INDIVIDUAL VARIATION IN THE EXTENT TO WHICH DRUG CUES ACQUIRE CONTROL OVER MOTIVATED BEHAVIOR

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in the University of Michigan
2014

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In memory of Seraphina “Sophie” Puglia

Acknowledgements

First, I would like to thank my graduate advisor, Terry Robinson, who has been a source of support throughout this entire process and who has taught me how to be a better scientist and writer. I would especially like to thank him for pushing me when I didn't think I could do it. I would also like to thank the rest of my dissertation committee- Kent Berridge, Wayne Aldridge, and Peggy Gnegy- for their input on this thesis. Finally, I would also like to thank Brandon Aragona for always having an open door to talk about data, life, or whatever might come up.

During my time in the Robinson Lab I've had the tremendous pleasure of working with many very talented individuals. First, I would like to thank Ben Saunders for being the best lab partner in crime. I would also like to thank Vedran Lovic for all of his advice (both personal and professional) over the years and for all of the rides to my car. I'd especially like to thank my two Lab Lizards for all of their help, especially over the last few months of writing. Liz- I can't even begin to tell you how much I will miss you and how boring my life will be without you. Remember, it is your job to keep Kawa in line now. Elizabeth, thank you for all of your help, both technically and emotionally, TER lab won't be the same once you leave. I would also like to thank Kyle Pitchers for lending me the immno bible (RIP) and helping me with troubleshooting- without your help I never would have finished my last experiment. I would also like to thank Paul Meyer, Bryan Singer, Jon Morrow, Jake Jedynak, Ken Wakabayashi, Kristi Pickup, and Alayna Czuj for their help and friendship over the years. A special thank you also goes to Shelly Flagel who has provided valuable input in both the design of some of the projects

in this dissertation as well as for providing feedback on my writing. Many undergraduates have helped along the way and I would like to thank them for all of their time and hard work. Specifically I would like to thank Joe Domino, Anu Dewasurendra, Lilly Bellfi, Patricia Checinski, Crystal Moreno, Jeremy Delor, and Kyle Eaton.

Outside of lab I have been fortunate to be surrounded by so many very wonderful people- Jocelyn Richard, Eila Roberts, Josie Urban, AJ Llaneza, Caitlin Orsini, Daniel Castro, Kirsten Porter-Stransky, Alexandra DiFeliceantonio, Matt Howe, Shanna Harkey, Elyse Aurbach, Morgan Gustison, Giovanna Paolone, Jeff Pettibone, Caitlin Vander Weele, Arif Hamid, Katy Goldey, and Alex Kawa. You guys have helped to make Michigan my home.

Finally, I couldn't have done any of this without the support of my family. Mom, Dad, and Stephen- I know that most of what I talk about doesn't usually make much sense to you guys, but thank you for sticking through this with me. And last, but certainly not least, I have to thank Zachary Chakan. I never in a million years thought I would meet someone like you. You have changed my outlook on life and I am forever grateful for your constant support, encouragement, and for helping me clean rat poop on your weekend visits. I love you with all of my heating. We did it!

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Abstract

Cues associated with natural or drug rewards can acquire such powerful control over behavior that individuals sometimes have difficulty resisting them. Indeed, the ability of reward-related cues to motivate excessive behavior has been implicated in drug addiction, obesity, and binge eating. For example, in human addicts and animal models of drug self-administration, drug cues are important for both maintaining and reinstating drug-seeking behavior. There is, however, considerable individual variation in the influence of reward-associated cues on behavior. We have argued that this is due, in part, to individual variation in the degree to which reward-related cues acquire incentive motivational properties (are attributed with incentive salience), and thus acquire the ability to act as incentive stimuli. For example, if a localizable stimulus (the conditioned stimulus, CS) is repeatedly paired with delivery of a food reward (the unconditioned stimulus, US) the food cue itself becomes attractive, eliciting approach and engagement with it in some rats (sign-trackers, STs). However, in other rats the food cue itself is not attractive, but instead upon CS presentation these animals approach the location where food will be delivered (goal-trackers, GTs). Yet other rats vacillate between the cue and the goal. Furthermore, a localizable food cue is a more effective conditioned reinforcer, and is more effective in reinstating food-seeking behavior, in STs than in GTs. Thus, only in some animals does a predictive cue also acquire the properties of an incentive stimulus – the ability to attract, the ability to act as a conditioned reinforcer, and to spur (motivate) seeking for its associated reward.

Recent studies suggest that the propensity of animals to attribute incentive salience to a food cue predicts the extent to which a drug cue acquires incentive properties. While there is now considerable evidence for individual variation in the extent to which a classically conditioned *food* cue is attributed with incentive salience, there is much less information concerning individual variation in the extent to which classically conditioned *drug* cues acquire incentive motivational properties. This dissertation will address the following questions: 1) Does individual variation in the tendency to attribute incentive value to a food cue predict the tendency to attribute incentive value to a classically conditioned drug cue? and 2) Are there differences in the ability of food and drug cues that are predictive of reward (i.e., GTs and STs) vs. ones that also acquire incentive motivational properties (i.e., STs) to engage brain reward systems?

In Chapter 2 I found that a classically conditioned cocaine cue became more attractive, in that it elicited greater approach behavior towards it, and more desired, in that it supported more robust drug-seeking behavior under extinction conditions, in individuals prone to attribute incentive salience to a food cue. In Chapter 3, I explored the extent to which an opioid cue became attractive and desired. I found that relative to GTs, STs were more attracted to the opioid cue and they also found it more desirable. Dopamine transmission within the nucleus accumbens core is necessary for conditioned approach behavior to an opioid cue. Furthermore, I found that in order for the mesocorticolimbic system to be engaged by either a food or opioid cue it must be imbued with incentive salience. Finally, in Chapter 4, I found that while a nicotine cue became attractive to both STs and GTs, it was a more effective conditioned reinforcer in STs than GTs. These studies have the potential to significantly shift how we think about individual vulnerability to addiction and relapse, and to point the way for better targeted interventions.

Chapter 1

Introduction

Attending to environmental stimuli (sights, sounds, smells, places) that are associated with rewards and dangers (i.e., cues) are of cardinal importance for survival. Animals live in an ever-changing environment and being able to determine where resources might be found or where a predator might be lurking could be the difference between life and death, and cues provide a mechanism for doing so as they carry information. Cues can aid animals in learning about whether an outcome will be good or bad, reinforcing actions leading to positive or appetitive outcomes and diminishing behaviors leading to negative or aversive outcomes, and thus contribute to decision-making. Furthermore, cues can also act as predictors of reward - signaling the location and/or the availability of reward. For example, the vivid red color of an apple indicating it is ready to be consumed or a hole in the ground where a hungry bird might find a worm. Although cues are important for guiding normal, adaptive, behaviors, such as seeking out food or a mate, they can also contribute to compulsive behaviors associated with disorders such as obesity, binge eating, gambling, and addiction.

Conditioned stimuli

Cues in the environment that are associated with rewards can influence behavior in multiple ways. One of the oldest, well known, and most studied ways is the ability of cues to act as conditioned stimuli (CS), which are capable of eliciting a conditioned response (CR, Pavlov 1927). This was first demonstrated in now classic studies conducted by Pavlov (1927). In

Pavlov's original experiments he paired the ticking of a metronome (the CS), an initially neutral stimulus, with the delivery of a food reward (the unconditioned stimulus, US). He found that after repeated pairings, presentation of the ticking metronome (the CS) alone could elicit salivation (the CR). Pavlov noticed that dogs initially salivated, a reflexive response, unconditionally when presented with the food reward (the US), and therefore termed the ability of the CS to evoke a CR as a conditioned reflex. The idea that the ability of a CS to evoke a CR was a simple reflexive response was a long held idea by many researchers.

Incentive stimuli

While CSs are capable of eliciting simple CRs, there have been many studies since Pavlov's initial work showing that reward cues not only acquire the ability to elicit simple reflexive or autonomic CRs, but they can also activate complex emotional and motivational states (Berridge 2001; Bindra 1978; Cardinal et al. 2002; Konorski 1967; Lajoie and Bindra 1976; Rescorla 1988; Toates 1986). In order for a reward cue to arouse a motivational state it must be attributed with incentive salience. Incentive salience refers to the "motivational… component of reward. Its attribution transforms mere sensory information about rewards and their cues (sights, sounds, and smells) into attractive, desired, riveting incentives" (Berridge and Robinson 2003, p. 510). Cues that have been attributed with incentive salience (incentive stimuli) acquire three fundamental properties. Incentive stimuli are: (1) attractive, eliciting approach towards them and biasing attention, (2) "wanted", in that individuals will work for them (i.e., they act as conditioned reinforcers) and, (3) can evoke a state of conditioned motivation that spurs the pursuit of their associated reward (Berridge 2001; Cardinal et al. 2002; Lovibond 1983; Milton and Everitt 2010).

Conditioned approach

The first feature of an incentive stimulus is the ability to grab one's attention and attract. This serves to bring the individual into close proximity with the cue and often also with the reward itself. In the lab this feature of an incentive stimulus is often measured by Pavlovian conditioned approach behavior. In a classic Pavlovian conditioned approach experiment, a cue (the CS) is paired with the delivery of a reward (the US) and after repeated pairings presentation of the CS often comes to elicit approach behavior towards it (Brown and Jenkins 1968; Hearst and Jenkins 1974). This is quite remarkable given that no action is required by the animal to receive reward delivery. Hearst and Jenkins (1974) originally termed this conditioned approach response "sign-tracking" because animals directed their behavior towards the cue, or "sign", which predicted reward delivery. It is important to note that the term "autoshaping" was originally used to describe this procedure (Brown and Jenkins 1968). However, this is actually a misnomer because with this procedure no responses are reinforced or "shaped". The ability of reward cues to elicit sign-tracking behavior has now been documented in a variety of species including birds, fish, primates, mice, rats, dogs and humans (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; Zener 1937). Importantly, sign-tracking behavior is not maintained by accidental reinforcement or superstitious behavior and is not due to stimulus substitution (Boakes 1977; Flagel et al. 2009; Harris et al. 2013; Holland 1977; Hollis 1982; Killeen 2003; Lajoie and Bindra 1976; Locurto et al. 1976; Timberlake and Grant 1975; Timberlake and Lucas 1985; Williams and Williams 1969).

Conditioned reinforcement

Secondly, incentive stimuli become desired ("wanted") and are sought after, in the sense that individuals will work for them. Put another way, incentive stimuli can serve as conditioned or secondary reinforcers. This feature of an incentive stimulus serves to support behavior over delays in reinforcement, maintain behavior in the absence of reward, and support the acquisition of new instrumental responding or instrumental chains (Fantino 1977; Fantino 2008; Hull 1943; Kelleher and Gollub 1962; Mackintosh 1974). In the lab, the ability of a cue to serve as a conditioned reinforcer is often measured by whether the cue will reinforce instrumental responding in the absence of any reward, such as in traditional extinction-reinstatement self-administration paradigms (for review see Nair et al. 2009; Shaham et al. 2003). A more stringent test of the ability of a cue to serve as a conditioned reinforcer is the ability of animals to acquire a novel instrumental response that has not previously been associated with reward (Mackintosh 1974).

Conditioned motivation

Finally, incentive stimuli acquire the ability to arouse a state of conditioned motivation that spurs reward seeking or energizes ongoing reward seeking behavior (Bindra 1968; Cardinal et al. 2002; Milton 2012; Milton and Everitt 2010). This feature of an incentive stimulus has traditionally been measured by Pavlovian-to-instrumental transfer (PIT) procedures (Estes 1943; 1948; Holmes et al. 2010; Lovibond 1983; Talmi et al. 2008). In a typical PIT experiment individuals first undergo Pavlovian training where a cue is paired with non-contingent reward delivery (i.e., no response is necessary to receive reward). Next, individuals undergo an instrumental training phase where they learn to perform an instrumental action (e.g., make a lever press) to earn a reward. Finally, during the test phase, instrumental responding is assessed

(usually under extinction conditions) during non-contingent presentation of the previously Pavlovian conditioned cue. Generally, presentation of the Pavlovian cue increases the rate or "vigor" of instrumental responding for the reward and this is thought to reflect a state of conditioned motivation (Bindra 1968; Holmes et al. 2010; Milton and Everitt 2010). In addition to invigorating an ongoing response, non-contingent cue presentations can also evoke seeking responses (Barker et al. 2012; Deroche-Gamonet et al. 2002).

Drug cues as incentive stimuli

The ability of incentive stimuli to motivate behavior is highly adaptive as they increase the likelihood that an animal will acquire rewards that are necessary for survival (e.g., food and water) and for propagation of the species (e.g., a receptive sexual partner). However, incentive stimuli can also motivate maladaptive behavior. For example, on a daily basis we all encounter cues, such as a Starbucks or McDonalds sign, that signal the availability of high-fat and sugary foods. Encounters with these types of cues can arouse motivation and contribute to over-eating and obesity (Berridge et al. 2010; Cornell et al. 1989; Jansen 1998). The idea that incentive stimuli can also contribute to disorders such as addiction, motivating continued drug use and also instigating relapse, has a long history. Indeed, Stewart et al. (1984) argued that the "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" (p. 251). It is, "the drug itself, or presentation of a stimulus previously paired with the drug, [that] acts to create a motivational state that facilitates drug-seeking behavior" (p. 256). There are now several theories of addiction that emphasize the importance of drug-associated cues (Di Chiara 1998; Milton and Everitt 2010; Robinson and Berridge 1993; Tomie 1996).

Milton and Everitt (2010) recently conceptualized the ability of drug cues to acquire properties of an incentive stimulus, and thus motivate behavior, in what they termed the "three routes to relapse" (Fig. 1.1, adapted from Milton and Everitt 2010). First, drug cues can attract attention, eliciting approach behavior towards locations where drugs will be found or towards devices used for drug delivery (also see Tomie 1996). Second, drug cues can act as conditioned reinforcers that can reinforce actions leading to the procurement of drugs, maintain drug-seeking behavior even when drug is not available, and reinstate extinguished drug-seeking behavior. Lastly, drug cues can evoke a state of conditioned motivation that can maintain ongoing drug-seeking and –taking behaviors and during a period of abstinence, instigate relapse. Thus, the ability of drug cues to act as incentive stimuli contribute to both continued drug use and relapse. It is important to note that while each of these properties of an incentive stimulus relies on dissociable psychological and neurobiological systems (Cardinal et al. 2002), and can be studied in isolation in the laboratory, they likely act in concert in the real world of addicts to promote relapse (Milton and Everitt 2010).

Conditioned approach

While there is now considerable evidence that food cues can be imbued with incentive salience, acquiring the ability to elicit approach (sign-tracking) towards them (Brown and Jenkins 1968; Davey and Cleland 1982; Hearst and Jenkins 1974), it has been less clear whether drug cues also support approach behavior. Tomie (1996) was amongst the first to point out that one reason that drug paraphernalia (e.g., needles, pipes, glassware, etc.) are problematic in the context of addiction is because these objects become attractive and facilitate approach and engagement, leading to continued drug use. However, initial attempts to demonstrate sign-

tracking to a cue associated with an intravenous (IV) injection of cocaine were unsuccessful (Kearns and Weiss 2004). This led Everitt and Robbins (2005) to speculate that,

> *"it might logically be thought that Pavlovian approach is involved in maladaptively attracting humans towards sources of addictive drug reinforcers…as emphasized in the incentive salience theory of addiction. However…approach to a CS predictive of a drug…has [not] been clearly demonstrated in laboratory studies…although…[it] is readily seen in animals responding for natural rewards. It may be that the experimental conditions for demonstrating [this] phenomena in a drug seeking setting have not yet been optimized, but it may also be that the behavioral influence of CSs associated with drugs and natural reinforcers differ fundamentally in this regard"* (p. 1482).

However, there have now been several reports, from several different labs and using drugs from a variety of different classes, that rats will approach a cue associated with drug delivery. For example, Tomie (2001) was the first report that rats would approach a cue associated with either an ethanol/saccharin or an amphetamine/saccharin solution. However, both of these studies utilized sweetened drug solutions and it was unclear, despite a number of controls, whether approach to the CS was driven by its association with the drug or with the sweet solution. Krank et al. (2008) recently followed up on Tomie's original studies and found that rats would also approach a cue associated with an unsweetened ethanol solution, supporting the notion that drug cues can elicit approach behavior. It wasn't until several years after the report of Kearns and Weiss (2004), that Uslaner et al. (2006) successfully demonstrated conditioned approach to a cue associated with an IV infusion of cocaine. Uslaner et al. (2006) suggested that the reason that Kearns and Weiss (2004) failed to show sign-tracking to a cocaine cue was because of issues with their training parameters. Kearns and Weiss (2004) used a very short inter-trial interval (average of 90 s between trials) and, unlike food, the neurobiological and interoceptive effects of cocaine last for a long period of time (at least longer than 90 s). It is therefore likely that upon subsequent CS-US pairings rats were still experiencing the effects of the previous injection,

making the rats' ability to form an association between the CS and the US difficult. In fact, Kearns and Weiss (2004) reported that their animals displayed signs of cocaine-induced stereotypy, which would make approach to the cocaine cue nearly impossible. There have now been several reports, all using longer inter-trial intervals, with drugs from different drug classes, including psychostimulants and opioids, that rats will also approach a cue associated with an IV drug injection (Aragona et al. 2009; Flagel et al. 2010; Madsen and Ahmed 2014; Peters and De Vries 2013; Yager and Robinson 2013). It remains to be seen whether conditioned approach is also observed in response to another highly abused drug, nicotine. This question will be explored in Chapter 4.

Conditioned reinforcement

Drug-associated cues also acquire the ability to act as conditioned reinforcers and this contributes to self-administration behavior in both humans and non-human animals. One reason drug-associated conditioned reinforcers are thought to contribute to persistent drug use is because of their ability to maintain drug-seeking behavior over long periods of time, even when drug is not available. This is often studied in the lab using second-order schedules of drug delivery, where responses are reinforced by presentation of the drug cue before any drug delivery (Arroyo et al. 1998; Everitt and Robbins 2000; Goldberg 1973; Goldberg and Tang 1977; Katz 1979; Schindler et al. 2002). Responding under this schedule can be maintained by very few, or even single, drug infusions over an extended period of time (Goldberg and Tang 1977; Spear and Katz 1991). Drug cues can also support the acquisition of multi-operant sequences or complex behavioral chains leading to drug reward (Olmstead et al. 2000; Thompson and Pickens 1969; Thompson and Schuster 1964). Perhaps an even more powerful demonstration of the ability of drug cues to serve as conditioned reinforcers is that animals will learn a novel instrumental

response to earn presentation of the drug cue alone (Bertz and Woods 2013; Davis and Smith 1976; Di Ciano and Everitt 2004; Palmatier et al. 2007) and this response is resistant to extinction- the drug cue alone can support responding for up to two months (Di Ciano and Everitt 2004). Furthermore, drug associated cues are important for maintaining self-administration behavior; removal of the cue drastically diminishes self-administration behavior even when drug is still available (Caggiula et al. 2001; Schenk and Partridge 2001). Finally, the ability of drug cues to act as conditioned reinforcers has been demonstrated many times by self-administration studies where a drug associated cue can reinstate extinguished drug-seeking behavior (de Wit and Stewart 1981; Nie and Janak 2003; See 2002; Shaham et al. 2003). Even cues associated with cocaine in just one self-administration session can increase cocaine seeking behavior a year later (Ciccocioppo et al. 2004).

Conditioned motivation

Lastly, drug cues can motivate drug-seeking behavior by eliciting a state of conditioned motivation or desire. In humans, this conditioned motivational state is often referred to as craving and can be measured by either implicit or subjective measures of desire or craving (Carter and Tiffany 1999; Hester et al. 2006; Rosenberg 2009). As mentioned previously, PIT procedures are typically used in non-human animals to assess cue-evoked incentive motivation. While there have been many demonstrations of PIT using natural rewards, few studies have attempted to use this procedure with a drug reward. The majority of studies to date that have used PIT to measure conditioned motivation have only used orally administered alcohol as the reward (Corbit and Janak 2007; Glasner et al. 2005; Krank 2003). It wasn't until recently that LeBlanc et al. (2012) expanded on this work and demonstrated successful transfer during the PIT test when using IV cocaine as the reward. It is also interesting to note that LeBlanc et al.

(2012) trained animals to self-administer cocaine using a two-action, seeking-taking chain. In this procedure rats must first respond on the "seeking" lever to gain access to the "taking" lever. Responses on the "taking" lever results in cocaine delivery. They found that during the PIT test presentation of the cocaine-cue increased responding during both the seeking and taking parts of the behavioral chain, suggesting that drug cues contribute to both the motivation to acquire and consume drugs.

Individual variation in the extent to which a food cue acquires properties of an incentive stimulus

While reward cues can acquire incentive motivational properties, and thus motivate behavior, there is considerable individual variation in the extent to which reward-associated cues can influence behavior (Barker et al. 2012; Beaver et al. 2006; Flagel et al. 2009; Mahler and de Wit 2010; Meyer et al. 2012a; Robinson and Flagel 2009; Saunders and Robinson ; Schachter 1968; Tomie et al. 2000). Next, I will review a series of studies that address the topic of individual variation in the propensity of animals to attribute incentive salience to food cues.

Conditioned approach

Zener (1937) was the first to systematically describe individual variation in the extent to which a food cue could become attractive and elicit approach. Using a procedure that was nearly identical to the classic studies conducted by Pavlov (1927), Zener (1937) paired the ringing of a bell (the CS) with food delivery (the US). However, unlike in most of Pavlov's studies, the dogs in Zener's experiment were not restrained. Zener (1937) reported that after training some dogs responded to presentation of the CS with a "small but definite movement of approach toward the conditioned stimulus… followed by a backing up later to a position to eat." Other dogs, however, would make "an initial glance at the bell" followed by "a constant fixation …to the food pan…"

(p. 391). There were also dogs that would vacillate their response, looking back and forth between the bell and the food pan.

After the initial work by Zener (1937), little attention was paid to individual variation in the topography of conditioned responses evoked by conditioned stimuli. It wasn't until 40 years later that Boakes (1977) described similar individual variation in rats. Boakes (1977) used a standard Pavlovian conditioning procedure where illumination of a lever (the CS) was paired with non-contingent delivery of a food pellet (the US). Similar to what Hearst and Jenkins (1974) had reported, Boakes found that upon illumination of the lever, some rats approached and engaged with the lever. Following the precedent set by Hearst and Jenkins (1974), Boakes referred to this CS-elicited approach to the cue, or sign, as a sign-tracking (ST) response. However, Boakes also found that some rats did not approach the lever, but instead presentation of the CS elicited approach to where the food would later be delivered and he referred to this as a goal-tracking (GT) response (Boakes 1977). Additionally, Boakes (1977) conducted several follow-up studies to determine what variables might affect behavior during autoshaping (i.e., variability of reward delivery, motivation level). He found that decreasing the probability of a trial being rewarded produced more sign-tracking behavior (see also Anselme et al. 2013) and that varying the level of food deprivation had no effect on either sign- or goal-tracking behavior.

