it (Yager and Robinson, 2012). Additionally, for STs, a cocaine cue is more desired, in the sense that it reinforces more robust drug-seeking behavior, relative to GTs (Saunders and Robinson, 2010; Saunders and Robinson, 2011b).

The ability of drug cues to serve as conditioned reinforcers has received considerable attention in preclinical studies. Indeed, in the vast majority of so-called "cue-induced" reinstatement studies, the drug cue does not actually *induce* behavior, but acts to reinforce actions already taken (Shaham et al., 2003). In addicts, however, drug cues most often appear *before* drug-seeking actions and *instigate* such actions by arousing a conditioned motivational state of "craving", which may influence brain and behavior implicitly (Berridge and Robinson, 2003; Childress et al., 2008; Fischman, 1989; Fischman and Foltin, 1992; Robinson and Berridge, 1993). Such conditioned motivational states can be very powerful, promoting drug seeking behavior even in the face of a conscious desire to remain abstinent and/or to avoid adverse consequences (Epstein and Preston, 2003; Midanik and Greenfield, 2000; Wallace, 1999). Under some conditions cue-evoked conditioned motivation can reach the level of

conscious awareness and may be experienced as a strong urge or desire (craving), and the intensity of craving can predict future drug intake and the likelihood of relapse (Carter and Tiffany, 1999; Ehrman et al., 1992; Epstein et al., 2009; Preston et al., 2009; Shiffman et al., 2002; Tiffany and Wray, 2012). The neural mechanisms underlying these conditioned motivational states are unclear, but several studies have demonstrated that the intensity of subjective craving elicited by drug cues is positively correlated with the magnitude of cue-evoked dopamine release in the striatum of addicts (Volkow et al., 2008; Wong et al., 2006), suggesting a role for dopamine.

It has proven difficult to examine drug cue-evoked conditioned motivation in preclinical studies, because researchers can only indirectly measure implicit "craving". In many studies, Pavlovian-to-instrumental transfer (PIT) procedures (Estes, 1943; Estes, 1948; Lovibond, 1983) have been used to infer conditioned motivation for food cues, but until recently, none have demonstrated such an effect with drug cues. LeBlanc et al. (2012), using a PIT paradigm, were first to successfully demonstrate that the noncontingent presentation of a cocaine cue can invigorate ongoing self-administration behavior (see Corbit and Janak, 2007b for a demonstration of orally-administered alcohol-cue PIT). However, attempts to model conditioned motivational mechanisms of *relapse* in rats, via the noncontingent presentation of drug cues, have so far been relatively unsuccessful (de Wit and Stewart, 1981; Deroche-Gamonet et al., 2002; Grimm et al., 2000). To that end, we utilized a novel procedure, adapted from Cooper et al. (2007), to access the ability of a cocaine cue to produce conditioned "craving" that spurs drug-seeking behavior in the face of adverse consequences. In this procedure, rats are faced with a choice between continuing to take drug, but suffering adverse consequences if they do (footshock), or abstaining. When the cost is sufficiently high rats will abstain, which then allows

the opportunity to test the ability of presentation of the drug cue to evoke drug-seeking, despite the continued presence of the adverse consequence. We hypothesized that in individuals for whom food cues acquire exaggerated incentive motivational value, a cocaine cue would be more effective at evoking drug-seeking. Furthermore, we examined the role of dopamine in cocaine cue-evoked "craving". We focused on dopamine signaling specifically within the nucleus accumbens core, because this region is critical for the expression of motivated behaviors (Cardinal et al., 2002a; Ikemoto and Panksepp, 1999), and dopamine signaling there is necessary for food-cue PIT to occur (Corbit and Balleine, 2011; Hall et al., 2001; Lex and Hauber, 2008).

Materials and Methods

Subjects

Male Sprague Dawley rats (N=71; Harlan, IN) weighing 275-325 grams at surgery were individually housed in a temperature and humidity controlled colony room on a 12-hr light/12-hr dark cycle (lights on at 0800 hr). Water and food were available ad libitum (i.e., rats were not food deprived at any time). After arrival rats were given one week to acclimate to the colony room before any testing began. During this period they were handled periodically by the experimenter. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus

Behavioral testing was conducted in standard (30.5 x 24.1 x 21 cm) test chambers (Med Associates Inc., St. Albans, VT, USA) located inside sound-attenuating cabinets. A ventilating fan masked background noise. For Pavlovian training each chamber had a food cup located in the center of one wall, 3 cm above a stainless steel grid floor. Head entries into the food cup were recorded by breaks of an infrared photobeam located inside. A retractable lever that could be

illuminated from behind was located 2.5 cm to the left or right of the food cup, approximately 6 cm above the floor. The location of the lever with respect to the food cup was counterbalanced across rats. On the wall opposite the food cup, a red house light remained illuminated throughout all experimental sessions. Responses were recorded using Med Associates software.

Surgery

Following Pavlovian training, rats were prepared with intravenous catheters as described previously (Crombag et al., 2000) under ketamine hydrochloride (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia. Following surgery, catheters were flushed daily with 0.2 ml sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO) to minimize infection and prevent occlusions. Catheter patency was tested at the end of testing by intravenous injection of 0.2 ml sodium thiopental (20 mg/ml in sterile water, Hospira, IL). Only rats that became ataxic within 5-10 s were considered to have patent catheters and included in the analyses. For Experiment 2, after receiving an intravenous catheter, rats were positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The skull of each rat was leveled and chronic guide cannulae (22 gauge stainless steel; Plastics One) were inserted bilaterally 2 mm above the target site in the nucleus accumbens core (relative to Bregma: anterior +1.8 mm; lateral +1.6 mm; ventral -5.0 mm). Guide cannulae were secured with skull screws and acrylic cement, and wire stylets (28 gauge, Plastics One) were inserted to prevent occlusion. After surgery, all rats received antibiotic and carprofen (5 mg/kg) for pain. Rats were allowed to recover from surgery for at least 7 days before testing began.

Microinjections

In Experiment 2, before the reinstatement test session, rats received a single microinjection of either vehicle (0.9% sterile saline) flupenthixol (20 µg in saline; Sigma, St. Louis, MO), a

relatively nonselective dopamine receptor antagonist, or d-amphetamine sulfate (10 µg in saline; Sigma, St. Louis, MO), an indirect dopamine agonist. Drug doses were based on previous studies (e.g., Di Ciano et al., 2001; Ito and Hayen, 2011; Saunders and Robinson, 2012; Wyvell and Berridge, 2000). Intracerebral microinjections were made through 28 gauge injector cannulae (Plastics One) lowered to the injection site in the nucleus accumbens core (ventral -7.0 mm relative to skull), 2 mm below the ventral tip of the guide cannulae. During infusions, rats were gently held by the experimenter. All infusions were administered bilaterally at a volume of 0.5 µl/side, delivered over 90 s using a syringe pump (Harvard Apparatus, Holliston, MA) connected to microinjection cannulae via PE-20 tubing. After infusions, the injectors were left in place for 60 s to allow for drug diffusion before being withdrawn and replaced with wire stylets. Rats received a microinjection of saline approximately 5 days before the reinstatement test, to acclimate them to the injection procedure.

Procedure

Experiment 1: Individual variation in cocaine cue-evoked reinstatement

Pavlovian training: Pavlovian training procedures were the similar to those described previously (Flagel et al., 2007; Saunders and Robinson, 2010). For two days prior to the start of training, 10 banana-flavored pellets (45 mg, BioServe, #F0059; Frenchtown, NJ) were placed in the home cages to familiarize the rats with this food. Approximately one week after arrival, rats were placed in the test chambers, with the lever retracted, and trained to retrieve pellets from the food cup by receiving 25 45-mg banana pellets on a variable time (VT) 30-sec schedule. All rats retrieved the pellets and began Pavlovian training the next day. Each trial consisted of insertion (and simultaneous illumination) of the lever (CS) into the chamber for 8 s, after which time the lever was retracted and a single food pellet (US) was immediately delivered into the adjacent

food cup. Each training session consisted of 25 trials in which CS-US pairings occurred on a variable time (VT) 90-s schedule (the time between CS presentations varied randomly between 30 and 150 s). Lever deflections, food cup entries during the 8-s CS period, latency to the first lever deflection, latency to first food cup entry during the CS period, and food cup entries during the inter-trial interval were measured.

Quantification of behavior using an index of Pavlovian conditioned approach (PCA): For some analyses rats were classed into three groups: (1) Those that preferentially interacted with the lever ("sign-trackers", STs), (2) those that preferentially interacted with the food cup during lever presentation ("goal-trackers", GTs), and (3) those that had no clear preference for the lever or food cup ("intermediates", INs). The extent to which behavior was lever (CS) or food-cup directed was quantified using a composite index (Lovic et al., 2011; Meyer et al., 2012a; Saunders and Robinson, 2011b) that incorporated three measures of Pavlovian conditioned approach: (1) the probability of either deflecting the lever or entering the food cup during each CS period [P(lever) - P(food cup)]; (2) the response bias for contacting the lever or the food cup during each CS period [(#lever deflections - #food-cup entries) / (#lever deflections + #food-cup entries)]; and (3) the latency to contact the lever or the food cup during the CS period [(lever deflection latency - food-cup entry latency) / 8]. Thus, the Pavlovian conditioned approach index (PCA Index) score consisted of [(Probability difference score + Responses bias score + Latency difference score) / 3]. This formula produces values on a scale ranging from -1.0 to +1.0, where scores approaching -1.0 represent a strong food cup-directed bias and scores approaching +1.0 represent strong lever-directed bias. The average PCA Index score for days 4 and 5 of training was used to class rats. Rats were designated STs if they obtained an average index score of +0.25 or greater, and as GTs if they obtained a score of -0.25 or less. The remaining rats within the -

0.24/+0.24 range were classed as INs. Note that in Experiment 2, INs were excluded from further study.

Self-administration

Acquisition: Self-administration sessions began one week after surgery in chambers outfitted with two nose ports, but no levers or food magazine. A nose poke into the active port resulted in an intravenous injection of cocaine HCl (0.4 mg (weight of the salt) per kg per infusion in 50 µl of saline delivered over 2.6 s) on a fixed ratio (FR) 1 schedule. Coincident with the start of an infusion was 20-s timeout period, during which the active nose poke light was illuminated. This light served as the CS signaling cocaine delivery. We utilized a training procedure that guaranteed all rats received exactly the same number of cocaine injections, by imposing an infusion criterion (IC) on self-administration sessions (i.e., session length was determined by how long it took each rat to reach the IC, not by an explicit time limit (see Saunders and Robinson, 2010; Saunders and Robinson, 2011b). Rats were initially allowed to take 10 infusions per session for three sessions, and this IC was then increased to 20 for three sessions and finally to 40 infusions for five sessions.

Imposition of adverse consequences to drug self administration: In most reinstatement studies, following self-administration acquisition animals are put through extinction training to reduce behavior to a low level before a reinstatement test (Shaham et al., 2003). However, we wanted to avoid extinction training, for two reasons. 1. Addicts do not undergo extinction training prior to relapse, but may be abstinent for a number of reasons, including adverse consequences of continued drug use. 2. Extinction training itself has important effects on brain and behavior (Knackstedt et al., 2010). Therefore, we adapted a procedure based one developed by Cooper et al. (2007), where adverse consequences (footshock) are imposed on drug seeking,

and gradually escalated until drug taking reaches near-abstinence levels (Figure 4.1). During this phase, after the acquisition of stable cocaine self administration behavior, sessions were no longer determined by number of infusions, but instead were limited to 30 min/day. Electric current was applied to the front two-thirds of the chamber floor throughout these sessions, such that rats were required to walk across the electrified portion of the floor in order to make a nose poke and receive cocaine infusions, which remained paired with the cocaine cue. Thus, in contrast to extinction procedures, drug and the drug CS remained available at all times, but rats were faced with a choice of whether or not to continue taking cocaine in the face of rising negative consequences. Initially, rats received a 30-min session with footshock set at 0 milliamps (mA), to establish baseline levels of behavior, followed by successive sessions with footshock set at 0.15, 0.20, and 0.25 mA. At this point, any rats that took fewer than 5 cocaine infusions received one additional session with the same shock intensity. For any rats that took more than 5 infusions, the footshock intensity was increased in subsequent sessions by increments of 0.05 mA, until they took fewer than 5 infusions. Following this adverse consequences training phase, rats were returned to their home cages for a two-week "incubation" period (Grimm et al., 2001) before the reinstatement test.

Reinstatement: After the incubation period, rats were returned to the self-administration chambers for a 30-min reinstatement test under extinction conditions. Importantly, during this session, the front two-thirds of chamber floor was electrified, as before, but at 50% of the intensity each rat reached at the end of shock training. Additionally, the cocaine-associated cue light was illuminated *noncontingently*, that is, independent of the rat's behavior, for 20 s every 3 minutes (thus, in this test, nose pokes did not produce the drug paired cue, and thus the cue did not act as a conditioned reinforcer). This was done in order to isolate the ability of the cocaine

cue to evoke drug-seeking behavior (to "goad actions", see Stewart et al., 1984), presumably because they generated a conditioned motivational state ("wanting"), from its ability to reinforce actions already emitted.

Experiment 2: The role of nucleus accumbens core dopamine in cocaine cue-evoked reinstatement

A separate cohort of rats was used in Experiment 2 (n=43). All procedures leading up to the reinstatement session were identical to Experiment 1.

Reinstatement: Following the incubation period, STs and GTs were assigned to one of three drug treatments: vehicle (saline), flupenthixol, or amphetamine, for a total of six independent groups: ST-VEH (n=8), ST-FLU (n=8), ST-AMPH (n=7), GT-VEH (n=6), GT-FLU (n=7), and GT-AMPH (n=7). On the day of the reinstatement test, each rat received a single microinjection, as described above, before being placed in the test chamber 10-15 min later. Reinstatement session parameters, including noncontingent cue presentations, were identical to Experiment 1.

Histology

After the completion of all behavioral testing, rats in Experiment 2 were anesthetized with an overdose of sodium pentobarbital and their brains were removed and flash frozen in isopentane chilled to approximately -30 C by a mixture of isopropyl alcohol and dry ice. Frozen brains were sectioned on a cryostat at a thickness of 60 μm, mounted on slides, air-dried, and stained with cresyl violet. Microinjection sites were verified by light microscopy and plotted onto drawings from a rat brain atlas (Paxinos and Watson, 2007).

Statistical Analyses

Linear mixed-models (LMM) analyses of variance (ANOVA) were used for all repeated-measures data. The best-fitting model of repeated measures covariance was determined by the

lowest Akaike information criterion score (Verbeke, 2009). Depending on the model selected, the degrees of freedom may have been adjusted to a non-integer value. Two-way ANOVAs were used to compare group responding during reinstatement tests. T tests were used for planned comparisons of group means. Pearson's correlations were used to compare reinstatement responding to PCA index scores in Experiment 1. Statistical significance was set at $p < 0.05$.

Results

Experiment 1 – Individual variation in Pavlovian conditioned approach (PCA) behavior

Figure 4.2 illustrates the degree of individual variation in PCA behavior in the rats used in Experiment 1 (n = 28), by plotting the distribution of individual rat PCA index scores. Similar to our previous reports (Flagel et al., 2007; Saunders and Robinson, 2012), we found large variation in the type of conditioned responses different rats made. Rats classed as STs, which preferentially directed their CR towards the CS (lever), were defined as those with PCA index scores ranging from +0.25 to +1.0. Rats classed at GTs, conversely, which preferentially directed their CR towards the food cup during the CS period, had PCA index scores ranging from -0.25 to -1.0. Finally, IN rats, that vacillated between CS-directed and food cup-directed CRs, were those with scores ranging from -0.24 to +0.24.

Acquisition of cocaine self-administration in STs, GTs, and INs

Consistent with previous reports (Saunders and Robinson, 2010; Saunders and Robinson, 2011b), there were no group differences in the acquisition of cocaine self administration behavior (Figure 4.3). STs, INs, and GTs did not differ in the number of active (no effect of group, $F_{(2,25)} = 0.413$, $p = 0.666$; Figure 4.3A) or inactive nose pokes (no effect of group, $F_{(2,25)} = 0.321$, $p = 0.729$) they made across the IC. To further examine the pattern of self administration behavior at the end of training, we analyzed within-session responding during the final two

sessions at IC 40. The pattern of cocaine intake did not differ across groups, with all showing consistent and uniform rates of cocaine intake (Figure 4.3B).

Cocaine self administration in the face of adverse consequences

Rats were next allowed to continue to self administer cocaine, but to do so were required to walk across an electrified portion of the chamber floor. As footshock intensity increased, STs, INs, and GTs all significantly decreased the number of cocaine infusions they took (effect of shock intensity, $F_{(5,26.18)} = 46.784$, $p < 0.001$; Figure 4.4A). Importantly, there were no group differences in the total number of cocaine infusions taken (effect of group, $F_{(2,15.18)} = 2.308$, $p = 0.133$; Figure 4.4A). Additionally, there were no group differences in the final shock intensity required to reduce responding to the criterion level of fewer than 5 infusions in a session (effect of group, $F_{(2,25)} = 0.711$, $p = 0.501$; Figure 4.4B). Thus, there was no indication that STs, GTs, or INs were differentially sensitive to footshock.

Individual variation in the ability of a cocaine cue to spur drug-seeking behavior in the face of adverse consequences

We next tested the ability of presentation of the cocaine cue (the light in the nose port), independent of any action, to spur drug-seeking behavior, as indicated by active nose pokes, even when making a nose poke response would still require crossing the electrified floor. Although all groups discriminated between active and inactive nose pokes (effect of port, $F_{(1,50)} = 50.204$, $p < 0.001$), there were significant group differences in the degree of cue-evoked drug-seeking behavior (group by nosepoke interaction, $F_{(2,50)} = 4.453$, $p = 0.017$; Figure 4.5A). Planned comparisons of the groups revealed that both STs ($t_{(18)} = 3.23$, $p = 0.002$) and INs ($t_{(16)} = 2.68$, $p = 0.008$) made significantly more active responses during the reinstatement test, relative to GTs. Additionally, we found that the vigor of cue-induced drug-seeking behavior was

significantly correlated with PCA index scores. Indeed, variation in PCA Index scores accounted for 25.3% of the variance in active responses during the reinstatement test ($R^2 = 0.253$, $p = 0.0032$; Figure 4.5B). We separately analyzed the number of active responses rats made specifically while the CS was illuminated (CS active responses), and found that this also was significantly correlated with PCA index scores (data not shown; $R^2 = 0.1980$; $p = 0.0088$). Thus, the degree to which a rat approached and engaged a food-associated cue during Pavlovian training predicted the extent to which a cocaine cue later evoked drug-seeking behavior in the face of adverse consequences.

Experiment 2 – The role of accumbens dopamine in cocaine cue-evoked reinstatement

Pavlovian training and acquisition of cocaine self administration were very similar to Experiment 1 so the data are not shown.

Cocaine self administration in the face of adverse consequences

As footshock intensity increased all groups in Experiment 2 decreased the number of cocaine infusions they took to a very low level (effect of shock intensity, $F_{(4, 62.25)} = 68.436$, $p < 0.001$) and there were no group differences in the total amount of cocaine infusions taken (no effect group, $F_{(5,41.59)} = 0.459$, $p = 0.804$). The data looked very much as in Experiment 1 and so are not shown. There were also no group differences in the final shock intensity required to reduce responding to criterion (no effect of group, $F_{(5,37)} = 0.180$, $p = 0.969$; Figure 4.6).

Dopamine receptor blockade in the nucleus accumbens core preferentially suppresses cue-evoked drug-seeking in STs

We first compared STs and GTs who received vehicle prior to the reinstatement test and found that STs reinstated to a greater degree than GTs, which replicates the finding from Experiment 1 (group by nosepoke interaction, $F_{(1,24)} = 6.893$, $p = 0.015$; Figure 4.7A).

Flupenthixol suppressed cue-evoked drug seeking relative to vehicle in both STs and GTs (effect of treatment, $F_{(1,25)} = 28.158$, $p < 0.001$; Figure 4.7A), but did so to a significantly greater degree in STs than GTs (group by treatment interaction, $F_{(1,25)} = 6.753$, $p = 0.015$; Figure 4.7A). Furthermore, in STs flupenthixol suppressed active responses to a greater extent than inactive responses, suggesting that the effect was not due to nonspecific motor impairments (treatment by nosepoke interaction for STs, $F_{(1,28)} = 39.686$, $p < 0.001$). Finally, we found that in STs, but not GTs, flupenthixol significantly reduced the number of active responses made during the 20-sec periods when the cocaine CS was present, relative to vehicle (STs, $t_{(14)} = 2.166$, $p = 0.024$;, GTs, ($t_{(11)} = 1.322$, $p = 0.106$; Figure 4.7B). We should note, however, that GTs made very few responses during the CS period even after vehicle administration, so there is likely a floor effect on their suppression.

Amphetamine in the accumbens core preferentially enhances cue-evoked drug-seeking in STs

Amphetamine increased total active responses relative to vehicle levels in both STs and GTs (effect of treatment, $F_{(1,24)} = 15.134$, $p = 0.001$; Figure 4.7C), and there was a trend towards preferential enhancement of active responding in STs, relative to GTs (trend for group by treatment interaction, $F_{(1,24)} = 4.083$, $p = 0.055$), though this effect did not reach statistical significance. Importantly, we analyzed the effect of amphetamine on active versus inactive responding, within each group. Amphetamine preferentially enhanced responding at the active nose poke in STs (treatment by nosepoke interaction for STs, $F_{(1,26)} = 10.512$, $p = 0.003$), but not GTs (no treatment by nosepoke interaction for GTs, $F_{(1,22)} = 4.165$, $p = 0.053$), suggesting that the increased reinstatement effect in STs was not due to a nonspecific enhancement of behavior. We separately analyzed the effects of amphetamine on CS active responses. Relative to vehicle,

amphetamine caused an increase in the number of CS active responses (effect of treatment, $F_{(1,24)} = 10.397$, $p < 0.004$; Figure 4.7D), but this potentiation effect was greater in STs (group by treatment interaction, $F_{(1,24)} = 5.028$, $p = 0.034$).

Histological verification of cannulae placements

Figure 4.8 illustrates the location of the microinjection tips within the nucleus accumbens core for rats used in Experiment 2.

Discussion

As recently summarized by Milton and Everitt (2010), drug-associated stimuli can promote relapse via three related but dissociable mechanisms: by motivating approach behavior, acting as conditioned reinforcers, or instigating conditioned motivational states that spur seeking. Most preclinical models of relapse utilize procedures that only directly assess the conditioned reinforcing properties of drug cues (de Wit and Stewart, 1981; Shaham et al., 2003). However, in addicts, Pavlovian drug cues often produce a conditioned motivational state that *instigates* and/or *invigorates* drug seeking actions, rather than reinforcing actions already taken. Using a novel reinstatement procedure, we report that a cocaine cue, presented independent of any action, instigated drug-seeking behavior behavior in rats, despite adverse consequences. This suggests that, as in addicts, cocaine cues can generate a conditioned motivational state of "craving" in rodents. To our knowledge, this represents the first clear demonstration of this phenomenon in rats (see below for further discussion). Importantly, we found large individual differences in the ability of the cocaine cue to evoke drug-seeking behavior. Cue-induced cocaine-seeking behavior was the greatest in rats that were also prone to attribute incentive salience to a food cue. Furthermore, we found that dopamine signaling within the nucleus accumbens core was necessary for cue-evoked cocaine seeking, and potentiation of dopamine signaling within the

core was sufficient to enhance cue-evoked cocaine-seeking. This suggests that the ability of a drug cue to arouse a conditioned motivational state of "craving" is strongly modulated by dopamine neurotransmission in the accumbens core. Our findings are consistent with a recent report showing that a Pavlovian cocaine cue can also invigorate ongoing self-administration behavior (LeBlanc et al., 2012). These results have a number of implications for thinking about the role of drug cues in relapse, as well as the contribution of dopamine to cue-elicited reward seeking.

Assessing the validity of preclinical relapse models

Traditional reinstatement studies clearly illustrate that drug cues can acquire powerful conditioned reinforcing properties, but there are limitations to their interpretability as a model of relapse. First, these studies do not present drug cues in the way that addicts typically encounter them. That is, in addicts cues usually provoke (motivate) actions to procure drugs, rather than reinforcing actions already taken. Few preclinical studies have addressed this relapse mechanism, however, and with mixed results. For example, following extinction of self-administration behavior drug cues presented noncontingently (i.e., independent of any action) are not very effective in producing reinstatement (de Wit and Stewart, 1981; Grimm et al., 2000; Kruzich et al., 2001) or produce only very low levels of responding (Deroche-Gamonet et al., 2002), which is why in most reinstatement studies drug-seeking actions are reinforced by presentation of the cue. Second, most reinstatement studies incorporate extinction training to reduce behavior to low levels before a test session. Human addicts rarely undergo extinction training, however, and typically forgo drug use due to rising negative costs (i.e., poor health, financial limitations, incarceration; Epstein and Preston, 2003). Additionally, extinction training, compared to abstinence alone, induces brain plasticity mechanisms, particularly within the

nucleus accumbens, that act to inhibit future drug seeking and may obscure drug-induced changes (Knackstedt et al., 2010). Furthermore, compared to abstinence, after extinction training, different neural structures become necessary for reinstatement (Fuchs et al., 2006). Thus, procedures utilizing extinction may inaccurately assess the ability of cues to produce reinstatement, and/or the brain systems that underlie reinstatement. In a recent study, Cooper et al. (2007) utilized an escalating negative consequences procedure, rather than extinction, similar to our study, but in their reinstatement test both response contingent and noncontingent cues were present. Thus, conditioned reinforcement and conditioned motivational mechanisms of drug-seeking were confounded.

With the current study, we avoided these issues, in order to isolate the ability of a drug cue to instigate drug-seeking by generating a conditioned motivational state. Not only was the cue sufficient to instigate responding, it did so even though rats were required to experience footshock in order to make a drug-seeking response. Importantly, however, we found considerable variation in the intensity of cue-evoked cocaine-seeking. This may be one reason it has proven difficult to promote robust drug-seeking behavior unless the cue was able to serve as a conditioned reinforcer. It also suggests that this procedure may be especially useful for examining individual differences in susceptibility to relapse. We found that the cocaine cue instigated much more robust cocaine-seeking behavior in rats prone to attribute incentive salience to a food cue (STs), than in those less prone to attribute incentive salience to a food cue (GTs). This is the first evidence that STs and GTs differ in the ability of a discrete reward cue to instigate conditioned motivation, extending our previous studies, which showed that discrete cocaine cues instigate greater approach behavior in STs, and that a discrete cocaine cue serves as a more robust conditioned reinforcer in STs (Saunders and Robinson, 2010; Yager and

Robinson, 2012). Additionally these results are consistent with another recent study, where we demonstrated that a cocaine "priming" injection reinstates cocaine seeking behavior to a greater degree in STs. The paradigm used in the current study is functionally equivalent to a traditional PIT procedure, in that a drug-associated CS, when presented independent of behavior, invigorated instrumental responding. Interestingly, our results fit together with a recent report by Barker et al. (2012), demonstrating that for rats that showed the greatest PIT in response to a food-associated cue an alcohol-associated cue produced greater reinstatement behavior.

The role of dopamine in cue-evoked drug seeking

Dopamine signaling has been implicated in underlying the incentive motivational properties of Pavlovian reward cues, in particular in transforming motivationally "cold" CSs into attractive and invigorating incentive stimuli (Berridge, 2007; Berridge, 2012; Robinson and Berridge, 1993). For example, dopamine-specific lesions or receptor blockade, both systemically and within the nucleus accumbens core, attenuate sign-tracking conditioned approach CRs, and conditioned reinforcement for food-associated stimuli (Cardinal et al., 2002b; Di Ciano et al., 2001; Flagel et al., 2011b; Hall et al., 2001; Saunders and Robinson, 2012; Taylor and Robbins, 1986). Potentiation of dopamine release, conversely, increases sign-tracking approach CRs behavior (Hitchcott et al., 1997; Holden and Peoples, 2010; Phillips et al., 2003a; but see Simon et al., 2009), and also increases the conditioned reinforcing effects of discrete food and drug-associated cues (Collins et al., 2012; Hill, 1970; Kelley and Delfs, 1991; Robbins, 1975, 1976; Taylor and Robbins, 1984).

Additionally, dopamine signaling is necessary for Pavlovian food cues to produce conditioned motivation, as measured with PIT procedures. For example, blockade of dopamine receptors, either systemically, or within the nucleus accumbens, attenuates PIT (Dickinson et al.,

2000; Lex and Hauber, 2008; Ostlund and Maidment, 2012; Wassum et al., 2011), while potentiation of dopamine transmission, via drug-induced sensitization or treatment with dopamine-enhancing drugs, increases PIT effects (Saddoris et al., 2011; Wyvell and Berridge, 2000, 2001). As recently put by LeBlanc et al. (2012), "It will be of interest to see whether dopamine plays a similar role in cocaine-motivated PIT…". Our results suggest that it does. Dopamine signaling within the accumbens core appears to be both necessary for cocaine-cue PIT, and sufficient on its own to enhance cocaine-cue PIT. Interestingly, amphetamine selectively amplified the number of cocaine-seeking responses made by STs, but not GTs, specifically during the brief cue presentations. Thus, manipulation of dopamine signaling within the accumbens core specifically enhanced the motivational value of the cocaine cue, and only in some rats, and did not simply alter the probability of making nose poke responses.

It is important to note that in addition to potentiating release and preventing reuptake of dopamine, amphetamine also has similar effects on other monoamines, such as serotonin and norepinephrine (Moore, 1978; Seiden et al., 1993). Thus, signaling of other neurochemicals may have been partly involved in the increased reinstatement we saw following amphetamine administration.

Pavlovian cues can instigate or invigorate instrumental behavior, as is often measured using PIT procedures, via two mechanisms. First, cues have general enhancement effects that are outcome independent, in that their presence facilitates instrumental responding for any appetitive outcome, even those the cue was never paired with (Balleine, 1994; Dickinson and Dawson, 1987). Second, outcome-specific enhancement can occur, where a cue biases instrumental actions that result in the same outcome with which the cue was previously paired (Colwill and Rescorla, 1988; Kruse et al., 1983). This cue-evoked reinstatement we found here is likely the

result of a general enhancement effect, for at least two reasons. One, no action besides active nose poking was ever associated with different cues or rewards, meaning that action bias was impossible. Two, the general, but not specific enhancing effects of Pavlovian cues on food-seeking behavior is dependent on dopamine signaling (Ostlund and Maidment, 2012) and activity in the nucleus accumbens core (Corbit and Balleine, 2011; Hall et al., 2001).

Clinical relevance

Our results provide preclinical evidence for suspected mechanisms of cue-induced conditioned motivational states, such as implicit "craving", in addicts. Importantly, conditioned motivational states can be elicited by drug-associated stimuli, and spur drug-seeking behavior in addicts, even outside of conscious awareness. For example, addicts will preferentially respond to receive an injection of a sub-conscious threshold dose of cocaine, over an injection of saline, even though they report having had no such preference (Fischman, 1989; Fischman and Foltin, 1992). Furthermore, drug cues can elicit brain activity in the striatum, amygdala, and other regions in cocaine addicts, even when the cues are presented in such a way that they escape conscious detection (Childress et al., 2008). Our results suggest that endogenous dopamine signaling, at least within the nucleus accumbens core, is critical for a drug-associated cue to evoke a conditioned "craving" that is powerful enough to spur drug seeking, even in the face of adverse consequences.

Unconscious "craving" states can, however, rise to the level of conscious awareness, where they can be measured as subjective craving (Fischman, 1989; Fischman and Foltin, 1992; Jaffe et al., 1989). Several human studies have shown that when addicts view drug-associated cues, dopamine surges within the striatum, and the magnitude of release is positively correlated with self-reported subjective craving (Boileau et al., 2007; Volkow et al., 2006; Wong et al., 2006;

Zijlstra et al., 2008). Thus, dopamine may have a role in drug-related conscious motivational states, but it remains unclear under what conditions these states implicitly motivate behavior, versus when addicts become consciously aware of them. We should also point out, however, that human studies indicate that dopamine has other, broader roles in processing drug cues. For example, dopamine signaling regulates the degree to which drug cues bias the attention of addicts (Ersche et al., 2010; Franken et al., 2004; Hitsman et al., 2008; Munafo et al., 2007). Thus, it is possible that an important factor mediating the ability of the cocaine cue to spur reinstatement in our study was the amount of attention the cue captured. Dopamine in the accumbens core may be involved in regulating cue attention and/or perceptual salience, in addition to generating the motivation to make a drug-seeking response. Consistent with this notion is a human study by Franken et al. (2004) who found that administration of haloperidol, a dopamine receptor antagonist, reduced attentional bias to heroin cues among heroin addicts.

In conclusion, we suggest that there are robust individual differences in the ability of drug cues to instigate conditioned motivational states that spur relapse, and that these differences are bidirectionally controlled by dopamine signaling within the nucleus accumbens core. This may be a key mechanism by which cues contribute to the persistent threat of relapse in addicts.

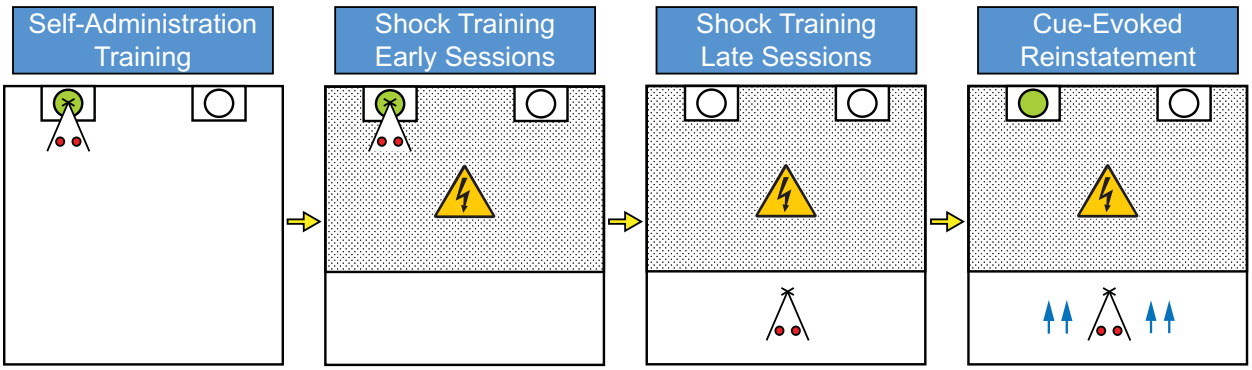


Figure 4.1. Overview of self administration and reinstatement procedures. Rats were first trained to self administer cocaine paired with a discrete visual cue (left panel). Following the acquisition of stable self administration, the front two-thirds of the chamber floor was electrified. Initially, footshock was applied at a low intensity, and rats continued to self administer cocaine (second panel). Footshock intensity was gradually escalated, resulting in a near complete discontinuation of cocaine self administration (third panel). During the reinstatement test, conducted under extinction conditions, the cocaine-associated cue was presented noncontingently throughout the session, which caused rats to cross the electrified floor and reinstate (right panel).

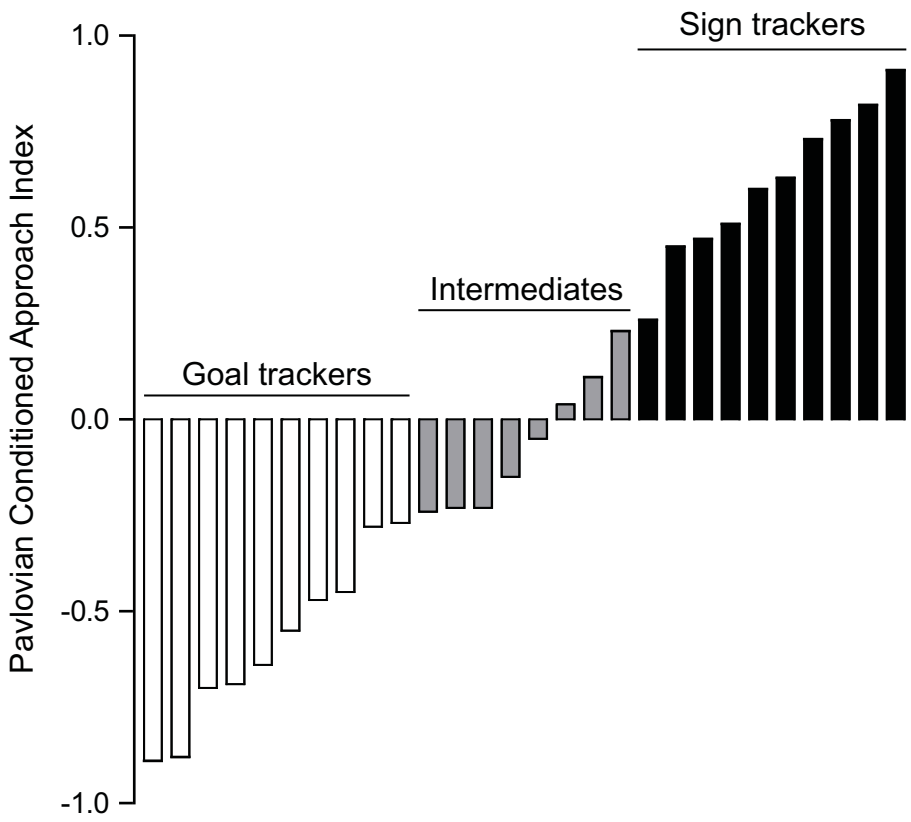
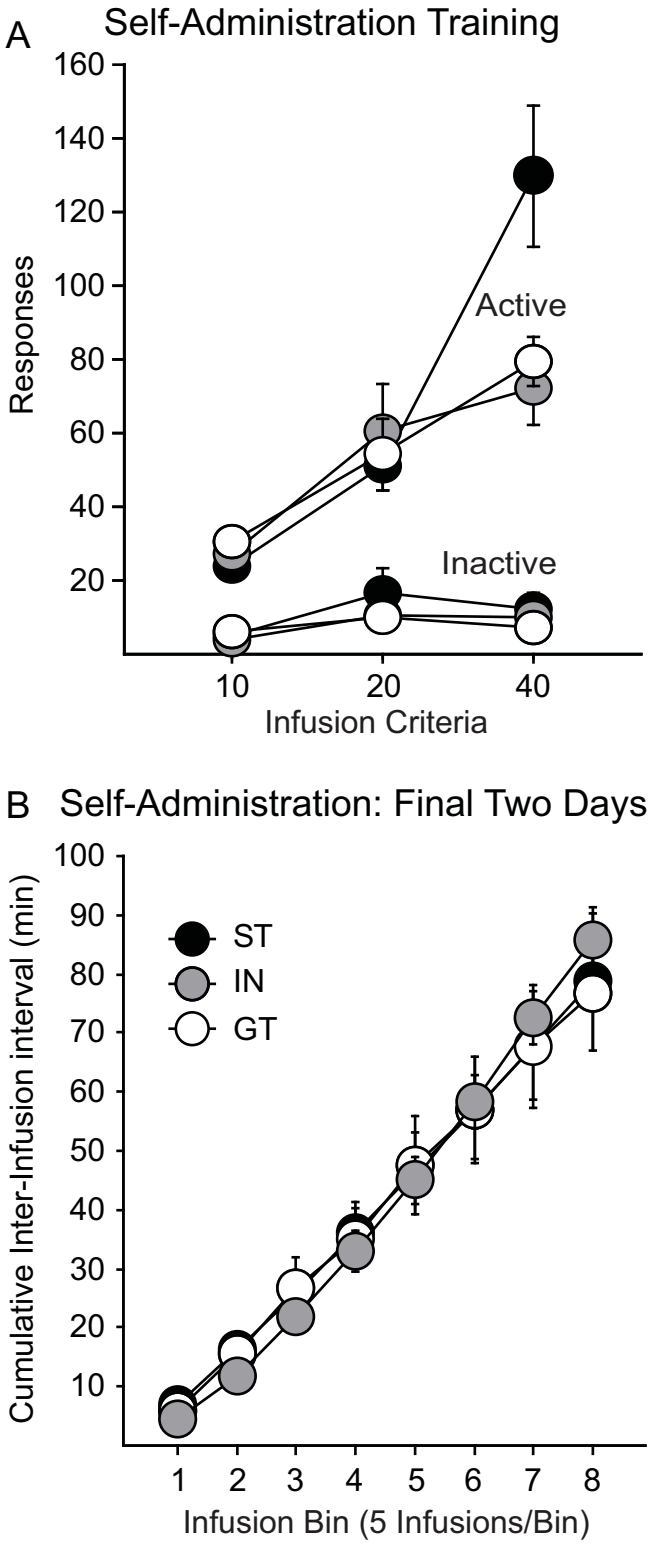


Figure 4.2. Individual variation in PCA behavior. PCA index scores for individual rats used in Experiment 1 are plotted. Rats receiving a score between +1.0 and +0.25 were classed as sign trackers (STs), those with a score between +0.24 and -0.24 were classed as intermediates (INs), and those with a score between -0.25 and -1.0 were classed as goal trackers (GTs).

Figure 4.3. Acquisition of cocaine (0.4 mg/kg) self-administration behavior in STs (n=10), INs (n=8), and GTs (n=10) in Experiment 1. A) The average number of active and inactive nose poke responses made at infusion criteria (IC) 10, 20, and 40. B) The average cumulative interinfusion interval during the last two self administration sessions at IC 40. Symbols represent the mean ± SEM.


A
Self-Administration Training
Responses
160
140
120
100
80
60
40
20
Active
Inactive
10
20
40
Infusion Criteria
B
Self-Administration: Final Two Days
Cumulative Inter-Infusion interval (min)
100
90
80
70
60
50
40
30
20
10
ST
IN
GT
1
2
3
4
5
6
7
8
Infusion Bin (5 Infusions/Bin)

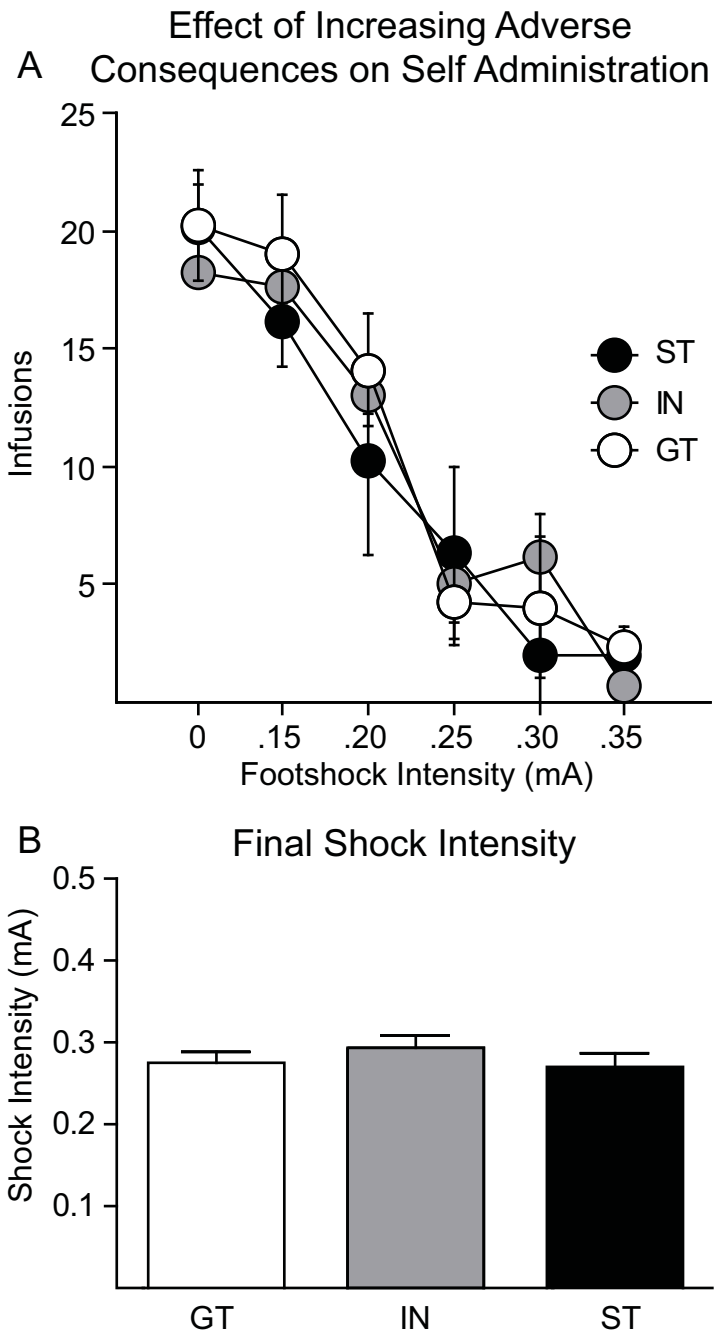


Figure 4.4. Self administration in the face of adverse consequences. Experiment 1. A) Average number of cocaine infusions taken at escalating footshock intensities for STs (n=10), INs (n=8), and GTs (n=10) in Experiment 1. B) Average final footshock intensity reached per group. Symbols represent the mean ± SEM.

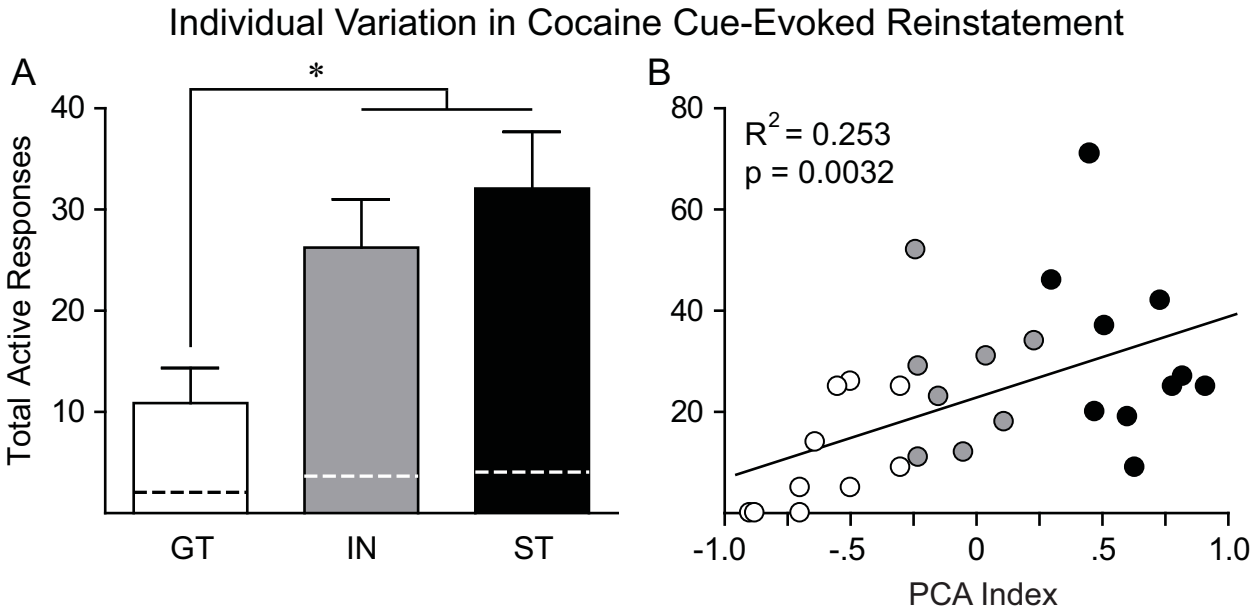


Figure 4.5. Individual variation in cue-evoked reinstatement. A) Average total active nose pokes made during the 30-min reinstatement test for STs (n=10), INs (n=8), and GTs (n=10) in Experiment 1. Dotted lines represent inactive nose pokes. B) Number of active responses made during the reinstatement test for each rat in Experiment 1, as a function of PCA index score. Error bars represent the mean ± SEM. *$P < 0.05$.

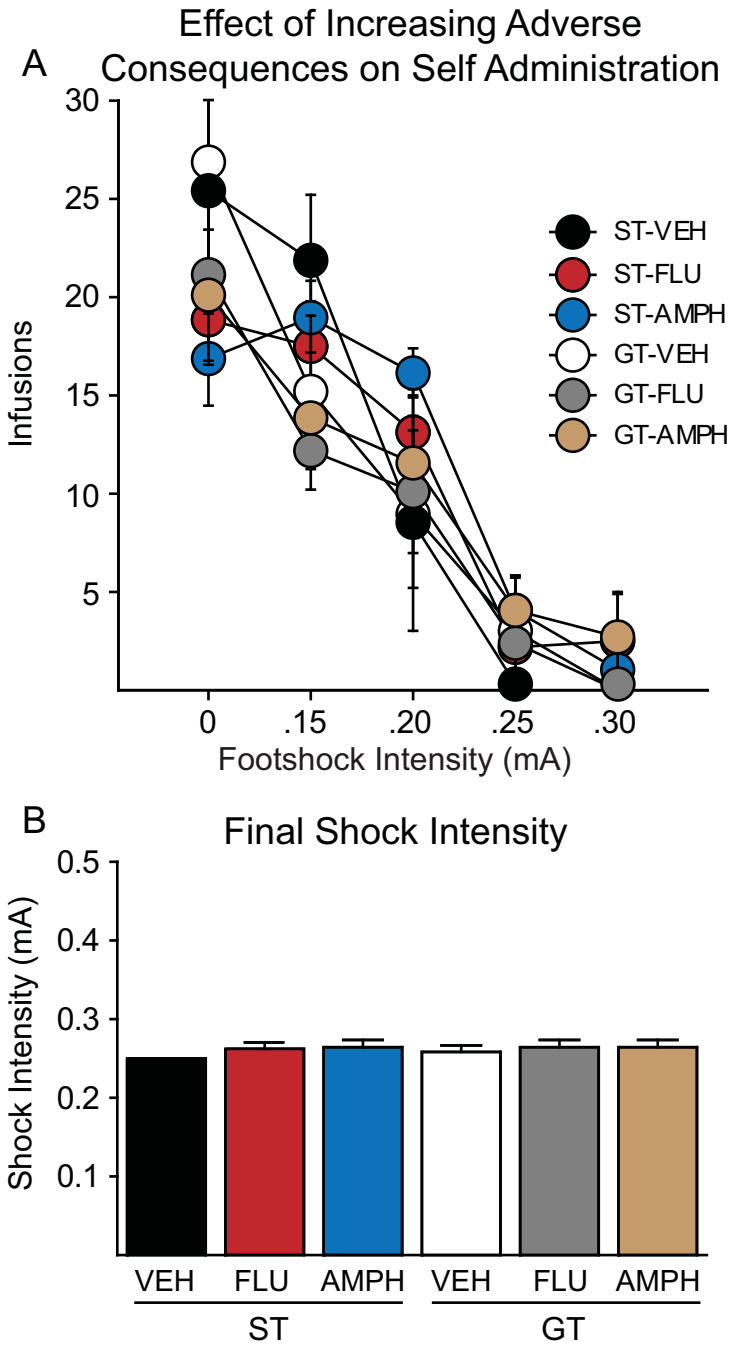


Figure 4.6. Self administration in the face of adverse consequences. Experiment 2. The average final shock intensity reached for rats grouped based on the drug treatment they received before the reinstatement test: ST-VEH (n=8), ST-FLU (n=8), ST-AMPH (n=7), GT-VEH (n=6), GT-FLU (n=7), and GT-AMPH (n=7). Symbols represent the mean ± SEM.

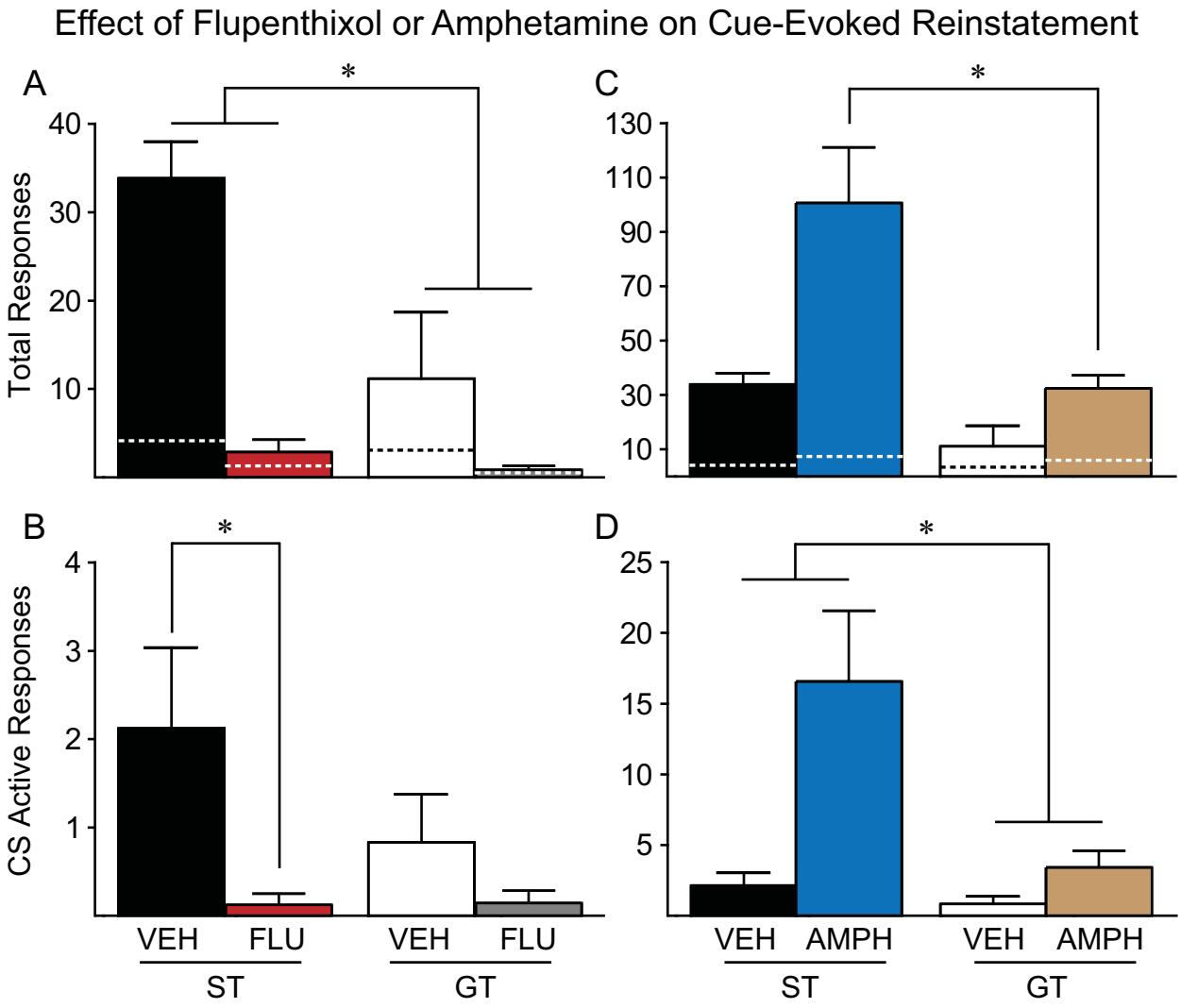


Figure 4.7. Effect of flupenthixol or amphetamine on cue-evoked reinstatement. A) Average total active responses made for rats that received either vehicle or flupenthixol (20 µg) prior to the reinstatement test. B) Average number of active responses made during CS presentations in the reinstatement test for vehicle and flupenthixol treated rats. C) Average total active responses made during the reinstatement test for rats treated with vehicle of amphetamine (10 µg). D) Average CS active responses for vehicle and amphetamine treated rats. Symbols represent the mean ± SEM. *$P < 0.05$.

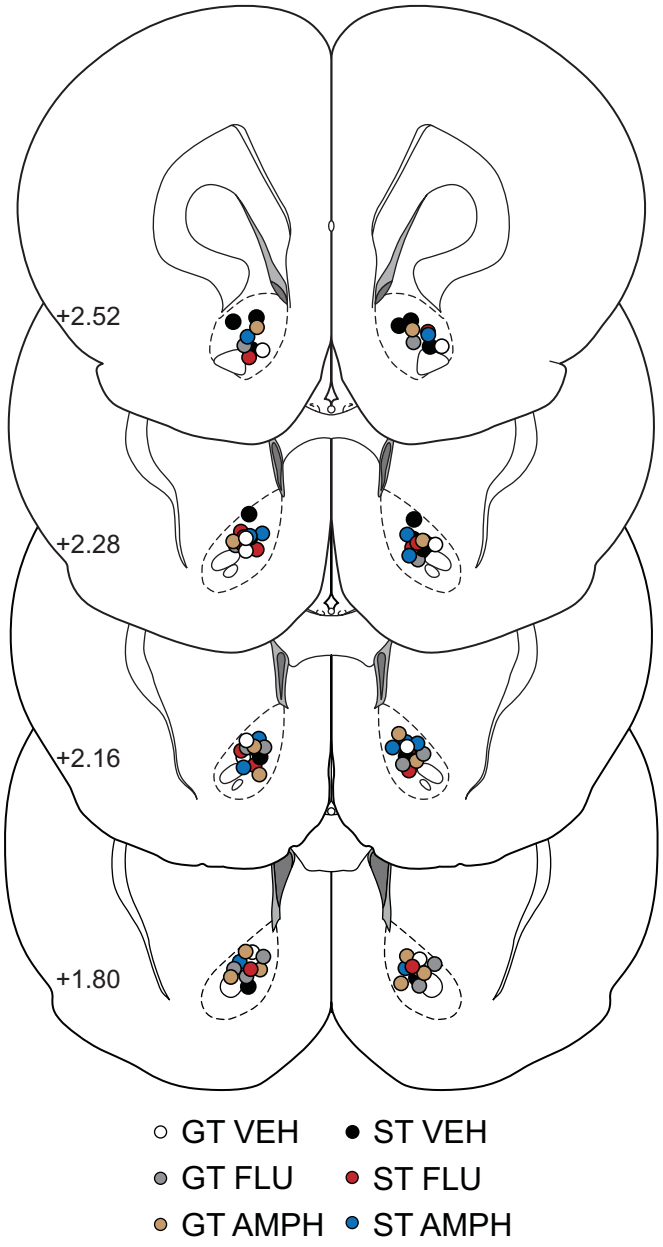


Figure 4.8. Location of microinjection tips within the nucleus accumbens core relative to Bregma for rats used in Experiment 2.

Chapter 5

Individual variation in the motivational properties of cocaine

Introduction

Cues associated with rewards can act as predictive conditional stimuli (CS), but if they are attributed with Pavlovian incentive motivational value ("incentive salience") they can also act as *incentive stimuli,* and thus exert considerable control over motivated behavior (Berridge, 2001; Bindra, 1978; Cardinal et al., 2002a; Flagel et al., 2009; Rescorla, 1988; Stewart et al., 1984). Indeed, incentive stimuli can acquire such a strong hold over behavior that some individuals have difficulty resisting them. For example, drug cues, including the places and paraphernalia associated with drug use, powerfully motivate behavior. In addicts, drug cues engage attention more powerfully than other stimuli (Duka and Townshend, 2004; Field and Cox, 2008; Schoenmakers et al., 2008), can provoke craving and relapse (Ehrman et al., 1992; O'Brien et al., 1992), and even stimulate "approach" behavior (Wiers et al., 2009). In non-human animals, drug cues are attractive and "wanted," facilitating approach and self-administration (Arroyo et al., 1998; Caggiula et al., 2001; Schenk and Partridge, 2001; Uslaner et al., 2006), and serve as powerful instigators of reinstatement/relapse of drug seeking (de Wit and Stewart, 1981; Milton and Everitt, 2010; Shaham et al., 2003). It is thought, therefore, that the high prevalence of continued drug use and relapse in addiction is due in part because addicts are hypersensitive to the incentive motivational properties of drug-associated cues (DeJong, 1994; Milton and Everitt, 2010; Robinson and Berridge, 1993; Stewart et al., 1984).

We have recently found, however, that there is considerable individual variation in the extent to which rats attribute incentive salience to reward cues (Flagel et al., 2007; Robinson and Flagel, 2009; see also Boakes, 1977; Zener, 1937). When a localizable cue is associated with the receipt of food reward, for some rats ("sign trackers", STs) the cue itself becomes attractive, eliciting approach and engagement with it (Hearst and Jenkins, 1974). For these rats the CS also serves as a potent conditioned reinforcer (i.e., STs will work to get it). Thus, in STs the cue comes to act as an incentive stimulus (Flagel et al., 2009; Robinson and Flagel, 2009). For other rats ("goal trackers", GTs; Boakes, 1977), the cue is equally predictive of reward (i.e., it serves as an effective CS), but they instead learn to approach the location of reward delivery, and for these rats the CS is relatively ineffective as a conditioned reinforcer (Robinson and Flagel, 2009; Yager and Robinson, 2010). This variation in the propensity to attribute incentive salience to food cues has considerable relevance to addiction-like behavior. For example, STs more readily acquire cocaine self administration behavior (Beckmann et al., 2011). Additionally, the removal of a discrete cocaine cue attenuates self administration in STs, while having little effect on GTs, and the same discrete cocaine cue robustly reinstates cocaine-seeking behavior in STs but not GTs (Saunders and Robinson, 2010).

Our previous studies focused on spatially discrete drug cues in the environment; however, it is unknown whether individual variation in propensity to attribute incentive salience to a discrete drug cue (Saunders and Robinson, 2010) generalizes to another type of drug cue – the internal interoceptive cues produced by a drug itself. This is an important question given that the interoceptive effects of drugs also powerfully motivate drug-seeking behavior. Human addicts report that drug craving is most intense in the moments just following drug use (Gawin and Kleber, 1986) and even a small "taste" (a prime) of a drug can significantly increase reported

drug craving (Jaffe et al., 1989) and future drug intake (de Wit and Chutuape, 1993). Priming doses of drugs also increase attentional bias to drug cues, suggesting that they generally enhance an individual's motivation for drug use (Duka and Townshend, 2004; Schoenmakers et al., 2008). Additionally, in non-human studies, a drug prime is a potent instigator of drug-seeking behavior (de Wit and Stewart, 1981; Shaham et al., 2003; Stretch et al., 1971). We asked, therefore, whether variation in the propensity to attribute incentive salience to a discrete food cue predicts variation in the motivational properties of cocaine itself, as assessed by performance on a progressive ratio test and cocaine-induced reinstatement.

Methods

Subjects and Housing

A total of 70 male Sprague-Dawley rats (Harlan, IN) weighing 250-300 g upon arrival were housed individually on a 12-hr light/12-hr dark cycle (lights on at 0800) in a climate-controlled colony room. Water and food were available *ad libitum*. After arrival rats were given one week to acclimate to the colony room before testing began. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus

Behavioral testing was conducted in standard (22x18x13 cm) test chambers (Med Associates Inc., St. Albans, VT, USA) located inside sound-attenuating cabinets. A ventilating fan masked background noise. For Pavlovian training each chamber had a food cup located in the center of one wall, 3 cm above a stainless steel grid floor. Head entries into the food cup were recorded by breaks of an infrared photobeam located inside the magazine. A retractable lever illuminated from behind was located 2.5 cm to the left or right of the food cup, approximately 6 cm above the floor. The location of the lever with respect to the food cup was counterbalanced across rats.

On the wall opposite the food cup, a red house light remained illuminated throughout all sessions. For self-administration sessions, the food cup and lever were removed and replaced with two nose-poke ports located 3 cm above the floor on the wall opposite the house light. A nose poke into the active port, detected by an infrared photobeam inside the hole, resulted in an intravenous cocaine infusion, delivered by an external pump through a tube connected to the rat's catheter back port. The infusion tube was suspended into the chamber via a swivel mechanism, allowing the rat free movement. Active and inactive nose-poke ports were counterbalanced to control for side bias. All measures were recorded using Med Associates software.

Pavlovian Conditioned Approach Training

Rats were first trained using a Pavlovian approach ('autoshaping') procedure similar to that described previously (Flagel et al., 2007). For two days prior to the start of training, 10 banana-flavored pellets (45 mg, BioServe, #F0059; Frenchtown, USA) were placed in the home cages to familiarize the rats with this food. The following day, rats were placed in the test chambers, with the lever retracted, and trained to retrieve pellets from the food cup by presenting fifty food pellets on a variable time (VT) 30-s schedule. After 2 days of pretraining, Pavlovian training commenced. Each training trial consisted of insertion (and simultaneous illumination) of the lever (CS) into the chamber for 8 s, after which the lever was retracted and a single food pellet (unconditional stimulus, US) was immediately delivered into the food cup. Each of five daily training sessions consisted of 25 response-independent trials in which CS-US pairing occurred on a variable time (VT) 90-s schedule (the time between CS presentations varied randomly between 30 and 150 s). Lever deflections, food cup entries, latency to lever deflection, and latency to food cup entry during CS presentation were measured.

Following Pavlovian training rats were assigned to one of three groups based on whether they (1) preferentially interacted with the lever-CS ("sign trackers", STs); (2) preferentially interacted with the food cup during lever-CS presentation ("goal trackers", GTs); or (3) had no preference for the lever-CS or food cup ("intermediate group", IG). This was quantified using a composite Pavlovian conditioned approach (PCA) index which was the average of three measures of conditioned approach behavior: (1) the *probability* of contacting either the lever-CS or food cup during the CS period [P(lever) – P(food cup)]; (2) the *response bias* for contacting the lever-CS or the food cup during the CS period [(#lever deflections – #food cup entries) / (#lever deflections + #food cup entries)]; and (3) the mean *latency* to deflect the lever or enter the food cup during the CS period [(food cup entry latency – lever deflection latency) / 8 (i.e., CS duration)]. This produces values on a scale ranging from -1.0 to +1.0. A score of +1.0 indicates a rat that on every trial deflected the lever-CS and never entered the food cup, a score of -1.0 indicates a rat that entered the food cup and never deflected the lever-CS, and a score of 0 indicates a rat that distributed his behavior equally between lever-CS and food cup. The average PCA index score for days 4 and 5 of training was used to classify animals. For the purposes of classification, we operationally defined animals as STs (n=16, 23%) if they obtained a PCA index score of +0.5 or greater (meaning they rapidly approached and vigorously engaged the lever-CS at least twice as much as to the food cup), and as GTs (n=19, 27%) if they obtained a score of -0.5 or less. The remaining animals within the -0.5/+0.5 range, those rats whose preference vacillated between lever-CS and food cup, were labeled as IGs (n=35, 50%). For this study, we were interested in comparing rats that clearly differed in their propensity to attribute incentive salience to reward cues, and therefore, only rats identified as STs or GTs were used in the rest of the study.

Intravenous Catheter Surgery

Next, ST and GT rats were prepared with intravenous catheters as described previously (Crombag et al., 2000) under ketamine hydrochloride (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia. Following surgery, catheters were flushed daily with 0.2 ml sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO) to minimize infection and prevent occlusions. Catheter patency was tested weekly by intravenous injection of 0.2 ml sodium thiopental (20 mg/ml in sterile water, Hospira, IL). Only rats that became ataxic within 5-10 s were considered to have patent catheters and included in the analyses.

Self-Administration: Acquisition

Self-administration sessions began 7 days after surgery in chambers outfitted with two nose ports as described above. A nose poke into the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline [0.2 mg (weight of the salt) per kg per infusion in 50 µl delivered over 2.6 s] on a fixed ratio (FR) 1 schedule (Carroll and Lac, 1997). Coincident with the start of an infusion was an unsignaled 20-s timeout period, during which nose pokes were recorded, but had no consequences. *No discrete cue in the environment (e.g., light or tone) was explicitly paired with drug delivery, to help insure that behavior was largely controlled by the interoceptive effects of the drug itself.* We also wanted all rats to receive exactly the same number of drug injections, and thus an infusion criterion (IC) was imposed on self-administration sessions (i.e., session length was determined by how long it took each rat to reach the IC, not by an explicit time limit; (Saunders and Robinson, 2010). Rats were initially allowed to take 10 infusions per session, and the IC was increased to 20, 40, and then 80 infusions. Rats were trained at IC 10, 20, and 40 for three consecutive sessions and at IC 80 for five consecutive sessions. A total of 27 rats (ST n=12, GT n=15) completed the self-

administration portion of the experiment. Rats were eliminated when they failed to acquire self administration (ST n=1, GT n=1) or lost catheter patency (ST n=3, GT n=4).

Self-Administration: Progressive Ratio Test

Following 5 sessions at IC 80 all rats received two consecutive sessions during which the reinforcement schedule was changed from an FR1 to a progressive ratio (PR) schedule. On the PR schedule the number of active nosepokes required to produce the next cocaine infusion increased after each infusion according to the following exponential progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40 …, derived from the formula $[(5\times e^{0.2n})-5]$, rounded to the nearest integer, where *n* is the position in the sequence of ratios (Roberts and Richardson, 1992). Progressive ratio sessions were terminated only after one hour had lapsed between completed ratios/infusions received. After the two PR sessions, rats were returned to an FR1 reinforcement schedule at IC 80 for three additional sessions to re-stabilize behavior.

Cocaine-Primed Reinstatement Test

After the last self-administration session at IC 80, rats underwent 8 60-min sessions of extinction training. In these sessions an active nosepoke response did not produce a cocaine infusion, though the infusion pump was turned on. The day after the final extinction session, rats were tested for cocaine-induced reinstatement of drug-seeking behavior. On this day, immediately before placement in the testing chamber, each rat received a 15 mg/kg i.p. injection of cocaine. Nosepokes were recorded, but had no consequences. Before testing, rats were habituated to the injection experience by receiving an i.p. injection of saline in their home cages.

Statistical Analysis

Linear mixed-models (LMM) analysis was used for all repeated measures data. The best-fitting model of repeated measures covariance was determined by the lowest Akaike information

criterion score (Verbeke, 2009). Depending on the model selected, the degrees of freedom may have been adjusted to a non-integer value. Analysis of variance was used to compare PR and reinstatement responding. Statistical significance was set at $p < .05$.

Results

Individual variation in Pavlovian conditioned approach behavior

Figure 5.1 shows the distribution of PCA index scores for all rats. As discussed above, GTs were operationally defined as rats with scores ranging between -1.0 and -0.5, STs between +0.5 and +1.0, and IGs between -0.5 and +0.5. There was some variation in index scores within the ST and GT groups, but most rats clustered near the extreme ends of the potential score range (i.e., the rats tested on self administration showed a strong preference for either the lever-CS or the food magazine during PCA training). Figure 5.2 shows the change in approach behavior as a function of training session in STs and GTs. With experience, STs developed a high probability of rapidly approaching and vigorously engaging the lever-CS (Figure 5.2 a-c). In contrast, GTs rarely engaged the lever-CS and instead came to rapidly approach the food magazine during lever-CS presentation (Figure 5.2 d-f). Thus, as in our previous studies (Flagel et al., 2007; Saunders and Robinson, 2010), the lever-CS developed the ability to evoke a conditioned response (CR) in both STs and GTs – it served as a predictive CS in both – but it was an attractive incentive stimulus only for STs.

Acquisition of cocaine self administration in STs and GTs

Rats were next trained to nose poke for an IV cocaine infusion under conditions in which no discrete cue (CS) was paired with drug delivery. Given that training sessions were limited to a fixed infusion criterion, group differences in acquisition would be evident in the number of cocaine infusions taken per minute (rate). There were no group differences in rate at any infusion

criterion [effect of group, $F_{(1,38.05)} = 0.022$, $p = 0.884$; Figure 5.3a]. Both groups made the same number of active nose pokes [$F_{(1,22.55)} = 1.80$, $p = 0.19$], which increased across training [effect of session, $F_{(13,19.86)} = 18.7$, $p < 0.0001$], and inactive nose pokes [$F_{(1,27.31)} = 3.274$, $p = 0.081$], which did not increase [no effect of session, $F_{(13,19.26)} = 1.297$, $p = 0.294$; Figure 5.3b]. To further examine baseline self-administration behavior, within-session responding was analyzed during the final two sessions of training at IC 80. The results of this analysis are shown in Figure 5.3c. The pattern of cocaine intake was the same in STs and GTs. By the end of training, both groups took infusions at a consistent, uniform rate [no group x infusion bin interaction, $F_{(1,44.14)} = 0.626$, $p = 0.838$]. Thus, using this procedure, there were no group differences in the acquisition self-administration behavior, as we have reported previously (Saunders and Robinson, 2010). However, using other procedures and doses STs have been reported to acquire cocaine self-administration more readily than GTs (Beckmann et al., 2011).