In a series of recent studies we expanded on this early work by Boakes and others, and characterized individual variation in the ability of a food cue to elicit either a sign-tracking or goal-tracking response in a population of over 4,000 rats (Fig. 1.2; Fitzpatrick et al. 2013; Flagel et al. 2007; Lovic et al. 2011; Meyer et al. 2012a; Morrow et al. 2011; Paolone et al. 2013; Robinson and Flagel 2009; Saunders and Robinson 2010; 2011; Saunders and Robinson 2012; Saunders et al. 2013; Yager and Robinson 2010). Based on these studies, it is clear that both STs

and GTs learn the CS-US association, and do so at a similar rate, as the CS reliably evokes an approach CR in both - the conditioned approach response is just directed at different locations (Robinson and Flagel 2009). Importantly, the difference in approach behavior between STs and GTs is not due to general differences in the ability to learn, as there are no differences between groups in learning a variety of tasks including fear conditioning and instrumental responding for both food and drug rewards (Morrow et al. 2011; Robinson and Flagel 2009; Saunders and Robinson 2010; Yager and Robinson 2010). Additionally, if the food cue is not explicitly paired with food delivery, rats do not learn either a ST or GT CR (Flagel et al. 2010; Lomanowska et al. 2011; Robinson and Flagel 2009).

What could account for differences in approach behavior? We have suggested that variation in the conditioned approach response is due to variation in the propensity to attribute incentive salience to reward cues and thus in the ability of reward cues to acquire properties of an incentive stimulus (Flagel et al. 2009; Meyer et al. 2012b; Robinson and Flagel 2009; Robinson et al. 2014). There are in fact several lines of evidence to suggest that the propensity to attribute incentive salience to reward cues represents a complex psychological trait (Meyer et al. 2012a). First, like many traits, the ST/GT behavioral phenotypes are heritable. Flagel et al. (2010) found that a rat line selectively bred for their locomotor response to a novel environment also varied in their propensity to attribute incentive salience to reward cues. As it turns out, when using food as the US, rats that showed high locomotor activity to a novel environment (bred high-responder rats, bHRs) were almost exclusively STs and rats that showed low locomotor activity to a novel environment (bred low-responder rats, bLRs) were almost exclusively GTs. The bHR/ST and bLR/GT phenotypes have continued to be selected together across many generations (Flagel et al. 2011b; Flagel et al. 2010) and the behavioral phenotypes of these

animals can be predicted prior to any Pavlovian training based on their breeding history. Additionally, after screening such a large population of rats, we began to notice a trend in the distribution of ST and GT CRs depending on which supplier, and even which colony within a supplier, we obtained the rats from. Recently, Fitzpatrick et al. (2013) analyzed the approach CRs of rats obtained from two commercial supplies, Harlan Laboratories and Charles River. They found that rats from Harlan are more likely to display a ST CR while rats from Charles River are more likely to display a GT CR. They also explored whether there were genetic differences between colonies, and found that colonies that were more genetically similar to one another were also more phenotypically similar. These data suggest that there are underlying genetic differences relating to the ST/GT phenotypes. Unfortunately, we do not know what specific genetic differences contribute to the ST/GT phenotypes and this remains an open field of questioning. Second, the ST/GT phenotypes can be influenced by environmental factors. For example, Lomanowska et al. (2011) showed the early life adversity (depravation of early life social experience) increased the proportion of STs in the population while Beckmann and Bardo (2012) reported that environmental enrichment shifted conditioned approach behavior towards goal-tracking. Therefore, like many psychological traits, these behavioral phenotypes are susceptible to gene by environment interactions. Third, there are biological differences between these behavioral phenotypes. For example, there are differences in the stress response of STs and GTs (Flagel et al. 2009) and there are differences in the dopamine system (see below for further discussion of this point). Finally, these phenotypes are stable over long periods of time (Robinson and Flagel 2009). Given that STs tend to approach reward cues because of a propensity to attribute incentive salience to such cues, we have asked whether variation in

conditioned approach predicts variation in the extent to which reward cues acquire other features of an incentive stimulus.

Conditioned reinforcement

In addition to incentive stimuli being attractive, they can also become desirable in the sense that they will reinforce actions to obtain them. Following the logic that CSs acquire properties of an incentive stimulus to a greater extent in STs than GTs, a CS should also be a more effective conditioned reinforcer in STs than in GTs. Indeed, we have shown that the food cue used during Pavlovian conditioning, which elicits approach towards it in STs but not in GTs, is a more effective conditioned reinforcer in STs than in GTs (Lomanowska et al. 2011; Meyer et al. 2012a; Robinson and Flagel 2009). Furthermore, I have also shown that a cue associated with food delivery in an instrumental task is more effective in reinstating food-seeking behavior, following extinction, in STs than GTs (Yager and Robinson 2010). Thus, a food-associated cue is both more attractive and more desired in STs than in GTs.

Conditioned motivation

While there is now considerable evidence for individual variation in the ability of food cues to attract and to serve as conditioned reinforcers, little is known about variation in the ability of a food cue to elicit a conditioned motivational state and thus motivate or spur behavior. As mentioned above, the ability of a cue to generate a conditioned motivational state is usually assessed using PIT. PIT experiments typically utilize an auditory stimulus during Pavlovian conditioning to avoid response competition during the PIT test because presentation of a localizable stimulus, such as a lever or light, would draw some individuals towards the cue thus competing with the instrumental portion of the task (Holmes et al. 2010; Tomie 1996). However, attempting this experiment in STs and GTs has been difficult because both STs and GTs attribute

incentive salience to an auditory cue (Meyer et al. 2014). While we have not performed a PIT experiment in STs and GTs, one recent study showed that mice vary in the extent to which a food-associated cue can invigorate food-seeking behavior in a PIT paradigm (Barker et al. 2012). Thus, it is likely that there is also large individual variation in the ability of a food cue to elicit a conditioned motivational state.

Individual variation in the extent to which discrete drug cues acquire properties of an incentive stimulus

While almost every person will try a potentially addictive substance at some point in their lives, only a small portion of individuals will ever become addicted (Anthony et al. 1994). We have suggested that this may be due, in part, to variation in the degree to which drug cues can gain motivational control over behavior. Here, I will review a series of studies where we investigated whether rats prone to attribute incentive salience to a food cue are also prone to attribute incentive salience to discrete drug cues.

Conditioned approach

Uslaner et al. (2006) were the first to report that a cue paired with an IV injection of cocaine is capable of eliciting an approach response (a ST CR). There was, however, considerable individual variation in the degree to which the cocaine cue became attractive. This individual variation may be due to differences in the propensity to attribute incentive salience to reward cues. To address this issue, Flagel et al. (2010) decided to take advantage of the bHR/ST and bLR/GT selectively-bred rat lines since their behavioral phenotypes were known without having to subject the rats to Pavlovian conditioned approach training. Flagel et al. (2010), using the selectively-bred rats as subjects, paired the presentation of a lever with an IV infusion of cocaine and measured approach responses. They reported that bHR/STs developed a sign-

tracking CR to a cue associated with an IV cocaine infusion whereas bLR/GTs did not. While this study was informative, it is also important to determine whether this effect is the same in outbred rats as there is no correlation between locomotor response to a novel environment and ST/GT behavior in outbred Sprague-Dawley rats (Beckmann et al. 2011; Robinson and Flagel 2009). Additionally, it was unclear whether bLR/GTs did not readily approach the cocaine cue because they did not attribute incentive salience to it or because they failed to learn the CS-US association. These issues are addressed in Chapter 2.

Conditioned Reinforcement

As mentioned above, we have reported in a number of different studies, and using different procedures, that a food cue is a more effective conditioned reinforcer in STs than in GTs (Lomanowska et al. 2011; Meyer et al. 2012a; Robinson and Flagel 2009; Yager and Robinson 2010). Thus, the next step was to determine whether a drug cue was also a more effective conditioned reinforcer in rats prone to attribute incentive salience to a food cue.

Saunders and Robinson (2010) first demonstrated individual variation in the conditioned reinforcing properties of a cocaine cue using a traditional drug self-administration procedure where instrumental responding resulted in delivery of both a light cue and cocaine. They found that after extinction training, STs made significantly more responses just for presentation of the cocaine cue than GTs. Furthermore, they also found that the cocaine cue was more important in maintaining self-administration behavior in STs than in GTs- removal of the cocaine cue drastically diminished self-administration behavior in STs, but not in GTs, even though cocaine was still available (Saunders and Robinson 2010). Additionally, Meyer et al. (2012b) reported similar findings when using a modified conditioned place preference procedure. In this procedure one tactile floor cue was paired with non-contingent cocaine injections while another

tactile floor cue was paired with saline injections. Meyer et al. (2012b) found that on the test day, when rats had access to both floors, STs spent significantly more time on the cocaine-associated floor than GTs. This effect was most likely due to the ability of the cocaine-associated floor to reinforce actions to maintain contact with it (i.e., the floor served as a conditioned reinforcer). Together these data suggest that cues associated with cocaine acquire incentive motivational properties to a greater degree in STs than in GTs, at least when assessed by conditioned reinforcement in the absence of any drug. Interestingly, these results are in line with a recent study by Barker et al. (2012). Barker et al. (2012) used a PIT paradigm to identify mice that attributed high or low levels of incentive salience to a food cue. They found that mice with the highest cue-motivated behavior during PIT showed the greatest cue-induced reinstatement of alcohol seeking. Thus, different measures of attribution of incentive salience to a food cue predict the extent to which a drug cue can serve as a conditioned reinforcer.

Conditioned Motivation

As mentioned previously, it is technically challenging to conduct a traditional PIT experiment in STs and GTs. To avoid the problems associated with a typical PIT experiment, Saunders et al. (2013) modified a conflict-based relapse model developed by Cooper et al. (2007) to measure the ability of a cocaine cue to produce a state of conditioned motivation that spurs drug-seeking behavior in the face of adverse consequences. In this procedure rats were first trained to self-administer cocaine, and cocaine injections were paired with illumination of the nose-poke port (the CS). Once rats were stably self-administering, the adverse consequence was imposed and they now had to make a choice: overcome the adverse consequence (footshock) to obtain drug or abstain. Once the cost was high enough, all rats abstained from taking drug. Then, on test day, the ability of non-contingent presentation of the cocaine cue to motivate or spur

behavior even in the continued presence of the adverse consequence was measured. Saunders et al. (2013) found that non-contingent presentation of the cocaine cue was more effective in arousing a state of conditioned motivation, and thus spurring drug-seeking behavior, to a greater degree in STs than in GTs or an unpaired control group.

Neural circuitry underlying individual variation in the attribution of incentive salience to reward cues

Neural circuitry mediating conditioned approach behavior

Currently, the underlying neurobiological differences accounting for individual variation in the attribution of incentive salience to food and drug cues are not well understood. To date, the focus has mainly been on differences in the dopamine (DA) system as there is considerable evidence that the DA system is involved in the assignment of incentive value to rewards and their associated stimuli (Berridge 2012; Berridge and Robinson 1998; Cardinal et al. 2002). For example, conditioned approach to either a food or drug associated cue is associated with dopamine release within the nucleus accumbens (Aragona et al. 2009; Day et al. 2007). Furthermore, lesions of the nucleus accumbens, which receives dense dopamine projections from the ventral tegmental area, as well as depletion of dopamine within the nucleus accumbens impairs acquisition of a conditioned approach CR (Chang et al. 2012; Dalley et al. 2002; Parkinson et al. 2002; Parkinson et al. 1999b).

As it turns out, STs and GTs differ on several measures of DA function. For example, the acquisition of a ST CR is dopamine-dependent whereas the acquisition of a GT CR is not (Danna and Elmer 2010; Flagel et al. 2011b). Furthermore, Flagel et al. (2011b) measured DA release within the nucleus accumbens core over the course of Pavlovian training with food as the US and found that there is a transfer of a phasic DA signal from the food-US to the lever-CS in STs but

not in GTs. Additionally, expression of a learned ST CR is dependent on intact DA signaling within the accumbens core whereas a GT CR is not (Saunders and Robinson 2012). STs also show greater expression of dopamine D1 receptor mRNA in the nucleus accumbens initially and lower levels of DA transporter and tyrosine hydroxylase in the ventral tegmental area and D2 mRNA in the nucleus accumbens relative to GTs following Pavlovian conditioned approach training (Flagel et al. 2007). Together these studies provide evidence that differences in DA signaling account for at least some of the behavioral differences observed in response to classically conditioned food cues; it remains to be seen whether the same is true of classically conditioned drug cues.

Individual variation in the engagement of brain reward systems by food and drug cues

Studies in both humans (using imaging techniques) and non-human animals (using immediate early gene expression) have shown that food and drug associated cues engage overlapping neural systems (Cardinal and Everitt 2004; Childress et al. 1999; Jentsch and Taylor 1999; Kelley 2004; Kelley et al. 2005; Kufahl et al. 2009; Schiltz et al. 2007; Schroeder et al. 2001; Tang et al. 2012; Volkow et al. 2008b; Zombeck et al. 2008). For example, presentation of food and drug cues engage brain regions such as the prefrontal cortex, dorsal and ventral striatum, thalamus, habenula, amygdala, ventral pallidum, and ventral tegmental area. These structures are located within the mesocorticolimbic dopamine pathway and other cortico-striatal-thalamic loops and comprise a so-called "motive circuit" (Kalivas and Volkow 2005). Activation of this "motive circuit" is associated with cue-induced drug craving in humans (Childress et al. 1999; Grant et al. 1996) and reinstatement of drug-seeking behavior in animal models of relapse (Kufahl et al. 2009; Zavala et al. 2008; Zhou et al. 2013). Even reward cues presented outside conscious awareness can engage brain reward pathways (Childress et al. 2008). However, as

mentioned above, reward cues acquire predictive value and, in some cases, can also acquire motivational value.

One recent study from our lab explored individual variation in the ability of a food cue to engage brain reward systems. Flagel et al. (2011a) used *in situ* hybridization to measure c-fos mRNA expression throughout the brains of STs and GTs after presentation of the food cue used during Pavlovian training. They found that presentation of the food cue induced greater c-fos mRNA expression in the orbitofrontal cortex, dorsal striatum, nucleus accumbens core and shell, lateral septum, lateral habenula, and the paraventricular, intermediodorsal, and central medial nuclei of the thalamus in STs relative to GTs or an unpaired control group. Furthermore, there were differences between STs and GTs in the degree to which c-fos mRNA expression was correlated between brain regions. For example, GTs were the only group to show a correlation between cortical and subcortical structures. These data suggest that in order for a food cue to engage brain reward systems it must be imbued with incentive salience – predictive value alone is not sufficient. This study provides evidence that food cues engage brain reward circuitry to a different degree in STs and GTs and it remains to be seen whether the same is true of drug cues, a topic that will be addressed in Chapter 3.

Summary of current studies

While there is now considerable evidence for individual variation in the extent to which a classically conditioned (Pavlovian) *food* cue is attributed with incentive salience, there is much less information concerning individual variation in the extent to which classically conditioned *drug* cues acquire incentive motivational properties. Prior to the work presented in this dissertation, there had only been one study assessing individual variation in the ability of a classically conditioned drug cue to acquire properties of an incentive stimulus, and they only

explored one property- the ability to attract (Flagel et al. 2010). The goals of this dissertation are twofold: 1) To determine whether the propensity of some animals to attribute incentive salience to a food cue *predicts* the ability of classically conditioned *drug* cues to control and motivate behavior. Additionally, in the past we have only examined one drug – cocaine – and here I will expand these findings to determine the generalizability of this phenomenon to other drug classes. 2) To examine the differences in the extent to which food and drug cues that are predictive vs. ones that also acquire incentive motivational properties engage brain reward systems. Thus, the overall aim of this dissertation is to explore the influences of Pavlovian conditioned drug cues on brain and behavior.

Chapter 2: A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue

Previous work has shown that a cocaine cue acquires greater control over self-administration behavior and produces more robust reinstatement of drug-seeking behavior (after extinction) in STs than GTs (Saunders and Robinson 2010). However, in this study, as in most studies of drug reinstatement, the cocaine cue acquired its motivational properties in an instrumental (self-administration) setting and there are many complex interacting psychological processes that contribute to behavior in an instrumental setting (Cardinal et al. 2002). While stimuli associated with drug self-administration do acquire incentive motivational properties, serving as conditioned reinforcers (Di Ciano and Everitt 2004) and spurring behavior (LeBlanc et al. 2012; Shaham et al. 2003), it is also important to determine whether purely Pavlovian drug cues can similarly motivate behavior, as these stimuli may be potent instigators of relapse in addicts. Thus, in this chapter (Yager and Robinson 2013), I investigated the extent to which a cocaine cue acquired incentive motivational properties when the cue is associated with cocaine

administration using classic Pavlovian conditioning procedures. I asked whether STs and GTs differed in the extent to which a classically conditioned cocaine cue came to (1) elicit approach towards it and (2) the degree to which it became itself desired, in that it reinforced actions to get it, using an extinction-reinstatement procedure.

Chapter 3: A classically conditioned opioid cue acquires greater control over motivated behavior and induces greater Fos protein expression in rats prone to attribute incentive salience to a food cue

Opiates are another class of drugs that are highly abused and readily self-administered by both humans and animals. Peters and De Vries (2013) recently reported that rats approached a cue associated with delivery of the opioid, heroin (see also Madsen and Ahmed 2014). There was, however, considerable individual variation in this response. The first aim of this chapter was to determine whether the extent to which an opioid cue is attractive (conditioned approach) and desired (conditioned reinforcement) is predicted by the propensity of an individual to attribute incentive salience to a food cue. The second aim of this chapter was to begin to explore whether the neural correlates underlying individual variation in the attribution of incentive salience to reward cues are similar between natural (food) and drug (opioid) associated cues. I first conducted a microinjection study where I administered the nonselective dopamine receptor antagonist flupenthixol into the nucleus accumbens (NAc) core to determine whether dopamine transmission within this structure is necessary for conditioned approach to an opioid cue. Next, I took a more broad approach and examined which brain regions within the mesocorticolimbic and cortico-striatal-thalamic systems were engaged by classically conditioned food and opioid cues. I further examined whether the degree to which these brain regions were engaged varied in animals for whom cues act as potent incentive stimuli (STs) relative to those for whom cues are

relatively devoid of incentive motivational properties (GTs). To do this, I used Fos protein expression as a marker of neural activity and quantified the extent to which presentation of either a food or an opioid cue elicited Fos expression throughout the brains of STs, GTs, and an Unpaired control group.

Chapter 4: A nicotine cue is equally attractive to sign-trackers and goal-trackers but differs in its conditioned reinforcing properties

While there is now evidence that rats will approach cues associated with alcohol, the opioid heroin, and the psychostimulant cocaine (Aragona et al. 2009; Krank et al. 2008; Madsen and Ahmed 2014; Peters and De Vries 2013; Tomie 2001; Uslaner et al. 2006), it remains to be seen whether this is also true for another highly abused drug: nicotine. My initial work (Yager and Robinson 2013) showed that rats that attribute incentive salience to a food cue also found a cocaine cue both attractive and desirable. Here, I asked whether there is similar variation in the extent to which a cue associated with intravenous nicotine delivery acquires the properties of an incentive stimulus. I asked whether STs and GTs differed in the extent to which 1) the nicotine cue became attractive by measuring conditioned approach and, 2) the extent to which the nicotine cue was desired, by measuring whether animals would learn an instrumental response (nose-poke) for presentation of the nicotine cue alone.

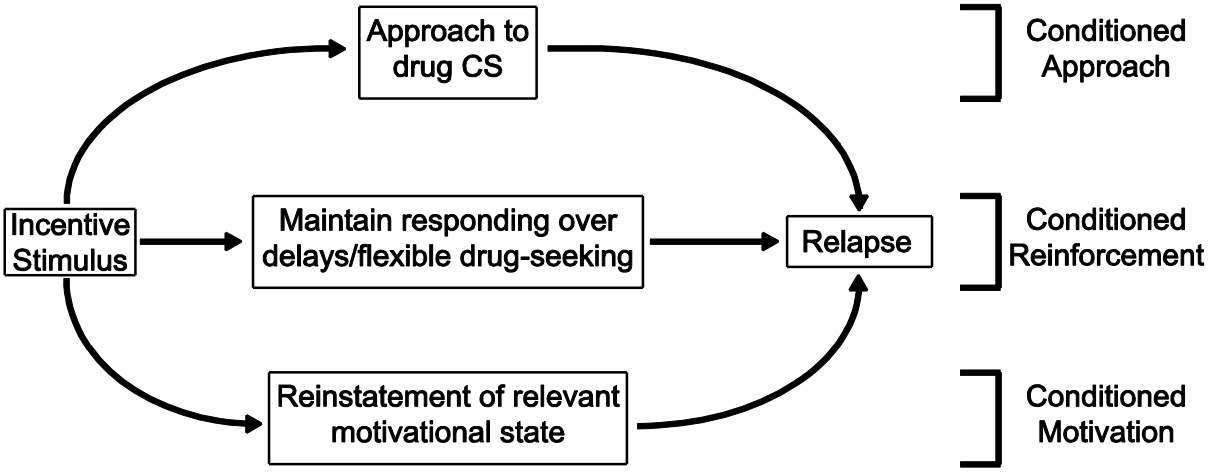


Figure 1.1. The "three routes to relapse". Adapted from Milton and Everitt (2010). Schematic representation of how Pavlovian conditioned stimuli associated with drugs can instigate relapse behavior in addicts. In the original, the term "conditioned stimulus" was used and here, we substituted "incentive stimulus".

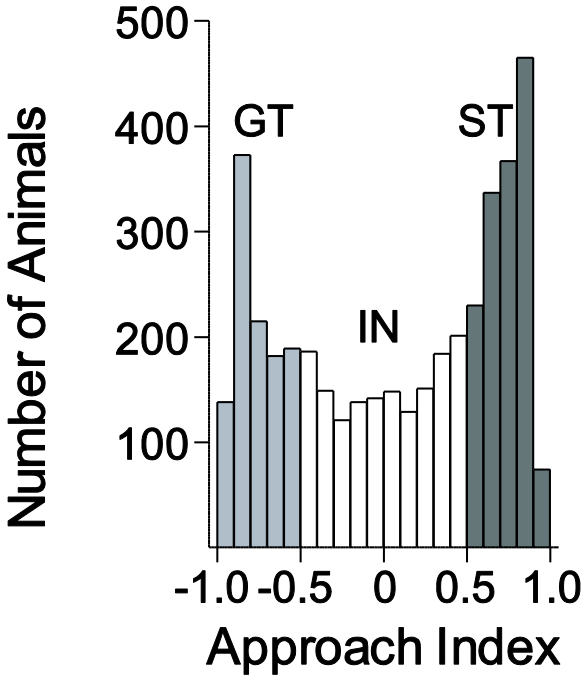


Figure 1.2. Variation in sign- vs. goal-tracking behavior in a large sample of the population (N =4149). A score of 1.0 reflects a complete bias for interaction with the lever-CS, and a score of -1.0 reflects complete bias for interaction with the food cup during lever-CS presentation. We operationally define STs as rats with a response bias of 0.5 to 1.0 and GTs as rats with a response bias of -1.0 to -0.5 (i.e., these subgroups are twice as likely to interact with either the CS or food tray, respectively). Rats with scores of -0.5 to 0.5 variably exhibit both ST and GT behavior.