STs are more motivated to obtain cocaine than GTs

By the end of self-administration training on a FR 1 schedule STs and GTs showed identical, stable behavior (Figure 5.3c). All rats were then transferred to a progressive ratio (PR) schedule for two consecutive sessions. We found no effect of PR session in either group, so the data from the two PR sessions were averaged together. STs made more active nose responses and attained higher final ratios ("breakpoints") than GTs [effect of group, $F_{(1,50)} = 9.632$, $p = 0.003$], reaching a final ratio nearly twice that of GTs (Figure 5.4a). Accordingly, STs received more cocaine injections [$F_{(1,50)} = 11.531$, $p = 0.001$], emitted more responses [$F_{(1,50)} = 8.534$, $p = 0.005$; Figure 5.4b], and took longer to reach breakpoint [$F_{(1,50)} = 6.916$, $p = 0.015$] than GTs. It should be emphasized that although STs worked nearly twice as much as GTs during PR testing, this amounted to, on average, only 3 more cocaine infusions relative to GTs. Given the large number

of infusions all rats received across training, this is a negligible difference in drug exposure that unlikely had any carryover effects on subsequent self-administration behavior (see below). Following PR test sessions, rats were returned to an FR1 schedule at IC 80 to re-stabilize behavior. During these sessions there were no group differences in the rate of self administration [$F_{(1,25.02)} = 0.001$, $p = 0.971$] or number of responses [$F_{(1,25.03)} = 0.209$, $p = 0.652$] (data not shown). After the resumption of baseline levels of self-administration, rats underwent 8 sessions of extinction training, during which nose pokes no longer produced cocaine. During this period STs and GTs decreased responding [effect of session, $F_{(7,43.65)} = 9.352$, $p < 0.0001$], and they did so at a similar rate [no group X session interaction, $F_{(1,43.65)} = 1.612$, $p = 0.157$; Figure 5.5].

Cocaine-induced reinstatement in STs and GTs

Following extinction training all rats were tested for cocaine-induced reinstatement of drug-seeking behavior. Rats were given a priming injection of cocaine (15 mg/kg, i.p.) immediately before being placed into the test chambers for a 60-min session under extinction conditions. STs showed more robust reinstatement of cocaine-seeking than GTs [$F_{(1,50)} = 4.657$, $p = 0.036$; Figure 5.6]. Although both groups discriminated between active and inactive nose pokes [effect of nose poke port, $F_{(1,50)} = 19.856$, $p < 0.0001$], STs reinstated to a greater degree than GTs [significant group X nose poke interaction, $F_{(1,50)} = 4.526$, $p = 0.038$; Figure 5.6]. We next assessed the relation between performance during PR testing and the degree of reinstatement following cocaine priming. For STs, performance during PR sessions was significantly correlated with performance during reinstatement (Spearman's $r = 0.504$, $p = 0.048$), but for GTs the correlation was not significant (Spearman's $r = 0.007$, $p = 0.490$). Thus, ST rats that made the most responses under a PR schedule tended to reinstate the most following a cocaine prime.

Discussion

We asked whether variation in propensity to attribute incentive salience to a localizable food cue predicts variation in the ability of cocaine to spur cocaine-seeking behavior. We found that it does. At the doses tested, STs worked harder to receive cocaine injections (i.e., had higher "breakpoints" on a progressive ratio schedule) and showed more robust cocaine-induced reinstatement, than GTs. These findings, together with our previous report (Saunders and Robinson, 2010), suggest that both interoceptive and exteroceptive drug cues acquire more potent incentive motivational properties, and thus the ability to spur drug-seeking behavior, in STs than GTs.

The ability of different types of drug cues to motivate behavior is especially important in the context of addiction, as drug-associated stimuli of many kinds can initiate a cascade of behaviors leading to relapse (de Wit, 1996; Milton and Everitt, 2010; Shaham et al., 2003). Cues gain this control over behavior if they come to act as *incentive stimuli*, which have three fundamental properties: 1) they attract and elicit approach; 2) they acquire conditioned reinforcing properties and become "wanted," in the sense that animals will work to obtain them; and 3) they energize ongoing instrumental actions (Berridge, 2001; Cardinal et al., 2002a; Milton and Everitt, 2010). There is, however, considerable individual variation in the propensity to attribute incentive salience to reward cues. A food cue acquires the properties of an incentive stimulus: it attracts (Flagel et al., 2007), it serves as an effective conditioned reinforcer (Robinson and Flagel, 2009), and it spurs food-seeking behavior (Yager and Robinson, 2010), to a greater extent in STs than GTs. This variation in incentive salience attribution extends to drug cues as well, as a discrete cocaine-associated cue motivates self administration and instigates more robust reinstatement in STs than GTs (Saunders and Robinson, 2010). Given the established differences between ST and GT rats, our current results suggest that cocaine itself also motivates drug-seeking behavior to a

greater degree in rats with a propensity to attribute incentive salience to reward-associated cues (i.e., STs).

There is more than one potential psychological process that can account for the variation in drug-seeking behavior exhibited by ST and GT rats in the current experiment. First, the subjective experience associated with cocaine, the constellation of *interoceptive cues* produced by cocaine, may be more pleasurable and/or attributed with greater incentive salience in STs than GTs. Thus, STs may come to "want" the experience of cocaine more than GTs (Robinson and Berridge, 2000). This interpretation is consistent with a recent finding that under some conditions STs acquire cocaine self-administration behavior more readily than GTs (Beckmann et al., 2011). With the procedure used here there was no difference between STs and GTs in the acquisition of cocaine self-administration (see also Saunders and Robinson, 2010), but our procedure was explicitly designed to limit any potential group difference in acquisition by limiting self-administration sessions by the number of cocaine infusions, not time. Indeed, when allowed to self administer cocaine unrestrained during PR sessions STs sought out more drug than GTs. Thus, the interoceptive cues produced by cocaine appear to motivate greater drug-seeking behavior in STs than GTs.

One limitation of the present study is that only one dose of cocaine was used. Extensive dose-effect studies would be required to determine if cocaine is more potent in STs (i.e., their dose-response function is shifted to the left) compared to GTs, or if cocaine has a larger maximum effect in STs (i.e., their dose-response function is shifted up). However, the opposite – that cocaine is more potent and/or efficacious in *GTs* – is very unlikely. In the context of our results, if this were the case, GTs would have to show more robust reinstatement compared to STs to a cocaine prime dose below 15 mg/kg, and then the opposite effect we observed at 15

mg/kg. There is no precedent in the reinstatement literature for this type of dose-effect relationship and, in general, cocaine-primed reinstatement is weak at doses below 15 mg/kg and maximal at doses of 15-20 mg/kg. Therefore, we think the data are consistent with the conclusion that cocaine is more potent and/or has greater maximum effect, in STs relative to GTs.

Variation in the motivational effects of cocaine described here in rats has parallels in humans. The ability of a drug prime to instigate relapse is well established, but there are also marked individual differences in both the subjective effects of drugs (de Wit et al., 1986; de Wit et al., 1987) and the drug priming effect (de Wit et al., 1987; Kirk and de Wit, 2000). Specifically, individuals reporting the greatest subjective drug effects have the highest motivation to obtain drug. This is supported by clinical reports indicating that not all drug users relapse after drug prime exposure (Lloyd and Salzberg, 1975). Our results suggest, therefore, that some of the variation in drug-induced relapse potential may be due to differences in the tendency of an individual to attribute incentive value to interoceptive drug cues.

A second, though not mutually exclusive interpretation of our results, is that cocaine enhances the motivational properties of *external cues* present during drug exposure to a greater degree in STs than GTs. Drugs can influence responding due to their direct subjective effects, but also by amplifying the motivational value of *other* stimuli. These effects are behaviorally dissociable but generally act in a synergistic manner (Caggiula et al., 2009; Palmatier et al., 2006). Stein (1964) and Hill (1970) originally conceptualized this enhancement effect that was later confirmed in experiments by Robbins (1975, 1976; 1984) and others (Beninger et al., 1981; Caggiula et al., 2009; Chaudhri et al., 2006; Palmatier et al., 2006; Phillips and Fibiger, 1990), who demonstrated the ability of drugs to enhance the conditioned reinforcing properties of

environmental stimuli. It is notable that there is considerable individual variation in the strength of this effect (Hill, 1970; Phillips and Fibiger, 1990; Robbins, 1975). Thus, the differences between STs and GTs in progressive ratio performance and cocaine-primed reinstatement may be due to differences in cocaine's ability to enhance the motivational value of noncontingent external cues independent of, or in addition to, cocaine's interoceptive effects. Perhaps STs responded more than GTs during PR testing and following cocaine priming in part because contextual stimuli continuously present during experience with cocaine acquired enhanced incentive salience – they became more motivating – and thus spurred greater drug-seeking in STs than GTs (Berridge, 2001; Robinson and Berridge, 1993). Although we took care to eliminate discrete drug-paired cues (CSs), there were stimuli – the context of the experimental chamber, the nose poke port – that remained present while the rats experienced cocaine. It is possible that the action of approaching and emitting an active nose poke became more likely in STs because cocaine potentiated the incentive value of these stimuli.

It is not known what neurobiological differences between STs and GTs account for their varying propensity to attribute incentive salience to food and drug cues. Of course, there is a wealth of evidence implicating ascending mesotelencephalic dopamine (DA) systems in the assignment of incentive motivational properties to rewards and associated stimuli (Berridge, 2007; Berridge and Robinson, 1998; Cardinal et al., 2002a; Di Chiara, 1998; Robbins et al., 1989). For example, blockade of DA receptors attenuates cocaine self-administration and reinstatement behavior (Pilla et al., 1999; Woolverton and Virus, 1989). Additionally, potentiation of DA activity in the nucleus accumbens (NAc) via local amphetamine injection increases the conditioned reinforcing properties of drug cues, whereas DA blockade in NAc attenuates it (Taylor and Robbins, 1984, 1986). Unfortunately, there has been little research on

potential neurobiological differences in STs and GTs, but recent evidence suggests that differences in DA systems do exist. For example, the acquisition of a sign-tracking CR is DA-dependent, but learning a goal-tracking CR is not (Flagel et al. 2011b). Furthermore, during Pavlovian training there is a transition of the phasic DA signal from a food US to a lever-CS in STs, but this transfer does not occur in GTs (Flagel et al. 2011b). These studies implicate differences in DA signaling in the behavioral differences seen with food-associated cues, but further research is needed to determine if DA system differences account for variation in the motivational properties of cocaine we report here.

In conclusion, we report that it is possible to predict, prior to any drug experience, which rats later will be more motivated to work for cocaine and to seek cocaine following a priming injection. Interestingly, Mahler and de Wit (2010) recently reported what may be a related phenomenon - smokers who show high craving to food cues when food deprived also show the highest craving to smoking cues. The results reported here extend our previous studies with STs and GTs and suggest that for some individuals not only do exteroceptive drug-associated cues acquire greater motivational control over behavior, but interoceptive drug cues do as well. Individuals for whom drug cues act as potent incentive stimuli will have more difficulty resisting them than individuals for whom cues merely act as predictors of reward. Thus, a complex set of external and internal cues may continually goad some individuals to action (Robinson and Berridge, 1993; Stewart et al., 1984), and therefore these individuals may be most susceptible to addiction. It will be important to further understand the psychological and neurobiological mechanisms that underlie variation in propensity to attribute incentive salience to reward cues because this might not only be relevant to the propensity for addiction, but for other impulse control disorders as well.

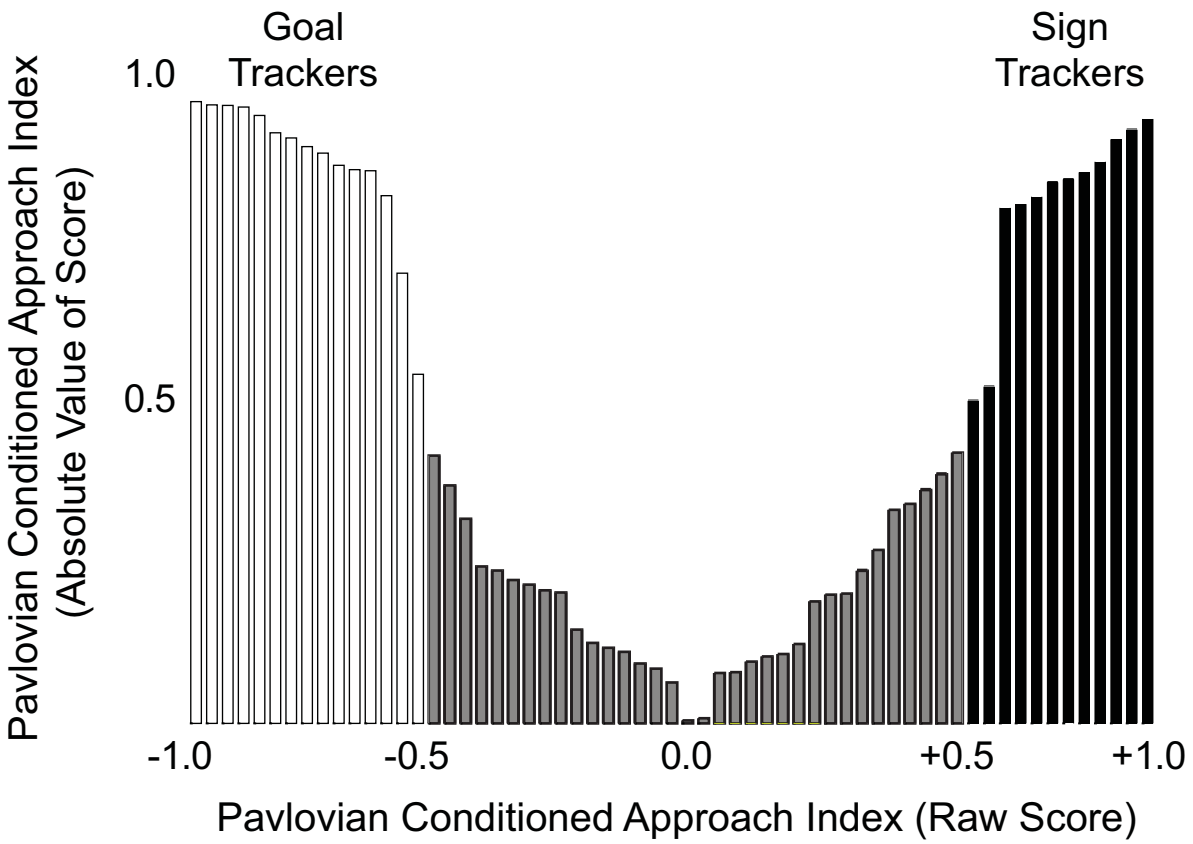


Figure 5.1. Distribution of Pavlovian conditioned approach (PCA) composite index scores based on behavior during sessions 4 and 5 of PCA training. Rats receiving a raw score of +0.5 to +1.0 were designated sign trackers (STs) and rats receiving a raw score of -0.5 to -1.0 were designated goal trackers (GTs). The remaining rats, with scores between -0.5 and +0.5 were designated intermediates (IGs). Raw scores are presented along the x-axis and corresponding absolute values along the y-axis. Index scores for IG rats are included to illustrate the bimodality of the index distribution, but IG rats were excluded from all further testing.

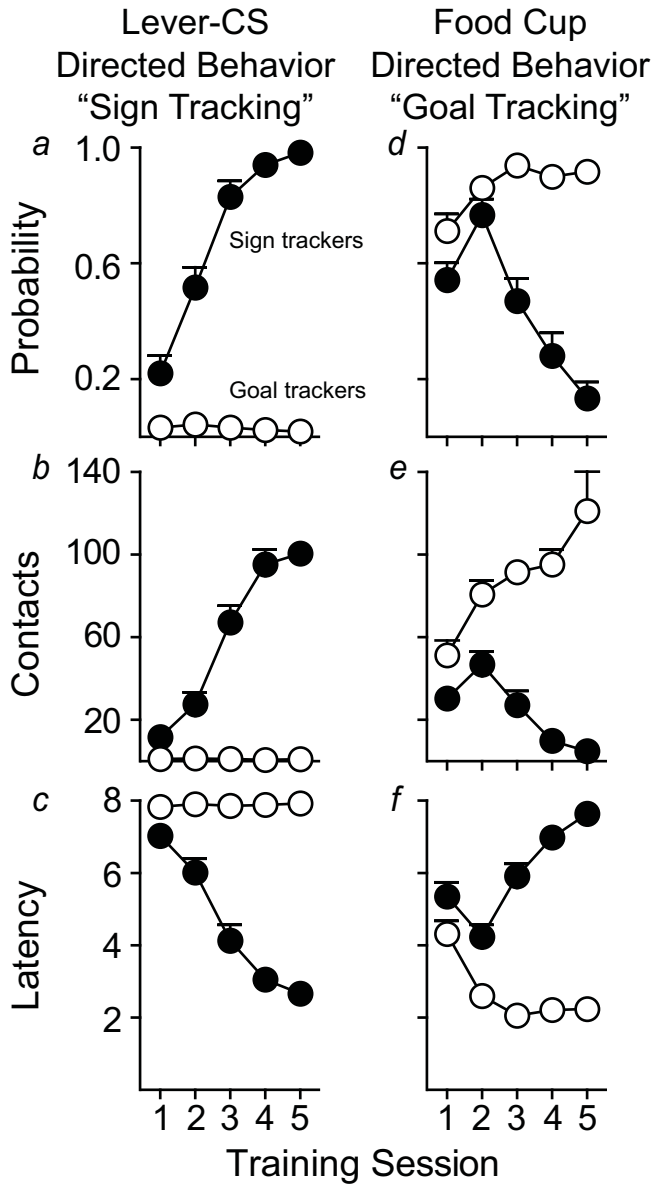


Figure 5.2. Behavior directed towards the lever-CS (sign tracking) is shown in panels a-c and that directed towards the food cup during CS presentation (goal tracking) is shown in panels d-f. Mean + SEM (a) probability of contacting the lever-CS [# trials with a lever-CS contact/#trials per session] during the 8-s CS period (b) number of lever-CS contacts made during the 8-s CS period, (c) latency to the first lever-CS contact, (d) probability of food cup entry [# trials with a food cup entry/#trials per session] during the 8-s CS period (e) number of food cup entries made during the 8-s CS period, (f) latency to the first food cup entry during the CS period. For all of these measures there was a significant effect of group (ST or GT), session, and a group x session interaction ($p<0.001$).

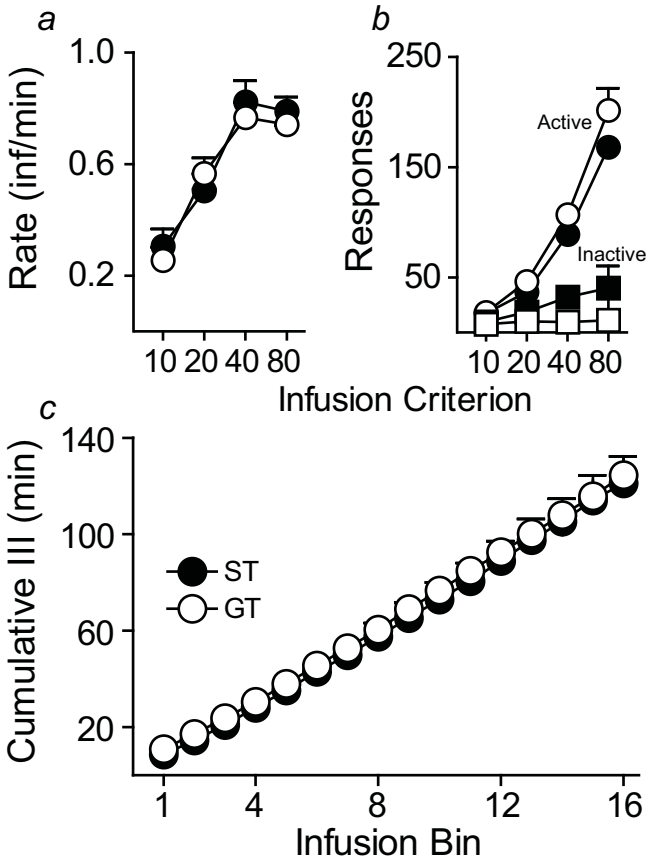


Figure 5.3. Acquisition of self-administration behavior in sign trackers (n=12) and goal trackers (n=15). (a) The mean ± SEM number of cocaine infusions per minute for infusion criteria 10, 20, 40, and 80 (0.2 mg/kg/inf). (b) The mean + SEM number of active (circles) and inactive (squares) nosepoke responses at each infusion criterion. (c) The mean + SEM cumulative interinfusion interval (III) during the final two self administration sessions at IC 80.

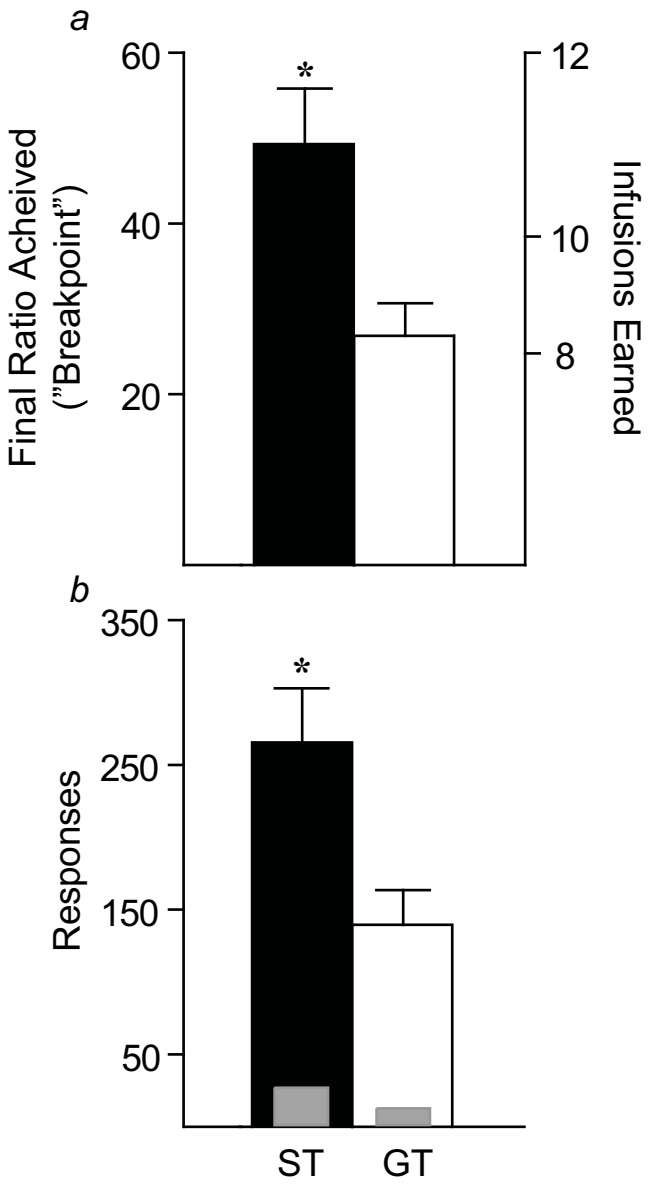


Figure 5.4. Self-administration behavior during progressive ratio (PR) testing for sign trackers and goal trackers. (a) The mean + SEM final PR ratio achieved ("breakpoint") and total infusions earned. (b) The mean + SEM number of active (thick bars) nosepoke responses made during PR testing. The inset gray bars represent the number of inactive nosepoke responses. *, indicates a significant group difference, $p < 0.05$.

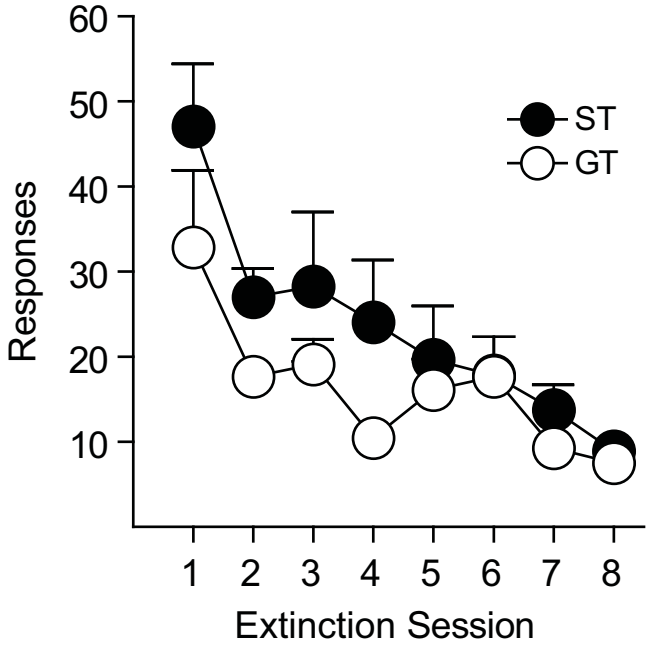


Figure 5.5. Extinction of responding for cocaine in sign trackers (n=12) and goal trackers (n=15). The mean + SEM number of active nosepoke responses is shown.

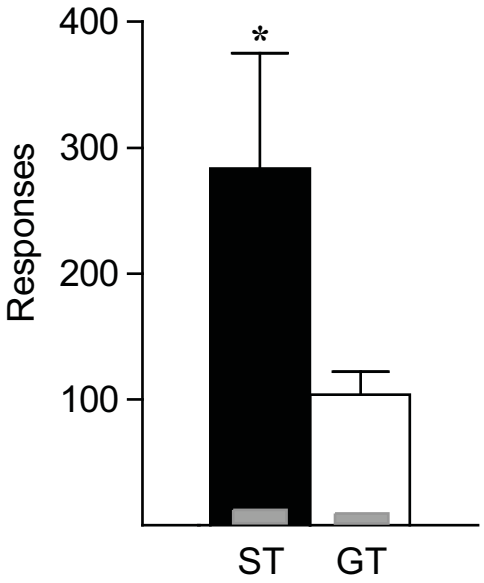


Figure 5.6. Cocaine-primed (15 mg/kg i.p. injection) reinstatement of drug-seeking behavior for sign trackers (n=12) and goal trackers (n=15) during a 60-min test session in which an active response had no consequences. The mean + SEM number of active nosepoke responses (thick bars) are shown. The inset gray bars represent the number of inactive nosepoke responses. *, indicates a significant group difference, $p < 0.05$.

Chapter 6

Individual variation in the motivational properties of a cocaine-associated context: role of dopamine in the accumbens core

Introduction

Drug-associated stimuli acquire powerful motivational control over the behavior of addicts, contributing to compulsive drug seeking and high rates of relapse. Such stimuli can be roughly classed into three broad categories: discrete cues that are explicitly paired with drug delivery (e.g., drug paraphernalia), static configurations of contextual cues (e.g., places where drugs are taken), and the interoceptive cues produced by drugs themselves. There is considerable evidence that behavior motivated by these different classes of cues is mediated by multiple psychological processes and by dissociable, although overlapping, neural systems (Cardinal et al., 2002a; Fuchs et al., 2008a; Holland, 1992; Holland and Bouton, 1999; Maren, 2011). Discrete drug cues are thought to usually promote drug seeking by eliciting approach behavior, reinforcing drug-seeking actions, and/or by generating conditioned motivational states that spur on drug-seeking actions (Milton and Everitt, 2010). Contexts and interoceptive stimuli can also directly evoke conditioned motivational states ("wanting"; Berridge, 2007; Crombag et al., 2008; Robinson and Berridge, 1993) that spur seeking behavior, but contexts are also often thought to function as "occasion setters", or discriminative stimuli, interacting with discrete stimuli to signal when drug is or is not available (Bouton, 2002; Bouton and Swartzentruber, 1991; Crombag et al., 2008; Fuchs et al., 2005; Holland, 1992). That is, the distinctions between these different types of cues

can be blurry, as they can simultaneously engage more than one process (for review see Crombag et al., 2008).

In a series of preclinical studies we have found that there is considerable individual variation in the degree to which discrete and interoceptive reward cues, including drug cues, acquire motivational properties. When a localizable Pavlovian conditional stimulus (CS), predicts the delivery of food reward (unconditional stimulus, US), only some rats (sign-trackers, STs; Hearst and Jenkins, 1974) find the cue attractive and desirable, in that they will avidly approach it and will work to obtain it. For others (goal-trackers, GTs; Boakes, 1977), the cue itself is less attractive, and it is less desired, but it nevertheless elicits conditioned approach directed at the location of food delivery (Flagel et al., 2007; Robinson and Flagel, 2009). Thus, the cue is an equally effective CS for both STs and GTs, in that it produces a conditioned response (CR) in both, but only for STs is it attributed with incentive salience, rendering it a motivationally potent incentive stimulus. Importantly, we have found that individual variation in the propensity to attribute incentive salience to a food cue, as measured by conditioned approach behavior, predicts the extent to which discrete drug cues motivate behavior. For example, for STs, a cocaine-associated cue is more attractive, reliably eliciting conditioned approach towards it (Yager and Robinson, 2012), and for STs, a discrete cocaine cue is more important for the maintenance of self administration behavior, and it is also more desired, in that STs make more drug seeking responses just to receive the cue, relative to GTs (Saunders and Robinson, 2010). Additionally, the interoceptive cues produced by a priming injection of cocaine instigate greater reinstatement in STs than GTs (Saunders and Robinson, 2011b).

It is currently unknown, however, if there is similar individual variation in the way STs and GTs respond to drug-associated *contextual* cues. Interestingly, very few preclinical studies (e.g.,

Fuchs et al., 2005; Fuchs et al., 2008a; Fuchs et al., 2008b) have specifically examined the ability of a drug-associated context to renew drug-seeking behavior. Indeed, the majority of so-called "context-induced" reinstatement studies use a procedure (Bouton and Bolles, 1979; Crombag et al., 2008) where rats are first trained to self-administer drug in the presence of a discrete drug cue, and drug seeking responses are then extinguished in the presence of the discrete cue, but in an alternate context (e.g., Bossert et al., 2004; Bossert et al., 2007). For the reinstatement test, rats are returned to the drug-associated context and allowed to respond for presentations of the discrete drug cue. Thus, with such procedures, the ability of the drug context alone to arouse a conditioned motivational state of "wanting" and its ability to reestablish the conditioned reinforcing effects of a discrete drug cue are confounded. One goal of the experiments reported here was to try and tease these two processes apart by utilizing procedures that excluded discrete drug-associated cues (see Fuchs et al., 2005). The second aim was to determine if individual variation in Pavlovian conditioned approach behavior predicts individual variation in the ability of a cocaine context to renew drug-seeking behavior. Finally, a third aim was to determine if dopamine (DA) in the core of the nucleus accumbens is necessary for contextual renewal of drug-seeking behavior.

Materials and Methods

Subjects

Male Sprague-Dawley rats (Harlan, IN) weighing 250-300 g upon arrival were housed individually on a 12-hr light/12-hr dark cycle (lights on at 0800) in a climate-controlled colony room. Water and food were available *ad libitum*. After arrival rats were given one week to acclimate to the colony room before testing began, during which the experimenters repeatedly

handled them. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus

Pavlovian training and self-administration testing were conducted in standard (22x18x13 cm) test chambers (Med Associates Inc., St. Albans, VT, USA) located inside sound-attenuating cabinets. A ventilating fan masked background noise. *Pavlovian conditioned approach training*: Chambers had a food cup located in the center of one wall, 3 cm above a stainless steel grid floor. Head entries into the food cup were recorded by breaks of an infrared photobeam located inside the magazine. A retractable lever illuminated from behind was located 2.5 cm to the left or right of the food cup, approximately 6 cm above the floor. The location of the lever with respect to the food cup was counterbalanced across rats. On the wall opposite the food cup, a red house light remained illuminated throughout all sessions. *Self-administration*: For these sessions, the food cup and lever were removed and replaced with two nose-poke ports located 3 cm above the floor on the wall opposite the house light. A nose poke into the active port, detected by an infrared photobeam inside the hole, resulted in an intravenous cocaine infusion, delivered by a syringe pump through a tube connected to the rat's catheter back port. The syringe pump was located outside the sound-attenuating chamber so it would not provide an auditory cue. The infusion tube was suspended into the chamber via a swivel mechanism, allowing the rat free movement. Active and inactive nose-poke ports were counterbalanced across rats. All measures were recorded using Med Associates software. *Cocaine-context conditioning*: In Experiment 1 the test chambers were constructed with black acrylic (67.5 length x 33 width x 60.5 cm tall). Cameras (SPE-57, CCTV Specialty Bullet Cameras, Lake Worth, FL, USA) were mounted 12 cm directly above the open ceiling of the chambers to record video. Fluorescent light tubes (32

W) covered with red filter shields (McMaster-Carr, Elmhurst, IL, USA) were illuminated over each chamber. In Experiment 2 the test chambers were clear acrylic chambers (43.5 length x 21.5 width x 21 cm tall). A smaller (23 long x 6 wide x 21 cm tall) clear acrylic insert was positioned in the center of the larger chamber, which restricted each rat's movement to the perimeter of the chamber. To record locomotor activity, we surrounded each chamber with a rectangular strip (47 long x 28 cm wide) containing 5 photocell pairs distributed along the longer side of the chamber. These photocells recorded movement within the chamber as total photocell beam breaks and chamber crossovers. Testing for Experiment 2 was also done under red light conditions.

Surgery

For Experiments 3 and 4, following Pavlovian training, rats were prepared with intravenous catheters as described previously (Crombag et al., 2000) under ketamine hydrochloride (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia. Following surgery, catheters were flushed daily with 0.2 ml sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO) to minimize infection and prevent occlusions. At the end of self-administration training, catheter patency was tested by intravenous injection of 0.2 ml sodium thiopental (20 mg/ml in sterile water, Hospira, IL). Only rats that became ataxic within 5-10 s were considered to have patent catheters and included in the analyses. For Experiment 4, after receiving an intravenous catheter, rats were positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The skull of each rat was leveled and chronic guide cannulae (22 gauge stainless steel; Plastics One) were inserted bilaterally 2 mm above the target site in the nucleus accumbens core (relative to Bregma: anterior +1.8 mm; lateral +1.6 mm; ventral -5.0 mm). Guide cannulae were secured with skull screws and acrylic cement, and wire stylets (28 gauge, Plastics One) were inserted to prevent

occlusion. After surgery, all rats received antibiotic and carprofen (5 mg/kg) for pain. Rats were given 7 days to recover from surgery before testing commenced.

Microinjections

In Experiment 4, before the reinstatement test session, rats received a microinjection of vehicle (0.9% sterile saline) or flupenthixol (15 µg in saline; Sigma, St. Louis, MO), a relatively nonselective dopamine receptor antagonist. Flupenthixol was chosen in order to antagonize all actions of endogenous dopamine near the injection site, to assess the general (i.e., not specific to a particular receptor) function of dopamine in context reinstatement. The flupenthixol dose was chosen based on previous studies (e.g., Ito and Hayen, 2011; Saunders and Robinson, 2012). Intracerebral microinjections were made through 28-gauge injector cannulae (Plastics One) lowered to the injection site in the nucleus accumbens core (ventral -7.0 mm relative to skull), 2 mm below the ventral tip of the guide cannulae. During infusions, rats were gently held by the experimenter. All infusions were administered bilaterally at a volume of 0.5 µl/side, delivered over 90 s using a syringe pump (Harvard Apparatus, Holliston, MA) connected to microinjection cannulae via PE-20 tubing. After infusions, the injectors were left in place for 60 s to allow for drug diffusion before being withdrawn and replaced with wire stylets. Rats received a microinjection of saline approximately 5 days before the reinstatement test, to acclimate them to the injection procedure.

Experimental design and procedures

Pavlovian training: Pavlovian training procedures were the similar to those described previously (Flagel et al., 2007; Saunders and Robinson, 2010). For two days prior to the start of training, 10 banana-flavored pellets (45 mg, BioServe, #F0059; Frenchtown, NJ) were placed in the home cages to familiarize the rats with this food. Approximately one week after arrival, rats

were placed in the test chambers, with the lever retracted, and trained to retrieve pellets from the food cup by receiving 25 45-mg banana pellets on a variable time (VT) 30-sec schedule. All rats retrieved the pellets and began Pavlovian training the next day. Each trial consisted of insertion (and simultaneous illumination) of the lever (CS) into the chamber for 8 s, after which time the lever was retracted and a single food pellet (US) was immediately delivered into the adjacent food cup. Each training session consisted of 25 trials in which CS-US pairings occurred on a variable time (VT) 90-s schedule (the time between CS presentations varied randomly between 30 and 150 s). Lever deflections, food cup entries during the 8-s CS period, latency to the first lever deflection, latency to first food cup entry during the CS period, and food cup entries during the inter-trial interval were measured.

Quantification of behavior using an index of Pavlovian conditioned approach (PCA): For some analyses rats were classed into three groups: (1) Those who preferentially interacted with the lever ("sign-trackers", STs), (2) those who preferentially interacted with the food cup during lever presentation ("goal-trackers", GTs), and (3) those who had no clear preference for the lever or food cup ("intermediates", INs). The extent to which behavior was lever (CS) or food-cup directed was quantified using a composite index (Lovic et al., 2011; Meyer et al., 2012a; Saunders and Robinson, 2011b) that incorporated three measures of Pavlovian conditioned approach: (1) the probability of either deflecting the lever or entering the food cup during each CS period [P(lever) - P(food cup)]; (2) the response bias for contacting the lever or the food cup during each CS period [(#lever deflections - #food-cup entries) / (#lever deflections + #food-cup entries)]; and (3) the latency to contact the lever or the food cup during the CS period [(lever deflection latency - food-cup entry latency) / 8]. Thus, the Pavlovian conditioned approach index (PCA Index) score consisted of [(Probability difference score + Responses bias score + Latency

difference score) / 3]. This formula produces values on a scale ranging from -1.0 to +1.0, where scores approaching -1.0 represent a strong food cup-directed bias and scores approaching +1.0 represent strong lever-directed bias. The average PCA Index score for days 4 and 5 of training was used to class rats. Rats were designated STs if they obtained an average index score of +0.3 or greater, and as GTs if they obtained a score of -0.3 or less. The remaining rats within the middle score range were classed as INs. Note that INs were excluded from further study because we wanted to focus on those animals that differed markedly on their propensity to attribute incentive salience to reward cues (Flagel et al., 2007; Meyer et al., 2012a). Overall, a large population of rats (N=410) received Pavlovian training, but only a subset (N=171; STs N=105, GTs N=66) was used in the experiments described below.

Experiment 1: Cocaine context conditioning

Experiment 1 was conducted using the apparatus described above. Following Pavlovian training, STs and GTs (N=48) were assigned to drug-paired or drug-unpaired conditions, resulting in four independent groups: GT-Unpaired (n=5), GT-Paired (n=6), ST-Unpaired (n=18), ST-Paired (n=19). Rats were initially placed in the conditioning chambers for a 40-min habituation session, before which no injections were given. The next day, six consecutive daily 30-min conditioning sessions commenced. Rats in the paired groups received an i.p. injection of cocaine (10 mg/kg) immediately prior to being placed in the chambers, followed by an injection of saline in the home cage, 20 min after removal from the conditioning chambers, and 50 min after the original injection. Rats in the unpaired groups received the opposite injection schedule, getting a saline injection before entering the conditioning chamber and cocaine 20 min after removal, in their home cage. On the 7^{th} day, all rats received an injection of saline before being placed in the conditioning chambers. Behavior was video recorded on the test day.

Experiment 2: Cocaine context conditioning (replication)

To verify the results obtained in Exp. 1, especially given that the N in the GT groups was relatively small, we conducted a replication of this study, but using a different apparatus (described above), in which motor activity was quantified by photocell beam breaks. Following Pavlovian conditioning, a separate cohort of rats (N=27) was conditioned using a similar procedure as Experiment 1. Rats were first habituated to the photocell conditioning chambers in two 40-min sessions, during which we recorded locomotor activity (photocell beam breaks). Next, rats were assigned to cocaine paired and unpaired conditioning groups, matched based on their locomotor response on the second day of habituation. Thus, four independent groups were tested: GT-Unpaired (n=5), GT-Paired (n=5), ST-Unpaired (n=8), ST-Paired (n=9). Next, five consecutive conditioning sessions were conducted. On each day, rats in the paired groups received an i.p. injection of cocaine (10 mg/kg) before being placed in the photocell chambers for 40-min session. Paired rats then received a saline injection in their home cage 40 min after the end of the conditioning session. Unpaired rats received the opposite injection schedule. On day 6, all rats received an injection of saline before a 40-min test session. A computer recorded total photocell beam breaks and chamber crossovers during these sessions.

Experiment 3: Individual variation in context-induced reinstatement of cocaine-seeking behavior

An independent cohort of rats (N=42) was used for Experiment 3. Pavlovian training was identical to Experiments 1 and 2.

Cocaine Self-Administration Training: Following Pavlovian training and surgery, cocaine self-administration training sessions were conducted in operant chambers outfitted with two nose pokes and configured to provide one of two unique environmental contexts, that differed along auditory, visual, olfactory, and tactile stimulus modalities (based on previous studies, e.g., Fuchs

et al., 2005). One context contained a continuous white house light, pine odor, and wire mesh floor. The other context contained a continuous red houselight, continuous white noise, vanilla odor, and a bar floor. These stimuli were present throughout the entire session, independent of behavior. A nose poke into the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA, MD) dissolved in 0.9% sterile saline [0.4 mg (weight of the salt) per kg per infusion in 50 µl delivered over 2.6 s] on a fixed ratio (FR) 1 schedule (Carroll and Lac, 1997). Coincident with the start of an infusion was an unsignaled 20-s timeout period, during which nose pokes were recorded, but had no consequences. *No discrete cues (e.g., light or tone) were explicitly paired with drug delivery at any point during testing*. Also note that infusion pumps were located on the outside of the sound-attenuating cabinets housing the experimental chambers, thus pump noise during cocaine infusions was minimized. Additionally, we imposed an infusion criterion (IC) on self-administration sessions (i.e., session length was determined by how long it took each rat to reach the IC, not by an explicit time limit) to ensure that all rats received the exact same amount of drug (Saunders and Robinson, 2010). Rats were initially allowed to take 5 infusions per session for three sessions, and the IC was then increased to 10, 20, and then 40 infusions for three sessions each, for a total of 12 training sessions.

Extinction training: Rats underwent 7 consecutive 2-hr extinction sessions, during which responses had no programmed consequences. Extinction sessions were conducted in either the self-administration context (COC context group) or in the other, alternate context, which they had never experienced before (ALT context group). This design resulted in four groups: GT-COC (n=5), GT-ALT (n=11), ST-COC (n=13), and ST-ALT (n=13). All rats received 7 sessions of extinction.

Reinstatement: The day after the final day of extinction, rats in the COC context groups were placed again in the cocaine-training context for one additional 2-hr test session, while rats in the ALT context groups were reintroduced to the context in which they previously self-administered cocaine. During this test session nose pokes were recorded but had no consequences. That is, a nose poke did not result in presentation of cocaine or any cocaine-associated cue. Note that this procedure is different than that used in most previous studies on context reinstatement (e.g., Bossert et al. 2007), because in these the operant resulted in presentation of a drug-associated cue.

Experiment 4: The role of accumbens core dopamine in cocaine context-induced reinstatement

A separate cohort of rats (N=54) was used for Experiment 4. All procedural details leading up to the reinstatement test were the same as for rats in Experiment 3.

Reinstatement: Following extinction training, ALT and COC STs and GTs were assigned to vehicle (saline) or flupenthixol treatment groups. The resulted in eight independent groups: GT-COC-VEH (n=7), GT-COC-FLU (n=7), GT-ALT-VEH (n=8), GT-ALT-FLU (n=7), ST-COC-VEH (n=5), ST-COC-FLU (n=4), ST-ALT-VEH (n=8), and ST-ALT-FLU (n=8). On the day of the reinstatement test sessions, rats received a microinjection, as described above, before being placed in the test chambers 10-15 min later.

Histology

After the completion of behavioral testing, rats in Experiment 4 were euthanized via carbon dioxide overdose and their brains were removed and flash frozen in isopentane chilled to approximately -30 C by a mixture of isopropyl alcohol and dry ice. Frozen brains were sectioned on a cryostat at a thickness of 60 μm, mounted on slides, air-dried, and stained with cresyl violet.

Microinjection sites were verified by light microscopy and plotted onto modified drawings from a rat brain atlas (Paxinos and Watson, 2007).

Statistical Analyses

Linear mixed-models (LMM) analyses of variance (ANOVA) were used for all repeated-measures data. The best-fitting model of repeated measures covariance was determined by the lowest Akaike information criterion score (Verbeke, 2009). Depending on the model selected, the degrees of freedom may have been adjusted to a non-integer value. Video captured for Experiment 1 was analyzed using video tracking software (Topscan, Clever Sys., Inc., Reston, VA). Two-way ANOVAs were used to compare group responding during locomotor and reinstatement tests. A Pearson's correlation was used to compare degree of sensitization with test day locomotor activity in Experiment 2. Statistical significance was set at $p < 0.05$.

Results

Individual variation in Pavlovian conditioned approach behavior

Figure 6.1 illustrates the degree of individual variation in Pavlovian conditioned approach behavior in all rats (N=410) screened through the Pavlovian training procedure for Experiments 1-4. Plotted along the x-axis are individual rat PCA index scores. Similar to our previous reports (Flagel et al., 2007; Saunders and Robinson, 2012), we found large variation in the type of conditioned responses different rats made. Rats classed as STs, which preferentially directed their CR towards the CS (lever), were defined as those with PCA index scores ranging from +0.3 to +1.0. Rats classed at GTs, conversely, which preferentially directed their CR towards the food cup during the CS period, had PCA index scores ranging from -0.3 to -1.0. The remaining rats, which vacillated between CS-directed and food cup-directed CRs, were those with scores ranging from -029 to +0.29. These rats were excluded from further analysis. In addition, note

that only a subset of the STs (N=105) and GTs (N=66) shown in Fig. 1 was used further analyses, as needed to form experimental groups. The remainder were used in other studies.

Experiments 1 and 2 – Individual variation in cocaine context conditioned hyperactivity

Experiment 1: Figure 6.2A shows that on the test day, when all groups received saline prior to placement in the test chamber, Paired GTs were significantly more active than Unpaired GTs ($p < 0.001$) – that is, they showed conditioned hyperactivity in the cocaine paired environment. In contrast, Paired and Unpaired STs did not differ ($p = 0.260$). Importantly, the phenotype by drug condition interaction was also significant ($F_{(1, 44)} = 8.730$, $p = 0.005$), indicating that GTs showed a greater conditioned response than STs.

Experiment 2: The results of Experiment 2 were qualitatively similar to Experiment 1 (Figure 6.2B). In this experiment both Paired STs and GTs showed conditioned hyperactivity (the Paired groups showed greater activity than the Unpaired groups), but as in Experiment 1, there was a significant phenotype by drug condition interaction ($F_{(1,23)} = 4.799$, $p = 0.039$), indicating that Paired GTs showed a greater conditioned response than Paired STs.

We also examined locomotor activity during the training (conditioning) phase. Unpaired rats that received saline in the test chamber had a low level of activity, relative to Paired rats that received cocaine, and this did not change across conditioning days. There were no differences in the initial locomotor response to cocaine, as measured by photocell breaks in Paired STs and GTs on day 1 (Figure 6.3A). By day 5, Paired rats showed evidence of sensitization, showing greater cocaine-induced locomotor activity than on day 1 ($F_{(1,24)} = 14.359$, $p = 0.001$). Although it appears as if GTs showed more robust sensitization than STs, this effect was not significantly significant (phenotype by day interaction, $F_{(1,24)} = 3.058$, $p = 0.093$). However, one-way ANOVAs comparing behavior on days 1 and 5 within each Paired group revealed that GTs

significantly increased the number of beam breaks between Days 1 and 5 ($F_{(1,24)} = 11.927$, $p = 0.002$), but STs did not ($F_{(1,24)} = 2.915$, 0.101). Additionally, among all Paired rats, the degree of change in activity between days 1 and 5, an index of sensitization, was significantly correlated with the amount of conditioned activity on the drug-free test day ($R^2 = 0.3944$, $p = 0.008$; Figure 6.3B).

Experiment 3

Acquisition of cocaine self-administration behavior

Figure 6.4 shows that, similar to previous reports (Saunders and Robinson, 2011b), STs and GTs did not differ in the acquisition of cocaine self-administration behavior. The groups did not differ in the number of active responses ($F_{(1,40)} = 2.024$, $p = 0.163$; Figure 6.4A), or inactive responses ($F_{(1,40)} = 0.055$, $p = 0.816$), and there were no group differences in the time required to complete self administration sessions ($F_{(1,40)} = 0.184$, $p = 0.670$; Figure 6.4B).

Extinction training

Following self-administration training, STs and GTs were extinguished in either the same cocaine-associated context, or a novel alternate context. Importantly, Figure 6.5 shows there were no group differences in the number of active responses during extinction training ($F_{(3,38)} = 0.758$, $p = 0.525$; Figure 6.5), and all groups extinguished at similar rates (no group by session interaction, $F_{(18,38)} = 1.64$, $p = 0.099$), reaching a low level of behavior by session 7.

Individual variation in context-induced reinstatement of cocaine seeking

Figure 6.6 illustrates the ability of the cocaine-associated context to renew cocaine-seeking behavior in STs vs. GTs. Similar to previous reports (e.g., Fuchs et al., 2005), rats that were extinguished in an alternate context, when returned to the cocaine-associated context, made significantly more cocaine-seeking responses, as measured by active nose pokes, than rats

extinguished in the cocaine training context (effect of context, $F_{(1,39)} = 28.058$, $p < 0.001$). Importantly, GTs showed more robust context renewal of cocaine seeking than STs, as indicated by a significant phenotype by context interaction ($F_{(3,38)} = 8.607$, $p = 0.006$).

Experiment 4

For Experiment 4, Pavlovian, self-administration, and extinction training were very similar to Experiment 3, and so those data are not shown.

Effects of flupenthixol on cocaine context-induced renewal of drug-seeking

We first compared rats that received vehicle before the test session. Figure 6.7 shows that GTs showed much more robust context renewal of cocaine seeking than STs, as indicated by a significant phenotype by context interaction ($F_{(1,24)} = 12.342$, $p = 0.002$). This replicates the results of Experiment 3. Flupenthixol suppressed renewal of cocaine seeking relative to vehicle in both GT and ST ALT groups (effect of treatment, $F_{(1,27)} = 41.403$, $p < 0.001$), but did so to a significantly greater degree in GTs relative to STs, as indicated by a significant phenotype by treatment interaction ($F_{(1,24)} = 17.712$, $p < 0.001$). Importantly, flupenthixol suppressed active responses to a greater extent than inactive responses among ALT GTs (significant treatment by nosepoke interaction for ALT GTs, $F_{(1,26)} = 31.624$, $p < 0.001$), but not ALT STs (no treatment by nosepoke interaction for ALT STs, $F_{(1,28)} = 2.994$, $p = 0.095$), suggesting that the effect of flupenthixol was to attenuate drug-seeking responses specifically, and was not due to nonspecific motor impairments. Within the COC groups, flupenthixol slightly decreased active nose pokes (effect of treatment, $F_{(1,19)} = 7.238$, $p = 0.014$), although this effect was not specific to one phenotype (no phenotype by treatment interaction, $F_{(1,19)} = 3.784$, p 0.067), suggesting that flupenthixol may have also attenuated any residual drug-seeking motivation in these rats.

Histological verification of cannulae placements

Figure 6.8 illustrates the location of the microinjection tips within the nucleus accumbens core for rats used in Experiment 4.

Discussion

We found that there is considerable individual variation in the ability of a cocaine-associated context to motivate behavior, as indicated by two different measures. In Experiments 1 and 2, we found that GTs exhibited greater cocaine context conditioned hyperactivity. In Experiment 3, we found that for GTs, a cocaine context also renews greater cocaine-seeking behavior. Finally, in Experiment 4, we report that contextual renewal of cocaine seeking requires dopamine signaling within the core of the nucleus accumbens. These findings, taken together with our other recent studies, have a number of implications for thinking about the neurobiological and psychological mechanisms that govern relapse induced by different types of reward-associated stimuli.

Distinct neural systems process discrete versus contextual cues

Dissociable neural systems are thought to mediate learning about different classes of stimuli associated with motivationally significant events. These cue processing distinctions have been particularly well characterized in the fear conditioning literature. For example, learning about aversive contextual cues is heavily dependent on the hippocampus, along with its projections to the amygdala, nucleus accumbens, and prefrontal cortex, while the hippocampus is somewhat less important for learning about discrete (e.g., a tone) fear cues (Fanselow, 2010; Levita et al., 2002; Maren, 2001b; Phillips and LeDoux, 1992). The amygdala and nucleus accumbens, conversely, appear to be similarly important for learning about both discrete and contextual fear cues (Holland and Gallagher, 1999; Levita et al., 2002; Maren, 1998, 2001a). Similar neuroanatomical distinctions exist for processing of different classes of cues associated with appetitive USs (Cardinal et al., 2002a; Shalev et al., 2002).

There is growing evidence that the reinstatement of extinguished drug seeking relies on somewhat dissociable brain systems, depending on the class of drug cue involved. For example, Fuchs et al. (2005) found that pharmacological inactivation of the dorsal hippocampus, via GABA receptor agonism, impaired drug context-induced reinstatement, but failed to alter reinstatement induced by a discrete drug cue or the interoceptive cues associated with a drug injection. Interestingly, inactivation of the basolateral amygdala (BLA) or prelimbic/anterior cingulate cortex attenuates not only context-induced reinstatement, but also blocks discrete cue reinstatement (McLaughlin and See, 2003). Additionally, while prelimbic inactivation also attenuates drug-primed reinstatement (McFarland and Kalivas, 2001), BLA inactivation does not (Grimm and See, 2000). The nucleus accumbens core appears to be generally important for reinstatement, as its inactivation attenuates reinstatement in response to contextual, discrete, and interoceptive drug cues (Fuchs et al., 2004; Fuchs et al., 2008b; McFarland and Kalivas, 2001). Conversely, neural activity in the shell sub region of the accumbens is necessary for context-induced, but not discrete cue or drug-induced reinstatement (Fuchs et al., 2004; Fuchs et al., 2008b; McFarland and Kalivas, 2001). Taken together, these studies indicate that a distributed set of interconnected brain regions including the hippocampus, amygdala, prefrontal cortex, and nucleus accumbens is involved generally in reinstatement, but the contribution of specific areas varies depending on the type of drug cue involved.

Psychological processes governing reinstatement to different drug cues

Given that at least partially distinct neural systems regulate reinstatement to different types of drug cues, it follows that the *psychological* processes governing reinstatement behavior differ depending on the type of cue involved. Discrete drug cues, for example, are thought to acquire incentive motivational value as a function of their association with the primary rewarding effects

of drugs (Berridge, 2007; Bindra, 1978; Cardinal et al., 2002a; Konorski, 1967; Toates, 1986; Young, 1966). This can promote drug-seeking via multiple mechanisms (Milton and Everitt, 2010). First, discrete cues that are localizable can instigate approach behavior, drawing individuals into close proximity with themselves. Second, they can act as conditioned reinforcers, in that individuals will work to obtain them. Finally, their presence can evoke conditioned motivational states ("wanting") that spur drug-seeking behavior. We have found that there are considerable individual differences in the ability of discrete drug cues to promote behavior via each of these mechanisms. For STs, compared to GTs, discrete and localizable cocaine cues more effectively motivate approach behavior, serve as more robust conditioned reinforcers, and instigate more powerful conditioned motivational states that reinstate drug seeking (Saunders and Robinson, 2010; Saunders and Robinson, 2011a; Yager and Robinson, 2012).

Interoceptive cues produced by drugs themselves also serve to motivate drug-seeking behavior and promote relapse, because they act as both primary and (after training) conditioned reinforcers (Stewart et al., 1984; Wise, 2004). Individuals will perform actions to obtain drugs in part because the interoceptive cues produced by the drug are desirable. Additionally, the interoceptive cues produced by drugs can evoke conditioned motivational states that spur on drug seeking. This has been demonstrated in drug "priming" studies, where exposure to a small amount of drug evokes intense subjective craving states in people, and reinstates drug seeking in rats (Jaffe et al., 1989; Shaham et al., 2003; Stewart et al., 1984). As with discrete cues, we have found that STs and GTs differ in the way they respond to the interoceptive cues produced by drugs. For example, STs achieve higher breakpoints on a progressive ratio schedule for cocaine relative to GTs, suggesting that cocaine-associated interoceptive cues acquire greater conditioned

reinforcing properties in STs. Additionally, it appears that the interoceptive cues associated with cocaine also produce greater conditioned motivation in STs, as they show more robust cocaine-primed reinstatement compared to GTs (Saunders and Robinson, 2011b).

Contextual cues are somewhat less sharply defined than other cue types, in that they are comprised of a multimodal set of static stimuli. Learning theorists have suggested that even though a context is made up of many individual stimuli, contextual learning involves the integration of these separate cues into a configural representation that is qualitatively different from the representation of individual discrete cues (Fanselow, 2010; Holland and Bouton, 1999; Nadel and Willner, 1980; Rudy and Sutherland, 1995). There are several ways in which contextual cues, including drug contexts, can affect behavior. In the absence of salient discrete cues, contexts themselves may acquire motivational value via direct association with a US (Rescorla and Wagner, 1972). This may cause a context to instigate approach behavior (such as in a conditioned place preference test) and/or evoke conditioned motivational states that spur drug-seeking CRs, similar to discrete and interoceptive stimuli (Di Ciano and Everitt, 2003; Fuchs et al., 2005; Robinson and Berridge, 2003). Similarly, drug context conditioned hyperactivity is thought to reflect expression of conditioned motivation for the drug, but given that there the animal has no opportunity to work for the drug, or to approach and engage a discrete cue, this motivation is expressed as hyperactivity (Beninger et al., 1981; Jones and Robbins, 1992). In other situations, when contextual and discrete cue information interact, "hierarchical" learning can take place, where a context becomes associated with a discrete cue that predicts a US (Holland and Bouton, 1999; Rescorla, 1980). Under these circumstances, contexts serve as "occasion setters", indirectly modulating the effects of discrete cues that occur

within them (Bouton, 2002; Bouton and Swartzentruber, 1991; Crombag et al., 2008; Holland, 1992).

The results of our current study suggest that, like with other types of drug cues, there is robust individual variation in the ability of contextual drug-associated cues to motivate behavior. Here, it is GTs who show greater conditioned hyperactivity and renewal of cocaine seeking when presented with a cocaine context. Interestingly, these results parallel a recent finding that GTs show greater contextual fear expression, relative to STs, while STs show greater discrete cue (e.g., tone) induced fear (Morrow et al., 2011). Together with our current results, this suggests that STs and GTs may preferentially assign motivational significance to discrete and contextual information, respectively, and this preference occurs regardless of the emotional valence of the associated US.

We should point out that the results from our conditioned locomotion study at first appear at odds with two recent studies. First, Flagel et al. (2008) concluded that STs show greater sensitization in response to repeated cocaine injections. Here, GTs tended to exhibit greater cocaine-induced hyperactivity than STs by the end of cocaine conditioning, although the effect was not statistically significant. Notably, in Flagel et al. (2008) there were no group differences in cocaine-induced locomotor activity at the dose we used here, but STs showed more head movements, which are thought to be an index of stereotyped behavior (Robinson and Becker, 1986). We did not record rotations or head movements, and so while GTs showed greater overall locomotion, it remains possible that STs exhibited greater cocaine-induced stereotyped behavior that we were unable to measure. Second, Meyer et al. (2012b), using a modified place conditioning test, recently demonstrated that STs form a more robust preference for a cocaine-associated stimulus than GTs. In this study, however, testing was conducted in the dark, and the

only difference between cocaine and saline-associated stimuli was the tactile sensation of the floor rats walked on after receiving injections. Thus, preference behavior in that case was not mediated by the formation of a configural representation of the cocaine context. Indeed, Meyer et al. (2012b) interpret their results in terms of a conditioned reinforcement mechanism, rather than place conditioning.

We can only speculate about the psychological processes that mediate the contextual effects seen here in GTs. It has been suggested (Clark et al., 2012; Saunders and Robinson, 2012) that GT behavior, within the Pavlovian training context, is governed by a process analogous to cognitive reward expectation (Balleine, 1994; Bindra, 1978; Dickinson and Balleine, 1994; Toates, 1986). This interpretation does not appear to explain my cocaine context data, however, for at least two reasons. One, goal-directed behavior mediated by cognitive expectancies are thought to rely on explicit act-outcome associations, but in our conditioned locomotion experiments, rats never perform an action to receive drug, and so presumably no action-outcome associations were formed. Second, behavior governed by explicit cognitive expectations has been shown to not require dopamine signaling (Dickinson et al., 2000; Lex and Hauber, 2010; Wassum et al., 2011), which was not the case for context renewal here. An alternative explanation is that in the current experiments, given that no discrete cues were present, the cocaine-associated context itself generated a greater conditioned motivational state in GTs than STs, which spurred either hyperactivity or renewed cocaine seeking. The ability of reward cues to spur conditioned motivational states, such as reward "craving", that invigorate behavior, is thought to depend on dopamine signaling (see below), particularly within the nucleus accumbens (Dickinson et al., 2000; Lex and Hauber, 2008; Ostlund and Maidment, 2012; Saunders and Robinson, 2011a; Wassum et al., 2011). Thus, it is possible that under these conditions the

psychological process mediating context reinstatement in GTs is very similar to that which we have suggested mediates drug primed or discrete cue-evoked cocaine seeking in STs (Meyer et al., 2012a; Saunders and Robinson, 2011a, b). It is somewhat unclear why these cue types both produce greater conditioned motivation in STs, but contextual cues produce greater conditioned motivation in GTs. We can only speculate at this point, but one commonality between the cues used in our previous studies (Saunders and Robinson, 2011a, b), and how they differ from contextual cues, is that they have either physically (i.e., a round light), or temporally (i.e., rapid drug actions), discrete elements.

Dopamine signaling is thought to be important for the assignment and maintenance of motivational value to reward-associated cues (Beninger et al., 1981; Berridge, 2007; Cardinal et al., 2002a; Robinson and Berridge, 1993). Indeed, dopamine is important for the acquisition and expression of a sign-tracking CR, as well as for the ability of discrete cues to serve as conditioned reinforcers and to instigate conditioned motivational states that drive reward seeking (Di Ciano et al., 2001; Dickinson et al., 2000; Flagel et al., 2011b; Kelley and Delfs, 1991; Saunders and Robinson, 2012; Taylor and Robbins, 1984; Wassum et al., 2011). Our results extend these studies, demonstrating that dopamine transmission within the core sub region of the nucleus accumbens is also necessary for a cocaine-associated *context* to promote reinstatement. This appears somewhat inconsistent with the results of Bossert et al. (2007), who demonstrated that inactivation of dopamine D1 receptors in the shell, but not core, attenuates context-induced reinstatement of heroin seeking in a the presence of discrete cues. Several important methodological differences between these studies, however, may account for the discrepancy. First, Bossert et al. (2007) tested reinstatement of heroin seeking, while we used cocaine, and there is growing evidence that the neural systems involved in self administration and

reinstatement for opiates are quite different from stimulant drugs (Badiani et al., 2011). Second, we administered flupenthixol into the core, which acts to antagonize activity at both D1- and D2-type dopamine receptors. Thus, inactivation of D1 receptors only in the core may be insufficient to attenuate context reinstatement. It is possible that context renewal of cocaine-seeking requires endogenous dopamine signaling within the core, which involves activation of all receptors types, or that signaling via D2 receptors alone is necessary. Third, in Bossert et al. (2007) the reinstatement test involved the presence of *both* heroin-associated contextual and discrete cues. This makes it difficult to determine what D1 receptor activity was not necessary for during the reinstatement test, because it is possible that multiple psychological processes were confounded.

Although we did not directly assess the role of dopamine in the conditioned hyperactivity effects reported in Experiments 1 and 2, there is reason to believe that conditioned locomotor behavior is dependent on dopamine signaling. Multiple studies (e.g., Gold et al., 1988; Jones and Robbins, 1992) have demonstrated that selective lesions of dopamine neurons projecting to the nucleus accumbens abolish conditioned hyperactivity following contextual conditioning to drugs such as amphetamine. This suggests that dopamine in the accumbens is involved not only in the acute psychomotor response to stimulant drugs, but also in attributing the drug-associated context with motivational value. Therefore, the conditioned hyperactivity expressed by GTs in a cocaine context may be interpreted as reflecting the dopamine-dependent incentive motivational influence of the context.

It is unclear why contextual information would be specifically important for GT hyperactivity and renewal of cocaine seeking, but there are possible mechanisms by which contextual information evokes a conditioned motivational state that is dopamine dependent. Interestingly, electrical stimulation of the hippocampus alone can evoke drug-seeking responses

(Vorel et al., 2001) that are dependent on glutamate signaling within the ventral tegmental area (VTA). Additionally, given that context-reinstatement is dependent on a functional hippocampus and nucleus accumbens core (Fuchs et al., 2007; Fuchs et al., 2005; Fuchs et al., 2008b), as well intact signaling between the hippocampus and VTA (Luo et al., 2011), it is possible that hippocampal projections modulate dopamine transmission into the accumbens to regulate context reinstatement. This circuit may be recruited more readily in GTs, which is a potential mechanism that can be explored in future studies. We should note, however, that it is possible that under other experimental conditions, such as those involving combined context and discrete cue manipulations, that contextual information may serve a different psychological role in GTs, perhaps acting as an occasion setter to regulate the meaning of discrete cues. Future studies that examine how STs and GTs differ under conditions where contextual and discrete cue information interact will be important for determining exactly how these individuals differentially process reward-related stimuli.

In conclusion, we report that there is considerable individual variation in the extent to which a cocaine-associated context motivates behavior. Taken together with our other recent studies, it appears that discrete and contextual stimuli acquire differential motivational properties in different individuals. Previously (Flagel et al., 2009; Meyer et al., 2012a), we have suggested that STs represent individuals who are particularly vulnerable to addition-like traits. The current results indicate that STs and GTs may instead have different vulnerabilities stemming from the way their brains process motivationally relevant information. Thus, there are likely multiple "pathways" to addiction-like behavior. It will be important to consider such multifaceted vulnerability for the development of effective individualized treatments.

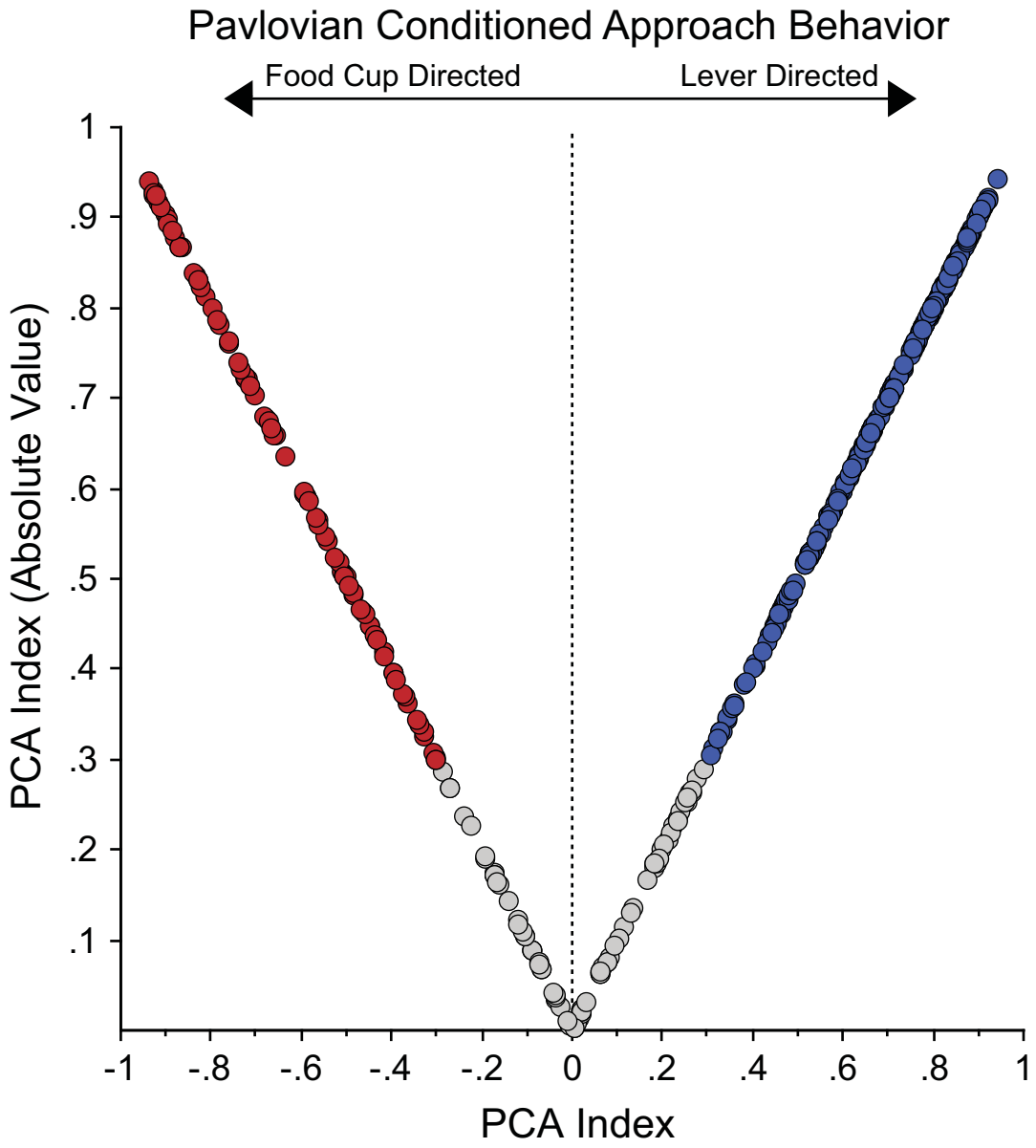
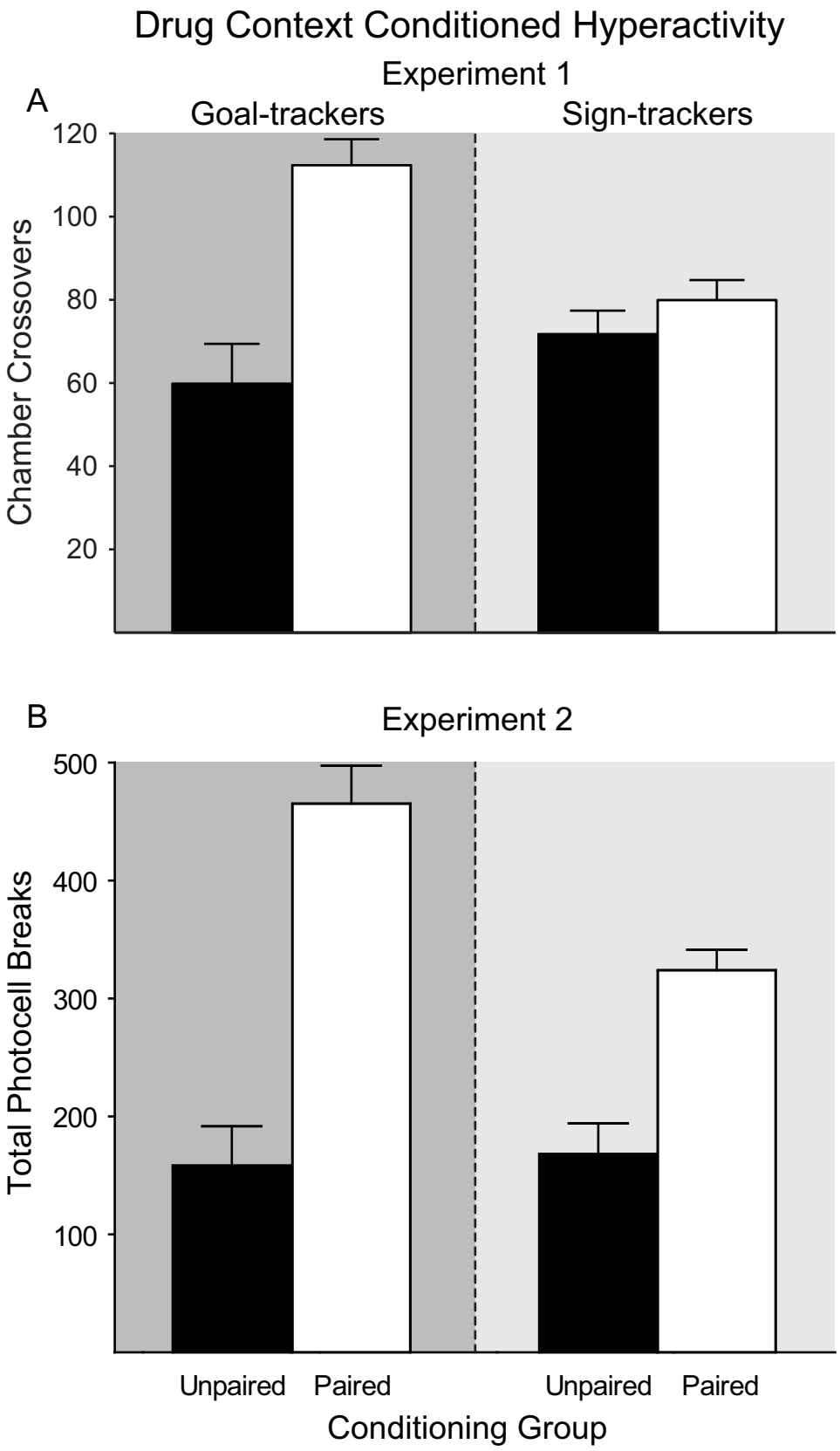


Figure 6.1. Individual variation in PCA behavior. PCA index scores for all individual rats screened (N=410) are plotted. Rats receiving a score between +1.0 and +0.3 were classed as sign trackers (STs), those with a score between +0.29 and -0.29 were classed as intermediates (INs), and those with a score between -0.3 and -1.0 were classed as goal trackers (GTs). Note that all INs were excluded from further analysis, and only a subset of all STs and GTs screened were used in the experiments described here.