Chapter 2

A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue

Introduction

There is considerable individual variation in the extent to which cues associated with rewards acquire motivational control over behavior (Boakes 1977; Johnson 1974; Mahler and de Wit ; Robinson and Flagel 2009; Schachter 1968; Tomie et al. 2000). In a series of studies using food as the unconditioned stimulus (US), we have shown that only in some rats ("sign-trackers", STs; Hearst and Jenkins 1974) does a food cue itself (conditioned stimulus, CS) become attractive, eliciting approach and engagement with it, and "wanted", in the sense that animals will work to get it (Robinson and Flagel 2009). In other rats ("goal-trackers", GTs; Boakes 1977) the cue evokes a conditioned response (CR), but the CR consists of approach behavior directed towards the location where food will be delivered, rather than towards the food cue itself, and in GTs a food cue is a less effective conditioned reinforcer (Robinson and Flagel 2009). We have suggested that this phenotypic variation is due, at least in part, to intrinsic individual variation in the propensity to attribute incentive motivational properties (incentive salience) to reward cues (Flagel et al. 2009; Flagel et al. 2011b; Meyer et al. 2012a; Robinson and Flagel 2009; Saunders and Robinson 2010; Yager and Robinson 2010).

Although there is now considerable evidence for individual variation in the extent to which a classically conditioned *food* cue is attributed with incentive salience, there is much less

information concerning individual variation in the extent to which *drug* cues acquire incentive motivational properties. We previously reported that a cocaine cue produces greater reinstatement of drug-seeking behavior (after extinction) in STs than GTs (Saunders and Robinson 2010), but in that study, as in most studies on drug reinstatement, the cocaine cue acquired its motivational properties in an instrumental (self-administration) setting (See 2005 for review). Although drug cues may reinstate drug-seeking behavior whether the cue was associated with drug administration in either an instrumental setting (i.e., contingent upon an action) or a Pavlovian setting (independent of an action) (Kruzich et al. 2001; See 2005) these may involve different psychological and even neurobiological processes (Cardinal et al. 2002; Everitt et al. 2001; Parkinson et al. 1999b). One purpose of the experiments reported here, therefore, was to determine if STs and GTs differ in the extent to which a cocaine cue acquires incentive motivational properties when the cue is associated with cocaine administration using classic Pavlovian conditioning procedures; i.e., independent of any action. We assessed two different properties of an incentive stimulus (Cardinal et al. 2002): (1) the extent to which it comes to elicit approach towards it (Uslaner et al. 2006), and (2) the degree to which the cue reinforces actions to get it, using an extinction-reinstatement procedure (Kruzich et al. 2001). In addition to measuring conditioned approach behavior we also quantified a second CR, conditioned orienting behavior, which may not require that the cue be attributed with incentive salience (Saunders and Robinson 2012). This allowed us to assess if rats learned the CS-US association, even if the CS failed to elicit approach into close proximity with it.

Materials and Methods

Subjects

A total of 207 (Exp. 1 initial N = 111, Exp. 2 initial N = 96) male Sprague-Dawley rats (Harlan, Haslett, Michigan) weighing 250-275g upon arrival were individually housed in a climate-controlled colony room on a 12-hr light/12-hr dark cycle (lights on at 0800 hr). Food and water were available *ad libitum*. After arrival, rats were given 1 week to acclimate to the colony room before testing commenced. All experiments followed the principles of laboratory animals care specified by *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* National Research Council (2003).

Pavlovian training using food as the US

A summary of the experimental design is shown in Figure 2.1.

Pavlovian training procedure. Rats were initially trained using a Pavlovian conditioned approach (PCA) procedure and equipment described previously (Flagel et al. 2007; Saunders and Robinson 2012). Briefly, rats were trained over 5 consecutive daily sessions consisting of 25 trials/session. Each trial consisted of insertion of an illuminated lever (lever-CS) into the chamber for 8 s. Retraction of the lever was immediately followed by the delivery of a single 45-mg banana-flavored pellet (the US) into the food magazine. CS-US pairings occurred on a variable time (VT) 90 (30-150 s) schedule. No instrumental response was required by the rat to initiate delivery of the food pellet. Lever deflections, magazine entries, latency to the first lever deflection, and latency to the first magazine entry during CS presentation were quantified.

Pavlovian conditioned approach (PCA) index. Following completion of Pavlovian training, animals were assigned to one of three groups based on whether they preferentially interacted with the lever-CS (‘sign-trackers’, STs), preferentially interacted with the food magazine during

the lever-CS presentation ('goal-trackers', GTs), or had no strong preference for the lever-CS or food magazine ('intermediate group', IG). This was quantified using a composite Pavlovian conditioned approach (PCA) index, based on performance on days 4 and 5 of training, as described previously (Lomanowska et al. 2011; Meyer et al. 2012a). Briefly, the PCA Index score consisted of the average of three measures of conditioned approach behavior: (1) the probability of contacting either the lever-CS or food magazine during a trial [P (lever)-P (food magazine)]; (2) the response bias for contacting the lever-CS or food magazine during a trial [(#lever deflections - #food magazine entries)/ (#lever deflections + #food magazine entries)]; and (3) the mean latency to contact the lever or enter the food magazine during a trial [(magazine contract latency - lever deflection latency)/8]. This produces values ranging from -1.0 to +1.0, where a score of +1 indicates an animal made a ST CR on every trial, a score of -1 that an animal made a GT CR on every trial and a score of 0 that an animal distributed ST and GT responses 50:50. For purposes of classification, rats with scores of -1.0 to -0.3 were operationally classed as GTs and rats with scores of +0.3 to +1.0 were classed as STs. Rats that were within the range of -0.29 to +0.29, whose behavior vacillated between the lever-CS and food magazine, were classified as intermediates (IGs) and were not used further because we were interested in comparing rats that differed strongly in their propensity to attribute incentive salience to food cues (Meyer et al. 2012a). Of the 207 rats screened for this experiment, 109 were classed STs, 45 IGs, and 53 GTs, and the distribution of PCA Index scores were similar to that reported previously (Meyer et al. 2012a; Saunders and Robinson 2011; Saunders and Robinson 2012).

Video analysis. For a subset of rats the 1st, 3rd, and 5th session were video recorded using a digital recording system and the video was subsequently scored offline and analyzed for orientation to the CS in 8 STs and 8 GTs. An orienting response was scored if a rat made a head

and/or body movement in the direction of the lever-CS during the CS period, even if it did not approach into close proximity the lever-CS. Of course, if a rat approached and engaged the CS, as indicated by a lever deflection, an orienting response would also be scored, as this always preceded approach. Thus, we were able to quantify the acquisition of two different CRs: 1) an orienting CR, and 2) an approach CR.

Experiment 1: Pavlovian approach using cocaine as the US

Surgery. Following Pavlovian training using food as the US, chronic indwelling catheters were implanted into the jugular vein of STs and GTs as described previously (Crombag et al. 2000).

Apparatus. Behavioral testing was conducted in chambers identical to those used to screen animals for ST and GT, except the food magazine and lever were removed from the chamber and two stimulus lights were placed on the left and right sides of the wall opposite the white houselight, 13.5 cm above the stainless steel grid floor. The side of the stimulus light designated to serve as a CS (i.e., to be paired with cocaine infusion) was counterbalanced between rats. A syringe pump, located outside the sound attenuating chamber and connected to rats' catheter back ports, delivered cocaine infusions. The infusion tubing was suspended into the chamber via a swivel mechanism, which allowed rats free movement in the chamber.

Pavlovian training procedures. Prior to training rats were assigned to either Paired (CS and US presented together) or Unpaired groups (US explicitly not paired with presentation of the CS). Before Pavlovian training began, rats were first habituated to the presentation of the stimulus light (light-CS) and infusion procedure to decrease otherwise high levels of responding to what were novel stimuli (Uslaner et al. 2006). The habituation session consisted of 25 individual trials (VT 90 s schedule) during which both stimulus lights were simultaneously illuminated for either 10 or 20 s (see below for further description) and coincided with activation of the infusion pump

and an intravenous (IV) infusion of saline (50 µL delivered in 2.8 s). Fifteen days of Pavlovian conditioning, using cocaine as the US, commenced the following day (see Fig. 2.1a for the overall design). Each session consisted of eight trials (CS-US presentations) occurring on a VT schedule with a mean of 900 s (840-960 s). For rats in the Paired group, each light-CS presentation was paired with an intravenous infusion of either 0.2 or 0.4 mg/kg of cocaine HCl (weight of the salt, dissolved in 0.9% saline in 50 µl over 2.8 s). Of course, no action was required to initiate either illumination of the light or the cocaine injection. For rats tested with 0.4 mg/kg, each trial consisted of illumination of the CS for 10 s and cocaine delivery coincided with the onset of the CS. This experiment was conducted first, and therefore, we had scored the video prior to conducting a second experiment with a lower dose. Based on the video we decided that it would be advantageous if rats had a little more time available to make an approach response. Thus, for the second experiment with a dose of 0.2 mg/kg we increased the length of time the stimulus light was illuminated to 20 s, and cocaine delivery began 10 s after CS onset. Rats in the Unpaired groups received non-contingent infusions of 0.4 mg/kg cocaine that were explicitly not paired with illumination of the CS (cocaine was administered on a VT schedule with a mean of 180 s after the CS was extinguished).

Video analysis. Video was scored offline by an observer blind to the experimental condition for two different conditioned responses (CRs). (1) *Conditioned Orientation*: an orienting response was scored if the rat made a head and/or body movement in the direction of the CS during the CS period, regardless of whether the rat approached the CS. (2) *Conditioned Approach*: an approach response was scored if during the CS period a rat moved towards the CS, bringing its nose to within 1cm of the light. To do this a rat had to rear, lifting both paws off the floor, towards the light. Thus, if an approach response were scored on a given trial an orienting response would

also be scored, as orienting always preceded approach. However, an orienting response could occur in the absence of an approach response. Rats were removed from analysis if their catheter lost patency (ST $n = 4$, GT $n = 1$).

It is also worth noting that the conditioned orientation response reported here (defined as head and/or body movement towards the cue) should not be confused with the conditioned orientation response defined by Holland and colleagues (defined as rearing close to the cue, Gallagher et al. 1990; Han et al. 1997; Holland 1977; McDannald et al. 2004). By our criteria, this CR would be defined as an approach response.

Experiment 2: Individual variation in Pavlovian cue-induced reinstatement of drug-seeking behavior

The basic experimental design for Experiment 2 is shown in Fig. 2.1b. For this experiment an independent cohort of rats were trained on the Pavlovian task using food as the US to identify STs and GTs, and subsequently prepared with IV catheters, exactly as described for experiment 1.

Apparatus. For self-administration, extinction, and reinstatement testing the food magazine and lever were removed from the chamber and replaced with two nose-poke ports located 3 cm above the floor on the left and right sides of the wall opposite the houselight. A stimulus light was mounted above each nose-poke port, 13.5 cm above the floor. Removable waste trays were filled with corn cob bedding, a pine scented air-freshener was placed in the chamber, a red houselight was used, and the floor was made of stainless steel bars. For Pavlovian training sessions, the nose-poke ports were removed from the chambers, the removable waste trays were emptied, a vanilla scented air-freshener was placed in the chamber, a white houselight was used, and the floor was made of wire mesh to create a context different from that used for self-

administration sessions. This was done to reduce any effect of context conditioning acquired during the Pavlovian conditioning sessions from influencing responding during the reinstatement test.

Self-administration training. Rats were trained to make an instrumental response (a nose poke) to receive an intravenous injection of cocaine (0.4 mg/kg/infusion over 2.8 s) on a fixed ratio (FR) 1 20 s time-out schedule of reinforcement. Responses into the active port during the time-out, or into the inactive port, had no programmed consequence. *Importantly, no explicit discrete cue was associated with the drug infusion during self-administration sessions*. Rather than restricting the length of the session, rats were required to earn a fixed number of infusions each day (infusion criterion, IC), which increased across days, as described previously (Saunders and Robinson 2010; 2011). This was done to ensure that all rats received exactly the same number of drug infusions.

Pavlovian conditioning procedures. Following acquisition of stable self-administration behavior over 12 days of training, the nose poke ports were removed and rats underwent two sessions of Pavlovian training with cocaine as the US. Each Pavlovian session was separated by three days of self-administration at IC 40 (see Fig. 2.1b). Prior to Pavlovian conditioning, rats were assigned to either Paired or Unpaired groups, matched based on the length of time to complete self-administration sessions averaged over the final two days of training at IC 40. Each Pavlovian training session consisted of 20 CS-US presentations. For rats in the Paired group, the light-CS was illuminated for 20 s and cocaine delivery (0.2 mg/kg over 2.8 s) coincided with the onset of the CS. Rats in the Unpaired group received non-contingent infusions of cocaine that were explicitly not paired with CS presentation (cocaine was administered on VT schedule with a mean of 120 s after the light-CS was extinguished). Following the second Pavlovian

conditioning session, rats were again allowed to self-administer cocaine at IC 40 for three additional days to re-stabilize behavior. Thus, in this experiment the CS that predicted cocaine delivery was not present during instrumental (self-administration) sessions, but was associated with cocaine in two separate Pavlovian training sessions, and the Pavlovian context was distinct from the self-administration context.

Extinction and reinstatement. After the last self-administration session at IC 40, rats underwent ten daily 60 min sessions of extinction training. During extinction, responses into the nose ports had no consequences. The day after the final extinction session, rats were tested for Pavlovian cue-induced reinstatement of drug-seeking behavior (Fig. 2.1b). During this session, responses into the active nose poke resulted in illumination of the cocaine cue (CS) for 5 s and activation of the infusion pump, but no cocaine delivery.

Statistical analysis

Linear mixed-models (LMM) analysis was used for all repeated measures data (Verbeke and Molenberghs 2000). The covariance structure was explored and modeled for each dependent variable. Analysis of variance was used to analyze dose-response data for conditioned orientation, conditioned approach, and to compare reinstatement responding. When main effects were found post hoc comparisons were made using Fisher's LSD test. Statistical significance was set at $p < 0.05$.

Results

Individual variation in Pavlovian conditioned approach behavior to a food cue

Two distinct phenotypes emerged as a result of Pavlovian training using food as the US, as reported previously (Robinson and Flagel 2009). For a subset of rats presentation of the lever-CS came to evoke a sign-tracking (ST) CR, consisting of reliable and rapid approach to the lever-CS

(Figs. 2.2a and 2.2c) and vigorous engagement with it (Fig. 2.2b). In contrast, for another subset of rats, presentation of the lever-CS rarely elicited approach to it. Rather, presentation of the lever-CS elicited a goal-tracking (GT) CR that consisted of reliable and rapid approach to the food magazine (Figs. 2.2d and 2.2f) and vigorous engagement with it (Fig. 2.2e). Individual variation in the topography of the conditioned approach responses that developed with Pavlovian training is clearly evident by examining the change in the PCA Index scores in STs and GTs over days of training (Fig. 2.3a).

Both STs and GTs learn a conditioned orienting response

In contrast to variation in the topography of conditioned approach behavior, *both* STs and GTs developed a conditioned orienting response to the lever-CS across sessions [$F(2, 25.52) = 31.95$, $p < 0.001$], before then approaching either the lever or the food magazine, respectively, and the two groups did not differ (Fig. 2.3b). Indeed, on trials when GTs had their head in the food magazine prior to presentation of the CS they would typically remove their head from the food magazine when the lever-CS was presented, glance at the lever, and then turn back to the food magazine. Thus, both STs and GTs learned a conditioned orienting response directed towards the lever-CS, but only in STs did the lever-CS become sufficiently attractive to draw a rat into close proximity with it, and only STs vigorously engaged the lever-CS (Figs. 2.2 and 2.3).

Individual variation in conditioned approach to a cocaine cue, but not conditioned orientation

When cocaine is used as a US, rather than food, rats typically do not physically engage a lever-CS, therefore, they do not reliably deflect it. Instead, a sign-tracking CR consists of approach to the CS, and sniffing and investigation of it (Flagel et al. 2010; Uslaner et al. 2006). Thus, when using cocaine as the US we scored a CS-directed approach response (a ST CR) if a rat brought its nose to within 1 cm of the light-CS during the CS period, which required it to rear. In contrast,

conditioned orientation was defined as a head and/or body movement in the direction of the light-CS upon CS presentation, regardless of whether the rat reared, bringing it into close proximity to the light.

Conditioned orientation (0.2 mg/kg). Figure 2.4a illustrates the probability of conditioned orientation across training sessions when using 0.2 mg/kg cocaine as the US. As can be seen in Fig. 2.4a, at this dose both Paired STs and GTs learned a conditioned orienting response, as indicated by a significant increase in the probability of orienting behavior across sessions [$F(2, 34.42) = 11.07$, $p < 0.001$], and there were no group differences. Additionally, both STs and GTs showed a significant increase in the probability of orienting to the cocaine cue across sessions, relative to their respective Unpaired control groups [pairing x session interactions; STs: $F(2, 60) = 5.67$, $p = 0.006$; GTs: $F(2, 33.74) = 3.91$; $p = 0.03$].

Conditioned approach (0.2 mg/kg). Figure 2.4b illustrates the probability of conditioned approach across training sessions when using 0.2 mg/kg cocaine as the US. Fig. 2.4b shows that STs and GTs differed in the extent to which the cocaine cue elicited an approach CR [effect of group, $F(1, 57) = 4.93$; $p = 0.03$]. At this dose STs continued to approach the CS across sessions, whereas GTs showed a significant decrease in the probability of approaching the cocaine cue across sessions [effect of session, $F(2, 10.51) = 4.38$, $p = 0.041$]. STs also had a higher probability of approaching the cocaine cue across sessions, relative to their Unpaired control group [pairing x session interaction, $F(2, 40) = 3.88$, $p = 0.029$], whereas the Paired and Unpaired GT groups did not differ, decreasing approach similarly across sessions.

Conditioned orientation (0.4 mg/kg): Fig. 2.5a shows that when using 0.4 mg/kg cocaine as the US both Paired STs and GTs acquired a conditioned orienting response, as indicated by a significant increase in the probability of orienting behavior across sessions [$F(2, 19) =$, $p <$

0.001], and the two groups did not differ. In addition, both STs and GTs showed a significant increase in probability of orienting to the cocaine cue across sessions, relative to their respective Unpaired control groups [pairing x session interactions; STs: $F(2, 16) = 14.1$, $p < 0.001$; GTs: $F(2, 50) = 10.84$, $p < 0.001$].

Conditioned approach (0.4 mg/kg). In contrast to conditioned orientation, Fig. 2.5b shows that STs did differ from GTs in the probability of approaching the CS [effect of group, $F(1, 52.17) = 4.44$, $p = 0.04$]. Indeed, the effect of session was statistically significant for STs [$F(2, 9) = 19.34$, $p = 0.001$] but not GTs. Finally, STs also showed a significant increase in the probability of approaching the cocaine cue across sessions, relative to their Unpaired control group [pairing x session interaction, $F(2, 22.77) = 8.52$, $p = 0.002$], whereas Paired and Unpaired GTs did not statistically differ. Importantly, neither STs nor GTs in the Unpaired group developed an orienting CR or an approach CR.

Figure 2.6 summarizes the dose-response functions for the probability of conditioned orientation (Panel a) and conditioned approach (Panel b) on the final day of training. For conditioned orienting a two-way analysis of variance (ANOVA) revealed that there were no differences between STs and GTs, and the probability of this CR increased as a function of dose in both groups [$F(1, 38) = 5.67$, $p = 0.022$]. However, Fig. 2.6b shows that the cocaine cue elicited greater approach behavior in STs than in GTs [$F(1, 38) = 6.03$, $p = 0.019$], although the probability of approach increased equally in STs and GTs as a function of dose. We separately analyzed dose-response data for STs and GTs and included unpaired control animals in this analysis. A one-way ANOVA showed a significant effect of treatment group for both STs and GTs on performance of a conditioned approach CR on the final day of training [STs, $F(2, 29) = 9.03$, $p = 0.001$; GTs, $F(2, 22) = 6.4$, $p = 0.006$]. However, post-hoc analysis (Fisher's LSD)

revealed that, on the final day of testing, Paired STs differed from Unpaired STs at both doses tested (p's < 0.05) while Paired GTs only differed from Unpaired GTs only at the highest dose tested [0.2 mg/kg, $p = 0.985$; 0.4 mg/kg, $p = 0.015$].

Acquisition and extinction of cocaine self-administration in STs and GTs

An independent cohort of rats underwent Pavlovian training with food as the US to identify STs and GTs as previously described (data not shown) and rats were subsequently prepared with IV catheters. Rats were then trained to nose poke for an IV cocaine infusion, but during self-administration sessions no cue was explicitly paired with drug delivery. Rats in each group received the same number of response-reinforcer pairings by requiring them to take a fixed number of drug injections each session. Thus, any differences in the acquisition of self-administration would be evident in the average number of cocaine infusions taken per minute (rate). There were no group differences in rate of responding at any infusion criterion (Fig. 2.7b). There were also no group differences in active or inactive responses/session and both groups learned to discriminate between the active and inactive ports (Fig. 2.7a). Following stable responding at IC 40 for two days, rats underwent two days of Pavlovian conditioning with cocaine as the US as described in the Methods. After each Pavlovian conditioning session, rats were returned to an FR1 schedule at IC 40 to re-stabilize behavior. During these sessions there were no group differences in the rate of self-administration or the number of responses (data not shown).

Following the final day of self-administration testing at IC 40, rats underwent ten sessions of extinction training during which responses into the active port no longer produced cocaine. Figure 2.8 shows that there were no group differences in the rate of extinction and all

groups extinguished to the same low level of responding [effect of session, $F(9, 37) = 13.24$, $p < 0.001$].

A Pavlovian cocaine cue produced more robust reinstatement of drug-seeking behavior in STs than GTs

Following extinction training all rats were tested for the ability of response-dependent presentation of the Pavlovian cocaine cue (light-CS) to reinforce drug-seeking behavior. During this test, during which no cocaine was delivered, responses into the active port produced presentation of the light-CS previously either paired or unpaired with cocaine injections. All groups reinstated responding, in that the number of responses into the active port were greater than those into the inactive port (Fig. 2.9). However, Paired STs showed greater reinstatement of responding than Paired GTs, as indicated by a significant group x pairing interaction [$F(1, 37) = 7.22$, $p = 0.011$].