Figure 6.2. Variation in cocaine-context-induced hyperactivity. A) Experiment 1 – Average number of chamber crossovers made on the drug-free test day for STs and GTs that received cocaine injections paired with the conditioning context (Paired groups), or unpaired home cage injections (Unpaired groups). B) Experiment 2 – Average number of total photocell beam breaks during the drug-free test day for STs and GT that received cocaine injections paired with the conditioning context, or unpaired home cage injections. Symbols represent the mean ± SEM.


Drug Context Conditioned Hyperactivity
Experiment 1
A
Goal-trackers
Sign-trackers
120
100
80
60
40
20
Chamber Crossovers
B
Experiment 2
500
400
300
200
100
Total Photocell Breaks
Unpaired
Paired
Unpaired
Paired
Conditioning Group

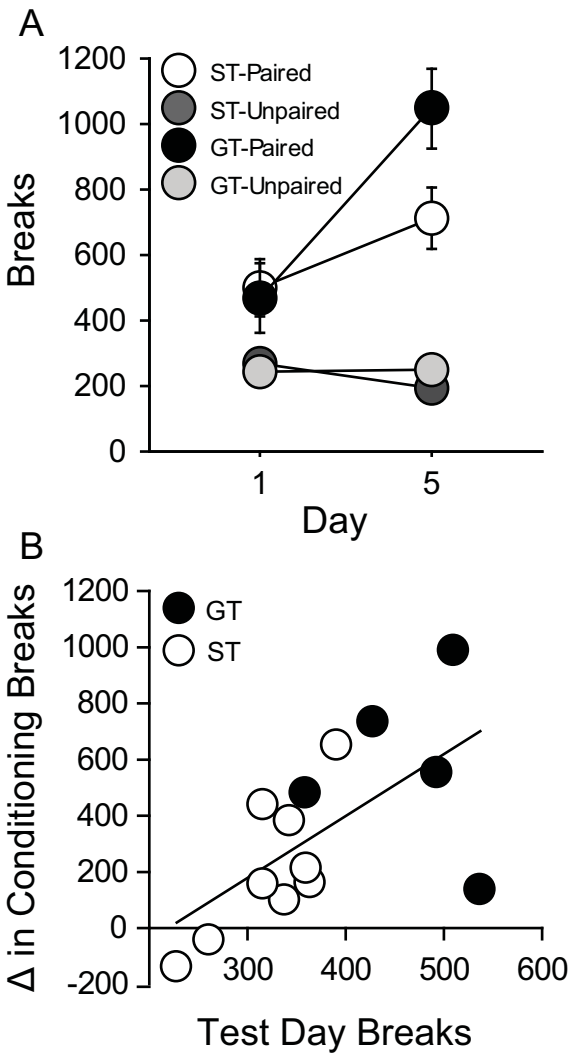


Figure 6.3. Individual variation in cocaine-induced locomotor activity. Experiment 2. A) Average number of photocell beam breaks for Paired and Unpaired STs and GTs on day 1 and day 5 of conditioning. B) The change in number of photocell breaks between day 1 and day 5 of conditioning as a function of number of beam breaks made on test day for individual Paired STs and GTs.

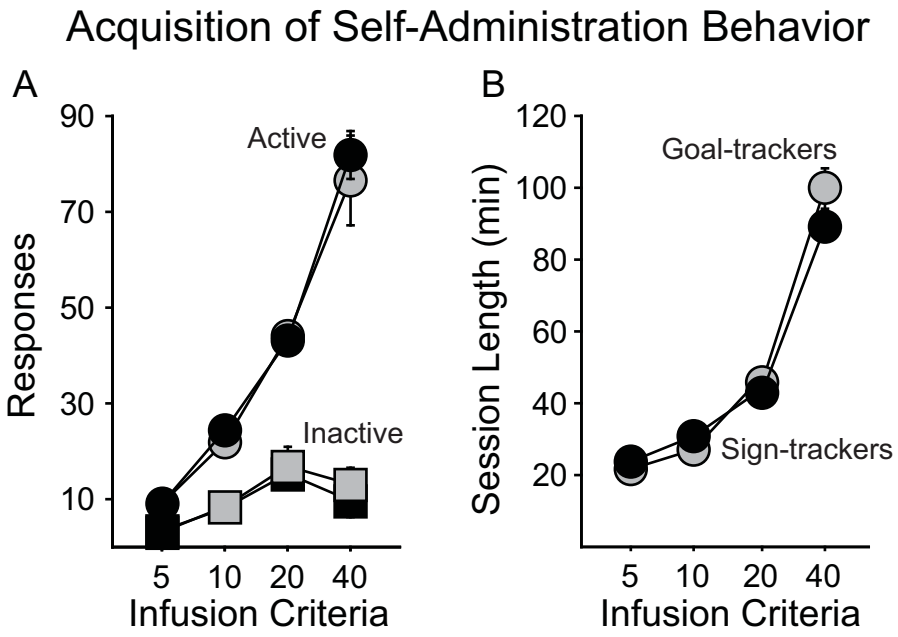


Figure 6.4. Acquisition of cocaine self-administration behavior. Experiment 3. A) Average number of active and inactive responses made across training IC for STs and GTs. B) Average time required to completed self administration sessions across training IC for STs and GT. Symbols represent the mean ± SEM.

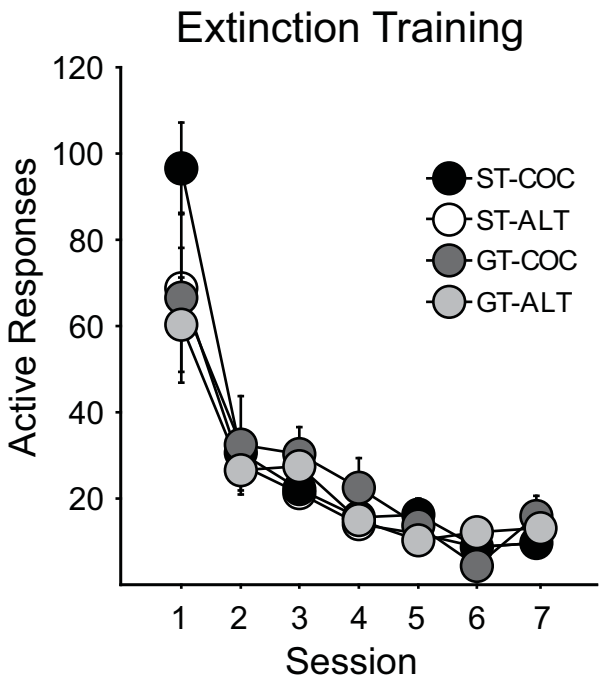


Figure 6.5. Extinction training. Experiment 3. Average number of active responses made for STs and GTs extinguished in either the cocaine training context (COC groups) or a novel, alternate context (ALT groups). Symbols represent the mean ± SEM.

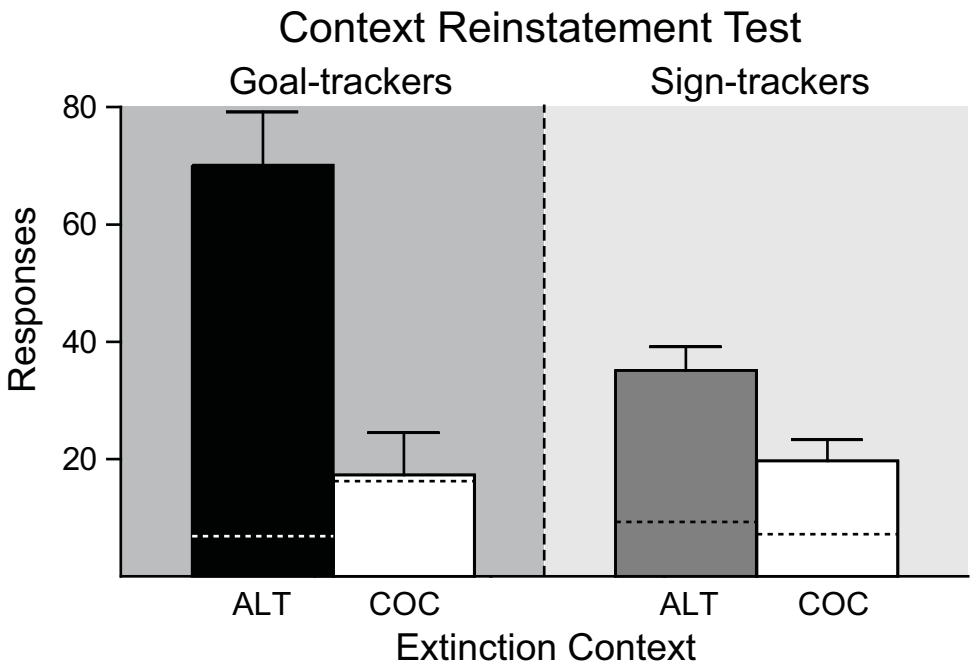


Figure 6.6. Variation in the ability of a cocaine-associated context to reinstate cocaine-seeking behavior. Experiment 3. Average number of responses made in a single 2-hr reinstatement test session in the cocaine training context for STs and GTs that had been extinguished in either the cocaine training context (COC groups) or a novel, alternate context (ALT groups). Bars represent active nose pokes and dotted lines represent inactive nose pokes. Symbols represent the mean ± SEM.

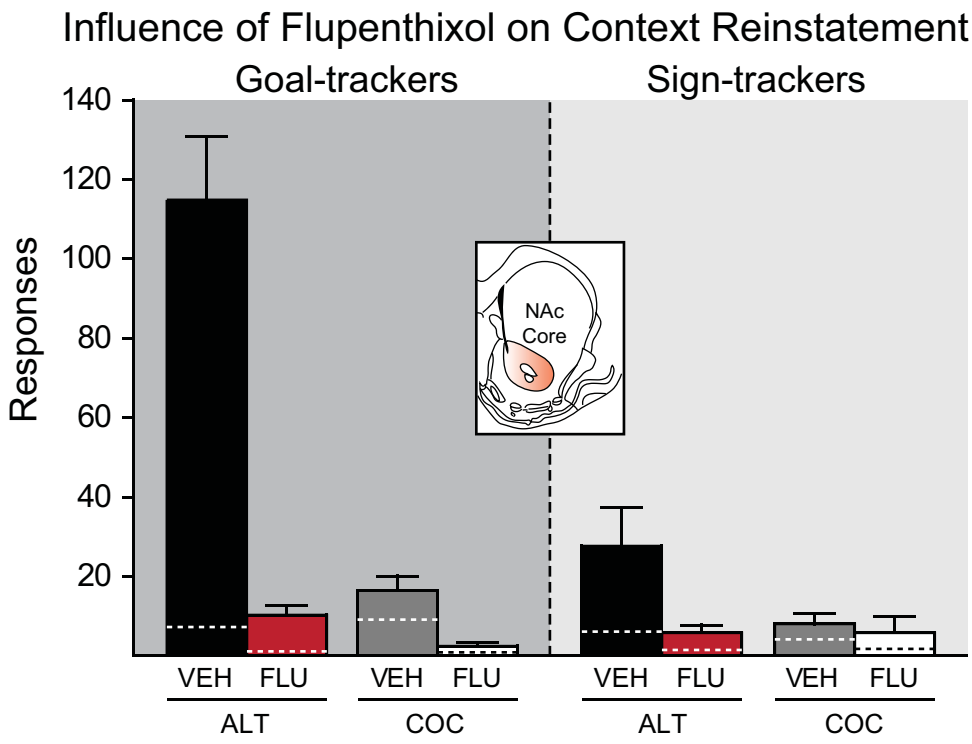


Figure 6.7. Effect of flupenthixol on context-induced reinstatement of cocaine seeking. Experiment 4. Average number of responses made in a single 2-hr reinstatement test session in the cocaine training context for STs and GTs that had been extinguished in either the cocaine training context (COC groups) or a novel, alternate context (ALT groups), and that had received either vehicle (VEH groups) or flupenthixol (15 µg; FLU groups) prior to the session. Bars represent active nose pokes and dotted lines represent inactive nose pokes. Bars represent active nose pokes and dotted lines represent inactive nose pokes. Symbols represent the mean ± SEM.

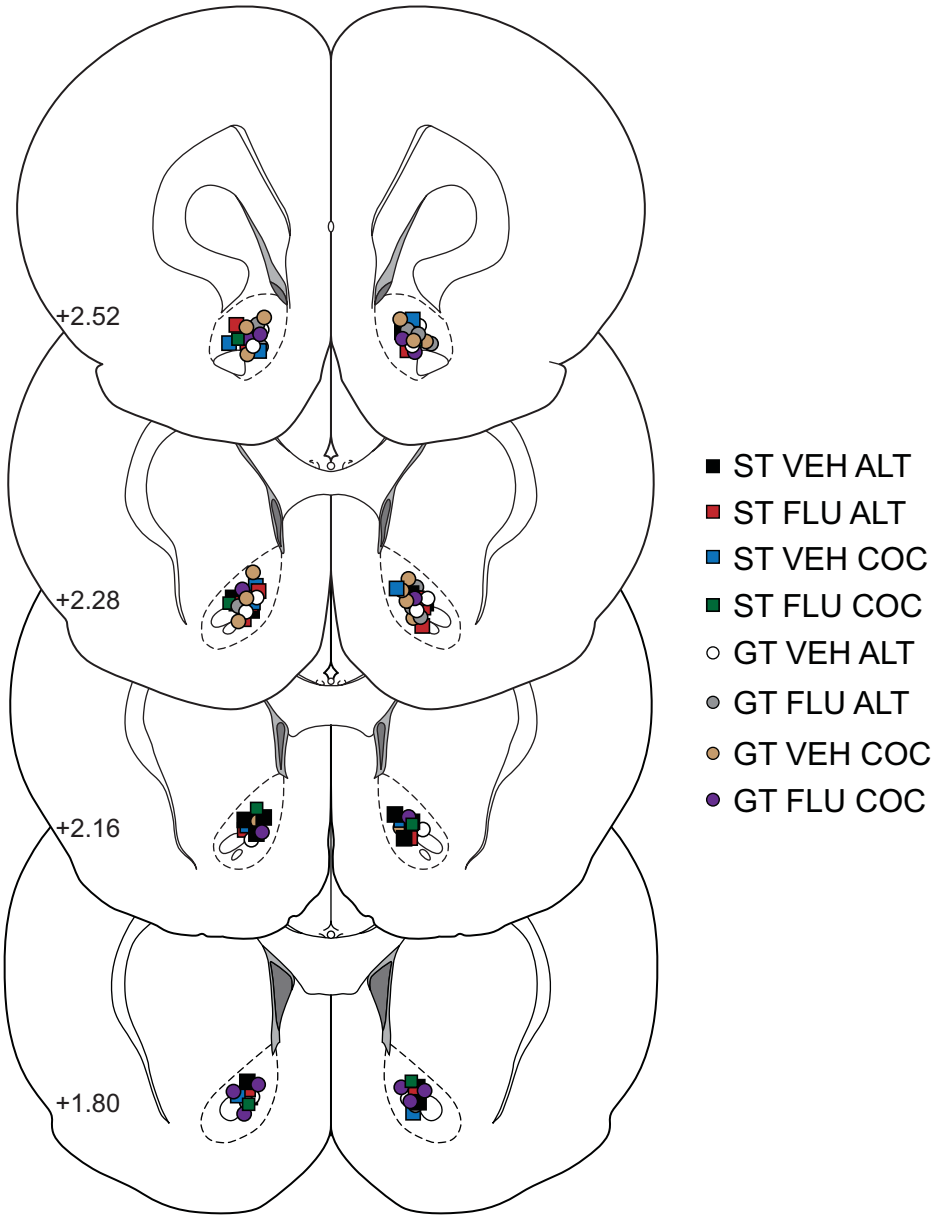


Figure 6.8. Location of microinjection tips within the nucleus accumbens core relative to Bregma for rats used in Experiment 4. Square symbols represent STs and circles represent GTs.

Chapter 7

General Discussion

In the experiments described in this dissertation, I explored 1) the role of dopamine in Pavlovian incentive motivational processes and 2) individual variation in the motivational properties of food- and drug-associated stimuli.

The role of dopamine in learning and incentive motivation

In chapter 2 (Saunders and Robinson, 2012), I explored the role of dopamine signaling within the nucleus accumbens core in the expression of three types of Pavlovian conditioned approach CRs: CS-directed "sign tracking" CRs, goal-location-directed "goal tracking" CRs, and an orienting CR.

In a recent study, Flagel et al. (2011b) used fast-scan cyclic voltammetry (FSCV) (Phillips et al., 2003b) to measure rapid dopamine signaling within the nucleus accumbens core during Pavlovian training in which a lever-CS was paired with food delivery, as described above. In rats that learned a sign-tracking CR the phasic dopamine signal transferred from the US to the CS, as a function of learning, similar to previous reports (see also Clark et al., 2012; Day et al., 2007). However, in rats that learned a goal-tracking CR, no such US-to-CS transfer occurred, even though for these rats the CS-US association was learned, as indicated by the fact that the CS came to reliably evoked a CR directed at the location of food delivery, as a function of training. Similarly, Parker et al. (2010) found that mice with disrupted phasic dopamine signaling learned

a goal-tracking CR normally, even though no clear US-to-CS transfer in dopamine signaling occurred. To test whether dopamine is necessary for learning a ST or GT CR, Flagel et al. (2011b) treated rats with systemic injections of the dopamine antagonist flupenthixol prior to each training session, which would block dopamine activity in all brain regions that receive a dopaminergic input. They found that flupenthixol blocked learning of a sign-tracking CR, but it had no effect on learning the CS-US association that underlies a goal-tracking CR (see also Danna and Elmer, 2010).

In their supplemental materials, Flagel et al. (2011b) also reported that systemic flupenthixol administration impaired the performance of *both* sign and goal tracking CRs. This result was difficult to interpret, however, because the effects occurred at doses that produced nonspecific reductions in motor activity. In my study (Saunders and Robinson, 2012), I circumvented that problem by injecting flupenthixol directly into the accumbens core, the same region where Flagel et al. (2011b) found increased cue-evoked dopamine release in STs. I found that blocking dopamine receptors in the accumbens core dose-dependently impaired the expression of sign-tracking, but not goal-tracking CRs. Furthermore, after administration of flupenthixol into the accumbens, sign-tracking behavior was fully impaired on the very first trial, before new learning (presumably via updated prediction-errors) could occur. Interestingly, similar learning-independent performance effects of dopamine manipulations have been found in recent studies of Parkinson's disease patients (e.g., Shiner et al., 2012). I also examined the effects of dopamine receptor blockade on the expression of another a CR, a conditioned orienting response in the direction of the CS. Importantly, both STs and GTs learn a conditioned orientation response to the lever, which indicates that it is dissociable from an approach CR, and rats that receive unpaired CS-US presentations do not develop conditioned orientation, suggesting that it is not a

simple reflexive reaction to the cue, but rather an indication of the learned CS-US association (see also Yager and Robinson, 2012). I found that in STs, dopamine antagonism did not attenuate performance of a conditioned orienting CR. This suggests that even in STs some stimulus-reward associations remained functional after dopamine blockade in the core of the accumbens. These results are consistent with views (e.g., Berridge, 2007) suggesting that dopamine is not necessary for the formation of stimulus-response associations, per se, but rather for imbuing learned cues with incentive salience, and thus making them attractive stimuli. The data in chapter 2 add to a growing literature regarding dopamine's role in incentive motivation versus prediction-error signaling.

Earlier studies provide further evidence that dopamine is not necessary for stimulus-reward learning. For example, Berridge and Robinson (1998) completely depleted dopamine in the dorsal and ventral striatum of rats using the neurotoxin 6-OHDA, and found they were still able to learn a new value of a food reward just as well as intact control rats. An important series of studies by Richard Palmiter and colleagues similarly demonstrated that genetically engineered dopamine-deficient (DD) mice, whose brains cannot produce dopamine, learned normally on a variety of tasks, such as conditioned place preference (Cannon and Palmiter, 2003; Hnasko et al., 2007; Hnasko et al., 2005; Robinson et al., 2005). From these studies, Robinson et al. (2005) concluded: "dopamine is not necessary for animals to learn to associate salient cues with rewards"…but it "is necessary for reward-related cues to attain motivational significance".

It is important to acknowledge that dopamine clearly has other functions in the brain besides regulating Pavlovian incentive motivation, and that the studies described in this dissertation only address some of potentially many roles dopamine has in behavior. For example, dopamine is implicated in arousal, action selection, cognitive flexibility, and behavioral effort, particularly

during instrumental conditioning (Beeler et al., 2012; Cools, 2008; Day et al., 2010; Redgrave et al., 1999; Robbins and Everitt, 1992; Salamone and Correa, 2012; Salamone et al., 2007; Wassum et al., 2012). I should note, though, that even for instrumental behaviors, dopamine can modulate responding by scaling performance vigor, or by regulating PIT effects (see below), independent of learning (Cagniard et al., 2006; Yin et al., 2006). Also, I have focused mostly on discussion of appetitive cue processing, but dopamine is also involved in processing aversive motivational states (Badrinarayan et al., 2012; Faure et al., 2008; Kapur et al., 2005; Oleson et al., 2012; Pezze and Feldon, 2004; Pezze et al., 2001; Richard and Berridge, 2011; Roitman et al., 2008).

Additionally, while I have emphasized dopamine signaling from the VTA to nucleus accumbens, midbrain dopamine neurons in the VTA, as well as substantia nigra, project to a variety of regions outside of the ventral striatum, such as the dorsal striatum, amygdala, prefrontal cortex, and hippocampus, and different dopamine neurons have different activity patterns and functions (Bromberg-Martin et al., 2010; Fields et al., 2007; Lammel et al., 2011; Lammel et al., 2012; Li et al., 2012; Margolis et al., 2006; Watabe-Uchida et al., 2012; Witten et al., 2011). Finally, dopamine is but one of many neurotransmitters systems involved in reward-related processes, and even in mediating the incentive motivational properties of reward cues (Bakshi and Kelley, 1993; Berridge, 2012; Cardinal et al., 2002a; Difeliceantonio and Berridge, 2012; Kelley et al., 2002; Mahler and Berridge, 2009; Novak et al., 2010; O'Connor et al., 2010; Puglisi-Allegra and Ventura, 2012; Smith et al., 2010; Ventura et al., 2007; Wassum et al., 2009). Thus in future research it will be necessary to fully investigate the contribution of these other systems, such as glutamate and endogenous opioids, in individual differences in reward cue processing, as well as their interactions with dopamine.

Sign trackers preferentially assign motivational value to discrete drug cues

Based on our recent studies (Flagel et al., 2009; Flagel et al., 2007; Robinson and Flagel, 2009), suggesting that for STs, a discrete food cue became attractive and served as a more robust conditioned reinforcer, in chapter 3 of this dissertation (Saunders and Robinson, 2010). I began to assess whether the variation in responsivity to a discrete food cue STs and GTs exhibit during Pavlovian training predicts variation in the ability of a discrete cocaine cue to maintain and reinstate drug seeking.

I trained STs and GTs to self administer cocaine, in sessions where a discrete visual cue was explicitly paired with drug infusions. Following the acquisition of stable self-administration, upon cue removal, there was a dramatic reduction in the rate of self administration of STs but not GTs, suggesting that the cocaine cue had acquired considerable motivational power of its own, but only in STs. Further evidence for such differences came in a follow-up experiment, where I found that STs also made more cocaine-seeking responses, under extinction conditions, for presentations of the discrete cocaine cue. This suggests that, as with a discrete food cue, a discrete cocaine cue serves as a more robust conditioned reinforcer for STs.

Consistent with my finding, Yager and Robinson (2012) found that a cocaine cue acquired greater ability to reinstate drug seeking behavior following extinction in STs than GTs, even if it was only ever paired with cocaine in separate Pavlovian conditioning sessions. Yager and Robinson also found that STs developed more robust conditioned approach behavior to a discrete cue that had been paired with noncontingent intravenous cocaine infusions compared to GTs. This result directly parallels the discrete cue-directed CRs that STs tend to make during Pavlovian training with a food cue. Furthermore, using a conditioned cue preference procedure, Meyer et al. (2012b) showed that STs preferred a tactile cue that had been paired with cocaine

injections to one paired with saline, while GTs did not. Importantly, in all of these experiments, both total drug intake and cue exposure were held equivalent across groups. Additionally, using these controlled procedures (e.g., Saunders and Robinson, 2010), I found STs and GTs acquired self administration behavior equally well, providing further evidence that any behavioral differences were not a reflection of differences in the learned predictive strength of the cues. I should note, however, one recent study reported that when total drug intake was not limited by the experimenter, rats that preferentially exhibit sign-tracking responses acquired self administration at a faster rate than rats that preferentially goal-track (Beckmann et al., 2011). However, this effect was found only at low doses and using a self-administration training procedure that is a modified version of Pavlovian approach training, so there is as yet no clear evidence that STs and GTs acquire drug self administration differently.

As noted above, experiments utilizing the traditional extinction-reinstatement procedure (e.g., Saunders and Robinson, 2010; Shaham et al., 2003) are thought to primarily assess the conditioned reinforcing properties of drug cues, given that animals respond to produce the cue. It is also possible, however, that behavior during such reinstatement tests is also maintained in part by a cue-evoked conditioned motivational state (Milton and Everitt, 2010). After the initial cue presentation, it is unknown if the cue promotes subsequent responses because it reinforced the preceding response, or if it evoked conditioned motivation. I addressed this in chapter 4.

Novel procedures to model drug "craving" in rodents

In chapter 4, I utilized a novel procedure to examine an individual variation in a discrete cocaine cue's ability to evoke drug seeking by producing a conditioned motivational state, as well as the role of accumbens core dopamine in this effect.

Drug-associated cues, as described above, produce relapse via multiple psychological mechanisms (Milton and Everitt, 2010). In this chapter, I wanted to directly explore relapse via cue-evoked conditioned motivation (states of "wanting" or "craving"; Berridge, 2007). To do this, I developed a novel procedure, based on a recent paper by Cooper et al. (2007). I trained STs and GTs to self administer cocaine, where a discrete visual cue was paired with drug infusions, and then, instead of extinction training, an aversive consequence (footshock) to drug seeking was introduced to eliminate self-administration behavior. When the consequences were high enough that responding fell to a low level, I assessed the ability of the cocaine cue to instigate drug seeking by presenting it noncontingently, under extinction conditions, but with the electric barrier still in place. This spurred more robust seeking behavior in STs than GTs. Thus, both cocaine (see below; Saunders and Robinson, 2011b) and discrete cocaine cues produce a state of conditioned motivation to a greater extent in some rats, and this motivational state is powerful enough to evoke drug-seeking behavior, even overcoming aversive consequences. The experiments in chapter 4 extend a recent study by Leblanc et al. (2012), who demonstrated that a discrete cocaine cue can invigorate *ongoing* cocaine-seeking behavior. Our results suggest that the cue itself can generate intense conditioned motivation, even in the absence of drug availability.

These studies represent some of the first successful attempts to model relapse in rodents using procedures other than the traditional extinction-reinstatement paradigm (Shaham et al., 2003). My experiment incorporated two important procedural distinctions. First, cocaine seeking was evoked, or instigated, by the noncontingent presentation of a discrete cocaine cue. This is important, given that in real addicts, relapse is often instigated by the incidental appearance of drug-associated stimuli. Additionally, in my study, rats were not extinguished, but rather

abstained from cocaine seeking when faced with escalating aversive costs. This better approximates the reasons that addicts discontinue drug use, which often occurs when they are faced with unacceptable physical or social costs (Epstein and Preston, 2003; Wallace, 1999). Furthermore, extinction training, relative to abstinence alone, alters the brain in ways that are relevant to relapse (Fuchs et al., 2006).

I next followed up on this behavioral study, training another group of rats on the same escalating adverse consequences procedure. I then injected these rats with drugs to either block or facilitate dopamine transmission within the nucleus accumbens core. I found that antagonism of dopamine signaling, via flupenthixol administration, blocked cue-evoked drug seeking, while potentiation of dopamine transmission, via amphetamine administration, increased cue-evoked seeking responses. This parallels a recent finding that the magnitude of cue-evoked dopamine release within the nucleus accumbens core is significantly correlated with intensity of sign-tracking responses rats made toward a discrete cocaine cue (Aragona et al., 2009). Thus, it is likely that accumbens core dopamine transmission has a direct role in discrete cue-evoked drug seeking.

These results from chapter 4 are in accordance with a series of studies that further suggest a causal role for dopamine signaling in incentive motivation for reward cues. For example, potentiation of dopamine release increases sign-tracking behavior (Hitchcott et al., 1997; Holden and Peoples, 2010; Phillips et al., 2003a; but see Simon et al., 2009), but not goal-tracking behavior (Doremus-Fitzwater and Spear, 2011), and also increases the conditioned reinforcing effects of food and drug-associated cues (Collins et al., 2012; Hill, 1970; Kelley and Delfs, 1991; Robbins, 1975, 1976; Taylor and Robbins, 1984). Additionally, injection of amphetamine increases the ability of a discrete Pavlovian cue to spur ongoing food-seeking behavior, as

measured by a general PIT procedure (Wyvell and Berridge, 2001). This is consistent with reports that administration of dopamine receptor antagonists suppresses general PIT effects (Dickinson et al., 2000; Ostlund and Maidment, 2012; Smith and Dickinson, 1998; Wassum et al., 2011), suggesting that dopamine signaling is necessary for Pavlovian CSs to invigorate instrumental responding. Dopamine appears to be somewhat less important for the outcome-selective version of PIT. For example, Yin et al. (2006) found that genetically-modified "hyperdopaminergic" mice failed to show elevated outcome-specific PIT, relative to wild type control mice (see also, Shiflett, 2012). Furthermore, Ostlund and Maidment (2012) reported that dopamine antagonists did not influence the ability of CSs to bias action selection for a specific outcome. Dopamine's role in mediating the conditioned motivational effects of CSs may be relatively localized to the ventral striatum, however, as elimination of dopamine cells projecting to the dorsal striatum had no effect on either general or outcome-specific PIT (Pielock et al., 2011).

Internal states motivate cocaine seeking differentially in sign and goal trackers

In chapter 5 (Saunders and Robinson, 2011b), I investigated variation in the motivational properties of cocaine in STs and GTs.

Internal subjective states associated with drug intake are clearly critical in the initial phases of drug taking, but they also acquire conditioned motivational properties that become important for the maintenance of drug seeking, and relapse (Jaffe et al., 1989; Stewart et al., 1984). When rats trained to self administer cocaine in the absence of any explicitly paired cues were tested on a progressive ratio schedule, with the goal of assessing motivation to obtain cocaine itself, STs made more drug seeking responses, reaching higher "breakpoints" that GTs. Thus, even though STs and GTs showed no initial differences in self-administration behavior, at least under these

restricted conditions, for STs, the conditioned effects of cocaine motivated more persistent drug seeking. Notably, this is one of the few instances in which I removed the stringent infusion criterion that was in place throughout these studies and let rats respond for cocaine without limits. While STs did reach significantly higher breakpoints, given the quickly escalating response requirements of the PR schedule, this only resulted in a few extra infusions, relative to GTs. This does suggest, however, that once STs have acquired stable self-administration, they may be more prone to escalating drug intake, relative to GTs. This can be addressed in future studies using long/extended access self-administration paradigms (Ahmed and Koob, 1998; Vanderschuren and Everitt, 2004; Wakabayashi et al., 2010).

In the second part of this experiment, I extinguished drug-seeking responses and then gave rats a "priming" injection of cocaine before an additional test day conducted under extinction conditions. This reinstated robust drug seeking behavior in STs, but relatively less behavior in GTs. Similar to the progressive ratio effect, this indicated that the conditioned interoceptive cues associated with cocaine generated greater conditioned motivation in STs. Combined with the data in chapter 4, it appears that both discrete, localizable drug stimuli and interoceptive drug stimuli acquire greater conditioned motivational value in STs. It is somewhat unclear why these cue types both produce greater conditioned motivation in STs, but contextual cues (see below) produce greater conditioned motivation in GTs. One commonality between the light cue I used in chapter 4 and the internal drug cues in chapter 5 is that they both have discrete elements, either physically (i.e., a circular light) and/or temporally (i.e., a limited time course of drug action).

Drug contexts differentially motivate sign and goal tracker behavior

In chapter 6 I investigated ST and GT responses to static, contextual cues - that are neither physically nor temporally discrete. Contextual information is thought to be a consolidation of the

separate elements of a US-associated environment into a configural representation that is qualitatively different from representations of discrete and interoceptive cues (Fanselow, 2010; Holland and Bouton, 1999). In chapter 6, I asked whether a cocaine-associated context acquires different motivational value in STs and GTs.

First, I found that GTs exhibited greater conditioned hyperactivity when placed into a context that had been previously associated with experimenter-delivered injections of cocaine. Drug-conditioned locomotor behavior is thought to reflect activation of conditioned motivation for the drug, but given that there is no opportunity for the animal to work for drug, or to approach and engage a discrete localizable cue, it is expressed as general hyperactivity (Beninger et al., 1981; Jones and Robbins, 1992; Robinson and Berridge, 1993). Though I did not assess the dopamine dependence of the conditioned hyperactivity effect reported in this chapter, there is reasonable evidence that the ability of drug-associated contexts to induce hyperactivity requires dopamine signaling. For example, Gold et al. (1988) found that repeated amphetamine injections in a specific context resulted in conditioned locomotion on a test day when rats received saline injections, but if dopamine cells projecting to the nucleus accumbens were selectively lesioned via infusion of 6-hydroxydopamine (6-OHDA), this conditioned hyperactivity was abolished, even though rats still showed acute unconditioned locomotion in response to amphetamine. The effect appears to be relatively specific to dopamine within the accumbens, as dopamine depletions in the caudate putamen and prefrontal cortex fail to block conditioned hyperactivity (Jones and Robbins, 1992). This suggests that dopamine in the accumbens is involved not only in the acute psychomotor response to stimulant drugs, but also in attributing the drug-associated context with motivational value. Therefore, the conditioned hyperactivity expressed by GTs in a

cocaine context may be interpreted as reflecting the dopamine-dependent incentive motivational influence of the context.

In a separate experiment, I found that GTs, when returned to a context where they had learned to self administer cocaine, following extinction in a novel context, renewed their cocaine-seeking behavior significantly more than STs. Notably, this result and the conditioned hyperactivity effect described above were the first examples of a reward cue-related behavioral effect that was larger in GTs. In accordance with this, we recently found, using a fear conditioning paradigm, that GTs show greater contextual conditioned fear than STs, but STs showed greater discrete cue-induced fear (Morrow et al., 2011). Together with my study, this raises the interesting possibility that STs and GTs preferentially assign incentive motivational value to discrete and contextual information, respectively, associated with emotional events, and regardless of the valence of the outcome.

It is unclear how STs and GTs might differ in a procedure like those used in most other drug context experiments (e.g., Bossert et al., 2004; Bossert et al., 2007; Bossert et al., 2009), where the context serves as an occasion setter (Crombag et al., 2008; Holland, 1992). Critically in these studies, discrete cues are associated with drug-seeking actions at all phases of the experiment, but during extinction, the context is changed. Rats learn that the extinction context means the discrete cues are no longer associated with drug. Then, when returned to the drug context, the contextual information indirectly drives behavior by reestablishing the conditioned reinforcing properties of the discrete cue. Several studies have shown that antagonism of dopamine receptors blocks reinstatement under these conditions (Bossert et al., 2007; Bossert et al., 2009; Crombag et al., 2002). Given that the procedure I used was different from these studies, in that no discrete cues were used (Fuchs et al., 2005), it is possible that under these conditions context-induced

cocaine seeking would not be dependent on dopamine. To test this, I replicated the context renewal study, but also administered flupenthixol into the nucleus accumbens core of some rats just prior to the renewal test. This manipulation blocked the context-induced cocaine seeking in GTs. Given that no discrete cues are present, the context itself may acquire Pavlovian incentive motivational properties that directly drive drug seeking (Crombag et al., 2008; Fuchs et al., 2005). Several studies, outlined here, suggest that dopamine signaling, particularly within the accumbens core, is necessary for reward cues to evoke conditioned motivational states that spur on actions. Thus, it is possible that the context-induced reinstatement in GTs I found in chapter 6 is dependent on dopamine specifically because I utilized a procedure that resulted in the context itself acquiring Pavlovian incentive motivational value, similar to how discrete cues did for STs in other experiments.

What psychological processes account for sign and goal tracker variation?

Taken together, the studies in my dissertation, and related ones from our laboratory, clearly show that reward-associated stimuli are processed very differently across different individuals. We find that behavioral differences on a simple Pavlovian conditioning procedure predict differences in responsivity to an assortment of food and drug-associated cues, in a variety of experimental paradigms. This suggests that the differences we see between STs and GTs are not likely to be an artifact of one particular procedure, but reflect real underlying psychological and neurobiological diversity. While we do not have a clear understanding of the sources of that variability, it is worth discussing some potential options.

First, however, I should point out that the psychological processes that govern the acquisition and/or expression of ST and GT conditioned approach – the behavioral measure we use to define them – and processes that may govern the behavior of STs and GTs in other tasks described in

this dissertation may or may not be the same. There need not be a unitary ST and GT psychological substrate, and it is likely that they utilize different psychological strategies in different reward-seeking situations.

Berridge and colleagues (Difeliceantonio and Berridge, 2012; Mahler and Berridge, 2009) have suggested that a more or less equivalent psychological process governs the behavior exhibited by STs and GTs during Pavlovian training. They argue that the tendency to attribute Pavlovian incentive motivational value to cues is equal in STs and GTs, the difference is onto which cues that value is attributed. STs, as we have suggested, come to find the lever cue attractive and desirable, which drives their approach towards it. GTs, conversely, attribute incentive salience equally, but to the food hopper instead – specifically, in the case of the Berridge studies – the dish where food is delivered. This hypothesis stems from studies demonstrating that pharmacological amplification of mu-opioid receptor signaling within the central amygdala enhances behaviors that occur once a rat has completed an approach CR, such number of nibbles and sniffs of either the lever, or the food dish while the lever is extended, and it does so similarly in STs and GTs (Difeliceantonio and Berridge, 2012; Mahler and Berridge, 2009). It is somewhat difficult to relate these studies to ours, due to differences in what behaviors are measured. Given that the data are based on hand-scored video of behavior that occurs once an approach CR has been made, is unclear in these studies whether or not the probability of actually making an approach CR itself – walking to the lever or food dish – is affected by opioid manipulations. For example, in the study by Mahler and Berridge (2009), mu-opioid receptor stimulation actually decreased computer-scored lever presses among sign trackers, which could be a result of a reduction in the probability of lever approach and/or and alteration of the topography of behavior once the lever had been approached. In a follow-up study, however,

Difeliceantonio and Berridge (2012) reported that mu opioid stimulation decreased the latency to make a contact with either the lever, or food dish, suggesting that this manipulation may enhance the vigor of approach behavior regardless of phenotype. We have shown that making a sign-tracking approach CR, but not a goal-tracking CR, is dependent on dopamine signaling (Flagel et al., 2011b; Saunders and Robinson, 2012). To this extent, at least, the neural systems responsible for getting STs and GTs to the location they end up approaching are different, suggesting that the psychological process and elicits approach may also be different. Once a rat has made his preferred CR, however, it is clear that the more "consummatory" terminal behaviors (e.g., grabbing, biting, sniffing, etc) are regulated by similar neural substrates (e.g., endogenous opioids) in STs and GTs, and thus are likely to be more similar from a psychological perspective, perhaps reflecting an incentive salience "wanting" mechanism.

Thus, we cannot rule out the possibility that the food dish has incentive motivational properties for GTs that are functionally similar to those STs appear to have for the lever. Future studies could clarify this. For example, it is unknown if GTs will respond in order to receive presentations of the food dish itself, which would be the complementary study to the lever conditioned reinforcement test that favors STs (Robinson and Flagel, 2009). As a final consideration, Difeliceantonio and Berridge (2012) have suggested more recently that similarities between STs and GTs may not exist under baseline testing conditions but surface only when internal motivational state is dramatically altered, such as under food deprivation or pharmacological activation. Indeed, under baseline conditions, we see low levels of physical interaction with the food dish in GTs (P. Meyer, unpublished observations).

The context results from chapter 6, along with other studies, suggest that in at least some circumstances, goal-tracker behavior is under the control of contextual information to a greater

degree than is sign-tracker behavior. At this point, however, we can only speculate whether goal-tracking CRs themselves are under significant contextual control. Interestingly, during Pavlovian training, GTs, though they clearly discriminate between discrete CS and non-CS periods (i.e., they respond in the food hopper at a much higher rate when the CS is present), tend to enter the food hopper overall more than STs during the intertrial interval (Meyer et al., 2012a). This might be evidence that the context of the chamber, which includes the food hopper, acquires conditioned motivational properties that drive GT responding independent of discrete cue presentations. However, we do know that intertrial food hopper entries for STs and GTs are not affected by antagonism of dopamine receptors in the accumbens core (Saunders and Robinson, 2012). To the extent that the ability of cues to evoke conditioned motivational states is dependent on dopamine signaling in the accumbens (see above), this interpretation is somewhat inconsistent with my findings in chapter 6, where core dopamine blockade prevented context-induced reinstatement in GTs. Given the differences in the experimental setting between these studies, however, we can potentially explain this discrepancy. In the Pavlovian training procedure, the context more likely serves as an occasion setter (Holland, 1992), indirectly modulating the effects of the discrete lever cue, while in the reinstatement paradigm, given that no discrete cues are present, the context may acquire Pavlovian incentive motivational properties that directly drive drug seeking (Crombag et al., 2008; Fuchs et al., 2005). These different psychological mechanisms of context-regulated behavior are subserved by distinct neural mechanisms (see below; Holland and Bouton, 1999).

Alternatively, contextual control of goal-tracking CRs may not depend on dopamine signaling, or may be controlled by dopamine signaling in areas outside the accumbens core (Flagel et al., 2011b; Saunders and Robinson, 2012). A final possibility is that goal-tracking CRs

are not under significant contextual control. This can be tested in future experiments by introducing context shifts during Pavlovian training. If goal-tracking behaviors are mediated by an occasion setting mechanism, for example, we would expect rats would exhibit attenuated goal tracking immediately after a context shift, and would have to relearn the CR in the new context.

We have previously speculated (Meyer et al., 2012a; Saunders and Robinson, 2012) that something akin to a cognitive reward expectancy process (Bindra, 1978; Dickinson and Balleine, 1994; Toates, 1986) may mediate goal-tracking CRs. If presentation of the CS evokes an explicit cognitive representation of the outcome (US), this could result in goal-directed approach to the food cup to await delivery of the expected reward. Goal-directed instrumental behavior governed by explicit cognitive expectations ("instrumental incentives") does not require dopamine (Dickinson et al., 2000; Lex and Hauber; Wassum et al., 2011; Yin et al., 2006), but instead may depend on endogenous opioid signaling (Wassum et al., 2009), which has also been implicated in some aspects of goal-tracking behavior, as described above (Difeliceantonio and Berridge, 2012; Mahler and Berridge, 2009). Therefore, the goal-directed approach seen here may be akin to behavior governed by an act-outcome association, which is thought to be dependent more on corticostriatal than mesolimbic circuits (Balleine and Dickinson, 1998; Daw et al., 2005).

In light of the results from my context studies, however, we can potentially refine this interpretation somewhat. GTs may use so-called 'model-based' forms of learning (Daw et al., 2005; Dayan and Balleine, 2002; Glascher et al., 2010; Tolman, 1948), which involve the formation of a kind of "cognitive map" of the environment that includes information about how specific events are related to one another (McDannald et al., 2012). Importantly, contextual information is an integral part of such representations (Nadel and Willner, 1980; Redish, 1999). In the Pavlovian setting in our experiments, contextual information may be integrated into the

"model space", such that when a GT enters the training context, it calls up specific representations of the outcomes associated with discrete cue presentation, and with the action of entering the magazine.

In summary, the psychological mechanisms of individual variation described in this dissertation are quite complex. Under some conditions, STs and GTs likely utilize dopamine-dependent Pavlovian incentive motivational processes to guide their reward-seeking behavior, but under others, differing processes may be engaged. Examination of intermediate rats, that exhibit a mix of sign and goal-tracking CRs, suggests that the psychological processes governing approach may switch on a trial-to-trial basis (Saunders and Robinson, 2012). Thus, it is likely that considerable variation also exists *within* individuals, in addition to *across* individuals. Many future studies are necessary to fully understand what these processes governing these behaviors are, the particular circumstances under which they diverge, and when they converge.

What neural circuitry underlies sign and goal-tracker phenotypes?

We have only just begun to parse the neural systems that 1) are involved in the acquisition and performance of sign and goal-tracker CRs and 2) contribute to the behavioral differences predicted by variation in approach CRs. One study recently investigated, at least indirectly, cue-induced neural activity of STs and GTs. Flagel et al. (2011a) measured c-fos mRNA expression – an indirect measure of neuronal activation – in the brains of STs and GTs following exposure to the same lever cue they had experienced during Pavlovian training (in this case, under extinction conditions). This cue exposure produced significant increases in c-fos mRNA expression in the nucleus accumbens core and shell, dorsal striatum, lateral habenula, lateral septum, OFC, and the paraventricular, mediodorsal, and central medial nuclei of the thalamus in STs, relative to rats who received an equivalent number of unpaired presentations of the CS and

US. Interestingly, in GTs c-fos mRNA expression in these regions was not different from unpaired control rats, even though for GTs the cue was an effective CS. This suggests that the acquisition of predictive value, via Pavlovian conditioning, is not sufficient for a discrete, localizable CS to significantly "engage" traditional brain reward systems.

Notably, however, in Flagel et al. (2011a), on the cue exposure day rats were only presented with the lever cue – the food hopper was removed from the chamber. Thus, this design did not allow for an assessment of c-fos expression in response to food hopper exposure. It is possible that in GTs, experience with the hopper (or the hopper in combination with the lever) would be sufficient to activate at least some of the brain regions examined in Flagel et al. (2011a). Additionally, Flagel et al. (2011a) did not examine c-fos expression in the hippocampus and related regions known to be important for processing contextual information (Holland and Bouton, 1999). In light of the context conditioning results from chapter 6, and those of Morrow et al. (2011), we might expect that exposure to the Pavlovian training context, in the absence of any discrete cues, would be sufficient to induce c-fos activity in these regions selectively in GTs. One possible difference in the way GT and ST brains process emotional information is that in GTs, hippocampal circuits are preferentially engaged, while in STs, the mesocorticolimbic circuitry described above is predominately engaged.

Therefore, in general, it will be important to assess the role of neural circuitry involved in contextual information processing in variation across STs and GTs. The hippocampus is critically involved in contextual conditioning, and its connectivity allows it to regulate key structures involved in motivated behavior. Importantly, the hippocampus is positioned to regulate dopamine signaling throughout the forebrain via direct modulation of dopamine terminal regions in the PFC, BLA, and nucleus accumbens core and shell, or indirectly, via

projections onto the VTA (Cardinal et al., 2002a; Floresco et al., 2001; Holland and Bouton, 1999; Lodge and Grace, 2005; Maren, 2011). These hippocampal output pathways have different functional roles (Corbit and Balleine, 2000; Fanselow, 2010; Fuchs et al., 2005; Gruber and McDonald, 2012; Holland and Bouton, 1999; Ito et al., 2005; Luo et al., 2011). The degree to which a given behavior is dopamine dependent in STs and GTs may depend on which of these systems is significantly engaged.

We have some evidence that the mesolimbic dopaminergic systems of STs and GTs are different, though the relevance of these differences for studies in this dissertation is unclear. In striatum, STs have higher levels of mRNA for the D1 dopamine receptor, and lower levels of dopamine transporter (DAT) mRNA, than GTs, which has the functional consequence of greater dopamine receptor activation (Flagel et al., 2007). Other studies, using selectively bred rats, have shown that within the nucleus accumbens core STs have more spontaneous dopamine release events ("transients"), and have a greater number of high affinity dopamine D2 receptors, relative to GTs (Flagel et al., 2010). These studies should be interpreted with caution, however, as selective breeding can result in many nonspecific genetic and neural distinctions.

Parallels between human and non-human animal studies

The overall goal of my dissertation has been to better understand individual variation in responsivity to reward-related cues, especially drug cues. Though it is often difficult to isolate particular psychological variables, there is extensive evidence that the processes I have described above in rodents that allow cues to guide adaptive reward-seeking behavior, but contribute to compulsive behaviors such as addiction, also exist in humans. In many cases, human data closely parallel the preclinical literature, suggesting that the individual differences we see in rodents can directly inform clinical investigations.

Humans find reward cues “attractive”, such that they receive greater perceptual and attentional resources, even outside volitional awareness (Hickey et al., 2010a; Hickey and van Zoest, 2012; Raymond and O'Brien, 2009). This is often measured by their ability to bias attention relative to neutral cues (Field and Cox, 2008). Interestingly, studies in humans demonstrate substantial individual variation in the degree to which discrete reward cues are allocated with visual and attentional resources. For example, Hickey et al. (2010b) found that individuals with “reward-seeking” personality characteristics, as measured by the Behavioral Inhibition/Activation Scale (Carver and White, 1994), showed greater reward-primed attentional bias for reward-associated visual stimuli. A few studies have even attempted to examine individual differences in discrete drug-cue approach tendencies in humans (Christiansen et al., 2012; Field et al., 2008; Field et al., 2005; Palfai, 2006; Thewissen et al., 2007; Van Gucht et al., 2008; Wiers et al., 2009). Direct measures of approach behavior are difficult to examine in people, so investigators have developed experimental paradigms that allow for approach to be inferred. For example, Field et al. (2005) found that individuals with high levels of alcohol craving had more pronounced “approach” to alcohol-related pictures, as measured by the speed at which they moved a character on a computer screen towards or away from the pictures. Wiers et al. (2009) found a similar relationship between alcohol drinking history and the tendency to use a computer joystick to “approach” alcohol-related images on a screen.

The degree to which humans find drug cues attractive, as measured by their ability to bias attention, relative to neutral cues, predicts subjective craving for drugs, prospective drug use, and likelihood of relapse (Cox et al., 2002; Field and Cox, 2008; Franken et al., 2000; Marissen et al., 2006; Simon et al., 2010; Waters et al., 2003). For example, Field and Eastwood (2005) found that when subjects were experimentally manipulated into exhibiting greater attentional bias to

discrete alcohol cues, they experienced greater subjective craving, and drank more alcohol later during a taste test. By training subjects to exhibit less attentional bias to alcohol cues, Fadardi and Cox (2009) reduced their subsequent alcohol consumption. Similarly, Attwood et al. (2008) found that smokers could be trained to show more or less attentional bias, and the degree of bias was positively associated with subjective craving. These studies suggest there is a direct correlation between the extent that a drug cue is attractive and attention grabbing and its ability to spur motivation to take drugs.

Additionally, a large number of studies have demonstrated that drug cue-induced craving is positively correlated with intensity of abuse history and/or future intake, and likelihood of relapse (for review, see Carter and Tiffany, 1999; Tiffany and Wray, 2012), though the relationship between craving and subsequent drug use is somewhat controversial, as some studies have also demonstrated weak or insignificant correlations between cue-induced craving and drug-related behaviors. Most of these studies, however, measure craving in the laboratory, where the context has never been associated with drug use, and thus may not be conducive to the generation of robust craving (Vezina and Leyton, 2009). Interestingly, recent studies have examined the relationship between craving and drug use in the addict's "natural environment". For example, Epstein et al. (2009) monitored use of cocaine and heroin in outpatient subjects, using an ecological momentary assessment method (Stone and Shiffman, 1994), where subjects themselves reported real-time behavioral and subjective data on handheld electronic devices. They found that cocaine use was predicted by a variety of antecedent "triggers", such as seeing the drug, or being reminded of drug use. Addicts who used more cocaine reported the most intense craving associated with these triggers (though this relationship was less clear for heroin use). Similar positive predictive associations between reported cue-induced craving and

subsequent real-life drug use have been found in other studies (Epstein et al., 2010; Preston et al., 2009; Shiffman, 2009; Shiffman et al., 2002).

Many of these studies demonstrate the considerable individual variation in the ability of drugs and drug cues to bias attention, produce craving, and instigate relapse in humans (Abrams et al., 1988; Carpenter et al., 2009; Carter and Tiffany, 1999; de Wit et al., 1986; de Wit et al., 1987; Kirk and de Wit, 2000; Lloyd and Salzberg, 1975; Niaura et al., 1998; Payne et al., 2006). Indeed, there is growing evidence that some humans are more reactive to certain types of cues, in that they bias attention and/or elicit craving for the reward to a greater extent. For example, Mahler and de Wit (2010) examined food and cigarette craving in a group of smokers. They found that those individuals that showed the highest craving in response to discrete food cues, when hungry, also showed the highest craving to discrete smoking cues, after a period of abstinence. Similar results were found in a more recent study (Styn et al., In press). This individual variation parallels that found in some of the studies with rats I described in this dissertation (e.g., Saunders and Robinson, 2010; Saunders and Robinson, 2011b), suggesting that some humans may be more "cue reactive", prone to assigning high motivational salience to discrete cues in general, regardless of the type of reward they are associated with. It is important to point out, however, that while more and more studies are examining the role of cues in disorders such as addiction, the majority of these studies exclusively focus on discrete cues, such as images or drug paraphernalia, rather than drug-associated contextual information per se. One conclusion that can be drawn from my experiments is that "cue reactive" is not a unitary concept, and it is likely that different individuals will be susceptible to the motivational effects of different types of reward-associated stimuli.

Dopamine regulates drug-cue responsivity in humans

As I demonstrated above in rodent studies, dopamine signaling is critically involved the attribution and expression of incentive motivational properties to multiple types of reward cues, and substantial evidence from human addiction studies, including many by Nora Volkow and colleagues, suggests that brain dopamine systems also play a key role regulating in the motivational impact of drug-related stimuli (primarily discrete cues) in addicts (Ersche et al., 2010; Franken et al., 2005; Franken et al., 2004; Goldstein et al., 2009; Laruelle et al., 1995; Leyton et al., 2002; Volkow et al., 2006; Volkow et al., 1994; Volkow et al., 2008; Wong et al., 2006). Dopamine signaling is often measured by displacement of the dopamine D2 receptor radiolabeled raclopride using PET imaging. For example, Volkow et al. (2006) found that when cocaine addicts view images of cocaine use, dopamine signaling surged within the striatum (see also Wong et al., 2006) and the magnitude of cue-evoked dopamine release correlated with subjective craving. Similar striatal dopamine increases have also been shown in response to discrete amphetamine-associated stimuli (Boileau et al., 2007), as well as heroin cues (Zijlstra et al., 2008).

Dopamine signaling in humans also has a broad role in attentional processing of reward cues, including drug cues. Increases in dopamine transmission produce enhancements in performance on behavioral tasks that require selective attention to stimuli, while reductions in dopamine, via pharmacological manipulations, or as seen among Parkinsonian patients, results in selective attention deficits (Clark et al., 1987; Franken et al., 2005; Hickey et al., 2010a; Nieoullon, 2002; Servan-Schreiber et al., 1998; Stam et al., 1993). A few recent studies have assessed the role of dopamine in attentional bias specifically for drug-related cues. For example, Franken et al. (2004) found that administration of haloperidol, a dopamine receptor antagonist, reduced attentional bias to discrete heroin cues among heroin addicts. Reductions in drug-cue attentional

bias were also found in smokers following acute tyrosine/phenylalanine depletion (Hitsman et al., 2008; Munafo et al., 2007). Complementary to this, administration of dopamine agonists increases drug-cue attentional bias (e.g., Ersche et al., 2010).

Dopamine signaling may also serve to regulate the responses of other brain regions associated with attentional bias for discrete drug cues, as recently demonstrated by Luijten et al. (2012). They found that, among smokers, haloperidol administration normalized brain activity within ACC and dorsolateral PFC associated with attentional bias to smoking cues. After haloperidol administration, smoker's cue-induced brain activity was identical to non-smoker controls. Consistent with this, Hermann et al. (2006) found that administration of the dopamine receptor antagonist anisulpride reduced alcohol cue-induced brain activity in the ACC and OFC in alcoholics, such that they were no longer different from control subjects. Indeed, many brain regions that receive dopaminergic innervation, such as the ACC, PFC, ventral striatum, and amygdala are implicated in attentional bias for drug-related cues (Ersche et al., 2010; Hester and Garavan, 2009; Janes et al., 2010; Luijten et al., 2012; Luijten et al., 2011), and striatal dopamine signaling, particularly in the ventral striatum, has been suggested to serve as an interface between so called "bottom-up" incentive motivational processes and "top-down" cognitive control of behavior (Aarts et al., 2010; Cools, 2008). Thus, it is possible that dopamine is involved in both the formation of attentional bias for drug cues, by "marking" them with incentive salience, and also in the maintenance of that bias, in part by regulating drug-cue detection that occurs in other brain regions. This has yet to be directly tested, however, and as Luijten et al. (2012) state, it will be important to "examine whether individual differences in dopaminergic activation…are associated with differences in attentional bias-related brain activation". Given that largely overlapping brain circuits are involved in the detection and processing of cues associated with

several classes of drugs (Kalivas and Volkow, 2005; Kuhn and Gallinat, 2011), dopamine likely has a fundamental role in drug-cue processing in humans.

The interaction between dopamine systems and other brain regions is complex, and not unidirectional. Dopamine systems, including the VTA, as well as its target regions, are also under regulation from fronto-cortical regions (Parikh and Sarter, 2008; Phillips et al., 2008; Takahashi et al., 2011; Volkow et al., 2005; Volkow et al., 2007). An extensive literature has implicated abnormal activity in frontal cortical brain systems in addiction-like behaviors (Bolla et al., 2004; Bolla et al., 2003; Feil et al., 2010; Goto et al., 2010; Hester and Garavan, 2004; Kalivas and Volkow, 2005; Lucantonio et al., 2012). It remains unclear, however, the extent that dysfunction within frontal cortical circuits seen in addicts is a cause or consequence of long-term drug use, and, of course, both could be true. Further research is needed to better understand how variation in frontal cortical activity may interact with dopaminergic variation to underlie cue-controlled maladaptive reward seeking.

Implications for treatment: a focus on individual differences

My results suggest that different individuals come to preferentially assign motivational value to different types of drug-related information. Specifically, some may be more motivated by discrete localizable drug cues, others by drug contexts. In either case, the presence of the type of cue that an individual is vulnerable to may facilitate maladaptive behavior, such as relapse. Thus, there are likely multiple susceptibility states across individuals, and as such, multiple "pathways" to addiction and related disorders. In a sense, the threat of addiction is a constantly moving target, where the particular factors that contribute to one individual's disease diverge from the next. This provides a somewhat daunting task for clinicians as they develop new treatment regiments, but one hope is that lessons from preclinical studies, like the ones described here, can

provide a guiding framework. In the development of future interventions, it may be useful for clinicians to consider 1) individual differences in the psychological factors that control pathological motivation for rewards, and 2) in susceptible individuals different types of drug cues may be especially insidious in instigating and maintaining drug-seeking behavior.

Some recent studies suggest potentially effective cue-based treatment strategies. For example, manipulating attentional bias to discrete drug cues via attentional control therapies may be an effective method for reducing some of the behavioral control those cues have over addicts (Attwood et al., 2008; Fadardi and Cox, 2009; Schoenmakers et al., 2010). These studies demonstrate that by training addicts to explicitly avoid paying attention to drug cues, or by extensively extinguishing drug cues by presenting them repeatedly and in different contexts without drug exposure, a cue's relapse-provoking abilities may be lessened. Additionally, drug cue "reappraisal" procedures, where subjects are instructed to reinterpret the meaning of a cue, to render it less motivationally salient, may be effective at reducing cue-induced craving (Zhao et al., 2012). These studies suggest that addicts may be able to exert some cognitive control over cue-induced urges, but important concerns exist over how generalizable and enduring such behavioral therapies are. The results of my studies suggest any one of these treatments will not be effective on every addict, given that the varying factors that spur their relapse behavior. Thus, a careful consideration of variation from subject to subject will be important for future development of targeted, patient-tailored interventions.

Conclusions

Given the enormous influence reward-related stimuli have on nearly every aspect of animal behavior, it is of great importance to understand the psychological and biological mechanisms by which emotional information in the environment gets translated into motivation, in addition to

the circumstances that result in the dramatic variation described in the studies above. Many questions remain about what appears to be the existence of different cue-processing "strategies", such as how they develop, and how they may evolve over time. It will require a synthesis of multiple levels of analysis, incorporating genetic, epigenetic, neurophysiological, and neural-systems-level investigations, to answer these questions. As answers begin to reveal themselves, we will understand a lot more about how the brain generates normal motivation, as well as psychopathology.

References

Aarts, E., Roelofs, A., Franke, B., Rijpkema, M., Fernandez, G., Helmich, R.C., Cools, R., 2010. Striatal dopamine mediates the interface between motivational and cognitive control in humans: evidence from genetic imaging. Neuropsychopharmacology 35, 1943-1951.

Abrams, D.B., Monti, P.M., Carey, K.B., Pinto, R.P., Jacobus, S.I., 1988. Reactivity to smoking cues and relapse: two studies of discriminant validity. Behav Res Ther 26, 225-233.

Ahmed, S.H., Koob, G.F., 1998. Transition from moderate to excessive drug intake: change in hedonic set point. Science 282, 298-300.

Anthony, J.C., Warner, L.A., Kessler, R.C., 1994. Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: Basic findings from the National Comorbidity Survey. Experimental and Clinical Psychopharmacology 2, 244-268.

Aragona, B.J., Day, J.J., Roitman, M.F., Cleaveland, N.A., Wightman, R.M., Carelli, R.M., 2009. Regional specificity in the real-time development of phasic dopamine transmission patterns during acquisition of a cue-cocaine association in rats. Eur J Neurosci 30, 1889-1899.

Arroyo, M., Markou, A., Robbins, T.W., Everitt, B.J., 1998. Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. Psychopharmacology 140, 331-344.

Attwood, A.S., O'Sullivan, H., Leonards, U., Mackintosh, B., Munafo, M.R., 2008. Attentional bias training and cue reactivity in cigarette smokers. Addiction 103, 1875-1882.

Badiani, A., Belin, D., Epstein, D., Calu, D., Shaham, Y., 2011. Opiate versus psychostimulant addiction: the differences do matter. Nature Reviews Neuroscience 12, 685-700.

Badrinarayan, A., Wescott, S.A., Vander Weele, C.M., Saunders, B.T., Couturier, B.E., Maren, S., Aragona, B.J., 2012. Aversive stimuli differentially modulate real-time dopamine transmission dynamics with the nucleus accumbens core and shell. J Neurosci 32, 15779-15790.

Bakshi, V.P., Kelley, A.E., 1993. Feeding induced by opioid stimulation of the ventral striatum: role of opiate receptor subtypes. Journal of Pharmacology and Experimental Therapeutics 265, 1253-1260.

Balleine, B., 1994. Asymmetrical interactions between thirst and hunger in Pavlovian-instrumental transfer. Q J Exp Psychol B 47, 211-231.

Balleine, B.W., Dickinson, A., 1998. Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37, 407-419.

Barker, J.M., Torregrossa, M.M., Taylor, J.R., 2012. Low prefrontal PSA-NCAM confers risk for alcoholism-related behavior. Nat Neurosci doi:10.1038/nn.3194.

Bayer, H.M., Glimcher, P.W., 2005. Midbrain dopamine neurons encode a quantitative reward prediction error signal. Neuron 47, 129-141.

Beckmann, J.S., Marusich, J.A., Gipson, C.D., Bardo, M.T., 2011. Novelty seeking, incentive salience and acquisition of cocaine self-administration in the rat. Behav Brain Res 216, 159-165.

Beckstead, R.M., Domesick, V.B., Nauta, W.J.H., 1979. Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Research 175, 191-217.
Beeler, J.A., Frazier, C.R., Zhuang, X., 2012. Putting desire on a budget: dopamine and energy expenditure, reconciling reward and resources. Front Integr Neurosci 6, 49.
Belin, D., Everitt, B.J., 2008. Cocaine seeking habits depend upon doparnine-dependent serial connectivity linking the ventral with the dorsal striatum. Neuron 57, 432-441.
Belin, D., Jonkman, S., Dickinson, A., Robbins, T.W., Everitt, B.J., 2009. Parallel and interactive learning processes within the basal ganglia: Relevance for the understanding of addiction. Behavioural Brain Research 199, 89-102.
Beninger, R.J., Hanson, D.R., Phillips, A.G., 1981. The acquisition of responding with conditioned reinforcement: effects of cocaine, (+)-amphetamine and pipradrol. Br J Pharmacol 74, 149-154.
Berendse, H.W., Graaf, Y.G.-D., Groenewegen, H.J., 1992. Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. The Journal of Comparative Neurology 316, 314-347.
Berke, J.D., Hyman, S.E., 2000. Addiction, dopamine, and the molecular mechanisms of memory. Neuron 25, 515-532.
Berridge, K.C., 2001. Reward learning: Reinforcement, incentives, and expectations, in: Medin, D.L. (Ed.), Psychology of Learning and Motivation: Advances in Research and Theory. Academic Press, San Diego, pp. 223-278.
Berridge, K.C., 2007. The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology (Berl) 191, 391-431.
Berridge, K.C., 2012. From prediction error to incentive salience: mesolimbic computation of reward motivation. Eur J Neurosci 35, 1124-1143.
Berridge, K.C., Robinson, T.E., 1998. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 28, 309-369.
Berridge, K.C., Robinson, T.E., 2003. Parsing reward. Trends Neurosci 26, 507-513.
Bindra, D., 1968. Neuropsychological interpretation of the effects of drive and incentive-motivation on general activity and instrumental behavior. Psychological Review 75, 1-22.
Bindra, D., 1978. How adaptive behavior is produced: A perceptual-motivation alternative to response reinforcement. Behav Brain Sci 1, 41-91.
Blaiss, C.A., Janak, P.H., 2009. The nucleus accumbens core and shell are critical for the expression, but not the consolidation, of Pavlovian conditioned approach. Behav Brain Res 200, 22-32.
Boakes, R.A., 1977. Performance on learning to associate a stimulus with positive reinforcement, in: Davis, H., Hurwitz, H. (Eds.), Operant-Pavlovian Interactions. Earlbaum, Hillsdale, NJ, pp. 67-97.
Boileau, I., Dagher, A., Leyton, M., Welfeld, K., Booij, L., Diksic, M., Benkelfat, C., 2007. Conditioned dopamine release in humans: a positron emission tomography [11C]raclopride study with amphetamine. J Neurosci 27, 3998-4003.
Bolla, K., Ernst, M., Kiehl, K., Mouratidis, M., Eldreth, D., Contoreggi, C., Matochik, J., Kurian, V., Cadet, J., Kimes, A., Funderburk, F., London, E., 2004. Prefrontal cortical dysfunction in abstinent cocaine abusers. J Neuropsychiatry Clin Neurosci 16, 456-464.
Bolla, K.I., Eldreth, D.A., London, E.D., Kiehl, K.A., Mouratidis, M., Contoreggi, C., Matochik, J.A., Kurian, V., Cadet, J.L., Kimes, A.S., Funderburk, F.R., Ernst, M., 2003. Orbitofrontal

cortex dysfunction in abstinent cocaine abusers performing a decision-making task. Neuroimage 19, 1085-1094.
Bolles, R.C., 1972. Reinforcement, expectancy and learning. Psychol Rev 79, 394-409.
Bossert, J.M., Liu, S.Y., Lu, L., Shaham, Y., 2004. A role of ventral tegmental area glutamate in contextual cue-induced relapse to heroin seeking. The Journal of Neuroscience 24, 10726-10730.
Bossert, J.M., Poles, G.C., Wihbey, K.A., Koya, E., Shaham, Y., 2007. Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. The Journal of Neuroscience 27, 12655-12663.
Bossert, J.M., Wihbey, K.A., Pickens, C.L., Nair, S.G., Shaham, Y., 2009. Role of dopamine D-1-family receptors in dorsolateral striatum in context-induced reinstatement of heroin seeking in rats. Psychopharmacology 206, 51-60.
Bouton, M.E., 2002. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biol Psychiatry 52, 976-986.
Bouton, M.E., Bolles, R.C., 1979. Contextual control of the extinction of conditioned fear. Learning and Motivation 10, 445-466.
Bouton, M.E., Swartzentruber, D., 1991. Sources of relapse after extinction in Pavlovian and instumental learning. Clinical Psychology Review 11, 123-140.
Breland, K., Breland, M., 1961. The misbehavior of organisms. American Psychologist 16, 681-684.
Brog, J.S., Salyapongse, A., Deutch, A.Y., Zahm, D.S., 1993. The patterns of afferent innervation of the core and shell in the “Accumbens” part of the rat ventral striatum: Immunohistochemical detection of retrogradely transported fluoro-gold. The Journal of Comparative Neurology 338, 255-278.
Bromberg-Martin, E.S., Matsumoto, M., Hikosaka, O., 2010. Dopamine in motivational control: rewarding, aversive, and alerting. Neuron 68, 815-834.
Brown, P.L., Jenkins, H.M., 1968. Auto-shaping of the pigeon's key-peck. J Exp Anal Behav 11, 1-8.
Burke, K.A., Franz, T.M., Miller, D.N., Schoenbaum, G., 2007. Conditioned reinforcement can be mediated by either outcome-specific or general affective representations. Front Integr Neurosci 1, 2.
Burke, K.A., Franz, T.M., Miller, D.N., Schoenbaum, G., 2008. The role of the orbitofrontal cortex in the pursuit of happiness and more specific rewards. Nature 454, 340-344.
Burns, M., Domjan, M., 1996. Sign tracking versus goal tracking in the sexual conditioning of male Japanese quail (Coturnix japonica). J Exp Psychol Anim Behav Process 22, 297-306.
Cabanac, M., 1979. Sensory Pleasure. The Quarterly Review of Biology 54, 1-29.
Caggiula, A.R., Donny, E.C., Palmatier, M.I., Liu, X., Chaudhri, N., Sved, A.F., 2009. The role of nicotine in smoking: a dual-reinforcement model. Nebr Symp Motiv 55, 91-109.
Caggiula, A.R., Donny, E.C., White, A.R., Chaudhri, N., Booth, S., Gharib, M.A., Hoffman, A., Perkins, K.A., Sved, A.F., 2001. Cue dependency of nicotine self-administration and smoking. Pharmacology Biochemistry and Behavior 70, 515-530.
Cagniard, B., Beeler, J.A., Britt, J.P., McGehee, D.S., Marinelli, M., Zhuang, X., 2006. Dopamine scales performance in the absence of new learning. Neuron 51, 541-547.
Cannon, C.M., Palmiter, R.D., 2003. Reward without dopamine. Journal of Neuroscience 23, 10827-10831.