Discussion

We previously reported that there is considerable individual variation in the extent to which a Pavlovian food cue acquires the properties of an incentive stimulus (Meyer et al. 2012a; Robinson and Flagel 2009; Yager and Robinson 2010). Here we asked whether variation in the propensity to attribute incentive salience to a food cue predicts the extent to which a classically conditioned cocaine cue acquires motivational properties. We found that a classically conditioned cocaine cue was more attractive, in that it elicited approach towards it, and more desirable, in that it reinforced actions to get it, in STs than GTs. Importantly, even though GTs did not reliably approach the cocaine cue they did learn the CS-US association, as indicated by acquisition of a conditioned orienting response, similar to that seen when a food cue is used as the US (Zener 1937, and the present study). These findings, together with our previous reports,

indicate that there is considerable individual variation in the extent to which a cocaine cue acquires motivational control over behavior (Flagel et al. 2010; Meyer et al. 2012b; Saunders and Robinson 2010).

One limitation when using intravenous drug as the US and approach as the CR is that there is no explicit "goal" to approach (this is also the case when using electrical brain stimulation as the US; Peterson et al. 1972). Thus, it can be difficult to determine whether GTs do not readily approach a cocaine cue because they do not learn the CS-US association as well as STs, or because the cocaine cue is not attributed with sufficient incentive salience to draw them into close proximity to it. To begin to address this issue we quantified, for the first time, acquisition of a conditioned orienting response as an alternative measure of learning the CS-US association (Grastyan and Vereczkei 1974; Sokolov 1963). Importantly, STs and GTs did not differ in the acquisition of an orienting CR, when either food or cocaine was used as the US. Conditioned orientation was not simply a reflexive reaction to a change in the environment (Sokolov 1963), as rats that received unpaired CS-US presentations did not orient to the cue. It has also been argued that sign-tracking behavior is simply an elaboration of a conditioned orienting response (Buzsaki 1982; Grastyan and Buzsaki 1979; Grastyan and Vereczkei 1974; Holland 1980). However, our data do not support this view because these two CRs were dissociable. When using food as the US, GTs oriented to the food cue, but then approached the food magazine, not the cue. Interestingly, Zener (1937) described a similar effect seventy-five years ago, observing that some dogs would respond to the CS with "an initial glance at the bell" before fixating on the food pan. Thus, absence of an approach CR does not mean that the CS-US association was not acquired. For example, when using an auditory CS, rats will orient to the sound source and display enhanced general activity but will not approach the auditory stimulus

(Cleland and Davey 1983; Holland 1977; Rescorla 1988). We suggest, therefore, that the reason GTs do not readily approach the cocaine cue is not because they fail to learn the CS-US association, but because they do not attribute sufficient incentive salience to the cue for it to become powerfully attractive.

Although STs and GTs did not differ in the acquisition of a conditioned orienting response they did differ in the extent to which the cocaine cue acquired incentive motivational properties, based on two independent measures. First, STs and GTs differed in the extent to which the cocaine cue evoked a conditioned approach response, defined as coming into close proximity with the cue. With a low dose of cocaine GTs did not show any evidence of conditioned approach, although with a higher dose they showed some approach, although less than that seen in STs. Second, the Pavlovian cocaine cue was more effective in reinforcing drug-seeking behavior following extinction of self-administration behavior. It is unlikely that these differences between STs and GTs were due to differences in the inherent reinforcing properties of cocaine during conditioning because they did not differ in the acquisition of self-administration behavior, as we have reported previously (Saunders and Robinson 2010; 2011). In addition, STs and GTs did not differ in exposure to cocaine or the number of cue-drug pairings, because we utilized procedures that held these variables constant.

It is interesting to note that during the Pavlovian cocaine cue reinstatement test, both Paired and Unpaired rats reinstated responding to some extent, in that responses in the active port were greater than in the inactive port in all groups, and indeed, in GTs there was no difference between Paired and Unpaired groups. However, in STs the cocaine cue did reinstate stronger drug-seeking behavior in Paired relative to Unpaired animals. Nevertheless, there are a number of reasons why Unpaired animals may have showed more active than inactive responses. First, a

light stimulus is itself inherently reinforcing and will sustain instrumental responding in the absence of any other reinforcer (Olsen and Winder 2009; Stewart 1960) and this may have been sufficient to maintain low levels of responding in all groups. Second, drugs have relatively long durations of action and so it is difficult to "unpair" a discrete CS and a drug US, unless the inter-trial interval is very long – well beyond the half-life of the drug. Although we used a relatively long inter-trial interval, it may have been short enough so that during the Pavlovian conditioning sessions brain levels of cocaine may have sometimes still been elevated during CS presentation, even in the Unpaired groups, resulting is some association between the CS and US. A third possibility is that some responding was maintained by context conditioning, that is, an association between cocaine and the context in which it was experienced. However, this seems less likely because the Pavlovian conditioning sessions were conducted in a context different from the self-administration and reinstatement context.

Our finding that a Pavlovian cocaine cue acquired greater incentive motivational value in STs than GTs is consistent with previous studies in which a cocaine cue acquired incentive motivational properties in an instrumental setting. Saunders and Robinson (2010) used a self-administration procedure, where responding resulted in both delivery of a light cue and cocaine, and reported that following extinction the light cue produced greater cue-induced reinstatement in STs than GTs. Furthermore, Meyer et al. (2012b) used a conditioned cue preference procedure, where a non-contingent cocaine injection was paired with a tactile floor cue (Cunningham et al. 1993), and found that only STs developed a preference for the cocaine-associated floor. With this procedure, approach to the cocaine-associated floor was likely due to its conditioned reinforcing properties. Our results are also consistent with studies using rats selectively bred for reactivity to a novel environment (Flagel et al. 2010). Selectively bred high

responder rats (bHRs) are almost exclusively STs and selectively bred low responder rats (bLRs) are almost exclusively GTs. Flagel et al. (2010) reported that bHR/STs developed a sign-tracking CR to a cue associated with an IV cocaine infusion whereas bLR/GTs did not.

The neurobiological basis of individual variation in the propensity to attribute incentive salience to food and drug cues is not known. There is, however, considerable evidence that dopamine (DA) systems are involved in the assignment of incentive value to rewards and their associated stimuli (Berridge 2012; Berridge and Robinson 1998; Cardinal et al. 2002), and STs and GTs do differ on some measures of DA function. For example, STs show greater expression of dopamine D1 receptor mRNA in the nucleus accumbens initially and lower levels of DA transporter and tyrosine hydroxylase in the ventral tegmental area and D2 mRNA in the nucleus accumbens relative to GTs following Pavlovian conditioned approach training (Flagel et al. 2007). Additionally, learning a sign-tracking CR is DA-dependent but learning a goal-tracking CR is not (Flagel et al. 2011b). Furthermore, Flagel et al. (2011b) showed that over the course of Pavlovian training with food as the US, there is a transfer of a phasic DA signal from the food-US to the lever-CS in STs but not in GTs, in the accumbens core. STs and GTs also differ in what brain regions are engaged by food cues. For example, presentation of a Pavlovian food cue elicits greater c-fos mRNA expression in STs in both the dorsal and ventral striatum as well as the orbitofrontal cortex and thalamus (Flagel et al. 2011a). Interestingly, in human studies, differences in dopamine transporter genotype is associated with differential activation of the mesocorticolimbic reward circuitry and behavioral responses elicited by smoking cues (Franklin et al. 2009). These studies are consistent with the hypothesis that individual variation in dopaminergic signaling may contribute to individual variation in the propensity to attribute incentive salience to reward cues, but this issue clearly requires further study.

The influence of drug cues on behavior have traditionally been studied using self-administration procedures, in which an animal works for both presentation of the cue *and* the delivery of the drug reward (de Wit and Stewart 1981; Shaham et al. 2003). However, there are many complex psychological processes that interact to contribute to behavior in an instrumental setting (e.g., see Fig. 2 in Cardinal et al. 2002). For example, it is difficult to parse whether a reward-associated cue is eliciting the next response or if it is reinforcing the prior response. Importantly, in the "real world" of substance abusers, drug cues often *precede* actions that result in acquiring and taking drugs. Indeed, the psychological and neurobiological processes controlling behavior may be quite different in Pavlovian vs. instrumental settings. For example, there is evidence that neural responses, as measured by immediate early gene expression, differ in response to the presentation of a Pavlovian CS vs. presentation of a CS acquired in an instrumental setting (Thomas et al. 2003; Thomas and Everitt 2001). Stimuli associated with drug self-administration do acquire incentive motivational properties, serving as conditioned reinforcers (Di Ciano and Everitt 2004) and spurring behavior (Shaham et al. 2003), but it is also important to determine whether purely Pavlovian drug cues can similarly motivate behavior, as these stimuli may be potent instigators of relapse in addicts. It has been reported that a Pavlovian cocaine cue can become both attractive, eliciting approach towards it (Uslaner et al. 2006), and desired, in that animals will work for presentation of the cue (Kruzich et al. 2001). However, in these studies there was considerable individual variation in the extent to which the cocaine cue acquired motivational control over behavior. The data reported here suggest that this variation is due, at least in part, to individual differences in the propensity to attribute incentive salience to a Pavlovian CS, transforming it from a mere CS into a potent incentive stimulus (Meyer et al. 2012a).

In conclusion, the extent to which a classically conditioned cocaine cue acquires motivational control over behavior is predicted by the propensity of an individual to attribute incentive salience to a food cue. Indeed, similar variation in the response to smoking cues was recently reported in humans - smokers who reported the highest craving to food cues when food deprived also reported the highest craving to smoking cues during abstinence (Mahler and de Wit 2010). Thus, some individuals are prone to attribute incentive salience to reward cues and this is true whether reward cues are presented in an instrumental or Pavlovian setting (Meyer et al. 2012b; Saunders and Robinson 2010; Yager and Robinson 2010). Individuals prone to attribute incentive salience to drug cues may be especially vulnerable to addiction, as in these individuals drug cues would most powerfully motivate drug-seeking and drug-taking behavior.

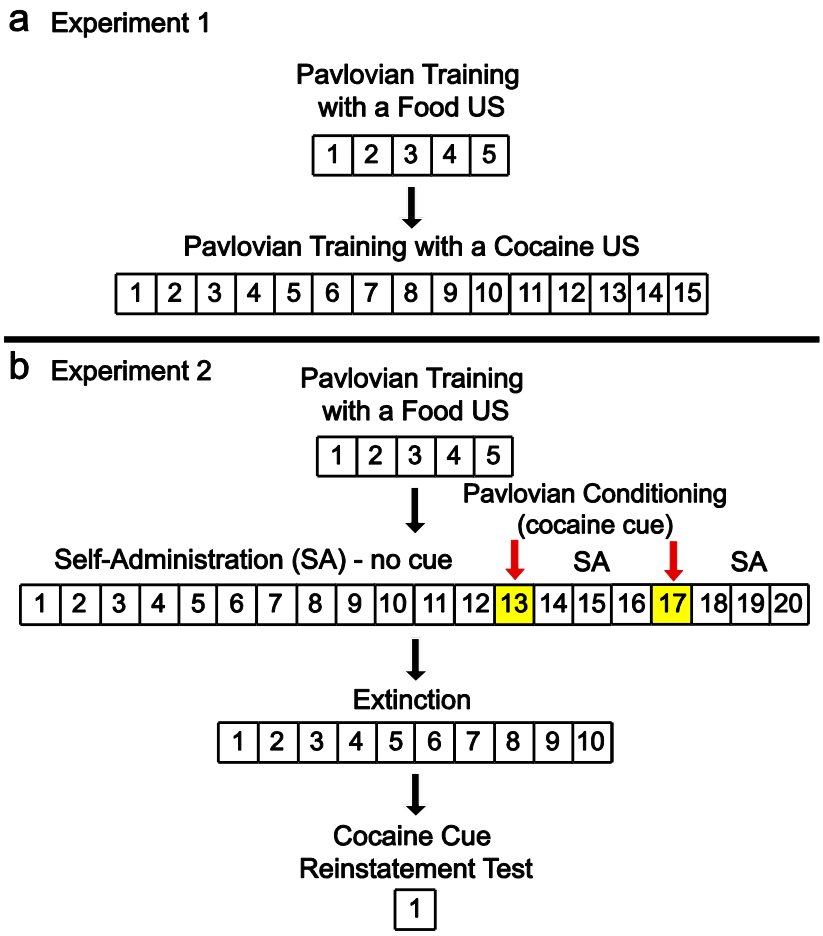
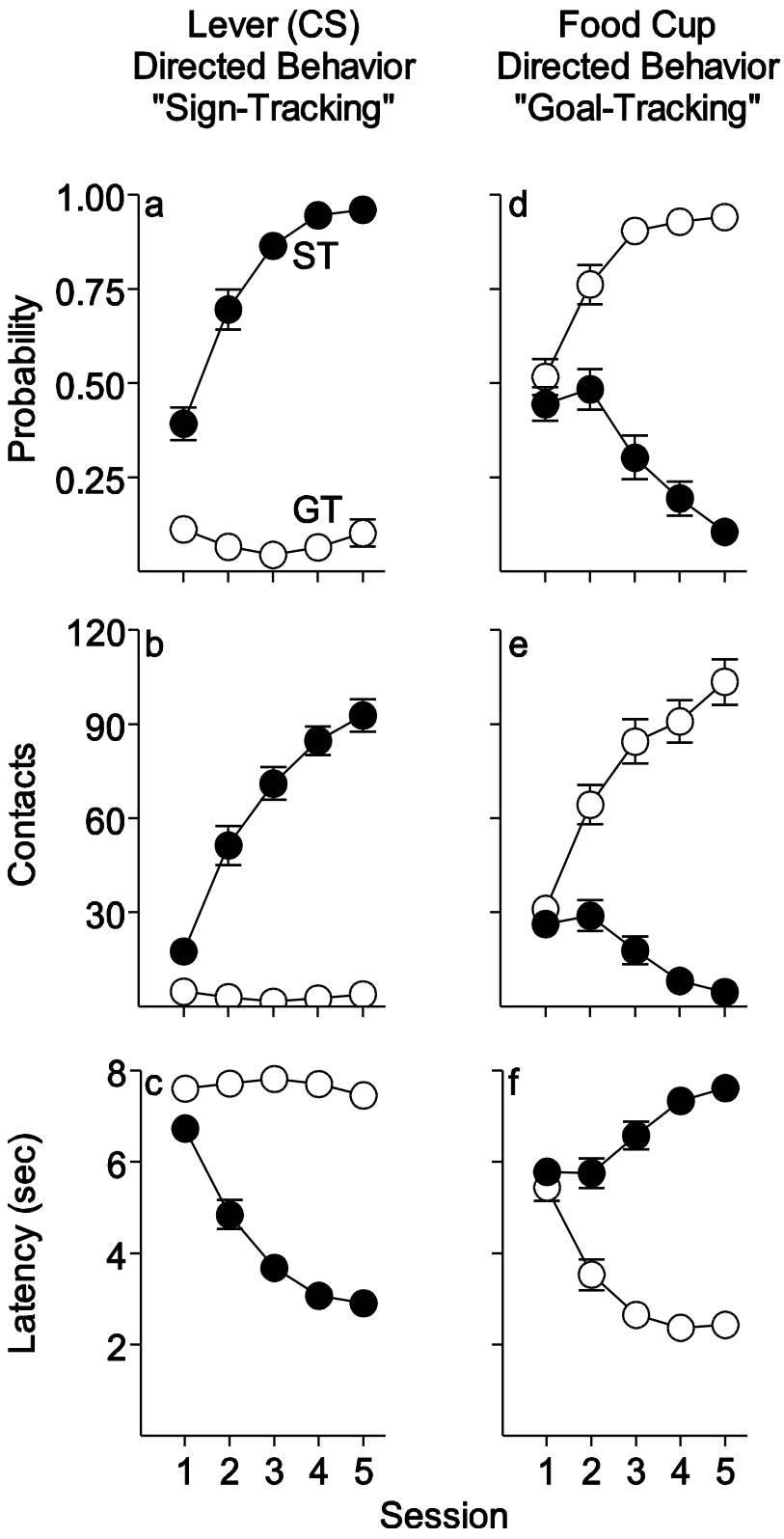


Figure 2.1. Schematic illustration of the experimental design. Independent groups of rats were used for each experiment. Each numbered box represents an individual session/day. (a) Following Pavlovian training with a food unconditioned stimulus (US), rats underwent subsequent Pavlovian training with a cocaine US during which rats received non-contingent cue-light (CS)-US presentations. (b) Following Pavlovian training with a food US, rats were trained to self-administer cocaine (US) in the absence of any explicit cue. During subsequent Pavlovian conditioning sessions rats received non-contingent cue-light (CS)- cocaine (US) presentations. Depending on the experimental phase, an active nose poke produced the US (acquisition), no US (extinction), or the CS but no US (reinstatement).

Figure 2.2. Behavior directed towards the lever-CS vs. the location of food delivery (the food magazine) during Pavlovian training in rats designated as sign-trackers (STs) or goal-trackers (GTs). The mean ± SEM for: (a) probability of approaching the lever-CS during the 8 s CS period, (b) number of lever contacts, (c) latency to first lever contact after CS presentation, (d) probability of approaching the food magazine during the 8 s CS period, (e) number of food magazine entries during the 8 s CS period, and (f) latency to the first food magazine entry after CS presentation. For all measures there was a significant effect of group (ST or GT), session, and a group x session interaction (*p's* < 0.001).


Lever (CS)
Directed Behavior
"Sign-Tracking"
Food Cup
Directed Behavior
"Goal-Tracking"
a
d
b
e
c
f
ST
GT
Probability
Contacts
Latency (sec)
1.00
0.75
0.50
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120
90
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Session

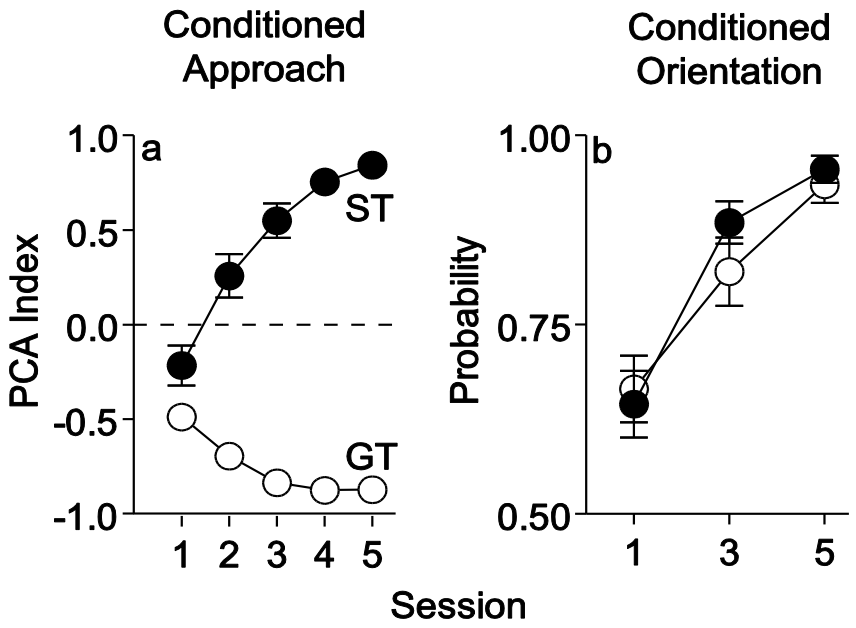


Figure 2.3. Comparison of development of conditioned approach and conditioned orientation towards the lever-CS during Pavlovian training in sign-trackers (STs, *n* =8) and goal-trackers (GTs, *n* =8). Data represent the means ± SEM. (a) PCA index scores for STs and GTs across 5 sessions of training. A score of 1.0 reflects a complete bias for interaction with the lever-CS (STs), a score of -1.0 reflects complete bias for interaction with the food magazine during lever-CS presentation (GTs), and a score of zero reflects that responses were directed equally to both locations. (b) Probability of orientation to the food cue (lever-CS), during the CS period, across training sessions.

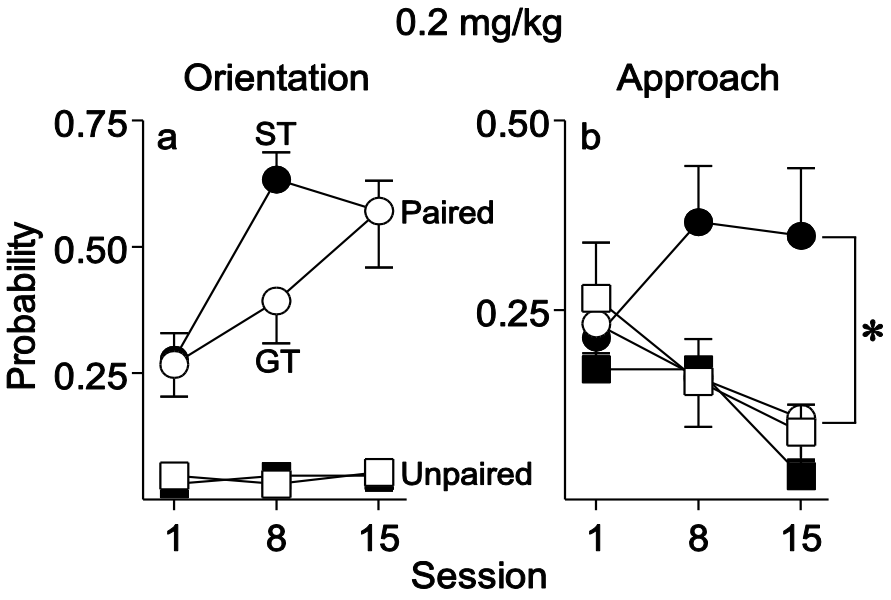


Figure 2.4. CS-directed orientation and approach to a cue associated with a non-contingent intravenous injection of 0.2 mg/kg cocaine in rats that received paired (ST *n* = 14, GT *n* = 7) or unpaired (ST *n* = 8, GT *n* = 8) cue-drug presentations. Data represent the means ± SEM. (a) The probability to orient to the cocaine cue during light-CS presentation. (b) The probability to approach the cocaine cue during the light-CS presentation. Asterisk, indicates a significant group difference between Paired STs and GTs, $p < 0.05$.

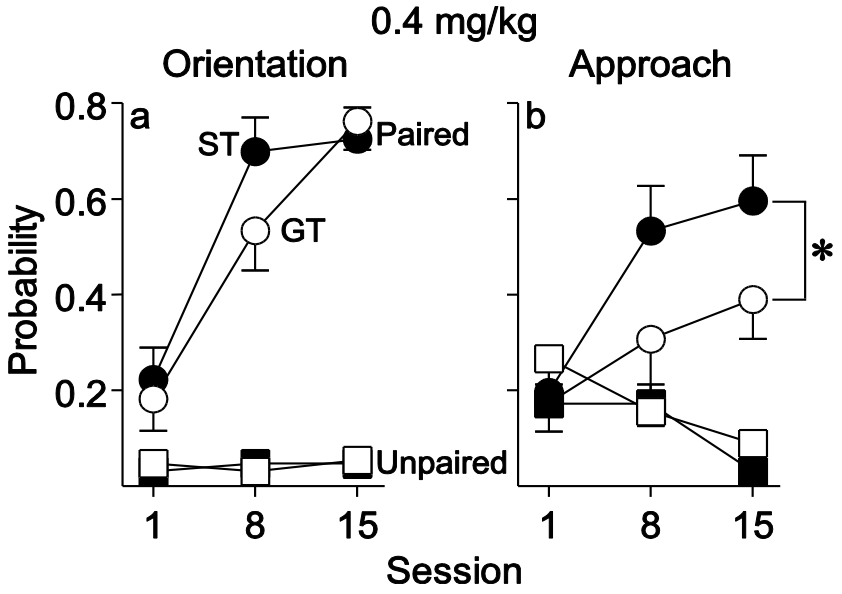


Figure 2.5. CS-directed orientation and approach to a cue associated with a non-contingent intravenous injection of 0.4 mg/kg cocaine in rats that received paired (ST *n* = 10, GT *n* = 11) or unpaired (ST *n* = 8, GT *n* = 8) cue-drug presentations. Data represent the means ± SEM. (a) The probability to orient to the cocaine cue during light-CS presentation. (b) The probability to approach the cocaine cue during the light-CS presentation. Asterisk, indicates a significant group difference between Paired STs and GTs, $p < 0.05$.

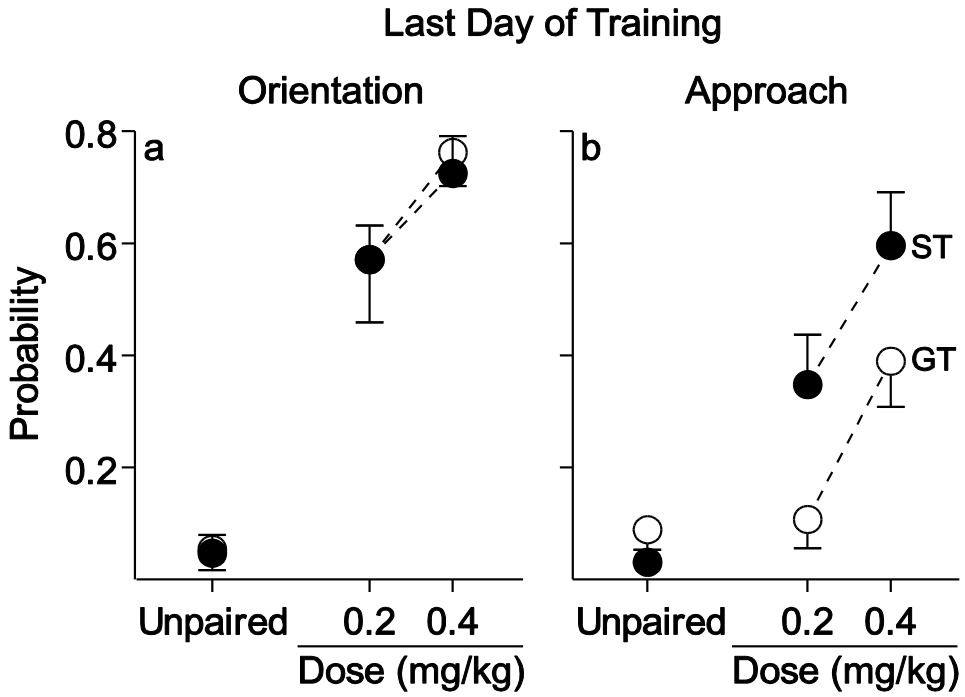


Figure 2.6. Dose-response functions. Probability of conditioned orientation (a) and conditioned approach (b) on the final day of training. Data represent the means ± SEM. Each data point represents an independent group of rats.

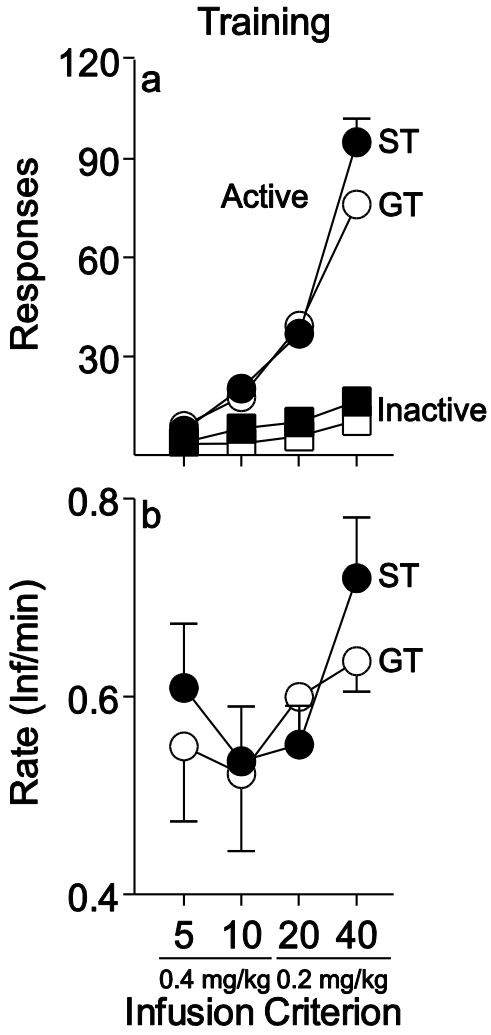


Figure 2.7. Acquisition of cocaine self-administration behavior in sign-trackers (*n* = 25) and goal-trackers (*n* = 16). (a) Mean ± SEM number of active and inactive responses for infusion criteria 5 and 10 (0.4 mg/kg/inf) and infusion criteria 20 and 40 (0.2 mg/kg/inf). (b) Mean ± SEM number of cocaine infusions/min at each infusion criterion.

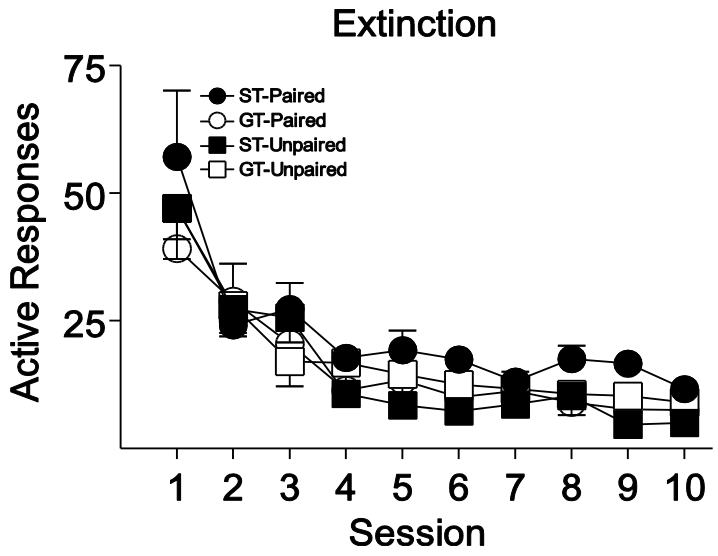


Figure 2.8. Extinction training. Mean ± SEM number of active responses during extinction of responding for cocaine in Paired sign-trackers (*n* = 14) and goal-trackers (*n* = 8) and Unpaired sign-trackers (*n* = 11) and goal-trackers (*n* = 8).

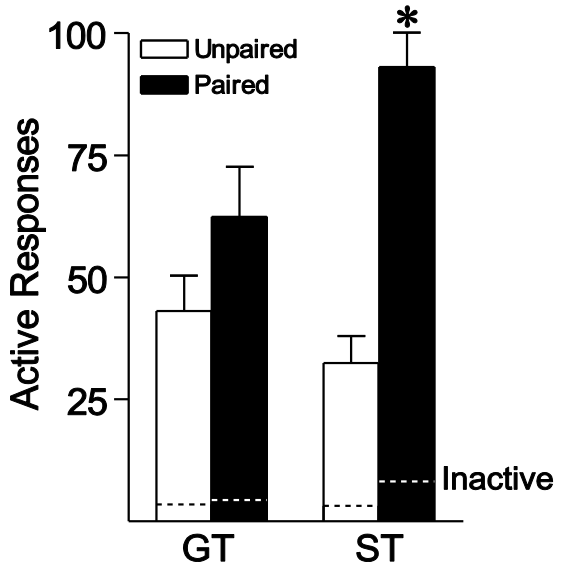


Figure 2.9. Cocaine cue reinstatement test. Following extinction, all animals were given a single 60 min cue reinstatement test session, in which active responses resulted in presentation of the cue previously paired or unpaired with non-contingent cocaine delivery. Mean ± SEM number of active responses in Unpaired (white bars) GTs (n = 8) and STs (n = 11) and Paired (black bars) GTs (n = 8) and STs (n = 14). The dashed lines indicate the mean number of responses in the inactive port. Asterisk, indicates significant difference, $p< 0.05$.

Chapter 3

A classically conditioned opioid cue acquires greater control over motivated behavior and induces greater Fos protein expression in rats prone to attribute incentive salience to a food cue

Introduction

Cues associated with natural or drug rewards can acquire such powerful motivational control over behavior that individuals sometimes have difficulty resisting them. There is, however, considerable individual variation in the ability of reward cues to gain such control over behavior (Mahler and de Wit 2010; Meyer et al. 2012b; Robinson and Flagel 2009; Schachter 1968; Styn et al. 2013). Evidence from a series of preclinical studies suggests that this variation is due, at least in part, to intrinsic individual variation in the extent to which reward cues are attributed with incentive salience and thus, in the ability of such cues to acquire properties of an incentive stimulus (Flagel et al. 2007; Meyer et al. 2012b; Robinson and Flagel 2009; Yager and Robinson 2010). For example, if a spatially discrete stimulus (the conditioned stimulus, CS) is repeatedly paired with delivery of a food reward (the unconditioned stimulus, US), in some rats ('sign-trackers', STs; Hearst and Jenkins 1974), the food cue itself becomes attractive, eliciting approach and engagement with it, and desired, in that rats will work to obtain it. In other rats ('goal-trackers', GTs; Boakes 1977) the food cue itself is less attractive, presentation of the CS instead elicits approach to the location where food will be delivered, and GTs do not work as avidly to gain access to the cue. Thus, only in some animals does a cue also acquire the

properties of an incentive stimulus – the ability to attract, the ability to act as a conditioned reinforcer, and to spur (motivate) seeking for its associated reward (for review see Robinson et al. 2014; Saunders and Robinson 2013).

Importantly, the propensity to attribute incentive salience to a food cue predicts the extent to which a discrete cocaine cue acquires motivational properties. For example, a cocaine cue is more attractive to STs, eliciting greater approach behavior (Flagel et al. 2010; Yager and Robinson 2013) and a cocaine cue is also more desired, in that STs will make more responses just for presentation of the cue, relative to GTs (Saunders and Robinson 2010; Yager and Robinson 2013). Finally, a cocaine cue spurs greater drug-seeking behavior in STs relative to GTs (Saunders et al. 2013). While we have shown that a cocaine cue acquires greater control over motivated behavior in STs relative to GTs, we currently do not know if this generalizes to other classes of drugs. Thus, the first aim of the experiments reported here was to determine whether the propensity of an individual to attribute incentive salience to a food cue predicts the extent to which cue associated with administration of an opioid drug acquires incentive motivational properties.

Currently, little is known about the neurobiological differences between STs and GTs that might account for differences in the propensity to attribute incentive salience to reward cues, though recent evidence points to differences in the mesolimbic dopamine system (Flagel et al. 2011a; Flagel et al. 2011b; Flagel et al. 2010; Flagel et al. 2007; Saunders and Robinson 2012). For example, recent work from our lab has shown that conditioned approach (sign-tracking) to a food cue is dependent on intact dopamine transmission within the nucleus accumbens (NAc) core (Saunders and Robinson 2012). Additionally, we have recently shown that in order for a food cue to engage the mesocorticolimbic system it must be imbued with incentive salience

(Flagel et al. 2011a). Therefore, our second aim was to begin to explore the neurobiological correlates underlying individual variation in the attribution of incentive salience to food and opioid cues. To do this we took a two-pronged approach. First, we assessed whether dopamine transmission within the NAc core is necessary for expression of conditioned approach to an opioid cue by pharmacologically blocking dopamine transmission within the NAc core. Next, we took a more broad approach and examined which brain regions were engaged by classically conditioned food and opioid cues and whether this varied by the degree to which individuals attributed incentive salience to reward cues (i.e., in STs vs. GTs). To do this we measured Fos protein expression elicited by presentation of either a food or opioid cue in brain structures located within the mesocorticolimbic and cortico-striatal-thalamic systems, as these systems have been shown to be engaged by both food and drug associated cues (Childress et al. 1999; Kelley et al. 2005; Schiltz et al. 2007; Schroeder et al. 2001; Tang et al. 2012).

Materials and Methods

Subjects

Male Sprague-Dawley rats weighing 250-275g upon arrival were purchased from Harlan (Haslett, MI) and Charles River (Portage, MI). Rats were individually housed in a climate-controlled colony room on a 12 hr light/12 hr dark cycle (lights on at 0800 hr) with *ad libitum* access to food and water (i.e., rats were not food restricted at any time). Following their arrival, rats were given one week to acclimate to the colony room before testing began. The experimenter gently handled rats several times during the acclimation period. The University of Michigan Committee on the Use and Care of Animals (UCUCA) approved all procedures.

Pavlovian training using food as the US

Apparatus. Behavioral testing was conducted in sixteen standard (22 x 18 x 13 cm) test chambers (Med Associates Inc., St. Albans, VT, USA) located in sound attenuating cabinets. Each cabinet was equipped with a ventilating fan that also served to mask background noise. Each chamber had a food cup located on the center of the front wall, 3 cm above a stainless steel grid floor. A retractable lever that could be illuminated from behind was located 6 cm above the floor either to the left or right of the food cup. The location of the lever was counterbalanced across rats. A red houselight was located on the wall opposite of the food cup and remained illuminated throughout the testing session.

Pavlovian training procedure. Pavlovian training procedures were similar to those described previously (Flagel et al. 2007; Meyer et al. 2012a). For 2 days prior to the start of training, 25 banana-flavored pellets (45 mg; BioServe) were placed into the home cage to familiarize the rats with this food. After the one week acclimation period, rats underwent one training session during which they learned to retrieve food pellets from the food cup. During this session the lever remained retracted and 25 pellets were delivered into the food cup according to a variable time (VT) 30 s (0-60 s) schedule of delivery. If a rat failed to consume all the pellets during this session, it was repeated again the next day. Subsequently, rats underwent 5 days (Experiments 1-3) or 7 days (Experiment 4) of Pavlovian conditioning (one session/day). Each session consisted of 25 trials during which the lever (lever-CS) was inserted into the chamber for 8 s, and during this time it was illuminated from behind. Immediately upon retraction of the lever a single 45-mg banana-flavored pellet (the US) was delivered into the food cup. CS-US pairings occurred on a VT 90 s (30-150 s) schedule. Importantly, no instrumental response was required by the rat to initiate delivery of the food pellet. Lever deflections, food cup entries, latency to the first lever

deflection, and latency to enter the food cup during each CS presentation were recorded using Med Associates software.

Pavlovian conditioned approach (PCA) index. Following completion of Pavlovian training, animals were classed into three groups: (1) Those that preferentially interacted with the lever-CS ('sign-trackers', STs), (2) those that preferentially interacted with the food cup during the lever-CS presentation ('goal-trackers', GTs), and (3) those that had no strong preference for either the lever-CS or food cup ('intermediate group', IG). The extent to which behavior was directed towards the lever-CS or the food cup was quantified using a composite Pavlovian conditioned approach (PCA) index, based on performance during days 4 and 5 of training, as previously described (Lomanowska et al. 2011; Meyer et al. 2012a). The PCA Index score incorporated three measures of conditioned approach behavior: (1) the probability of contacting either the lever-CS or food cup during a trial [P(lever)-P(food cup)]; (2) the response bias for contacting the lever-CS or food cup during a trial [(#lever deflections - #food cup entries)/ (#lever deflections + #food cup entries)]; and (3) the mean latency to contact the lever or enter the food cup during a trial [(food cup contact latency - lever deflection latency)/8]. This formula produces values ranging from -1.0 to +1.0, where a score of +1 indicates an animal made a ST CR on every trial, a score of -1 that an animal made a GT CR on every trial and a score of 0 that an animal distributed ST and GT responses equally. For purposes of classification, rats with scores of -1.0 to -0.5 were operationally defined as GTs and rats with scores of +0.5 to +1.0 were defined as STs (i.e., these subgroups were twice as likely to interact with either the food cup or lever-CS, respectively). Rats that were within the range of -0.49 to +0.49, whose behavior vacillated between the lever-CS and food magazine, were classified as intermediates (IGs) and were not used further because we were interested in comparing rats that differed strongly in their

propensity to attribute incentive salience to reward cues (Meyer et al. 2012a). A total of 283 rats were screened for ST and GT behavior, but only a subset (N= 102; STs *n*= 58, GTs *n*= 44) were used in the experiments described below

Experiment 1: Individual variation in Pavlovian conditioned approach using remifentanil as the US.

Surgery. Following completion of Pavlovian training using food as the US, chronic indwelling catheters were implanted into the jugular vein of STs and GTs as described previously (Crombag et al. 2000) under ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p) anesthesia. Post-operative pain was managed with carprofen (5 mg/kg). Following surgery, catheters were flushed daily with 0.2 ml of sterile saline containing 5 mg/ml gentamicin sulfate (Vedco) to prevent occlusions and minimize infections. Catheter patency was tested before the first day of training and again after the last day of training by intravenous (IV) injection of 0.2 ml of methohexital sodium (10 mg/ml in sterile water; JHP Pharmaceuticals). Rats were removed from the analysis if they failed to become ataxic within 5 s of injection (ST *n*= 6, GT *n*= 3).

Apparatus. Behavioral testing was conducted in chambers identical to those used to screen animals for ST and GT, except the food cup and lever were removed from the chamber and two stimulus lights were placed on the left and right sides of the wall opposite the white houselight, 13.5 cm above the stainless steel grid floor. The location of the stimulus light designated to serve as the CS (i.e., to be paired with remifentanil infusions) was counterbalanced between rats. Rats received remifentanil infusions via a syringe pump that was located outside the sound-attenuating chamber. The infusion tubing was suspended into the chamber via a swivel mechanism, which allowed rats' free movement in the chamber.

Pavlovian training procedures. Prior to training, rats were assigned to either Paired (CS and US presented together) or Unpaired groups (US explicitly not paired with presentation of the CS). After recovery from surgery, rats were first habituated to the presentation of the stimulus light (light-CS) and infusion procedure to decrease otherwise high levels of responding to a novel stimulus (Uslaner et al. 2006). This single habituation session consisted of 25 trials during which both stimulus lights were illuminated for 10 s and coincided with activation of the infusion pump and an IV infusion of saline (50 µl delivered over 2.8 s). Trials occurred on a VT 90 s (60-120 s) schedule. Starting the next day, rats underwent 8 days of Pavlovian conditioning using remifentanil as the US.

We used remifentanil in these studies, rather than morphine or heroin, because remifentanil is: (1) a potent µ-opioid receptor agonist (James et al. 1991; Michelsen and Hug 1996), (2) is readily self-administered by humans and animals (Baylon et al. 2000; Ko et al. 2002; Levine and Bryson 2010; Panlilio and Schindler 2000), and most importantly, (3) it has a very short half-life of approximately 45 s (Haidar et al. 1997). The short half-life of remifentanil is very advantageous for Pavlovian conditioning studies because it allows for frequent CS-US pairings (see Uslaner et al. 2006).

Each session consisted of 22 trials (CS-US presentations) occurring on a VT schedule with a mean of 360 s (300-420 s). This inter-trial interval (ITI) was chosen based on the half-life of remifentanil as well as pilot testing to determine the amount of time necessary for rats to recover from the locomotor suppressant effects of remifentanil. For rats in the Paired groups, each trial consisted of illumination of the stimulus light designated as the CS (light-CS) for 10 s, which coincided with an IV infusion of 1.6 or 3.2 µg/kg of remifentanil hydrochloride (weight of the salt, dissolved in 0.9% saline in 50 µl). The drug injection began at the same time the light-

CS was illuminated, but occurred over only 2.8 sec. Remifentanil was obtained from the hospital pharmacy of the University of Michigan Health system (Ultiva brand, GlaxoSmithKline; Uxbridge, Middlesex, UK). Again, no instrumental response was required by the rat to initiate either illumination of the light or the remifentanil infusion. Independent groups of rats were used for each dose of remifentanil tested. Rats in the Unpaired group received non-contingent infusions of 3.2 µg/kg remifentanil that were explicitly not paired with illumination of the light-CS (remifentanil was delivered on a VT schedule with a mean of 150 s after the CS was extinguished). We only tested rats in the Unpaired group with the higher dose of remifentanil as this dose produced the most behavior in the Paired rats.

Experiment 2: Individual variation in the conditioned reinforcing properties of a Pavlovian conditioned remifentanil cue

One week following the last PCA session with remifentanil as the US, rats from Experiment 1 underwent a single 40 min test for conditioned reinforcement. During this test the chamber was reconfigured such that the cue light was now located in the middle of the front wall and was flanked by two nose-poke ports. Responses into one port (Active) resulted in illumination of the remifentanil cue (light-CS) for 2s. Responses into the other port (Inactive) had no consequence. The side of the Active and Inactive ports was counterbalanced between rats. No remifentanil was delivered during this test.

Experiment 3: The role of nucleus accumbens core dopamine in the expression of Pavlovian conditioned approach to a remifentanil cue

An independent cohort of rats (N= 32) was used for Experiment 3. Rats were trained on the Pavlovian task using food as the US to identify STs. Only STs were used for this experiment as we were interested in whether dopamine transmission within the core was necessary for

conditioned approach to a remifentanil cue and based on our results from Experiment 1, GTs did not readily approach the remifentanil cue.

Surgery. Rats were first prepared with IV catheters as described in Experiment 1. Rats were then positioned in a stereotaxic apparatus (David Kopf Instruments). Bilateral 22-gauge stainless steel guide cannulas (Plastics One) were inserted 2 mm above the target site in the nucleus accumbens (NAc) core (relative to bregma: AP +1.8 mm; ML ±1.5 mm; DV -5.0 mm). Guide cannula were secured to the skull with three screws and dental acrylic. Stainless steel obturators, flush with the end of the cannula, were inserted to prevent occlusion.

Microinjections. Dopamine receptor blockade was achieved with local microinjections of the non-specific dopamine receptor antagonist flupenthixol (Sigma). We used a non-specific antagonist because we wanted to block all actions of endogenous dopamine (i.e., not to assess which receptor subtypes are more important) within the NAc core. Flupenthixol was administered in four doses: 0, 5, 10, and 20 µg in 0.9% sterile saline. Drug doses were based on a previous study where we showed that these doses of flupenthixol dose dependently decreased conditioned approach to a food cue (Saunders and Robinson 2012). On test days, stainless steel 28-gauge injectors (Plastics One) attached to PE-20 polyethylene tubing were inserted into the guide cannulas and extended 2 mm beyond the tip of the guide cannula. The experimenter gently held rats during infusions. The infusion volume of 0.5 µl per side was delivered over 60 s using a syringe pump (Harvard Apparatus, Holliston, MA, USA). Injectors remained in place for an additional 60 s to allow for drug diffusion before being removed and replaced with obturators. Following microinjections, rats were placed in holding chambers for 35 min before being moved to the testing chambers for the start of the session. This delay between drug injection and testing was imposed to account for the delayed onset of drug action (Saunders and Robinson 2012). All

microinjections were separated by 2 days of additional training to re-stabilize performance. Rats received a microinjection of saline ~4 days prior to the first test session to habituate them to the infusion procedure.