Cardinal, R.N., Parkinson, J.A., Hall, J., Everitt, B.J., 2002a. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26, 321-352.
Cardinal, R.N., Parkinson, J.A., Lachenal, G., Halkerston, K.M., Rudarakanchana, N., Hall, J., Morrison, C.H., Howes, S.R., Robbins, T.W., Everitt, B.J., 2002b. Effects of selective excitotoxic lesions of the nucleus accumbens core, anterior cingulate cortex, and central nucleus of the amygdala on autoshaping performance in rats. Behavioral Neuroscience 116, 553-567.
Carpenter, M.J., Saladin, M.E., DeSantis, S., Gray, K.M., LaRowe, S.D., Upadhyaya, H.P., 2009. Laboratory-based, cue-elicited craving and cue reactivity as predictors of naturally occurring smoking behavior. Addict Behav 34, 536-541.
Carr, D.B., Sesack, S.R., 2000. Projections from the Rat Prefrontal Cortex to the Ventral Tegmental Area: Target Specificity in the Synaptic Associations with Mesoaccumbens and Mesocortical Neurons. The Journal of Neuroscience 20, 3864-3873.
Carroll, M.E., Lac, S.T., 1997. Acquisition of i.v. amphetamine and cocaine self-administration in rats as a function of dose. Psychopharmacology (Berl) 129, 206-214.
Carter, B.L., Tiffany, S.T., 1999. Meta-analysis of cue-reactivity in addiction research. Addiction 94, 327-340.
Carver, C.S., White, T.L., 1994. Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. Journal of Personality and Social Psychology 67, 319-333.
Chang, S.E., Wheeler, D.S., Holland, P.C., 2012. Roles of nucleus accumbens and basolateral amygdala in autoshaped lever pressing. Neurobiol Learn Mem 97, 441-451.
Chaudhri, N., Caggiula, A.R., Donny, E.C., Booth, S., Gharib, M.A., Craven, L.A., Allen, S.S., Sved, A.F., Perkins, K.A., 2005. Sex differences in the contribution of nicotine and nonpharmacological stimuli to nicotine self-administration in rats. Psychopharmacology 180, 258-266.
Chaudhri, N., Caggiula, A.R., Donny, E.C., Palmatier, M.I., Liu, X., Sved, A.F., 2006. Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. Psychopharmacology (Berl) 184, 353-366.
Childress, A.R., Ehrman, R.N., Wang, Z., Li, Y., Sciortino, N., Hakun, J., Jens, W., Suh, J., Listerud, J., Marquez, K., Franklin, T., Langleben, D., Detre, J., O'Brien, C.P., 2008. Prelude to Passion: Limbic Activation by "Unseen" Drug and Sexual Cues. Plos One 3, 7.
Christiansen, P., Cole, J., Goudie, A., Field, M., 2012. Components of behavioural impulsivity and automatic cue approach predict unique variance in hazardous drinking. Psychopharmacology 219, 501-510.
Chudasama, Y., Robbins, T.W., 2003. Dissociable contributions of the orbitofrontal and infralimbic cortex to Pavlovian autoshaping and discrimination reversal learning: Further evidence for the functional heterogeneity of the rodent frontal cortex. Journal of Neuroscience 23, 8771-8780.
Ciccocioppo, R., Martin-Fardon, R., Weiss, F., 2004. Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. Nature Neuroscience 7, 495-496.
Clark, C.R., Geffen, G.M., Geffen, L.B., 1987. Catecholamines and attention. II: Pharmacological studies in normal humans. Neurosci Biobehav Rev 11, 353-364.
Clark, J.J., Hollon, N.G., Phillips, P.E., 2012. Pavlovian valuation systems in learning and decision making. Curr Opin Neurobiol doi:10.1016/j.conb.2012.06.004.

Cleland, G.G., Davey, G.C., 1983. Autoshaping in the rat: The effects of localizable visual and auditory signals for food. J Exp Anal Behav 40, 47-56.
Cohen, J.Y., Haesler, S., Vong, L., Lowell, B.B., Uchida, N., 2012. Neuron-type-specific signals for reward and punishment in the ventral tegmental area. Nature 482, 85-88.
Cole, P.D., Adamo, S.A., 2005. Cuttlefish (Sepia officinalis: Cephalopoda) hunting behavior and associative learning. Anim Cogn 8, 27-30.
Collins, G.T., Cunningham, A.R., Chen, J., Wang, S., Newman, A.H., Woods, J.H., 2012. Effects of pramipexole on the reinforcing effectiveness of stimuli that were previously paired with cocaine reinforcement in rats. Psychopharmacology (Berl) 219, 123-135.
Colwill, R.M., Rescorla, R.A., 1988. Associations between the discriminative stimulus and the reinforcer in instrumental learning. Journal of Experimental Psychology: Animal Behavior Processes 14, 155-164.
Cools, R., 2008. Role of dopamine in the motivational and cognitive control of behavior. Neuroscientist 14, 381-395.
Cooper, A., Barnea-Ygael, N., Levy, D., Shaham, Y., Zangen, A., 2007. A conflict rat model of cue-induced relapse to cocaine seeking. Psychopharmacology 194, 117-125.
Corbit, L., Janak, P., 2007a. Inactivation of the lateral but not medial dorsal striatum eliminates the excitatory impact of Pavlovian stimuli on instrumental responding. J Neurosci 27, 13977 - 13981.
Corbit, L.H., Balleine, B.W., 2000. The role of the hippocampus in instrumental conditioning. J Neurosci 20, 4233-4239.
Corbit, L.H., Balleine, B.W., 2005. Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. Journal of Neuroscience 25, 962-970.
Corbit, L.H., Balleine, B.W., 2011. The general and outcome-specific forms of Pavlovian-instrumental transfer are differentially mediated by the nucleus accumbens core and shell. J Neurosci 31, 11786-11794.
Corbit, L.H., Janak, P.H., 2007b. Ethanol-associated cues produce general pavlovian-instrumental transfer. Alcoholism: Clinical and Experimental Research 31, 766-774.
Corbit, L.H., Janak, P.H., Balleine, B.W., 2007. General and outcome-specific forms of Pavlovian-instrumental transfer: the effect of shifts in motivational state and inactivation of the ventral tegmental area. Eur J Neurosci 26, 3141-3149.
Corbit, L.H., Muir, J.L., Balleine, B.W., 2001. The role of the nucleus accumbens in instrumental conditioning: Evidence of a functional dissociation between accumbens core and shell. J Neurosci 21, 3251-3260.
Cox, W.M., Hogan, L.M., Kristian, M.R., Race, J.H., 2002. Alcohol attentional bias as a predictor of alcohol abusers' treatment outcome. Drug Alcohol Depend 68, 237-243.
Crombag, H.S., Badiani, A., Maren, S., Robinson, T.E., 2000. The role of contextual versus discrete drug-associated cues in promoting the induction of psychomotor sensitization to intravenous amphetamine. Behavioural Brain Research 116, 1-22.
Crombag, H.S., Bossert, J.M., Koya, E., Shaham, Y., 2008. Context-induced relapse to drug seeking: a review. Philosophical Transactions of the Royal Society B-Biological Sciences 363, 3233-3243.
Crombag, H.S., Grimm, J.W., Shaham, Y., 2002. Effect of dopamine receptor antagonists on renewal of cocaine seeking by reexposure to drug-associated contextual cues. Neuropsychopharmacology 27, 1006-1015.

Cunningham, C.L., Patel, P., 2007. Rapid induction of Pavlovian approach to an ethanol-paired visual cue in mice. Psychopharmacology (Berl) 192, 231-241.
Danna, C.L., Elmer, G.I., 2010. Disruption of conditioned reward association by typical and atypical antipsychotics. Pharmacol Biochem Behav 96, 40-47.
Davey, G.C., Cleland, G.G., 1982. Topography of signal-centered behavior in the rat: Effects of deprivation state and reinforcer type. J Exp Anal Behav 38, 291-304.
Davis, W.M., Smith, S.G., 1976. Role of conditioned reinforcers in initiation, maintenance and extinction of drug-seeking behavior. Pavlovian Journal of Biological Science 11, 222-236.
Daw, N.D., Niv, Y., Dayan, P., 2005. Uncertainty-based competition between prefrontal and dorsolateral striatal systems for behavioral control. Nat Neurosci 8, 1704-1711.
Day, J.J., Jones, J.L., Wightman, R.M., Carelli, R.M., 2010. Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. Biol Psychiatry 68, 306-309.
Day, J.J., Roitman, M.F., Wightman, R.M., Carelli, R.M., 2007. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci 10, 1020-1028.
Dayan, P., Balleine, B.W., 2002. Reward, motivation, and reinforcement learning. Neuron 36, 285-298.
de Wit, H., 1996. Priming effects with drugs and other reinforcers. Experimental and Clinical Psychopharmacology 4, 5-10.
de Wit, H., Chutuape, M.A., 1993. Increased ethanol choice in social drinkers following ethanol preload. Behav Pharmacol 4, 29-36.
de Wit, H., Stewart, J., 1981. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 75, 134-143.
de Wit, H., Uhlenhuth, E.H., Johanson, C.E., 1986. Individual-differences in the reinforcing and subjective effects of amphetamine and diazepam. Drug and Alcohol Dependence 16, 341-360.
de Wit, H., Uhlenhuth, E.H., Pierri, J., Johanson, C.E., 1987. Individual differences in behavioral and subjective responses to alcohol. Alcohol Clin Exp Res 11, 52-59.
DeJong, W., 1994. Relapse prevention: an emerging technology for promoting long-term drug abstinence. Int J Addict 29, 681-705.
Deneau, G., Yanagita, T., Seevers, M.H., 1969. Self-administration of psychoactive substances by the monkey. Psychopharmacology 16, 30-48.
Deroche-Gamonet, V., Piat, F., Le Moal, M., Piazza, P.V., 2002. Influence of cue-conditioning on acquisition, maintenance and relapse of cocaine intravenous self-administration. European Journal of Neuroscience 15, 1363-1370.
Di Chiara, G., 1998. A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. Journal of Psychopharmacology 12, 54-67.
Di Ciano, P., Cardinal, R.N., Cowell, R.A., Little, S.J., Everitt, B.J., 2001. Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of Pavlovian approach behavior. J Neurosci 21, 9471-9477.
Di Ciano, P., Everitt, B.J., 2003. Differential control over drug-seeking behavior by drug-associated conditioned reinforcers and discriminative stimuli predictive of drug availability. Behavioral Neuroscience 117, 952-960.

Di Ciano, P., Everitt, B.J., 2004. Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. Neuropharmacology 47, Supplement 1, 202-213.
Di Ciano, P., Everitt, B.J., 2005. Neuropsychopharmacology of drug seeking: Insights from studies with second-order schedules of drug reinforcement. European Journal of Pharmacology 526, 186-198.
Dickinson, A., Balleine, B., 1994. Motivational control of goal-directed action. Anim Learn Behav 22, 1-18.
Dickinson, A., Balleine, B., 2002. The role of learning in the operation of motivational systems, in: Pashler, H., Gallistel, R. (Eds.), Steven's handbook of experimental psychology: Learning, Motivation, and Emotion, 3 ed. Wiley, New York, pp. 497-533.
Dickinson, A., Dawson, G.R., 1987. Pavlovian processes in the motivational control of instrumental performance. Quarterly Journal of Experimental Psychology B Comparative and Physiological Psychology 39, 201-213.
Dickinson, A., Smith, J., Mirenowicz, J., 2000. Dissociation of pavlovian and instrumental incentive learning under dopamine antagonists. Behav Neurosci 114, 468-483.
Difeliceantonio, A.G., Berridge, K.C., 2012. Which cue to 'want'? Opioid stimulation of central amygdala makes goal-trackers show stronger goal-tracking, just as sign-trackers show stronger sign-tracking. Behav Brain Res 230, 399-408.
Doremus-Fitzwater, T.L., Spear, L.P., 2011. Amphetamine-induced incentive sensitization of sign-tracking behavior in adolescent and adult female rats. Behav Neurosci 125, 661-667.
Duka, T., Townshend, J.M., 2004. The priming effect of alcohol pre-load on attentional bias to alcohol-related stimuli. Psychopharmacology (Berl) 176, 353-361.
Ehrman, R.N., Robbins, S.J., Childress, A.R., O'Brien, C.P., 1992. Conditioned-responses to cocaine-related stimuli in cocaine abuse patients. Psychopharmacology 107, 523-529.
Epstein, D., Preston, K., 2003. The reinstatement model and relapse prevention: a clinical perspective. Psychopharmacology 168, 31-41.
Epstein, D.H., Marrone, G.F., Heishman, S.J., Schmittner, J., Preston, K.L., 2010. Tobacco, cocaine, and heroin: Craving and use during daily life. Addictive Behaviors 35, 318-324.
Epstein, D.H., Willner-Reid, J., Vahabzadeh, M., Mezghanni, M., Lin, J.L., Preston, K.L., 2009. Real-time electronic diary reports of cue exposure and mood in the hours before cocaine and heroin craving and use. Arch Gen Psychiatry 66, 88-94.
Ersche, K.D., Bullmore, E.T., Craig, K.J., Shabbir, S.S., Abbott, S., Muller, U., Ooi, C., Suckling, J., Barnes, A., Sahakian, B.J., Merlo-Pich, E.V., Robbins, T.W., 2010. Influence of compulsivity of drug abuse on dopaminergic modulation of attentional bias in stimulant dependence. Arch Gen Psychiatry 67, 632-644.
Estes, W.K., 1943. Discriminative conditioning. I. A discriminative property of conditioned anticipation. Journal of Experimental Psychology 32, 150-155.
Estes, W.K., 1948. Discriminative conditioning. II. Effects of a Pavlovian conditioned stimulus upon a subsequently established operant response. Journal of Experimental Psychology 38, 173-177.
Everitt, B.J., Dickinson, A., Robbins, T.W., 2001. The neuropsychological basis of addictive behaviour. Brain Research Reviews 36, 129-138.
Everitt, B.J., Robbins, T.W., 2000. Second-order schedules of drug reinforcement in rats and monkeys: measurement of reinforcing efficacy and drug-seeking behaviour. Psychopharmacology (Berl) 153, 17-30.

Everitt, B.J., Robbins, T.W., 2005. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nature Neuroscience 8, 1481-1489.
Fadardi, J.S., Cox, W.M., 2009. Reversing the sequence: reducing alcohol consumption by overcoming alcohol attentional bias. Drug Alcohol Depend 101, 137-145.
Fanselow, M.S., 2010. From contextual fear to a dynamic view of memory systems. Trends in Cognitive Sciences 14, 7-15.
Fantino, E., 1977. Conditioned reinforcement: Choice and information, in: Honig, W.K., Staddon, J.E.R. (Eds.), Handbook of operant behavior. Prentice-Hall, Englewood Cliffs, NJ.
Faure, A., Reynolds, S.M., Richard, J.M., Berridge, K.C., 2008. Mesolimbic dopamine in desire and dread: enabling motivation to be generated by localized glutamate disruptions in nucleus accumbens. J Neurosci 28, 7184-7192.
Feil, J., Sheppard, D., Fitzgerald, P.B., Yucel, M., Lubman, D.I., Bradshaw, J.L., 2010. Addiction, compulsive drug seeking, and the role of frontostriatal mechanisms in regulating inhibitory control. Neurosci Biobehav Rev 35, 248-275.
Field, M., Cox, W.M., 2008. Attentional bias in addictive behaviors: A review of its development, causes, and consequences. Drug and Alcohol Dependence 97, 1-20.
Field, M., Eastwood, B., 2005. Experimental manipulation of attentional bias increases the motivation to drink alcohol. Psychopharmacology (Berl) 183, 350-357.
Field, M., Kiernan, A., Eastwood, B., Child, R., 2008. Rapid approach responses to alcohol cues in heavy drinkers. J Behav Ther Exp Psychiatry 39, 209-218.
Field, M., Mogg, K., Bradley, B.P., 2005. Craving and cognitive biases for alcohol cues in social drinkers. Alcohol Alcohol 40, 504-510.
Fields, H.L., Hjelmstad, G.O., Margolis, E.B., Nicola, S.M., 2007. Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. Annu Rev Neurosci 30, 289-316.
Fischman, M.W., 1989. Relationship between self-reported drug effects and their reinforcing effects: studies with stimulant drugs. NIDA Res Monogr 92, 211-230.
Fischman, M.W., Foltin, R.W., 1992. Self-administration of cocaine by humans: a laboratory perspective. Cocaine: Scientific and social dimensions 166, 165-180.
Flagel, S.B., Akil, H., Robinson, T.E., 2009. Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. Neuropharmacology 56, 139-148.
Flagel, S.B., Cameron, C.M., Pickup, K.N., Watson, S.J., Akil, H., Robinson, T.E., 2011a. A food predictive cue must be attributed with incentive salience for it to induce c-fos mRNA expression in cortico-striatal-thalamic brain regions. Neuroscience 196, 80-96.
Flagel, S.B., Clark, J.J., Robinson, T.E., Mayo, L., Czuj, A., Willuhn, I., Akers, C.A., Clinton, S.M., Phillips, P.E., Akil, H., 2011b. A selective role for dopamine in stimulus-reward learning. Nature 469, 53-57.
Flagel, S.B., Robinson, T.E., Clark, J.J., Clinton, S.M., Watson, S.J., Seeman, P., Phillips, P.E.M., Akil, H., 2010. An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: Implications for addiction. Neuropsychopharmacology 35, 388-400.
Flagel, S.B., Watson, S.J., Akil, H., Robinson, T.E., 2008. Individual differences in the attribution of incentive salience to a reward-related cue: Influence on cocaine sensitization. Behavioural Brain Research 186, 48-56.

Flagel, S.B., Watson, S.J., Robinson, T.E., Akil, H., 2007. Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. Psychopharmacology (Berl) 191, 599-607.
Floresco, S.B., Todd, C.L., Grace, A.A., 2001. Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. The Journal of Neuroscience 21, 4915-4922.
Franken, I.H., Booij, J., van den Brink, W., 2005. The role of dopamine in human addiction: from reward to motivated attention. Eur J Pharmacol 526, 199-206.
Franken, I.H., Hendriks, V.M., Stam, C.J., Van den Brink, W., 2004. A role for dopamine in the processing of drug cues in heroin dependent patients. Eur Neuropsychopharmacol 14, 503-508.
Franken, I.H., Kroon, L.Y., Hendriks, V.M., 2000. Influence of individual differences in craving and obsessive cocaine thoughts on attentional processes in cocaine abuse patients. Addict Behav 25, 99-102.
Fuchs, R.A., Branham, R.K., See, R.E., 2006. Different neural substrates mediate cocaine seeking after abstinence versus extinction training: A critical role for the dorsolateral caudate-putamen. Journal of Neuroscience 26, 3584-3588.
Fuchs, R.A., Eaddy, J.L., Su, Z.I., Bell, G.H., 2007. Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug contextâ€ induced reinstatement of cocaineâ€ seeking in rats. European Journal of Neuroscience 26, 487-498.
Fuchs, R.A., Evans, K.A., Ledford, C.C., Parker, M.P., Case, J.M., Mehta, R.H., See, R.E., 2005. The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology 30, 296-309.
Fuchs, R.A., Evans, K.A., Parker, M.C., See, R.E., 2004. Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 176, 459-465.
Fuchs, R.A., Lasseter, H.C., Ramirez, D.R., Xie, X., 2008a. Relapse to drug seeking following prolonged abstinence: the role of environmental stimuli. Drug Discovery Today: Disease Models 5, 251-258.
Fuchs, R.A., Ramirez, D.R., Bell, G.H., 2008b. Nucleus accumbens shell and core involvement in drug context-induced reinstatement of cocaine seeking in rats. Psychopharmacology 200, 545-556.
Gamzu, E., Schwam, E., 1974. Autoshaping and automaintenance of a key-press response in squirrel monkeys. J Exp Anal Behav 21, 361-371.
Gawin, F.H., Kleber, H.D., 1986. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. Arch Gen Psychiatry 43, 107-113.
Geisler, S., Zahm, D.S., 2005. Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. The Journal of Comparative Neurology 490, 270-294.
Glascher, J., Daw, N., Dayan, P., O'Doherty, J.P., 2010. States versus rewards: dissociable neural prediction error signals underlying model-based and model-free reinforcement learning. Neuron 66, 585-595.
Goddard, B., Leri, F., 2006. Reinstatement of conditioned reinforcing properties of cocaine-conditioned stimuli. Pharmacology Biochemistry and Behavior 83, 540-546.
Gold, L.H., Swerdlow, N.R., Koob, G.F., 1988. The role of mesolimbic dopamine in conditioned locomotion produced by amphetamine. Behavioral Neuroscience 102, 544.

Goldberg, S.R., Tang, A.H., 1977. Behavior maintained under second-order schedules of intravenous morphine injection in squirrel and rhesus monkeys. Psychopharmacology 51, 235-242.
Goldstein, R.Z., Tomasi, D., Alia-Klein, N., Honorio Carrillo, J., Maloney, T., Woicik, P.A., Wang, R., Telang, F., Volkow, N.D., 2009. Dopaminergic response to drug words in cocaine addiction. J Neurosci 29, 6001-6006.
Goto, Y., Yang, C.R., Otani, S., 2010. Functional and dysfunctional synaptic plasticity in prefrontal cortex: roles in psychiatric disorders. Biol Psychiatry 67, 199-207.
Grimm, J.W., Hope, B.T., Wise, R.A., Shaham, Y., 2001. Neuroadaptation - Incubation of cocaine craving after withdrawal. Nature 412, 141-142.
Grimm, J.W., Kruzich, P.J., See, R.E., 2000. Contingent access to stimuli associated with cocaine self-administration is required for reinstatement of drug-seeking behavior. Psychobiology 28, 383-386.
Grimm, J.W., See, R.E., 2000. Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology 22, 473-479.
Groenewegen, H.J., 1988. Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. Neuroscience 24, 379-431.
Gruber, A.J., McDonald, R.J., 2012. Context, emotion, and the strategic pursuit of goals: interactions among multiple brain systems controlling motivated behavior. Front Behav Neurosci 6, 50.
Haber, S.N., Fudge, J.L., McFarland, N.R., 2000. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20, 2369-2382.
Haber, S.N., Knutson, B., 2010. The reward circuit: linking primate anatomy and human imaging. Neuropsychopharmacology 35, 4-26.
Hall, J., Parkinson, J.A., Connor, T.M., Dickinson, A., Everitt, B.J., 2001. Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. European Journal of Neuroscience 13, 1984-1992.
Hall, J.F., 1951. Studies in secondary reinforcement: I. Secondary reinforcement as a function of the frequency of primary reinforcement. Journal of Comparative and Physiological Psychology 44, 246-251.
Han, J.S., McMahan, R.W., Holland, P., Gallagher, M., 1997. The role of an amygdalo-nigrostriatal pathway in associative learning. J Neurosci 17, 3913-3919.
Hearst, E., Jenkins, H., 1974. Sign-tracking: The stimulus-reinforcer relation and directed action. Monograph of the Psychonomic Society, Austin.
Hebb, D.O., 1955. Drives and the C. N. S. (conceptual nervous system). Psychological Review 62, 243-254.
Heimer, L., Zahm, D.S., Churchill, L., Kalivas, P.W., Wohltmann, C., 1991. Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 41, 89-125.
Hermann, D., Smolka, M.N., Wrase, J., Klein, S., Nikitopoulos, J., Georgi, A., Braus, D.F., Flor, H., Mann, K., Heinz, A., 2006. Blockade of cue-induced brain activation of abstinent alcoholics by a single administration of amisulpride as measured with fMRI. Alcohol Clin Exp Res 30, 1349-1354.
Hester, R., Garavan, H., 2004. Executive dysfunction in cocaine addiction: evidence for discordant frontal, cingulate, and cerebellar activity. J Neurosci 24, 11017-11022.

Hester, R., Garavan, H., 2009. Neural mechanisms underlying drug-related cue distraction in active cocaine users. Pharmacology Biochemistry and Behavior 93, 270-277.
Hickey, C., Chelazzi, L., Theeuwes, J., 2010a. Reward changes salience in human vision via the anterior cingulate. J Neurosci 30, 11096-11103.
Hickey, C., Chelazzi, L., Theeuwes, J., 2010b. Reward guides vision when it's your thing: trait reward-seeking in reward-mediated visual priming. PLoS One 5, e14087.
Hickey, C., van Zoest, W., 2012. Reward creates oculomotor salience. Current Biology 22, R219-R220.
Hill, R.T., 1970. Facilitation of conditioned reinforcement as a mechanism of psychomotor stimulation, in: Costa, E., Garattini, S. (Eds.), Amphetamine and related compounds. Raven, New York, pp. 781-795.
Hitchcott, P.K., Harmer, C.J., Phillips, G.D., 1997. Enhanced acquisition of discriminative approach following intra-amygdala d-amphetamine. Psychopharmacology 132, 237-246.
Hitsman, B., MacKillop, J., Lingford-Hughes, A., Williams, T.M., Ahmad, F., Adams, S., Nutt, D.J., Munafo, M.R., 2008. Effects of acute tyrosine/phenylalanine depletion on the selective processing of smoking-related cues and the relative value of cigarettes in smokers. Psychopharmacology (Berl) 196, 611-621.
Hnasko, T., Sotak, B., Palmiter, R., 2007. Cocaine-conditioned place preference by dopamine-deficient mice is mediated by serotonin. J Neurosci 27, 12484 - 12488.
Hnasko, T.S., Sotak, B.N., Palmiter, R.D., 2005. Morphine reward in dopamine-deficient mice. Nature 438, 854-857.
Hodgson, R., Rankin, H., Stockwell, T., 1979. Alcohol dependence and the priming effect. Behaviour Research and Therapy 17, 379-387.
Holden, J.M., Peoples, L.L., 2010. Effects of acute amphetamine exposure on two kinds of Pavlovian approach behavior. Behav Brain Res 208, 270-273.
Holland, P.C., 1977. Conditioned stimulus as a determinant of the form of the Pavlovian conditioned response. J Exp Psychol Anim Behav Process 3, 77-104.
Holland, P.C., 1992. Occasion setting in Pavlovian conditioning. The psychology of learning and motivation 28, 69-125.
Holland, P.C., Bouton, M.E., 1999. Hippocampus and context in classical conditioning. Current Opinion in Neurobiology 9, 195-202.
Holland, P.C., Gallagher, M., 1999. Amygdala circuitry in attentional and representational processes. Trends in Cognitive Sciences 3, 65-73.
Holland, P.C., Gallagher, M., 2003. Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. European Journal of Neuroscience 17, 1680-1694.
Holmes, N.M., Marchand, A.R., Coutureau, E., 2010. Pavlovian to instrumental transfer: a neurobehavioural perspective. Neurosci Biobehav Rev 34, 1277-1295.
Hull, C.L., 1943. Principles of behavior: an introduction to behavior theory. Appleton-Century, Oxford, England.
Hurley, K.M., Herbert, H., Moga, M.M., Saper, C.B., 1991. Efferent projections of the infralimbic cortex of the rat. The Journal of Comparative Neurology 308, 249-276.
Ikemoto, S., 2007. Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbensâ€“olfactory tubercle complex. Brain Research Reviews 56, 27-78.

Ikemoto, S., 2010. Brain reward circuitry beyond the mesolimbic dopamine system: A neurobiological theory. Neurosci Biobehav Rev 35, 129-150.
Ikemoto, S., Panksepp, J., 1999. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Brain Res Rev 31, 6-41.
Ito, R., Everitt, B.J., Robbins, T.W., 2005. The hippocampus and appetitive Pavlovian conditioning: Effects of excitotoxic hippocampal lesions on conditioned locomotor activity and autoshaping. Hippocampus 15, 713-721.
Ito, R., Hayen, A., 2011. Opposing roles of nucleus accumbens core and shell dopamine in the modulation of limbic information processing. J Neurosci 31, 6001-6007.
Jaffe, J.H., Cascella, N.G., Kumor, K.M., Sherer, M.A., 1989. Cocaine-induced cocaine craving. Psychopharmacology 97, 59-64.
Janes, A.C., Pizzagalli, D.A., Richardt, S., de, B.F.B., Chuzi, S., Pachas, G., Culhane, M.A., Holmes, A.J., Fava, M., Evins, A.E., Kaufman, M.J., 2010. Brain reactivity to smoking cues prior to smoking cessation predicts ability to maintain tobacco abstinence. Biol Psychiatry 67, 722-729.
Jenkins, H.M., Moore, B.R., 1973. The form of the auto-shaped response with food or water reinforcers. J Exp Anal Behav 20, 163-181.
Jones, G., Robbins, T., 1992. Differential effects of mesocortical, mesolimbic, and mesostriatal dopamine depletion on spontaneous, conditioned, and drug-induced locomotor activity. Pharmacology Biochemistry and Behavior 43, 887-895.
Kalivas, P., Volkow, N., 2005. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162, 1403 - 1413.
Kalivas, P.W., 1993. Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Research Reviews 18, 75-113.
Kapur, S., 2003. Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. Am J Psychiatry 160, 13 - 23.
Kapur, S., Mizrahi, R., Li, M., 2005. From dopamine to salience to psychosis--linking biology, pharmacology and phenomenology of psychosis. Schizophr Res 79, 59-68.
Katz, J.L., 1979. A comparison of responding maintained under second-order schedules of intramuscular cocaine injection or food presentation in squirrel monkeys. J Exp Anal Behav 32, 419-431.
Kelleher, R.T., 1966. Conditioned reinforcement in second-order schedules. J Exp Anal Behav 9, 475-485.
Kelleher, R.T., Goldberg, S.R., 1977. Fixed-interval responding under second-order schedules of food presentation or cocaine injection. J Exp Anal Behav 28, 221-231.
Kelleher, R.T., Gollub, L.R., 1962. A review of positive conditioned reinforcement. J Exp Anal Behav 5, 543-597.
Kelley, A., Delfs, J., 1991. Dopamine and conditioned reinforcement. Psychopharmacology 103, 187-196.
Kelley, A.E., 2004a. Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44, 161-179.
Kelley, A.E., 2004b. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. Neuroscience and Biobehavioral Reviews 27, 765-776.

Kelley, A.E., Bakshi, V.P., Haber, S.N., Steininger, T.L., Will, M.J., Zhang, M., 2002. Opioid modulation of taste hedonics within the ventral striatum. Physiology & Behavior 76, 365-377.
Kelley, A.E., Berridge, K.C., 2002. The neuroscience of natural rewards: Relevance to addictive drugs. Journal of Neuroscience 22, 3306-3311.
Kelley, A.E., Domesick, V.B., 1982. The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: An anterograde and retrograde-horseradish peroxidase study. Neuroscience 7, 2321-2335.
Kelley, A.E., Domesick, V.B., Nauta, W.J.H., 1982. The amygdalostriatal projection in the rat: an anatomical study by anterograde and retrograde tracing methods. Neuroscience 7, 615-630.
Kelley, A.E., Schiltz, C.A., Landry, C.F., 2005. Neural systems recruited by drug- and food-related cues: studies of gene activation in corticolimbic regions. Physiol Behav 86, 11-14.
Kenny, P.J., 2011. Common cellular and molecular mechanisms in obesity and drug addiction. Nat Rev Neurosci 12, 638-651.
Killeen, P.R., 2003. Complex dynamic processes in sign tracking with an omission contingency (negative automaintenance). J Exp Psychol Anim Behav Process 29, 49-61.
Kippin, T.E., Fuchs, R.A., See, R.E., 2006. Contributions of prolonged contingent and noncontingent cocaine exposure to enhanced reinstatement of cocaine seeking in rats. Psychopharmacology 187, 60-67.
Kirk, J.M., de Wit, H., 2000. Individual differences in the priming effect of ethanol in social drinkers. J Stud Alcohol 61, 64-71.
Knackstedt, L.A., Moussawi, K., Lalumiere, R., Schwendt, M., Klugmann, M., Kalivas, P.W., 2010. Extinction training after cocaine self-administration induces glutamatergic plasticity to inhibit cocaine seeking. J Neurosci 30, 7984-7992.
Konorski, J., 1967. Integrative activity of the brain: An interdisciplinary approach. University of Chicago Press, Chicago.
Koob, G.F., Le Moal, M., 2001. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24, 97-129.
Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction. Neuropsychopharmacology 35, 217-238.
Krank, M.D., O'Neill, S., Squarey, K., Jacob, J., 2008. Goal- and signal-directed incentive: conditioned approach, seeking, and consumption established with unsweetened alcohol in rats. Psychopharmacology (Berl) 196, 397-405.
Kruse, J.M., Overmier, J.B., Konz, W.A., Rokke, E., 1983. Pavlovian conditioned stimulus effects upon instrumental choice behavior are reinforcer specific. Learning and Motivation 14, 165-181.
Kruzich, P.J., Congleton, K.M., See, R.E., 2001. Conditioned reinstatement of drug-seeking behavior with a discrete compound stimulus classically conditioned with intravenous cocaine. Behav Neurosci 115, 1086-1092.
Kuhn, S., Gallinat, J., 2011. Common biology of craving across legal and illegal drugs - a quantitative meta-analysis of cue-reactivity brain response. Eur J Neurosci 33, 1318-1326.
Lajoie, J., Bindra, D., 1976. An interpretation of autoshaping and related phenomena in terms of stimulus-incentive contingentcies alone. Canadian Journal of Psychology 30, 157-173.
Lammel, S., Ion, D.I., Roeper, J., Malenka, R.C., 2011. Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. Neuron 70, 855-862.

Lammel, S., Lim, B.K., Ran, C., Huang, K.W., Betley, M.J., Tye, K.M., Deisseroth, K., Malenka, R.C., 2012. Input-specific control of reward and aversion in the ventral tegmental area. Nature 491, 212-217.
Laruelle, M., Abi-Dargham, A., Van Dyck, C.H., Rosenblatt, W., Zea-Ponce, Y., Zoghbi, S.S., Baldwin, R.M., Charney, D.S., Hoffer, P.B., Kung, H.F., Innis, R.B., 1995. SPECT imaging of striatal dopamine release after amphetamine challenge. Journal of Nuclear Medicine 36, 1182-1190.
LeBlanc, K.H., Ostlund, S.B., Maidment, N.T., 2012. Pavlovian-to-Instrumental Transfer in Cocaine Seeking Rats. Behavioral Neuroscience doi:10.1037/a0029534.
Levita, L., Dalley, J.W., Robbins, T.W., 2002. Disruption of Pavlovian contextual conditioning by excitotoxic lesions of the nucleus accumbens core. Behavioral Neuroscience 116, 539.
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.
Lex, B., Hauber, W., 2010. The role of nucleus accumbens dopamine in outcome encoding in instrumental and Pavlovian conditioning. Neurobiol Learn Mem 93, 283-290.
Leyton, M., Boileau, I., Benkelfat, C., Diksic, M., Baker, G., Dagher, A., 2002. Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[11C]raclopride study in healthy men. Neuropsychopharmacology 27, 1027-1035.
Li, X., Qi, J., Yamaguchi, T., Wang, H.-L., Morales, M., 2012. Heterogeneous composition of dopamine neurons of the rat A10 region: molecular evidence for diverse signaling properties. Brain Structure and Function, 1-18.
Lindesmith, A., 1968. Addiction and opiates, 2nd ed. Aldine, Chicago.
Lloyd, R.W., Jr., Salzberg, H.C., 1975. Controlled social drinking: an alternative to abstinence as a treatment goal for some alcohol abusers. Psychol Bull 82, 815-842.
Lodge, D.J., Grace, A.A., 2005. The hippocampus modulates dopamine neuron responsivity by regulating the intensity of phasic neuron activation. Neuropsychopharmacology 31, 1356-1361.
Lomanowska, A.M., Lovic, V., Rankine, M.J., Mooney, S.J., Robinson, T.E., Kraemer, G.W., 2011. Inadequate early social experience increases the incentive salience of reward-related cues in adulthood. Behav Brain Res 220, 91-99.
Lovibond, P.F., 1983. Facilitation of instrumental behavior by a Pavlovian appetitive conditioned stimulus. J Exp Psychol Anim Behav Process 9, 225-247.
Lovic, V., Saunders, B.T., Yager, L.M., Robinson, T.E., 2011. Rats prone to attribute incentive salience to reward cues are also prone to impulsive action. Behav Brain Res 223, 255-261.
Lucantonio, F., Stalnaker, T.A., Shaham, Y., Niv, Y., Schoenbaum, G., 2012. The impact of orbitofrontal dysfunction on cocaine addiction. Nat Neurosci 15, 358-366.
Luijten, M., Veltman, D.J., Hester, R., Smits, M., Pepplinkhuizen, L., Franken, I.H., 2012. Brain Activation Associated with Attentional Bias in Smokers is Modulated by a Dopamine Antagonist. Neuropsychopharmacology, doi: 10.1038/npp.2012.1143.
Luijten, M., Veltman, D.J., van den Brink, W., Hester, R., Field, M., Smits, M., Franken, I.H., 2011. Neurobiological substrate of smoking-related attentional bias. Neuroimage 54, 2374-2381.
Luo, A.H., Tahsili-Fahadan, P., Wise, R.A., Lupica, C.R., Aston-Jones, G., 2011. Linking context with reward: a functional circuit from hippocampal CA3 to ventral tegmental area. Science 333, 353-357.
Mackintosh, N.J., 1974. The psychology of animal learning. Academic Press, London.