Pavlovian training and microinjection tests. Rats underwent Pavlovian training with remifentanil as the US for 8 days exactly as described for Experiment 1. Prior to the 9th training session, rats were given a vehicle microinjection. Subsequently, rats received microinjections of flupenthixol (5, 10, and 20 µg) in a counterbalanced order, followed by a second vehicle injection before the final session. It is important to note that each test session was identical to the training sessions (i.e., both CS and US were presented).

Histology. After completion of behavioral testing, rats were anesthetized with an overdose of pentobarbital sodium and their brains were extracted and flash-frozen in isopentane. Brains were sectioned at 60 µm on a cryostat throughout the extent of the accumbens core, mounted on slides, air-dried, and stained with Cresyl violet. Cannula placements were examined on sections using light microscopy and mapped onto schematics from a rat brain atlas (Paxinos and Watson 1998).

Experiment 4: Individual variation in Fos expression elicited by Pavlovian conditioned food and remifentanil cues

A separate cohort of rats was used in Experiment 4 (*n*= 76). An additional 4 rats were used as transport controls for this portion of the study. These control rats were moved daily from the colony room to the testing chambers concurrently with rats undergoing Pavlovian conditioning. Control rats were left undisturbed in the testing chambers, with the houselight on, for the length of each Pavlovian conditioning session.

Pavlovian training procedures for food cue groups. Prior to training, rats were randomly assigned to either the Paired or Unpaired group. Rats in the Paired group were trained on the Pavlovian task using food as the US and the lever as the CS to identify STs and GTs as described above. Rats in the Unpaired group received pseudorandom CS and US presentations during each session. Pavlovian training was conducted over seven consecutive days.

Pavlovian training procedures for remifentanil (REMI) cue groups. Rats were first trained on the Pavlovian task using food as the US, over seven consecutive days, to identify STs and GTs and subsequently prepared with I.V. catheters. Rats were then assigned to either the Paired or Unpaired group prior to Pavlovian training using remifentanil as the US and the cue light as the CS. Pavlovian conditioning with remifentanil as the US was identical to that for Experiment 1 except that training was conducted over seven days and each session consisted of 25 trials. This was done so that all rats received the same number of CS-US pairings for the cue that would be presented on test day.

Forced abstinence. Following completion of Pavlovian conditioning, rats were left undisturbed in their home cages for 10 days. This period of abstinence was necessary as Fos expression has been shown to diminish after repeated drug exposures. We have previously shown that after a 10 day drug-free period the ability of drug (amphetamine) to induce c-fos mRNA is restored (Ostrander et al. 2003). Rats in both the food and REMI groups underwent the 10 day abstinence period so that the time between the last Pavlovian training session and the test day was held constant across groups.

Context exposure sessions. To minimize the influence of contextual cues, all rats were placed into the test chambers for 30 min on days 8-10 following completion of Pavlovian conditioning, during which time the houselight was illuminated but neither the lever-CS nor the light-CS was

presented. Test chambers remained in the same configuration as during conditioning (i.e., the food cup remained in the chamber for the food cue group). Rats in the REMI cue group were tethered to the infusion line during these sessions.

Test day: re-exposure to the CS. The day following the last context exposure session (i.e., day 11 following the final Pavlovian conditioning session) rats were placed into the chambers, the houselight was illuminated, and following a 5-min habituation period, either the illuminated lever-CS was inserted into the chamber (for the food cue group) or the light-CS was illuminated (for the REMI cue group) for 4 s a total of 10 times (once per minute). Rats in the REMI cue group were tethered to the infusion line during this session. Note that no food or remifentanil was delivered during this test.

Tissue preparation. After the last CS presentation, rats were returned to their home cages. Approximately 60 min later, rats were anesthetized with pentobarbital sodium (390 mg/kg, i.p.) and perfused transcardially with 25 ml of 0.9 % saline followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were harvested and post-fixed for 1 hr at room temperature in the same fixative, then stored in 20% sucrose and 0.01% sodium azide in 0.1 M PB at 4ºC. Coronal sections (35 µm) were cut on a freezing microtome (CM 2000R, Leica) and stored in a cryoprotectant solution (30% sucrose and 30% ethylene glycol in 0.1 M PB). Sections were obtained through the brain in four parallel series. Tissue was stored at -20ºC until further processing.

Immunohistochemistry. All incubations were performed at room temperature with gentle agitation. Free-floating sections were washed three times (5 min) with 0.1 M Phosphate-buffered saline (PBS) between incubations. Sections were incubated in 1% H_2O_2 for 10 min and then blocked in an incubation solution (PBS containing 0.1% bovine serum albumin, Fisher; and 0.4%

Triton X-100, Sigma-Aldrich) for 1 hr. Next, tissue was incubated overnight with a rabbit polyclonal antibody against c-Fos (1:500; ab7963, lot GR126599; Abcam, Cambridge, MA, USA). Sections were then incubated in biotin-conjugated goat anti-rabbit IgG (1:500 in PBS containing 0.1% bovine serum albumin and 0.4% Triton X-100; Vector Laboratories) for 1 hr followed by a 1 hr incubation in avidin-biotin-horseradish peroxidase (1:1000 in PBS; ABC elite; Vector Laboratories). This was visualized using 0.02% 3,3´-diaminobenzidine tetrahydrochloride (10 min; Sigma-Aldrich) with 0.02% nickel sulfate in 0.1 M PB with hydrogen peroxide (0.015%). Sections were mounted onto Superfrost plus glass slides (Fisher) and coverslipped with dibutyl phthalate xylene.

Fos immunoreactivity analysis. Digital images were captured with a CCD camera (FX1520, SPOT Imaging Solutions, Sterling Heights, MI, USA) attached to a Leica microscope (DM400B, Leica, Wetzlar, Germany) with fixed camera settings for all subjects (using 10x objectives). Fos immunoreactive cells were counted by an individual blind to treatment conditions and were identified by black oval-shaped nuclei. Using National Institutes of Health ImageJ software, areas of analysis were defined based on landmarks unique for each brain region (Paxinos and Watson 1998). The total number of Fos immunoreactive cells was quantified from the left and right hemispheres of each animal for each region of interest and counts were averaged per animal. The orbitofrontal cortex (OFC) was sampled at +3.2 from bregma with a sampling area of 400 x 600 µm. The nucleus accumbens (NAc) core and shell subregions were sampled at +1.6 mm from bregma with a sampling area of 400 x 600 µm. The dorsolateral (DL) and dorsomedial (DM) dorsal striatum were sampled at +0.8mm from bregma with a sampling area of 600 x 600 µm. The basolateral amygdala (BLA) and central nucleus of the amygdala (CeA) were sampled at -2.56 from bregma with a sampling area of 400 x 600 µm. The paraventricular nucleus of the

thalamus (PVT) was sampled at -3.14 mm from bregma with a sampling area of 650 x 500 µm. The AP coordinates for each brain region were selected based on previous work looking at the induction of c-fos mRNA in STs and GTs in response to presentation of a food cue (Flagel et al. 2011a).

Video analysis

All Pavlovian conditioning sessions with remifentanil as the US were video recorded. Video was scored offline by an observed blind to treatment condition for two different conditioned responses (CRs) as described previously (Yager and Robinson 2013). (1) *Conditioned Orientation*: an orienting response was scored if the rat made a head and/or body movement in the direction of the CS during the CS period, regardless of whether the rat approached the CS. (2) *Conditioned Approach*: an approach response was scored if the rat moved towards the CS during the CS period, bringing its nose to within 1 cm of the light. Given the location of the cue light within the chamber, a rat had to rear, lifting both paws off the floor, in order to bring its nose within 1 cm of the light. It is worth noting that if an approach response was scored on a given trial an orienting response would also be scored, as orienting always preceded approach. However, an orienting response could occur in the absence of an approach response (e.g., an orienting response could occur in the absence of rearing). Additionally, to assess the effects of flupenthixol on locomotor behavior, we also scored locomotor activity during each test session that rats received a microinjection. To do this, video was scored every 5 min for 30 sec and the number of cage crosses made during this time were recorded.

Statistics

Linear mixed-models (LMM) analysis was used for all repeated measures data (Verbeke and Molenberghs 2000). The covariance structure was explored and modeled for each dependent

variable. Two way (group: ST/GT by dose) ANOVAs were used to analyze dose-response data for conditioned orientation and conditioned approach. Two way repeated measures (group: ST/GT by port: Active/Inactive) ANOVAs were used to compare groups during the conditioned reinforcement test. One-way ANOVAs were used to examine group differences in behavior upon re-exposure to the CS on test day and the average amount of Fos expression for each region of interest. Transport control rats were not included in this analysis as their Fos expression was not significantly different from that of the Unpaired rats, except in the CeA. The relationship between Fos expression and behavior upon re-exposure to the CS was examined using correlation Z-tests with a 95% confidence interval. When main effects were found, post hoc comparisons were made using Fisher's LSD test. Statistical significance was set at $p<0.05$.

Results

Experiment 1

Individual variation in Pavlovian conditioned approach to a food cue

As reported previously, two distinct phenotypes emerged as a result of Pavlovian training using food as the US (Flagel et al. 2007; Meyer et al. 2012a; Robinson and Flagel 2009). Across successive days of training, presentation of the lever-CS came to primarily evoke a sign-tracking (ST) CR in some rats. A ST CR consisted of reliable and rapid approach to the lever-CS (Fig. 3.1a, c) followed by engagement with it, as indicated by lever deflections (Fig. 3.1b). Conversely, for other rats, presentation of the lever-CS came to primarily evoke a goal-tracking (GT) CR. A GT CR consisted of reliable and rapid approach to the food cup (Fig. 3.1d, f), followed by engagement with it, as indicated here by repeated photocell beam breaks (Fig. 3.1e). It has also been reported that GTs lick, nibble, and bite the food cup after approaching it (see DiFeliceantonio and Berridge 2012; Mahler and Berridge 2009 for a more detailed description of

this behavior). Other rats (intermediates) showed a high incidence of both CRs. Their data are not shown because these animals were not used further.

Individual variation in conditioned approach to a remifentanil cue, but not conditioned orientation

When a drug is used as the US, rather than food, rats often do not physically engage a lever-CS. Instead, a sign-tracking CR consists of approach to the CS followed by sniffing and investigation of it (Flagel et al. 2010; Uslaner et al. 2006; Yager and Robinson 2013). This behavior is very similar to what is observed when rewarding electrical brain stimulation is used as the US (Peterson et al. 1972). Thus, when using remifentanil as the US, we scored a CS-directed approach response (a ST CR) if a rat brought its nose to within 1 cm of the light-CS during the CS period. In contrast, an orientation response was scored if a rat moved its head and/or body in the direction of the light-CS upon CS presentation, regardless of whether the rat approached it.

Conditioned orientation (1.6 µg/kg). Figure 3.2a illustrates the probability of conditioned orientation across training sessions when using 1.6 µg/kg remifentanil as the US. With this dose both Paired STs and GTs acquired a conditioned orientation response, as indicated by a significant increase in the probability of orientation behavior across sessions [$F(2, 39.25)= 23.59$, $p< 0.001$]. While both STs and GTs acquired a conditioned orientation response, and did so at a similar rate as indicated by a non-significant group by session interaction, STs oriented more than GTs [main effect of group, $F(1, 51.83)= 7.439$, $p= 0.009$]. However, both STs and GTs showed a significant increase in the probability of orienting to the remifentanil cue across sessions, relative to their Unpaired control groups [pairing x session interaction; STs: $F(2, 42.92)= 23.99$, $p< 0.001$; GTs: $F(2, 36.92)= 4.81$, $p= 0.01$].

Conditioned approach (1.6 µg/kg). Figure 3.2b illustrates the probability of conditioned approach across training sessions when using 1.6 µg/kg remifentanil as the US. Figure 3.2b shows that Paired STs and GTs differed in the extent to which the remifentanil cue elicited an approach CR [effect of group, $F(1, 45.04)= 15.17$, $p< 0.001$]. Indeed, STs approached the remifentanil cue significantly more across sessions relative to GTs [group x session interaction, $F(2, 41.38)= 3.84$, $p= 0.03$]. Finally, Paired STs also showed a significant increase in probability of approaching the remifentanil cue across sessions, relative to their Unpaired control group [pairing x session interaction, $F(2, 41.81)= 6.12$, $p= 0.005$], whereas Paired and Unpaired GTs did not statistically differ.

Conditioned orientation (3.2 µg/kg). Figure 3.2c illustrates that when using 3.2 µg/kg remifentanil as the US both Paired STs and GTs acquired a conditioned orientation response, as indicated by a significant increase in the probability of orientation behavior across sessions [$F(2, 18)= 99.62$, $p< 0.001$], and the two groups did not differ. In addition, both Paired STs and GTs showed a significant increase in probability of orienting to the remifentanil cue across sessions relative to their respective Unpaired control groups [pairing x session interaction; STs: $F(2, 24.02)= 20.4$; $p< 0.001$; GTs: $F(2, 17)= 33.01$, $p< 0.001$].

Conditioned approach (3.2 µg/kg). In contrast to conditioned orientation, Fig. 3.2d shows that Paired STs did differ from Paired GTs in the probability of approaching the CS when using 3.2 µg/kg remifentanil as the US [effect of group, $F(1, 45.59)= 20.18$, $p< 0.001$]. Indeed, the effect of session was statistically significant for STs [$F(2, 11.66)= 19.51$, $p< 0.001$] but not GTs. Additionally, both Paired STs and GTs showed a significant increase in probability of approaching the remifentanil cue across sessions, relative to their respective Unpaired control groups [pairing x session interaction; STs: $F(2, 21.5)= 14.73$, $p< 0.001$; GTs: $F(2, 31.64)= 3.4$,

p= 0.046]. Importantly, neither STs nor GTs in the Unpaired group acquired an orientation or approach CR.

Dose-response analysis. Figures 3.2e and 3.2f summarize the dose-response functions for the probability of conditioned orientation and conditioned approach on the final day of training. Two-way ANOVAs revealed that there was an overall difference between Paired STs and GTs in both probability of conditioned orientation [main effect of group, $F(1, 37)= 7.66$, $p= 0.009$] and probability of conditioned approach [main effect of group, $F(1, 37)= 27.47$, $p< 0.001$]. However, the probability of both of these CRs did not change as a function of dose. We separately analyzed conditioned approach dose-response data for STs and GTs and included Unpaired control animals in this analysis. A one-way ANOVA revealed a significant effect of treatment group for STs and a non-significant trend for GTs on performance of a conditioned approach CR on the final day of training [STs: $F(2, 30)= 27.96$, $p< 0.001$; GTs: $F(2, 26)= 3.19$, $p= 0.058$]. Post-hoc analysis (Fisher's LSD) revealed that, on the final day of training, Paired STs differed from Unpaired STs at both doses tested (p's< 0.001).

Experiment 2

A remifentanil cue is a more effective conditioned reinforcer in STs than GTs

Following a one week period of forced abstinence, all rats from Experiment 1 underwent a single test for conditioned reinforcement. During this test the chamber was reconfigured such that the remifentanil cue (light-CS) was relocated to the middle of the wall and was flanked by two nose-poke ports. Responses into the Active port produced presentation of the light-CS, which had previously either been paired or unpaired with remifentanil infusions, while responses into the Inactive port had no consequence. No remifentanil was delivered during this test. Figure 3.3 shows the mean difference in nose pokes into the Active minus Inactive port during the

conditioned reinforcement test. As can be seen in Figure 3.3, when either 1.6 (Fig. 3.3a) or 3.2 µg/kg (Fig. 3.3b) remifentanil was used as the US during Pavlovian conditioning, STs made more Active (relative to Inactive) responses than did GTs, as indicated by a significant group x port interaction [1.6 µg/kg: $F(1, 17)= 5.506$, $p= 0.031$; 3.2 µg/kg: $F(1, 20)= 5.516$, $p= 0.029$]. For rats in the Unpaired condition, there was no significant difference between groups.

Experiment 3

Pavlovian training with food as the US was very similar to Experiment 1, so for the sake of simplicity these data are not shown. It is important to point out that this experiment only utilized rats identified as STs.

Acquisition of conditioned orientation and approach to a remifentanil cue

As in Experiment 1, STs acquired orientation and approach CRs. As shown in Figure 3.5a, STs increased both orientation towards and approach to the remifentanil cue across sessions [main effect of session; orientation: $F(2, 18.03)= 54.29$, $p< 0.001$; approach: $F(2, 17.06)= 26.99$, $p< 0.001$].

Dopamine receptor blockade in the nucleus accumbens core suppresses conditioned approach, but not conditioned orientation, to a remifentanil cue

Upon review of video of the test sessions, we found that the 20 µg dose of flupenthixol produced non-specific effects on locomotor behavior, significantly decreasing the number of cage crosses at this dose [$F(3, 24)= 4.78$, $p= 0.01$; Fig. 3.4], by seemingly interacting with remifentanil to reduce locomotion, so data using this dose were not included in any further analysis. We first examined the effect of flupenthixol (0, 5, or 10 µg) on conditioned orientation and approach across the entire session. As can be seen in Figure 3.5b, flupenthixol dose-dependently decreased approach to the remifentanil cue [$F(2, 15.22)= 47.409$, $p< 0.001$] without affecting conditioned

orientation [$F(2, 14)= 3.565$, $p= 0.17$]. We next examined whether flupenthixol decreased approach on the very first trial (i.e., in the absence of any new learning). Indeed, as shown in Figure 3.5c, flupenthixol decreased approach on the very first trial [$F(2, 16.973)= 4.98$, $p= 0.02$]. Post-hoc analysis, however, revealed that there was only a significant decrease in conditioned approach on the first trial, relative to vehicle, with the higher dose of flupenthixol [5 µg: $p= 0.42$; 10 µg: $p= 0.03$].

Histological verification of cannula placements

Figure 3.6 illustrates the location of microinjection tips within the accumbens core for rats used in Experiment 3.

Experiment 4

Pavlovian training

Food cue group: Conditioned approach with food as the US. Rats were again classified as STs and GTs based upon their preference for either interacting with the lever-CS or the food cup. Across sessions, STs increased the probability of contacting the lever-CS (Fig. 3.7a) while GTs increased the probability of entering the food cup during the CS presentation (Fig. 3.7b). Rats in the Unpaired (UP) group did not develop a preference for either the lever-CS or the food cup. Analysis of the number contacts with the lever and food cup and the latency to approach them were very similar to the probability of approaching the lever and food cup, so for the sake of simplicity these data are not shown.

REMI cue group: Conditioned orientation and approach with remifentanil as the US. Following identification as STs and GTs, rats were conditioned with 3.2 µg remifentanil as the US in a manner very similar to Experiment 1. Figure 3.8a illustrates that when using 3.2 µg/kg remifentanil as the US both STs and GTs acquired a conditioned orienting response, as indicated

by a significant increase in the probability of orientation behavior across sessions [$F(2, 10)= 52.92$, $p< 0.001$], and the two groups did not differ. In addition, both STs and GTs showed a significant increase in the probability of orientation to the remifentanil cue across sessions relative to the UP group [pairing x session interaction; STs: $F(2, 18.96)= 41.22$; $p< 0.001$; GTs: $F(2, 21.82)= 28.94$, $p< 0.001$].

Figure 3.8b illustrates the probability of conditioned approach across training sessions when using 3.2 µg/kg remifentanil as the US. Figure 3.8b shows that STs and GTs differed in the extent to which the remifentanil cue elicited an approach CR [effect of group, $F(1, 25.77)= 45.91$, $p< 0.001$]. Indeed, STs approached the remifentanil cue significantly more across sessions relative to GTs [group x session interaction, $F(2, 16.96)= 14.29$, $p< 0.001$]. STs also showed a significant increase in probability of approaching the remifentanil cue across sessions, relative to the Unpaired group [pairing x session interaction, $F(2, 16.38)= 27.92$, $p< 0.001$]. While GTs approach more overall than Unpaired rats [main effect of pairing, $F(1, 29.44)= 22.4$, $p< 0.001$], this did not change across sessions.

Test day behavior: re-exposure to the CS

Food cue. Figures 3.7c and 3.7d illustrate behavior during the test session, during which the food cue was presented under extinction conditions (i.e., no food was delivered). There were significant group differences in the probability of approaching both the lever-CS [$F(2,16)= 4.82$, $p= 0.026$] and the food cup during the CS presentation [$F(2, 16)= 4.887$, $p= 0.025$]. STs approached the lever-CS more than GTs ($p= 0.009$) and there was a non-significant trend for STs to approach the lever-CS more then UP rats ($p= 0.06$). Conversely, GTs approach the food cup more than STs ($p= 0.008$), and UP rats did not differ from either STs or GTs. There were no significant correlations between the number of lever contacts, for STs, and magazine entries, for

GTs, upon re-exposure to the lever-CS and the number of Fos cells in any brain region examined.

Remifentanil cue. Figures 3.8c and 3.8d show orientation and approach to the remifentanil cue on test day, where the remifentanil cue was presented under extinction conditions (i.e., no remifentanil was delivered). There were significant group differences in conditioned orientation upon presentation of the CS [$F(2, 17)= 67.39$, $p< 0.001$]. As during training, STs and GTs both oriented more than UP rats ($p's< 0.001$). There were also group differences in approach to the remifentanil cue [$F(2, 17)= 13.52$, $p< 0.001$]. STs approached the remifentanil cue more than GTs or UP rats ($p's< 0.05$), which did not differ from one another. Correlational analyses revealed no significant relationship for STs between the probability of approaching the light-CS and the number of Fos cells in any brain region examined.

Fos immunoreactivity

Data in Figures 3.9-3.12 represent the mean (± SEM) number of Fos-positive cells in STs and GTs, exposed to either the food or the REMI cue, expressed as a percent of Fos-positive cells in the relevant Unpaired (UP) control group. However, the actual cell counts for each group are shown in Table 3.1, and one-way ANOVAS were conducted on the actual number of Fos cells as a function of group, and not the percent data. The graphs depict the data as a percent of the Unpaired group to decrease the number of bars used in each graph and to thus facilitate visually making group comparisons.

Orbitofrontal cortex (OFC). There were no significant group differences in Fos expression in the OFC elicited by either the food or REMI cue [food cue: $F(2, 14)= 2.707$, $p= 0.101$; REMI cue: $F(2, 15)= 1.922$, $p= 0.181$; Fig. 3.9].

Nucleus accumbens. The nucleus accumbens was divided into the core and shell subregions. There were significant group differences in Fos expression in the shell among both the food and REMI cue groups [food cue: $F(2, 14)= 43.933$, $p< 0.001$; REMI cue: $F(2, 15)= 18.968$, $p< 0.001$; Fig. 3.10a]. STs showed greater Fos expression in response to the food cue relative to GTs or the UP group (*p's*< 0.001). Additionally, presentation of the food cue increased Fos expression in GTs relative to the UP group ($p= 0.039$). Presentation of the REMI cue induced greater Fos expression in STs relative to GTs or the UP group (*p's*< 0.001), which did not differ from one another.

There were also significant group differences in Fos expression in the core among both the food and REMI cue groups [food cue: $F(2, 14)= 53.541$, $p< 0.001$; REMI cue: $F(2, 15)= 26.363$, $p< 0.001$; Fig. 3.10b]. STs showed greater Fos expression in response to both the food and REMI cues relative to GTs or the UP groups (*p's*< 0.001), which did not differ from one another.

Dorsal Striatum. The dorsal striatum was divided into dorsolateral and dorsomedial subdivisions. There were significant group differences in Fos expression in the dorsomedial portion of the dorsal striatum among both the food and REMI cue groups [food cue: $F(2, 14)= 21.602$, $p< 0.001$; REMI cue: $F(2, 15)= 8.563$, $p= 0.003$; Fig. 3.10c]. STs showed greater Fos expression in response to the food cue relative to GTs or the UP group (*p's*< 0.002). Additionally, presentation of the food cue increased Fos expression in GTs relative to the UP group ($p= 0.04$). In STs, presentation of the REMI cue induced greater Fos expression relative to GTs or the UP group (*p's*< 0.02), which did not differ from one another.

There were also significant group differences in Fos expression in the dorsolateral portion of the dorsal striatum after exposure to either the food and REMI cue [food cue: $F(2, 14)=$

11.631, p= 0.001; REMI cue: $F(2, 15)$= 11.812, p= 0.001; Fig. 3.10d]. In STs, presentation of either the food or REMI cue increased Fos expression in the dorsolateral striatum relative to GTs or the UP group (p's< 0.009), which did not differ from one another.

Amygdala. The amygdala was divided into the basolateral nucleus (BLA) and the central nucleus (CeA). In the CeA there were significant group differences in Fos expression elicited by the food cue, but not by the REMI cue, [food cue: $F(2, 14)$= 6.055, p= 0.013; REMI cue: $F(2, 15)$= 0.565, p= 0.58; Fig. 3.11a]. STs, in response to presentation of the food cue, showed an increase in Fos expression relative to the UP group (p= 0.004). While GTs and UP rats were not statistically different, there was a trend towards increased Fos expression in GTs relative to the UP group (p= 0.065).

Presentation of both the food and REMI cue evoked greater Fos expression in the BLA [food cue: $F(2, 14)$= 37.193, p< 0.001; REMI cue: $F(2, 15)$= 9.273, p= 0.002] in STs relative to GTs or the UP groups (p's< 0.008), which did not differ from one another (Fig. 3.11b).

Paraventricular nucleus of the thalamus. There were significant group differences in Fos expression in the paraventricular nucleus of the thalamus after exposure to both the food and REMI cue [food cue: $F(2, 14)$= 13.056, p= 0.001; REMI cue: $F(2, 15)$= 8.888, p= 0.003; Fig. 3.12]. In response to both the food and REMI cue, STs had greater Fos expression than either GTs or the UP groups (p's< 0.004), which did not differ from one another.

Discussion

We found that there is considerable individual variation in the extent to which a cue associated with an intravenous injection of the μ-opioid agonist, remifentanil, acquires properties of an incentive stimulus. First, relative to GTs, STs were more attracted to a classically conditioned remifentanil cue, in that they showed a higher probability of approaching into close

proximity with it. Second, STs found the remifentanil cue more desirable, in that it was more effective in reinforcing actions to get access to it in STs than GTs. We next wanted to begin to explore the neural circuitry underlying these behavioral differences. In experiment 3, we found that conditioned approach to a remifentanil cue requires intact dopamine signaling within the nucleus accumbens core. Finally, in experiment 4, we report that in order for either a food- or a remifentanil-associated cue to engage mesocorticolimbic and cortico-striatal-thalamic circuitry it must be imbued with incentive salience, as indicated by the fact that such cues induced Fos protein expression preferentially in STs in all brain regions studied.

Individual variation in the extent to which an opioid cue acquires incentive salience

We have reported previously that a discrete, localizable cocaine cue is more effective at eliciting approach behavior, is a more effective conditioned reinforcer, and is more effective in evoking a state of conditioned motivation that spurs drug-seeking behavior, in STs than GTs (Flagel et al. 2010; Saunders and Robinson 2010; Saunders et al. 2013; Yager and Robinson 2013). We do not know, however, the extent to which this effect generalizes across drug classes. It was recently established that rats will approach a cue associated with an intravenous injection of heroin (Madsen and Ahmed 2014; Peters and De Vries 2013) and that rats will acquire a novel response (i.e., nose poke) that produces a cue previously associated with the opioid remifentanil (i.e., it served as a conditioned reinforcer; Bertz and Woods 2013). However, in these studies, there was considerable variation in the behavioral responses they measured and we hypothesized that some of this variation may be due to differences in the extent to which the opioid cue acquired incentive salience. To begin to address this question, we asked whether variation in the propensity to attribute incentive salience to a food cue predicts the propensity to attribute incentive salience to a cue associated with the opioid remifentanil. First, we found that STs and

GTs differed in the extent to which the remifentanil cue became attractive as measured by the ability of the cue to elicit approach into close proximity to it. We found that with the low dose of remifentanil GTs did not acquire a conditioned approach response, although with a higher dose they did begin to approach, although to a lesser extent than STs. Second, the remifentanil cue was a more effective conditioned reinforcer in STs than GTs in that STs made more responses to earn presentation of the remifentanil cue. These results suggest that like cues associated with food and cocaine, there is individual variation in the extent to which an opioid cue comes to motivate behavior, and this is predicted by the extent to which a food cue is attributed with incentive salience.

While we show that STs and GTs differ in the extent to which an opioid cue becomes attractive and desired, one question that remains is whether these differences are due to the ability of STs and GTs to learn the CS-US association. As we have previously discussed, one limitation when using an intravenous injection of drug as the US and approach as the CR is that there is no "goal" to approach (Yager and Robinson 2013). Thus, it is difficult to determine whether GTs do not readily approach the drug associated cue because it was not attributed with sufficient incentive salience or if they failed to learn the CS-US association. To address this issue, we quantified another conditioned response, conditioned orientation, as an index of whether GTs learned the CS-US association, because previous studies suggest that performance of this CR does not require that the CS be attributed with incentive salience (Yager and Robinson 2013). For example, we recently showed that when cocaine was used as the US, STs and GTs did not differ in the acquisition of an orientation CR. We report here that there are also no differences between STs and GTs in the acquisition of an orientation CR to presentation of a remifentanil cue, though there were differences in the extent to which the cue elicited approach

behavior into close proximity to it. Therefore, we suggest the reason GTs did not readily approach the remifentanil cue is not because they failed to learn the CS-US association, as they acquired an orientation CR, but because the remifentanil cue was not attributed with sufficient incentive salience to attract animals to it.

Opiates, dopamine, and Pavlovian conditioned approach

It is generally assumed that the primary rewarding effects of all drugs of abuse are mediated by an increase in dopamine release (Luscher and Ungless 2006; Nestler 2005; Pierce and Kumaresan 2006). While it has been established that the primary rewarding effects of psychostimulants, such as cocaine and amphetamine, are mediated by dopamine release within the nucleus accumbens (Roberts et al. 1980), it appears that the same might not be true for the primary rewarding effects of opiates (for review see Badiani et al. 2011). For example, systemic blockade of dopamine receptors or selective lesions of dopamine terminals within the nucleus accumbens effects cocaine self-administration but has little to no effect on heroin self-administration (Ettenberg et al. 1982; Pettit et al. 1984). While the primary reinforcing effects of opiates may not be dopamine dependent, dopamine may be required for the secondary (conditioned) reinforcing effects of cues associated with opiate delivery. Therefore, we tested the hypothesis that dopamine transmission within the nucleus accumbens core may be necessary for the conditioned incentive motivational effects of an opioid (remifentanil) cue. Here, we report that endogenous dopamine signaling within the NAc core is in fact necessary for maintaining the motivational properties of a remifentanil cue, which makes it an attractive stimulus.

In addition to the debate over whether the rewarding effects of opiates are mediated by dopamine, there is also debate over the role of dopamine in learning. Schultz and colleagues have suggested that phasic dopamine signaling acts as a prediction-error signal, which is

necessary for stimulus-reward learning (Montague et al. 1996; Schultz 1998; Schultz et al. 1997; Waelti et al. 2001). Conversely, others have argued that dopamine is instead involved in transforming motivationally "cold", informational cues (CSs) into "hot", attractive, and desired incentives (Berridge 2007; 2012; Berridge and Robinson 1998; Zhang et al. 2009). Recently, we have taken advantage of natural individual variation in the extent to which reward cues acquire predictive (GTs) vs. incentive motivational properties (STs) to parse out the role of dopamine in stimulus-reward learning and motivated behavior. In two recent studies we showed that dopamine transmission is necessary for both the acquisition and expression of a ST CR but not a GT CR (Flagel et al. 2011b; Saunders and Robinson 2012). Together these data suggest that dopamine transmission is important in regulating the attribution of incentive salience to reward cues. We do not know, however, whether dopamine plays a similar role in the ability of drug cues to motivate conditioned approach behavior. Our results suggest that endogenous dopamine signaling within the accumbens core is also necessary for maintaining the motivational properties of drug cues. Furthermore, we also assessed the extent to which flupenthixol administration suppressed conditioned approach on the very first trial (i.e., in the absence of any new learning via an updated prediction-error signal). We found that flupenthixol suppressed approach behavior on the first trial, suggesting that our observed effects were not due to new learning but to degradation in the motivational properties of the cue.

Importantly, we also examined the effect of flupenthixol on conditioned orientation behavior. We did this in order to determine whether flupenthixol decreased approach behavior because dopamine transmission within the accumbens core is necessary for (1) maintaining the motivational properties of the remifentanil cue or (2) for maintaining the learned association between illumination of the light-CS and remifentanil delivery. We found that conditioned

orientation was not affected by flupenthixol treatment, which is consistent with previous results (Saunders and Robinson 2012). This suggests that the CS-US association remained intact and that flupenthixol decreased approach behavior because it degraded the motivational properties of the cue. These findings, together with our previous reports, suggest that DA transmission within the nucleus accumbens core is necessary for maintaining the motivational properties of both food and drug associated cues that make them powerful incentives (see also Saunders et al. 2013).

Individual variation in the engagement of brain reward circuitry by food and drug cues

There is now a wealth of evidence that cues associated with different classes of rewards (e.g., food, drugs, and sex) engage overlapping neural systems, including the mesocorticolimbic dopamine system and other cortico-striatal-thalamic loops that comprise a so-called "motive circuit" (Childress et al. 1999; Kalivas and Volkow 2005; Kelley et al. 2005; Kuhn and Gallinat 2011; Schroeder et al. 2001; Tang et al. 2012; Volkow et al. 2008b; Zombeck et al. 2008). For example, contextual cues associated with either food or drugs (i.e., morphine or nicotine) elicit similar neuronal activation patterns throughout the prefrontal cortex (Kelley et al. 2005; Schroeder et al. 2001; Schroeder et al. 2000). However, we have recently shown that there is individual variation in the extent to which a food cue can engage this "motive circuit". Flagel et al. (2011a) used *in situ* hybridization to measure cue-induced expression of c-fos mRNA throughout the brains of STs and GT. They found that after Pavlovian training with food as the US, presentation of the lever-CS, under extinction conditions, induced greater c-fos mRNA expression in the orbitofrontal cortex, dorsal striatum, NAc core and shell, lateral septum, lateral habenula, and the paraventricular, intermediodorsal, and central medial nuclei of the thalamus in STs relative to GTs or a control group, who received the same number of lever-CS and food presentations but in an unpaired manner. These findings suggest that the predictive value of the

food cue alone is not sufficient to engage this reward circuitry, but the cue must also be attributed with incentive salience. Here, we replicate and expand these findings and show similar expression patterns, this time using Fos protein expression as a marker of neuronal activation, in response to either a food or remifentanil cue. In almost every region we examined, both the food and remifentanil cue elicited greater Fos expression in STs relative to GTs or rats that received unpaired (UP) CS-US presentations. Furthermore, there were a number of regions (e.g., NAc core, DL striatum, PVT, BLA) where presentation of either the food or remifentanil cue had no effect on Fos expression in GTs (i.e., they did not differ from the UP group) while presentation of either cue produced robust Fos expression in STs. Together these data support the idea that the predictive value of cues is not sufficient to engage the "motive circuit"- the cue must also be attributed with incentive salience to do so.

It is important to note that on the cue exposure test day in Flagel et al. (2011a), the food cup was removed from the chamber to isolate the ability of the food cue to elicit c-fos mRNA expression. Thus, they could not assess c-fos mRNA expression when a GT CR was made. It is possible that approach to the food cup might be sufficient to activate at least some of the same brain regions in GTs as in STs. Indeed, some have argued that the food cup may also have incentive value (DiFeliceantonio and Berridge 2012; Mahler and Berridge 2009). For this reason, we decided to leave the food cup in the chamber on the test day to allow GTs to express their CR. In our analysis, we did not find any brain region where Fos expression was greater in GTs than in STs. However, we did find that in response to the food cue, Fos expression in the NAc shell and dorsomedial striatum was higher in GTs compared to the UP group. We think that this difference in Fos expression may be due to the psychological processes underlying goal-tracking behavior. We have previously speculated that a more cognitive reward expectation process

underlies goal-tracking, and that cognitive representation of the reward produces approach to the location of reward (food) delivery which may be more dependent on endogenous opioid signaling (Flagel et al. 2011a; Meyer et al. 2012a; Saunders and Robinson 2012; Wassum et al. 2009). Interestingly, Schiltz et al. (2007) reported that presentation of a food cue increased expression of enkephalin mRNA only in the striatum and DiFeliceantonio et al. (2012) recently found that enkephalin release within the dorsomedial striatum is involved in the motivation to eat. Therefore it is possible that enkephalin release within the striatum may be elicited by a cognitive expectation of the food reward, eliciting approach towards the goal in GTs. It is also necessary to point out that on each of the three days prior to the cue exposure test day, rats were placed into the chambers (with the food cup present) to minimize the influence of any contextual cues. These habituation sessions may have decreased the amount of goal-tracking observed on the test day, which may have led to less overall Fos expression in GTs.

It is important to note that we took precautions to minimize any behavioral differences on the test day that might confound the interpretation of our results by presenting the cues very briefly. However, we did see behavioral differences during the cue re-exposure test. We found that STs approached both the food and remifentanil cues while GTs approach the food cup and did not approach the remifentanil cue. To determine whether the differences we saw in Fos expression were due to differences in motor activity, we conducted correlational analyses between Fos expression and the probability of approaching either the food or remifentanil cue or approaching the food cup, and found no significant relationship in any brain region we examined. Additionally, given that both STs and GTs learn a conditioned orientation CR but UP animals do not, we expected to find at least one region where STs and GTs both differed from the UP group across both cues (food and remifentanil). In our current analysis, we did not find

any region that fulfilled this criterion. There are several candidate regions that may be involved in performance of an orientation CR, including the superior colliculus and the medial prefrontal cortex (Hasselmo and Sarter 2010; Overton and Dean 1988; Parikh et al. 2007) as these regions have been implicated in attention and disengaging animals from ongoing behavior. However, it remains to be seen whether the degree to which these regions are engaged by food and drug cues are different between STs, GTs, and UP controls.

Conclusions

In conclusion, we report that the propensity of an individual to attribute incentive salience to a food cue predicts the extent to which a classically conditioned opioid cue becomes attractive and desired. The results reported here extend our previous behavioral studies with STs and GTs and suggest that the ability of drug cues to acquire control over motivated behavior is similar across various classes of drugs (i.e., psychostimulants and opiates). We have only begun to understand the neural mechanisms underlying these behavioral differences. Here, we expand on our previous work with food cues and show that the ability of an opioid cue to motivate approach behavior is also dependent on intact dopamine transmission within the NAc core and that in order for either a food or an opioid cue to engage mesocorticolimbic and cortico-striatal-thalamic systems they must be imbued with incentive salience. These data suggest that there are specific neural patterns of activity associated with attribution of incentive salience to reward cues. Further work will need to be done to determine the functional role of regions outside the NAc core underlying individual variation in the attribution of incentive salience to reward cues. Our data support the notion that different classes of reward cues engage overlapping neural circuitry, and this will be an important consideration for developing pharmacological and cognitive-

behavioral treatments for a variety of impulse control disorders such as addiction, obesity, and gambling.

Figure 3.1. Individual variation in the development of behavior directed towards the lever-CS (sign-tracking) vs. behavior directed towards the food cup during the CS presentation (goal-tracking). Data are presented as means ±SEM. Panels on the left show the three measures of behavior directed towards the lever (sign-tracking behavior). (a) Probability of approaching the lever-CS during the 8 s CS period. (b) Number of lever contacts. (c) Latency to the first lever contact after the CS presentation. Panels on the right show the three measures of behavior directed towards the food cup (goal-tracking behavior). (d) Probability of approaching the food cup during the 8 s CS period. (e) Number of food cup entries during the 8 s CS period. (f) Latency to the first food cup entry after the CS presentation. For all measures there was a significant effect of group (ST or GT), session, and a group x session interaction (*p's*< 0.001).

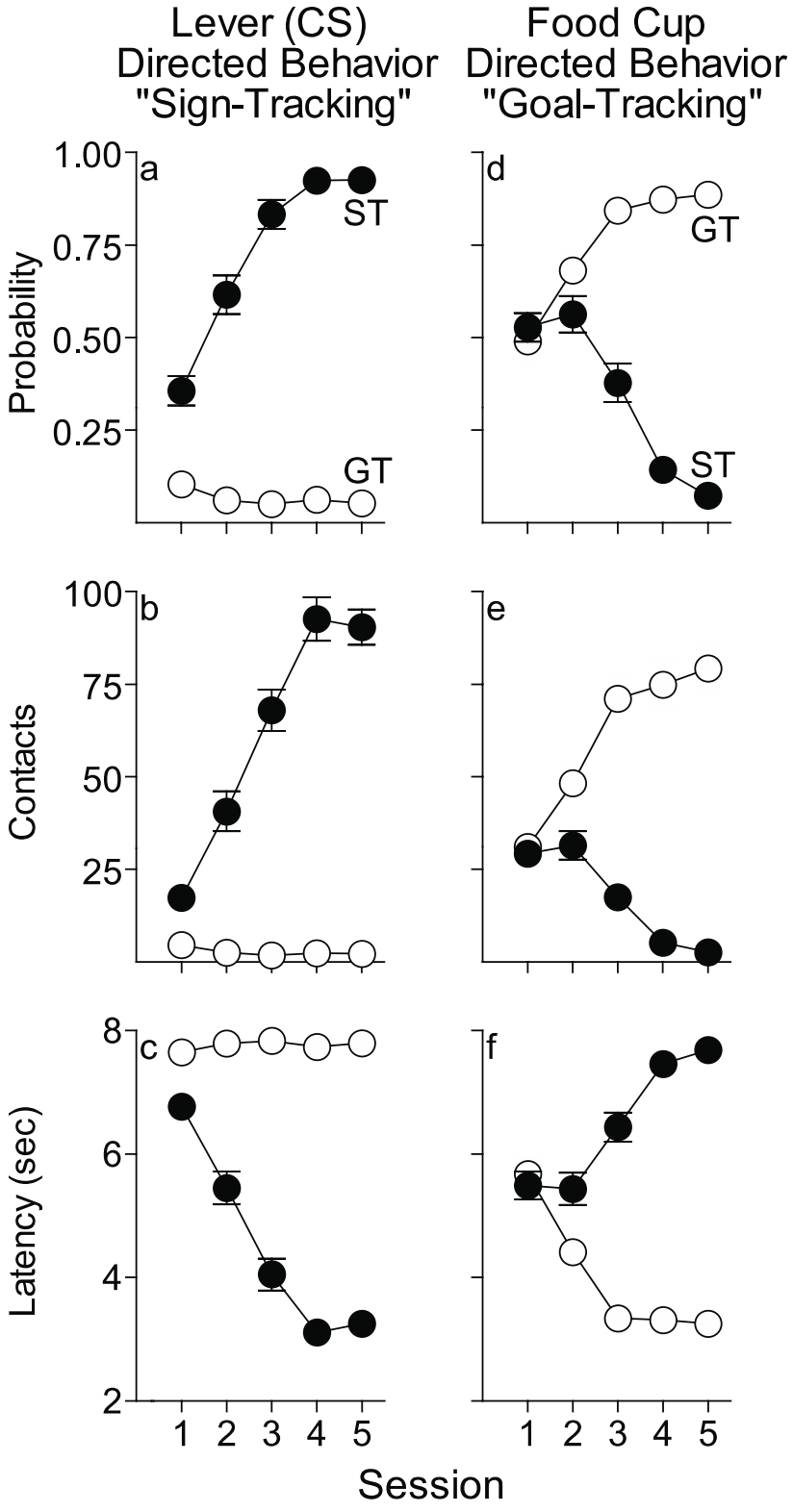
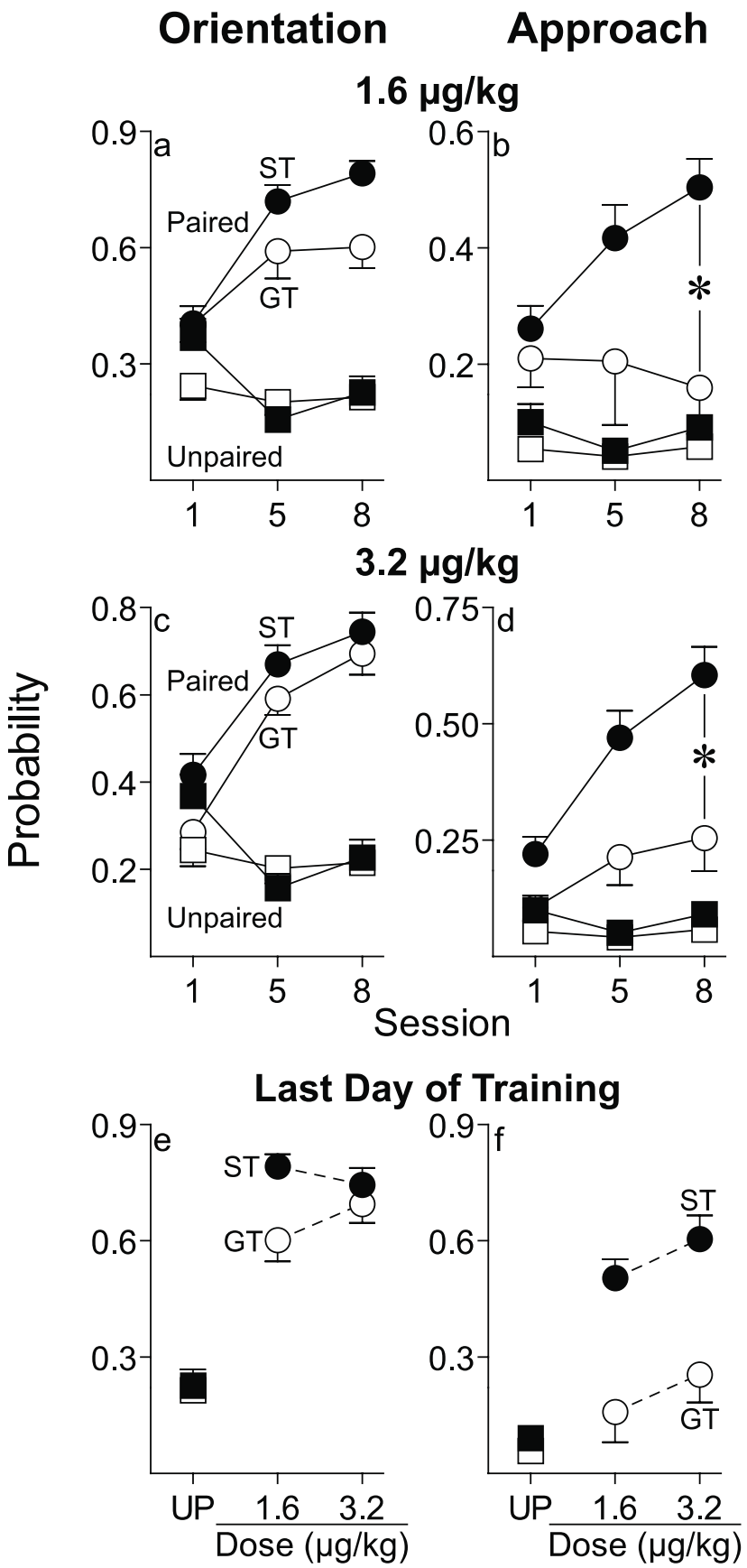

Lever (CS)
Directed Behavior
"Sign-Tracking"
Food Cup
Directed Behavior
"Goal-Tracking"
a
d
b
e
c
f
ST
GT
GT
ST
Probability
Contacts
Latency (sec)
1.00
0.75
0.50
0.25
100
75
50
25
8
6
4
2
1 2 3 4 5
1 2 3 4 5
Session

Figure 3.2. CS-directed orientation and approach to a cue associated with a non-contingent intravenous injection of remifentanil. All Unpaired rats were trained with 3.2 µg/kg remifentanil (STs *n*= 10, GTs *n*= 11). Data represent means ± SEM. Probability of orientation (a) and approach (b) to the remifentanil cue in rats that received 1.6 µg/kg remifentanil as the US (Paired STs *n*= 11, GTs *n*= 8). Probability of orientation (c) and approach (d) to the remifentanil cue in rats that received 3.2 µg/kg remifentanil as the US (Paired STs *n*= 12, GTs *n*=10). Dose-response functions for the probability of conditioned orientation (e) and approach (f) on the final day of training where each data point represents an independent group of rats. UP= unpaired. *, indicates a significant group difference between Paired STs and GTs, $p < 0.05$.