Mahler, S.V., Berridge, K.C., 2009. Which cue to "want?" Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue. J Neurosci 29, 6500-6513.
Mahler, S.V., de Wit, H., 2010. Cue-reactors: individual differences in cue-induced craving after food or smoking abstinence. PLoS One 5, e15475.
Maren, S., 1998. Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. The Journal of Neuroscience 18, 3088-3097.
Maren, S., 2001a. Is there savings for pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? Neurobiology of Learning and Memory 76, 268-283.
Maren, S., 2001b. Neurobiology of Pavlovian fear conditioning. Annual review of neuroscience 24, 897-931.
Maren, S., 2011. Seeking a spotless mind: extinction, deconsolidation, and erasure of fear memory. Neuron 70, 830-845.
Margolis, E.B., Lock, H., Hjelmstad, G.O., Fields, H.L., 2006. The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? J Physiol 577, 907-924.
Marissen, M.A., Franken, I.H., Waters, A.J., Blanken, P., van den Brink, W., Hendriks, V.M., 2006. Attentional bias predicts heroin relapse following treatment. Addiction 101, 1306-1312.
McClure, S.M., Daw, N.D., Montague, P.R., 2003. A computational substrate for incentive salience. Trends Neurosci 26, 423-428.
McDannald, M.A., Lucantonio, F., Burke, K.A., Niv, Y., Schoenbaum, G., 2011. Ventral striatum and orbitofrontal cortex are both required for model-based, but not model-free, reinforcement learning. J Neurosci 31, 2700-2705.
McDannald, M.A., Takahashi, Y.K., Lopatina, N., Pietras, B.W., Jones, J.L., Schoenbaum, G., 2012. Model-based learning and the contribution of the orbitofrontal cortex to the model-free world. Eur J Neurosci 35, 991-996.
McFarland, K., Kalivas, P.W., 2001. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. The Journal of Neuroscience 21, 8655-8663.
McLaughlin, J., See, R.E., 2003. Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology 168, 57-65.
Meyer, P.J., Lovic, V., Saunders, B.T., Yager, L.M., Flagel, S.B., Morrow, J.D., Robinson, T.E., 2012a. Quantifying individual variation in the propensity to attribute incentive salience to reward cues. PLoS ONE 7, e38987.
Meyer, P.J., Ma, S.T., Robinson, T.E., 2012b. A cocaine cue is more preferred and evokes more frequency-modulated 50-kHz ultrasonic vocalizations in rats prone to attribute incentive salience to a food cue. Psychopharmacology (Berl) 219, 999-1009.
Midanik, L., Greenfield, T., 2000. Trends in social consequences and dependence symptoms in the United States: the National Alcohol Surveys, 1984-1995. American journal of public health 90, 53.
Milton, A.L., Everitt, B.J., 2010. The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. Eur J Neurosci 31, 2308-2319.

Mogenson, G.J., 1987. Limbic-motor integration. Progress in psychobiology and physiological psychology 12, 117-170.
Moltz, H., 1965. Contemporary instinct theory and the fixed action pattern. Psychological Review 72, 27-47.
Montague, P.R., Dayan, P., Sejnowski, T.J., 1996. A framework for mesencephalic dopamine systems based on predictive Hebbian learning. J Neurosci 16, 1936-1947.
Moore, K.E., 1978. Amphetamines: biochemical and behavioral actions in animals, in: Iverson, S.D., Snyder, S.H. (Eds.), Handbook of psychopharmacology. Plenum, New York, pp. 41-98.
Morrow, J.D., Maren, S., Robinson, T.E., 2011. Individual variation in the propensity to attribute incentive salience to an appetitive cue predicts the propensity to attribute motivational salience to an aversive cue. Behav Brain Res 220, 238-243.
Munafo, M.R., Mannie, Z.N., Cowen, P.J., Harmer, C.J., McTavish, S.B., 2007. Effects of acute tyrosine depletion on subjective craving and selective processing of smoking-related cues in abstinent cigarette smokers. J Psychopharmacol 21, 805-814.
Murschall, A., Hauber, W., 2006. Inactivation of the ventral tegmental area abolished the general excitatory influence of Pavlovian cues on instrumental performance. Learning & Memory 13, 123-126.
Nadel, L., Willner, J., 1980. Context and conditioning: A place for space. Physiological Psychology; Physiological Psychology.
Nair, S.G., Adams-Deutsch, T., Epstein, D.H., Shaham, Y., 2009. The neuropharmacology of relapse to food seeking: methodology, main findings, and comparison with relapse to drug seeking. Prog Neurobiol 89, 18-45.
Nauta, W.J.H., Smith, G.P., Faull, R.L.M., Domesick, V.B., 1978. Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. Neuroscience 3, 385-401.
Nesse, R.M., Berridge, K.C., 1997. Psychoactive drug use in evolutionary perspective. Science 278, 63-66.
Niaura, R., Shadel, W.G., Abrams, D.B., Monti, P.M., Rohsenow, D.J., Sirota, A., 1998. Individual differences in cue reactivity among smokers trying to quit: effects of gender and cue type. Addict Behav 23, 209-224.
Nieoullon, A., 2002. Dopamine and the regulation of cognition and attention. Prog Neurobiol 67, 53-83.
Nilsson, J., Kristiansen, T.S., Fosseidengen, J.E., Ferno, A., van den Bos, R., 2008. Sign- and goal-tracking in Atlantic cod (Gadus morhua). Anim Cogn 11, 651-659.
Novak, M., Halbout, B., O'Connor, E.C., Rodriguez Parkitna, J., Su, T., Chai, M., Crombag, H.S., Bilbao, A., Spanagel, R., Stephens, D.N., Schutz, G., Engblom, D., 2010. Incentive learning underlying cocaine-seeking requires mGluR5 receptors located on dopamine D1 receptor-expressing neurons. J Neurosci 30, 11973-11982.
O'Brien, C.P., Childress, A.R., Ehrman, R., Robbins, S.J., 1998. Conditioning factors in drug abuse: can they explain compulsion? Journal of Psychopharmacology 12, 15-22.
O'Brien, C.P., Childress, A.R., McLellan, A.T., Ehrman, R.N., 1992. Classical Conditioning in Drug-Dependent Humans. Annals of the New York Academy of Sciences 654, 400-415.
O'Connor, E.C., Crombag, H.S., Mead, A.N., Stephens, D.N., 2010. The mGluR5 antagonist MTEP dissociates the acquisition of predictive and incentive motivational properties of reward-paired stimuli in mice. Neuropsychopharmacology 35, 1807-1817.

Oleson, E.B., Gentry, R.N., Chioma, V.C., Cheer, J.F., 2012. Subsecond Dopamine Release in the Nucleus Accumbens Predicts Conditioned Punishment and Its Successful Avoidance. The Journal of Neuroscience 32, 14804-14808.
Ongur, D., Price, J.L., 2000. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cerebral Cortex 10, 206-219.
Ostlund, S.B., Balleine, B.W., 2007. Orbitofrontal cortex mediates outcome encoding in pavlovian but not instrumental conditioning. Journal of Neuroscience 27, 4819-4825.
Ostlund, S.B., Balleine, B.W., 2008. Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. Journal of Neuroscience 28, 4398-4405.
Ostlund, S.B., Maidment, N.T., 2012. Dopamine receptor blockade attenuates the general incentive motivational effects of noncontingently delivered rewards and reward-paired cues without affecting their ability to bias action selection. Neuropsychopharmacology 37, 508-519.
Palfai, T.P., 2006. Activating action tendencies: The influence of action priming on alcohol consumption among male hazardous drinkers. J Stud Alcohol 67, 926-933.
Palmatier, M.I., Evans-Martin, F.F., Hoffman, A., Caggiula, A.R., Chaudhri, N., Donny, E.C., Liu, X., Booth, S., Gharib, M., Craven, L., Sved, A.F., 2006. Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. Psychopharmacology (Berl) 184, 391-400.
Pan, W.X., Schmidt, R., Wickens, J.R., Hyland, B.I., 2005. Dopamine cells respond to predicted events during classical conditioning: evidence for eligibility traces in the reward-learning network. J Neurosci 25, 6235-6242.
Panlilio, L., Schindler, C., Weiss, S., 1996. Cocaine self-administration increased by compounding discriminative stimuli. Psychopharmacology 125, 202-208.
Panlilio, L.V., Yasar, S., Nemeth-Coslett, R., Katz, J.L., Henningfield, J.E., Solinas, M., Heishman, S.J., Schindler, C.W., Goldberg, S.R., 2005. Human cocaine-seeking behavior and its control by drug-associated stimuli in the laboratory. Neuropsychopharmacology 30, 433-443.
Parikh, V., Sarter, M., 2008. Cholinergic mediation of attention: contributions of phasic and tonic increases in prefrontal cholinergic activity. Ann N Y Acad Sci 1129, 225-235.
Parker, J.G., Zweifel, L.S., Clark, J.J., Evans, S.B., Phillips, P.E., Palmiter, R.D., 2010. Absence of NMDA receptors in dopamine neurons attenuates dopamine release but not conditioned approach during Pavlovian conditioning. Proc Natl Acad Sci U S A 107, 13491-13496.
Parkinson, J., Roberts, A., Everitt, B., Di Ciano, P., 2005. Acquisition of instrumental conditioned reinforcement is resistant to the devaluation of the unconditioned stimulus. Q J Exp Psychol B 58, 19 - 30.
Parkinson, J.A., Crofts, H.S., McGuigan, M., Tomic, D.L., Everitt, B.J., Roberts, A.C., 2001. The role of the primate amygdala in conditioned reinforcement. J Neurosci 21, 7770-7780.
Parkinson, J.A., Olmstead, M.C., Burns, L.H., Robbins, T.W., Everitt, B.J., 1999. Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-Amphetamine. Journal of Neuroscience 19, 2401-2411.

Parkinson, J.A., Robbins, T.W., Everitt, B.J., 2000a. Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. European Journal of Neuroscience 12, 405-413.
Parkinson, J.A., Willoughby, P.J., Robbins, T.W., Everitt, B.J., 2000b. Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: Further evidence for limbic cortical-ventral striatopallidal systems. Behavioral Neuroscience 114, 42-63.
Pavlov, I.P., 1927. Conditioned Reflexes. Dover, New York, NY.
Pavlov, I.P., 1932. The reply of a physiologist to psychologists. Psychological Review 39, 91-127.
Paxinos, G., Watson, C., 2007. The rat brain in stereotaxic coordinates, 6th ed. Academic Press, New York, NY.
Payne, T.J., Smith, P.O., Adams, S.G., Diefenbach, L., 2006. Pretreatment cue reactivity predicts end-of-treatment smoking. Addict Behav 31, 702-710.
Pezze, M.A., Feldon, J., 2004. Mesolimbic dopaminergic pathways in fear conditioning. Prog Neurobiol 74, 301-320.
Pezze, M.A., Heidbreder, C.A., Feldon, J., Murphy, C.A., 2001. Selective responding of nucleus accumbens core and shell dopamine to aversively conditioned contextual and discrete stimuli. Neuroscience 108, 91-102.
Phillips, A.G., Fibiger, H.C., 1990. Role of reward and enhancement of conditioned reward in persistence of responding for cocaine. Behav Pharmacol 1, 269-282.
Phillips, A.G., McDonald, A.C., Wilkie, D.M., 1981. Disruption of autoshaped responding to a signal of brain-stimulation reward by neuroleptic drugs. Pharmacol Biochem Behav 14, 543-548.
Phillips, A.G., Vacca, G., Ahn, S., 2008. A top-down perspective on dopamine, motivation and memory. Pharmacol Biochem Behav 90, 236-249.
Phillips, G.D., Setzu, E., Hitchcott, P.K., 2003a. Facilitation of appetitive Pavlovian conditioning by d-amphetamine in the shell, but not the core, of the nucleus accumbens. Behavioral Neuroscience 117, 675-684.
Phillips, P.E., Robinson, D.L., Stuber, G.D., Carelli, R.M., Wightman, R.M., 2003b. Real-time measurements of phasic changes in extracellular dopamine concentration in freely moving rats by fast-scan cyclic voltammetry. Methods Mol Med 79, 443-464.
Phillips, R., LeDoux, J., 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behavioral Neuroscience 106, 274.
Pielock, S.M., Lex, B., Hauber, W., 2011. The role of dopamine in the dorsomedial striatum in general and outcome-selective Pavlovian-instrumental transfer. European Journal of Neuroscience 33, 717-725.
Pilla, M., Perachon, S., Sautel, F., Garrido, F., Mann, A., Wermuth, C.G., Schwartz, J.C., Everitt, B.J., Sokoloff, P., 1999. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. Nature 400, 371-375.
Pithers, R.T., 1985. The roles of event contingencies and reinforcement in human autoshaping and omission responding. Learning and Motivation 16, 210-237.
Preston, K.L., Vahabzadeh, M., Schmittner, J., Lin, J.L., Gorelick, D.A., Epstein, D.H., 2009. Cocaine craving and use during daily life. Psychopharmacology (Berl) 207, 291-301.
Puglisi-Allegra, S., Ventura, R., 2012. Prefrontal/accumbal catecholamine system processes high motivational salience. Front Behav Neurosci 6, 31.

Ray, J.P., Price, J.L., 1992. The organization of the thalamocortical connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain–prefrontal cortex topography. The Journal of Comparative Neurology 323, 167-197.
Raymond, J.E., O'Brien, J.L., 2009. Selective visual attention and motivation: the consequences of value learning in an attentional blink task. Psychol Sci 20, 981-988.
Redgrave, P., Prescott, T.J., Gurney, K., 1999. Is the short-latency dopamine response too short to signal reward error? Trends Neurosci 22, 146-151.
Redish, A.D., 1999. Beyond the cognitive map: From place cells to episodic memory. MIT Press.
Rescorla, R.A., 1980. Pavlovian second-order conditioning: Studies in associative learning. L. Erlbaum Associates.
Rescorla, R.A., 1988. Pavlovian conditioning - It's not what you think it is. American Psychologist 43, 151-160.
Rescorla, R.A., Skucy, J.C., 1969. Effect of response-independent reinforcers during extinction. Journal of Comparative and Physiological Psychology 67, 381-389.
Rescorla, R.A., Solomon, R.L., 1967. 2-Process learning theory - Relationships between Pavlovian conditioning and instrumental learning. Psychological Review 74, 151-&.
Rescorla, R.A., Wagner, A.R., 1972. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement, in: Black, A.H., Prokasy, W.F. (Eds.), Classical Conditioning. Appleton-Century-Crofts, New York, pp. 64-99.
Richard, J.M., Berridge, K.C., 2011. Nucleus accumbens dopamine/glutamate interaction switches modes to generate desire versus dread: D(1) alone for appetitive eating but D(1) and D(2) together for fear. J Neurosci 31, 12866-12879.
Robbins, T.W., 1975. The potentiation of conditioned reinforcement by psychomotor stimulant drugs. A test of Hill's hypothesis. Psychopharmacology 45, 103-114.
Robbins, T.W., 1976. Relationship between reward-enhancing and stereotypical effects of psychomotor stimulant drugs. Nature 264, 57-59.
Robbins, T.W., Cador, M., Taylor, J.R., Everitt, B.J., 1989. Limbic-striatal interactions in reward-related processes. Neurosci Biobehav Rev 13, 155-162.
Robbins, T.W., Everitt, B.J., 1992. Functions of dopamine in the dorsal and ventral striatum. Sem Neurosci 4, 119-127.
Roberts, D.C.S., Richardson, N.R., 1992. Self-administration of psychomotor stimulants using progressive ratio schedules of reinforcement, in: Boulton, A., Baker, G., Wu, P.H. (Eds.), Neuromethods. Humana, Totowa, pp. 223-269.
Robinson, S., Sandstrom, S., Denenberg, V., Palmiter, R., 2005. Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. Behav Neurosci 119, 5 - 15.
Robinson, T.E., Becker, J.B., 1986. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 396, 157-198.
Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18, 247-291.
Robinson, T.E., Berridge, K.C., 2000. The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95, S91-S117.
Robinson, T.E., Berridge, K.C., 2001. Incentive-sensitization and addiction. Addiction 96, 103-114.

Robinson, T.E., Berridge, K.C., 2003. Addiction. Annual Review of Psychology 54, 25-53.
Robinson, T.E., Flagel, S.B., 2009. Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. Biol Psychiatry 65, 869-873.
Roitman, M., Wheeler, R., Wightman, R., Carelli, R., 2008. Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. Nat Neurosci 11, 1376 - 1377.
Rudy, J.W., Sutherland, R.J., 1995. Configural association theory and the hippocampal formation: An appraisal and reconfiguration. Hippocampus 5, 375-389.
Saddoris, M.P., Stamatakis, A., Carelli, R.M., 2011. Neural correlates of Pavlovian-to-instrumental transfer in the nucleus accumbens shell are selectively potentiated following cocaine self-administration. European Journal of Neuroscience 33, 2274-2287.
Salamone, J.D., Correa, M., 2012. The Mysterious Motivational Functions of Mesolimbic Dopamine. Neuron 76, 470-485.
Salamone, J.D., Correa, M., Farrar, A., Mingote, S.M., 2007. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology (Berl) 191, 461-482.
Salamone, J.D., Correa, M., Nunes, E.J., Randall, P.A., Pardo, M., 2012. The behavioral pharmacology of effort-related choice behavior: dopamine, adenosine and beyond. J Exp Anal Behav 97, 125-146.
Saunders, B.T., Richard, J.M., 2011. Shedding light on the role of ventral tegmental area dopamine in reward. J Neurosci 31, 18195-18197.
Saunders, B.T., Robinson, T.E., 2010. A cocaine cue acts as an incentive stimulus in some but not others: Implications for addiction. Biol Psychiatry 67, 730-736.
Saunders, B.T., Robinson, T.E., 2011a. A cue evokes relapse in the face of adverse consequences preferentially in rats prone to attribute incentive salience to reward cues. Society for Neuroscience Abstracts.
Saunders, B.T., Robinson, T.E., 2011b. Individual variation in the motivational properties of cocaine. Neuropsychopharmacology 36, 1668-1676.
Saunders, B.T., Robinson, T.E., 2012. The role of dopamine in the accumbens core in the expression of Pavlovian-conditioned responses. Eur J Neurosci 36, 2521-2532.
Schachter, S., 1968. Obesity and eating. Science.
Schenk, S., Partridge, B., 2001. Influence of a conditioned light stimulus on cocaine self-administration in rats. Psychopharmacology (Berl) 154, 390-396.
Schiltz, C.A., Bremer, Q.Z., Landry, C.F., Kelley, A.E., 2007. Food-associated cues alter forebrain functional connectivity as assessed with immediate early gene and proenkephalin expression. BMC Biol 5, 16.
Schindler, C.W., Panlilio, L.V., Goldberg, S.R., 2002. Second-order schedules of drug self-administration in animals. Psychopharmacology (Berl) 163, 327-344.
Schneirla, T.C., 1959. An evolutionary and developmental theory of biphasic processes underlying approach and withdrawal, Nebraska symposium on motivation, 1959. Univer. Nebraska Press, Oxford, England, pp. 1-42.
Schoenmakers, T., Wiers, R.W., Field, M., 2008. Effects of a low dose of alcohol on cognitive biases and craving in heavy drinkers. Psychopharmacology (Berl) 197, 169-178.

Schoenmakers, T.M., de Bruin, M., Lux, I.F., Goertz, A.G., Van Kerkhof, D.H., Wiers, R.W., 2010. Clinical effectiveness of attentional bias modification training in abstinent alcoholic patients. Drug Alcohol Depend 109, 30-36.
Schultz, W., 1998. Predictive reward signal of dopamine neurons. J Neurophysiol 80, 1-27.
Schultz, W., 2007. Multiple dopamine functions at different time courses. Annu Rev Neurosci 30, 259-288.
Schultz, W., Dayan, P., Montague, P.R., 1997. A neural substrate of prediction and reward. Science 275, 1593-1599.
Schwartz, B., Williams, D.R., 1972. The role of the response-reinforcer contingency in negative automaintenance. J Exp Anal Behav 17, 351-357.
Seiden, L.S., Sabol, K.E., Ricaurte, G.A., 1993. Amphetamine: effects on catecholamine systems and behavior. Annual review of pharmacology and toxicology 33, 639-676.
Servan-Schreiber, D., Carter, C.S., Bruno, R.M., Cohen, J.D., 1998. Dopamine and the mechanisms of cognition: Part II. D-amphetamine effects in human subjects performing a selective attention task. Biol Psychiatry 43, 723-729.
Shaham, Y., Shalev, U., Lu, L., de Wit, H., Stewart, J., 2003. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology 168, 3-20.
Shalev, U., Grimm, J.W., Shaham, Y., 2002. Neurobiology of relapse to heroin and cocaine seeking: A review. Pharmacological Reviews 54, 1-42.
Shiffman, S., 2009. Ecological momentary assessment (EMA) in studies of substance use. Psychol Assess 21, 486-497.
Shiffman, S., Gwaltney, C.J., Balabanis, M.H., Liu, K.S., Paty, J.A., Kassel, J.D., Hickcox, M., Gnys, M., 2002. Immediate antecedents of cigarette smoking: an analysis from ecological momentary assessment. J Abnorm Psychol 111, 531-545.
Shiflett, M.W., 2012. The effects of amphetamine exposure on outcome-selective Pavlovian-instrumental transfer in rats. Psychopharmacology (Berl) doi:10.1007/s00213-012-2724-y.
Shiner, T., Seymour, B., Wunderlich, K., Hill, C., Bhatia, K.P., Dayan, P., Dolan, R.J., 2012. Dopamine and performance in a reinforcement learning task: evidence from Parkinson's disease. Brain 135, 1871-1883.
Simon, J.J., Walther, S., Fiebach, C.J., Friederich, H.C., Stippich, C., Weisbrod, M., Kaiser, S., 2010. Neural reward processing is modulated by approach- and avoidance-related personality traits. Neuroimage 49, 1868-1874.
Simon, N.W., Mendez, I.A., Setlow, B., 2009. Effects of prior amphetamine exposure on approach strategy in appetitive Pavlovian conditioning in rats. Psychopharmacology (Berl) 202, 699-709.
Skinner, B.F., 1938. The behavior of organisms: an experimental analysis. Appleton-Century, Oxford, England.
Skinner, B.F., 1948. Superstition in the pigeon. J Exp Psychol 38, 168-172.
Smith, J.W., Dickinson, A., 1998. The dopamine antagonist, pimozide, abolishes Pavlovian-instrumental transfer. Journal of Psychopharmacology 12, A6.
Smith, K.S., Berridge, K.C., Aldridge, J.W., 2011. Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. Proc Natl Acad Sci U S A 108, E255-E264.
Smith, K.S., Mahler, S.V., Pecina, S., Berridge, K.C., 2010. Hedonic hotspots: Generating sensory pleasure in the brain, in: Kringelbach, M.L., Berridge, K.C. (Eds.), Pleasures of the Brain. Oxford University Press, pp. 62-73.

Solomon, R.L., Corbit, J.D., 1974. An opponent-process theory of motivation: I. Temporal dynamics of affect. Psychological Review 81, 119-145.
Stam, C.J., Visser, S.L., Op de Coul, A.A., De Sonneville, L.M., Schellens, R.L., Brunia, C.H., de Smet, J.S., Gielen, G., 1993. Disturbed frontal regulation of attention in Parkinson's disease. Brain 116 (Pt 5), 1139-1158.
Stein, L., 1964. Amphetamine and neural reward mechanisms, in: Steinberg, H., de Reuck, A.V.S., Knight, J. (Eds.), Animal behaviour and drug action. Churchill, London, pp. 91-118.
Stewart, J., de Wit, H., Eikelboom, R., 1984. Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 91, 251-268.
Stone, A.A., Shiffman, S., 1994. Ecological momentary assessment (EMA) in behavorial medicine. Annals of Behavioral Medicine 16, 199-202.
Stretch, R., Gerber, G.J., 1973. Drug-induced reinstatement of amphetamine self-administration behaviour in monkeys. Can J Psychol 27, 168-177.
Stretch, R., Gerber, G.J., Wood, S.M., 1971. Factors affecting behavior maintained by response-contingent intravenous infusions of amphetamine in squirrel monkeys. Can J Physiol Pharmacol 49, 581-589.
Styn, M.A., Bovbjerg, D.H., Lipsky, S., Erblich, J., In press. Cue-induced cigarette and food craving: A common effect? Addictive Behaviors.
Sutton, R.S., 1988. Learning to predict by the methods of temporal differences. Mach Learn 3, 9-44.
Sutton, R.S., Barto, A.G., 1998. Reinforcement learning: An introduction. MIT Press, Cambridge, MA.
Swanson, L.W., 1982. The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Research Bulletin 9, 321-353.
Takahashi, Y.K., Roesch, M.R., Wilson, R.C., Toreson, K., O'Donnell, P., Niv, Y., Schoenbaum, G., 2011. Expectancy-related changes in firing of dopamine neurons depend on orbitofrontal cortex. Nat Neurosci 14, 1590-1597.
Taylor, J.R., Robbins, T.W., 1984. Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacology (Berl) 84, 405-412.
Taylor, J.R., Robbins, T.W., 1986. 6-Hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. Psychopharmacology (Berl) 90, 390-397.
Thewissen, R., Havermans, R.C., Geschwind, N., van den Hout, M., Jansen, A., 2007. Pavlovian conditioning of an approach bias in low-dependent smokers. Psychopharmacology (Berl) 194, 33-39.
Tiffany, S.T., Wray, J.M., 2012. The clinical significance of drug craving. Annals of the New York Academy of Sciences 1248, 1-17.
Timberlake, W., Lucas, G.A., 1985. The basis of superstitious behavior: chance contingency, stimulus substitution, or appetitive behavior? J Exp Anal Behav 44, 279-299.
Tindell, A.J., Berridge, K.C., Zhang, J., Pecina, S., Aldridge, J.W., 2005. Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. Eur J Neurosci 22, 2617-2634.
Tindell, A.J., Smith, K.S., Berridge, K.C., Aldridge, J.W., 2009. Dynamic computation of incentive salience: "wanting" what was never "liked". J Neurosci 29, 12220-12228.

Toates, F., 1986. Motivational Systems. Cambridge University Press, Cambridge, UK.
Tolman, E.C., 1948. Cognitive maps in rats and men. Psychol Rev 55, 189-208.
Tomie, A., 1996. Locating reward cue at response manipulandum (CAM) induces symptoms of drug abuse. Neuroscience and Biobehavioral Reviews 20, 505-535.
Tomie, A., Aguado, A.S., Pohorecky, L.A., Benjamin, D., 2000. Individual differences in Pavlovian autoshaping of lever pressing in rats predict stress-induced corticosterone release and mesolimbic levels of monoamines. Pharmacology Biochemistry and Behavior 65, 509-517.
Tomie, A., Grimes, K.L., Pohorecky, L.A., 2008. Behavioral characteristics and neurobiological substrates shared by Pavlovian sign-tracking and drug abuse. Brain Research Reviews 58, 121-135.
Tomie, A., Lincks, M., Nadarajah, S.D., Pohorecky, L.A., Yu, L., 2012. Pairings of lever and food induce Pavlovian conditioned approach of sign-tracking and goal-tracking in C57BL/6 mice. Behav Brain Res 226, 571-578.
Trowill, J.A., Panksepp, J., Gandelman, R., 1969. An incentive model of rewarding brain stimulation. Psychological Review 76, 264-281.
Uslaner, J.M., Acerbo, M.J., Jones, S.A., Robinson, T.E., 2006. The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. Behavioural Brain Research 169, 320-324.
Uslaner, J.M., Dell'Orco, J.M., Pevzner, A., Robinson, T.E., 2008. The influence of subthalamic nucleus lesions on sign-tracking to stimuli paired with food and drug rewards: Facilitation of incentive salience attribution? Neuropsychopharmacology 33, 2352-2361.
Uslaner, J.M., Robinson, T.E., 2006. Subthalamic nucleus lesions increase impulsive action and decrease impulsive choice - mediation by enhanced incentive motivation? European Journal of Neuroscience 24, 2345-2354.
Uslaner, J.M., Yang, P.W., Robinson, T.E., 2005. Subthalamic nucleus lesions enhance the psychomotor-activating, incentive motivational, and neurobiological effects of cocaine. Journal of Neuroscience 25, 8407-8415.
Van Gucht, D., Vansteenwegen, D., Van den Bergh, O., Beckers, T., 2008. Conditioned craving cues elicit an automatic approach tendency. Behaviour Research and Therapy 46, 1160-1169.
Vanderschuren, L., Everitt, B.J., 2004. Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 305, 1017-1019.
Ventura, R., Morrone, C., Puglisi-Allegra, S., 2007. Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward- and aversion-related stimuli. Proc Natl Acad Sci U S A 104, 5181 - 5186.
Verbeke, G., 2009. Linear mixed models for longitudinal data. Springer, New York, NY.
Vezina, P., Leyton, M., 2009. Conditioned cues and the expression of stimulant sensitization in animals and humans. Neuropharmacology 56 Suppl 1, 160-168.
Volkow, N., Wang, G., Telang, F., Fowler, J., Logan, J., Childress, A., Jayne, M., Ma, Y., Wong, C., 2006. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J Neurosci 26, 6583 - 6588.
Volkow, N.D., Wang, G.J., Fowler, J.S., Logan, J., Schlyer, D., Hitzemann, R., Lieberman, J., Angrist, B., Pappas, N., MacGregor, R., Burr, G., Cooper, T., Wolf, A.P., 1994. Imaging endogenous dopamine competition with [11C]raclopride in the human brain. Synapse 16, 255-262.

Volkow, N.D., Wang, G.J., Ma, Y.M., Fowler, J.S., Wong, C., Ding, Y.S., Hitzemann, R., Swanson, J.M., Kalivas, P., 2005. Activation of orbital and medial prefrontal cortex by methylphenidate in cocaine-addicted subjects but not in controls: Relevance to addiction. Journal of Neuroscience 25, 3932-3939.
Volkow, N.D., Wang, G.J., Telang, F., Fowler, J.S., Logan, J., Childress, A.R., Jayne, M., Ma, Y., Wong, C., 2008. Dopamine increases in striatum do not elicit craving in cocaine abusers unless they are coupled with cocaine cues. Neuroimage 39, 1266-1273.
Volkow, N.D., Wang, G.J., Telang, F., Fowler, J.S., Logan, J., Jayne, M., Ma, Y., Pradhan, K., Wong, C., 2007. Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. J Neurosci 27, 12700-12706.
Volkow, N.D., Wise, R.A., 2005. How can drug addiction help us understand obesity? Nat Neurosci 8, 555-560.
Vorel, S.R., Liu, X., Hayes, R.J., Spector, J.A., Gardner, E.L., 2001. Relapse to cocaine-seeking after hippocampal theta burst stimulation. Science 292, 1175-1178.
Waelti, P., Dickinson, A., Schultz, W., 2001. Dopamine responses comply with basic assumptions of formal learning theory. Nature 412, 43-48.
Wakabayashi, K.T., Weiss, M.J., Pickup, K.N., Robinson, T.E., 2010. Rats markedly escalate their intake and show a persistent susceptibility to reinstatement only when cocaine is injected rapidly. J Neurosci 30, 11346-11355.
Wallace, J.M., 1999. The social ecology of addiction: race, risk, and resilience. Pediatrics 103, 1122-1127.
Wassum, K., Ostlund, S., Maidment, N., Balleine, B., 2009. Distinct opioid circuits determine the palatability and the desirability of rewarding events. Proc Natl Acad Sci U S A 106, 12512 - 12517.
Wassum, K.M., Ostlund, S.B., Balleine, B.W., Maidment, N.T., 2011. Differential dependence of Pavlovian incentive motivation and instrumental incentive learning processes on dopamine signaling. Learn Mem 18, 475-483.
Wassum, K.M., Ostlund, S.B., Maidment, N.T., 2012. Phasic mesolimbic dopamine signaling precedes and predicts performance of a self-initiated action sequence task. Biol Psychiatry 71, 846-854.
Watabe-Uchida, M., Zhu, L., Ogawa, S.K., Vamanrao, A., Uchida, N., 2012. Whole-brain mapping of direct inputs to midbrain dopamine neurons. Neuron 74, 858-873.
Waters, A.J., Shiffman, S., Sayette, M.A., Paty, J.A., Gwaltney, C.J., Balabanis, M.H., 2003. Attentional bias predicts outcome in smoking cessation. Health Psychol 22, 378-387.
Weingarten, H.P., 1983. Conditioned cues elicit feeding in sated rats - a role for learning meal initiation. Science 220, 431-433.
Wiers, R.W., Rinck, M., Dictus, M., van den Wildenberg, E., 2009. Relatively strong automatic appetitive action-tendencies in male carriers of the OPRM1 G-allele. Genes Brain Behav 8, 101-106.
Wilcove, W.G., Miller, J.C., 1974. CS-USC presentations and a lever: human autoshaping. J Exp Psychol 103, 868-877.
Williams, D.R., Williams, H., 1969. Auto-maintenance in the pigeon: sustained pecking despite contingent non-reinforcement. J Exp Anal Behav 12, 511-520.
Wise, R.A., 2004. Dopamine, learning and motivation. Nat Rev Neurosci 5, 483-494.
Wise, R.A., Spindler, J., deWit, H., Gerberg, G.J., 1978. Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. Science 201, 262-264.

Witten, I.B., Steinberg, E.E., Lee, S.Y., Davidson, T.J., Zalocusky, K.A., Brodsky, M., Yizhar, O., Cho, S.L., Gong, S., Ramakrishnan, C., Stuber, G.D., Tye, K.M., Janak, P.H., Deisseroth, K., 2011. Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. Neuron 72, 721-733.

Wong, D.F., Kuwabara, H., Schretlen, D.J., Bonson, K.R., Zhou, Y., Nandi, A., Brasic, J.R., Kimes, A.S., Maris, M.A., Kumar, A., Contoreggi, C., Links, J., Ernst, M., Rousset, O., Zukin, S., Grace, A.A., Lee, J.S., Rohde, C., Jasinski, D.R., Gjedde, A., London, E.D., 2006. Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. Neuropsychopharmacology 31, 2716-2727.

Woods, J.H., Schuster, C.R., 1971. Opiates as reinforcing stimuli, in: Thompson, T., Pickens, R. (Eds.), Stimulus properties of drugs. Appleton-Century-Crofts, New York.

Woolverton, W.L., Virus, R.M., 1989. The effects of a D1 and a D2 dopamine antagonist on behavior maintained by cocaine or food. Pharmacol Biochem Behav 32, 691-697.

Wyvell, C.L., Berridge, K.C., 2000. Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: Enhancement of reward "wanting" without enhanced "liking" or response reinforcement. Journal of Neuroscience 20, 8122-8130.

Wyvell, C.L., Berridge, K.C., 2001. Incentive sensitization by previous amphetamine exposure: Increased cue-triggered "wanting" for sucrose reward. J Neurosci 21, 7831-7840.

Yager, L.M., Robinson, T.E., 2010. Cue-induced reinstatement of food seeking in rats that differ in their propensity to attribute incentive salience to food cues. Behav Brain Res 214, 30-34.

Yager, L.M., Robinson, T.E., 2012. A classically conditioned cocaine cue acquires greater control over behavior in rats prone to attribute incentive salience to a food cue. Psychopharmacology.

Yin, H., Zhuang, X., Balleine, B., 2006. Instrumental learning in hyperdopaminergic mice. Neurobiol Learn Mem 85, 283 - 288.

Young, P.T., 1959. The role of affective processes in learning and motivation. Psychological Review 66, 104-125.

Young, P.T., 1966. Hedonic organization and regulation of behavior. Psychological Review 73, 59-86.

Zahm, D.S., 2000. An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neurosci Biobehav Rev 24, 85-105.

Zahm, D.S., 2006. The evolving theory of basal forebrain functional anatomical macrosystems. Neurosci Biobehav Rev 30, 148-172.

Zener, K., 1937. The significance of behavior accompanying conditioned salivary secretion for theories of the conditioned response. American Journal of Psychology 50, 384-403.

Zhang, J., Berridge, K.C., Aldridge, J.W., 2012. Computational models of incentive-sensitization in addiction: Dynamic limbic transformation of learning into motivation, in: Gutkin, B., Ahmed, S.H. (Eds.), Computational Neuroscience of Drug Addiction. Springer, New York, NY, pp. 189-203.

Zhang, J., Berridge, K.C., Tindell, A.J., Smith, K.S., Aldridge, J.W., 2009. A neural computational model of incentive salience. PLoS Comput Biol 5, e1000437.

Zhao, L.-Y., Tian, J., Wang, W., Qin, W., Shi, J., Li, Q., Yuan, K., Dong, M.-H., Yang, W.-C., Wang, Y.-R., Sun, L.-L., Lu, L., 2012. The Role of Dorsal Anterior Cingulate Cortex in the Regulation of Craving by Reappraisal in Smokers. PLoS One 7, e43598.

Zijlstra, F., Booij, J., van den Brink, W., Franken, I.H., 2008. Striatal dopamine D2 receptor binding and dopamine release during cue-elicited craving in recently abstinent opiate-dependent males. Eur Neuropsychopharmacol 18, 262-270.