Orientation
Approach
1.6 µg/kg
3.2 µg/kg
Last Day of Training
a
b
c
d
e
f
ST
GT
Paired
Unpaired
Probability
Session
Dose (µg/kg)
UP
1.6
3.2
1
5
8
*

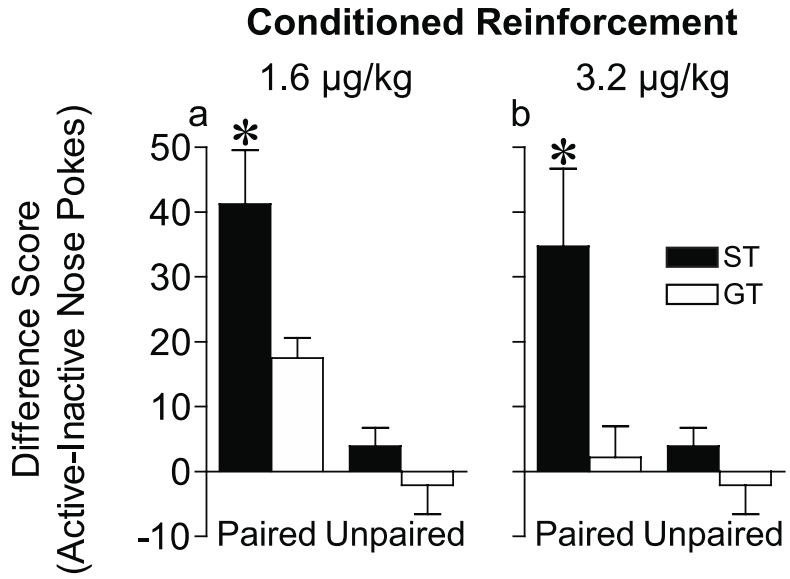


Figure 3.3. Performance during the conditioned reinforcement test. In this test, a nose poke into one port (Active) resulted in 2 s presentation of the cue either previously paired or unpaired with non-contingent remifentanil delivery. Nose pokes into the other port (Inactive) had no consequence. All unpaired rats were trained with 3.2 µg/kg remifentanil (STs *n*= 10, GTs *n*= 11). Data represent the means ± SEM difference in nose pokes into the Active minus Inactive port for rats that were trained with (a) 1.6 µg/kg remifentanil (Paired STs *n*= 11, GTs *n*= 8) or (b) 3.2 µg/kg remifentanil (Paired STs *n*= 12, GTs *n*=10). *, indicates a significant group difference.

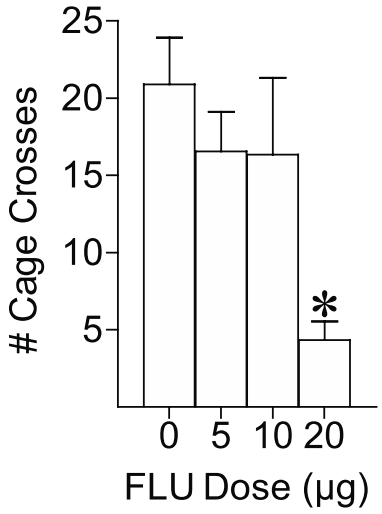


Figure 3.4. Effect of flupenthixol (FLU) on locomotor behavior in STs (*n*= 9). *, indicates a significant difference relative to 0 µg dose.

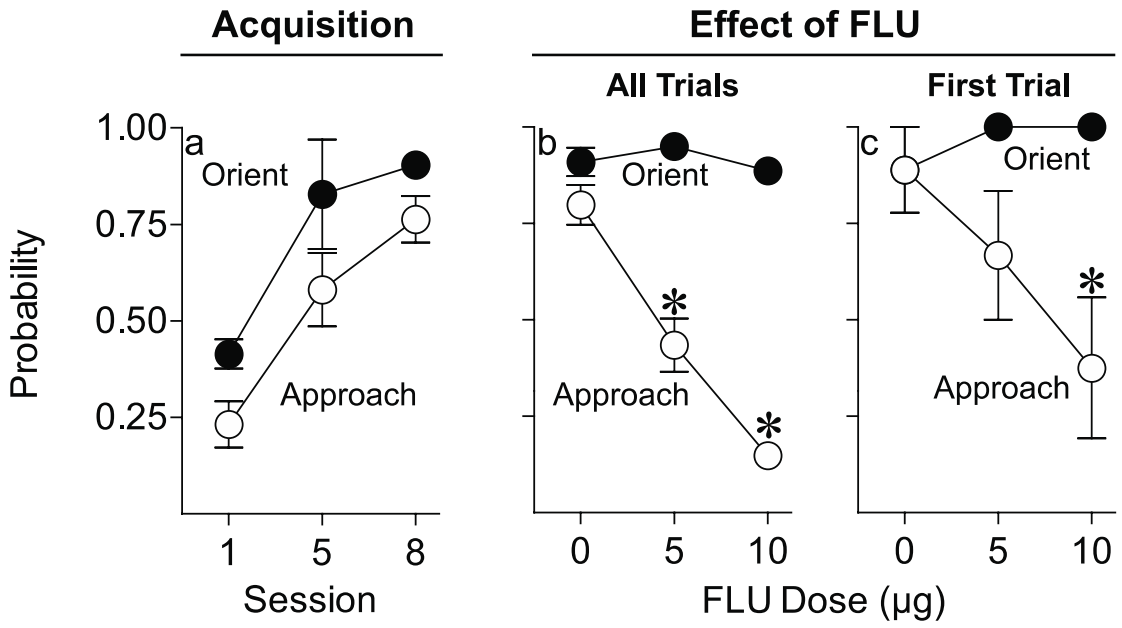


Figure 3.5. Effect of flupenthixol (FLU) in STs (*n*= 9) on performance of conditioned orientation and approach to a remifentanil cue. Data are presented as the mean ± SEM. (a) Acquisition of CS-directed orientation and approach to a cue associated with a non-contingent intravenous injection of 3.2 µg/kg remifentanil in rats that were classified as STs. (b) Probability of approaching the remifentanil cue. (c) Probability of approaching the remifentanil cue on the very first trial. *, indicates significant difference relative to vehicle. *p's*< 0.05.

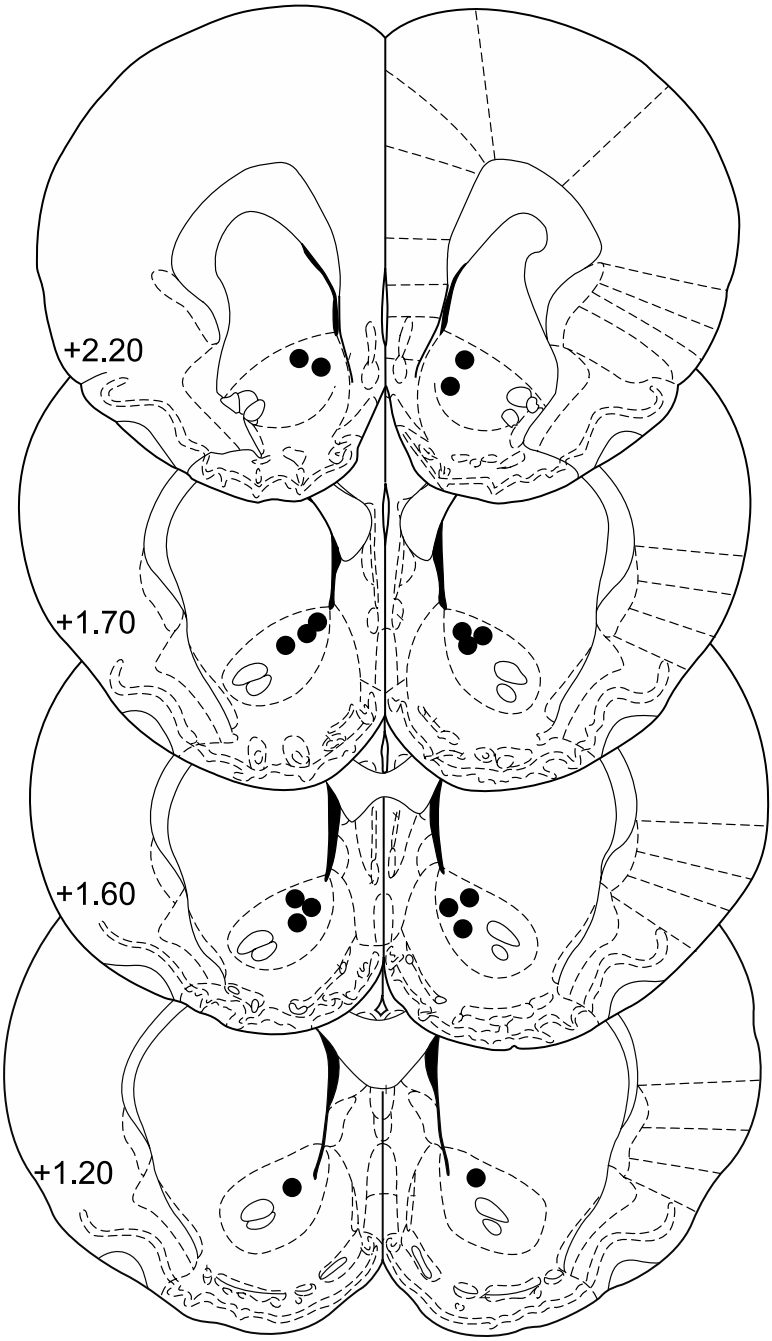


Figure 3.6. Location of microinjection tips within the NAc core relative to bregma for STs (*n*= 9) used in experiment 3.

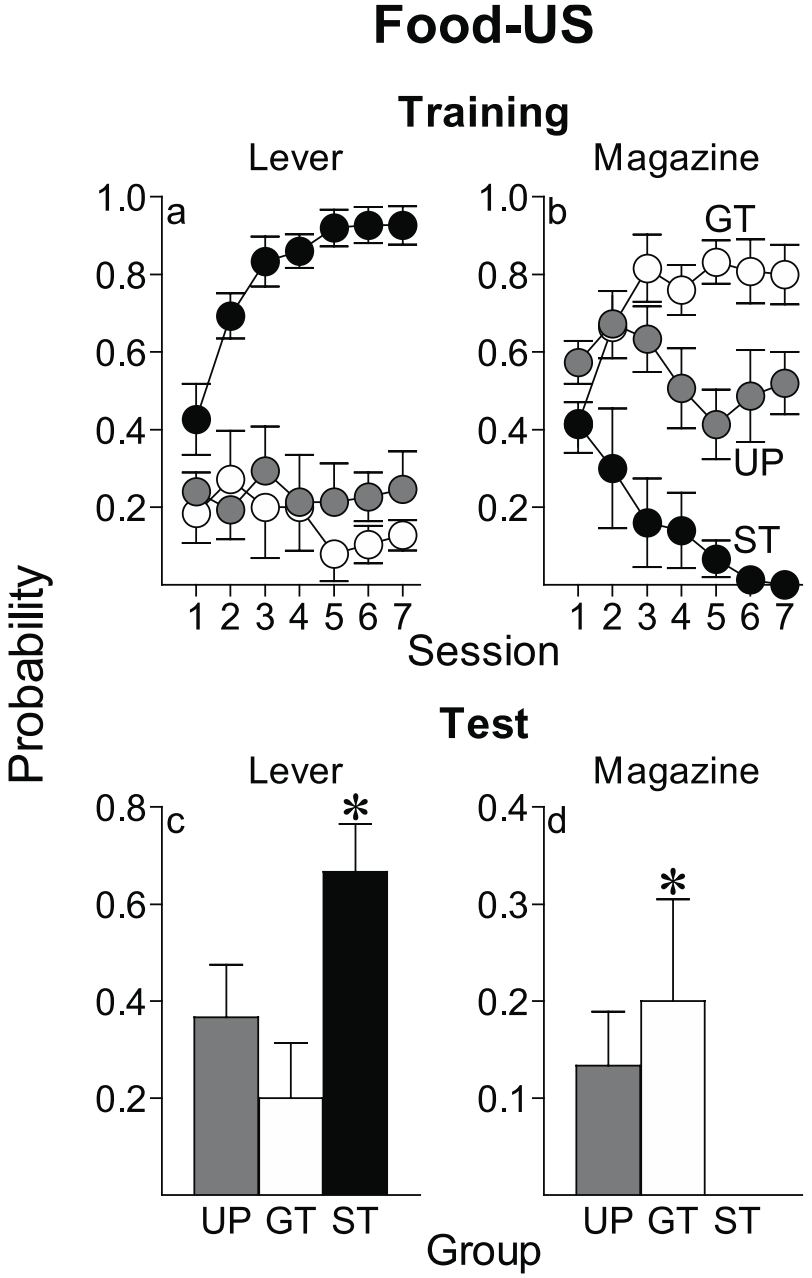


Figure 3.7. Behavior directed towards the lever-CS vs. the location of food delivery (food magazine) in rats that were classified as STs (*n*= 6) or GTs (*n*= 5) or that received unpaired (*n*= 6) cue-food presentations. Data represent the means ± SEM. (a) The probability of approaching the food cue (lever-CS) across training sessions. (b) The probability of approaching the food magazine during the 8 s CS period. On test day, rats were presented with the food cue (lever-CS) 10 times under extinction conditions (i.e., no food was delivered). (c) The probability of approaching the food cue on test day. (d) The probability of approaching the food magazine during the CS presentation on test day. UP= unpaired. *, indicates a significant difference between STs and GTs. *p's*< 0.05.

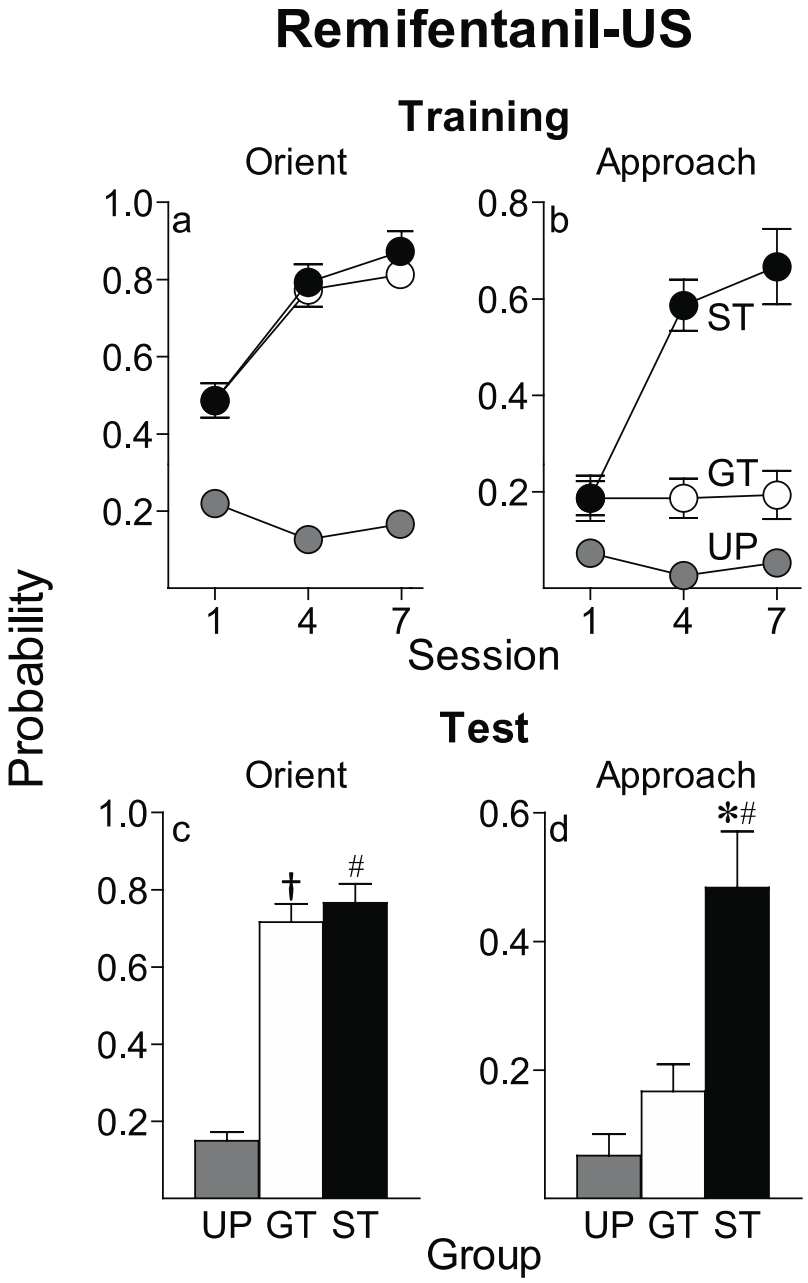


Figure 3.8. CS-directed orientation and approach to a cue associated with a non-contingent intravenous injection of 3.2 µg/kg remifentanil in rats that received paired (STs *n*= 6, GTs *n*= 6) or unpaired (*n*= 6) cue-drug presentations. Data represent the means ± SEM. (a) The probability of orientation to the remifentanil cue across training sessions. (b) The probability to approach the remifentanil cue across training sessions. On test day, rats were presented with the remifentanil cue (light-CS) 10 times under extinction conditions (i.e., no remifentanil was delivered during this test). (c) The probability of orientation to the remifentanil cue on test day. (d) The probability to approach the remifentanil cue on test day. UP= unpaired. *, indicates a significant difference between STs and GTs. #, indicates a significant difference between STs and UP rats. †, indicates a significant difference between GTs and UP rats. *p's*< 0.05.

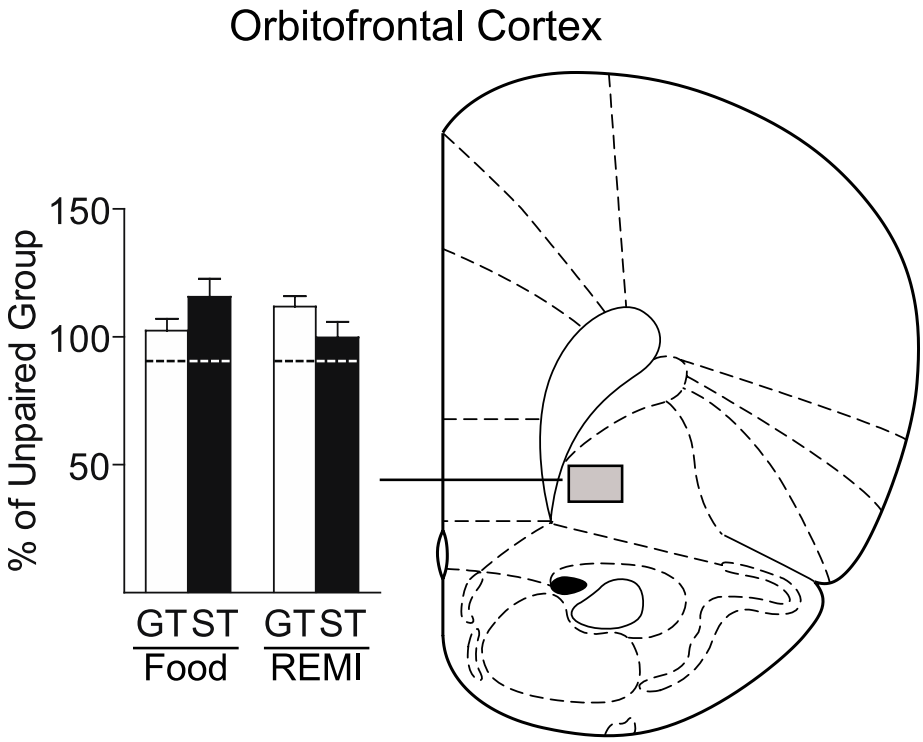
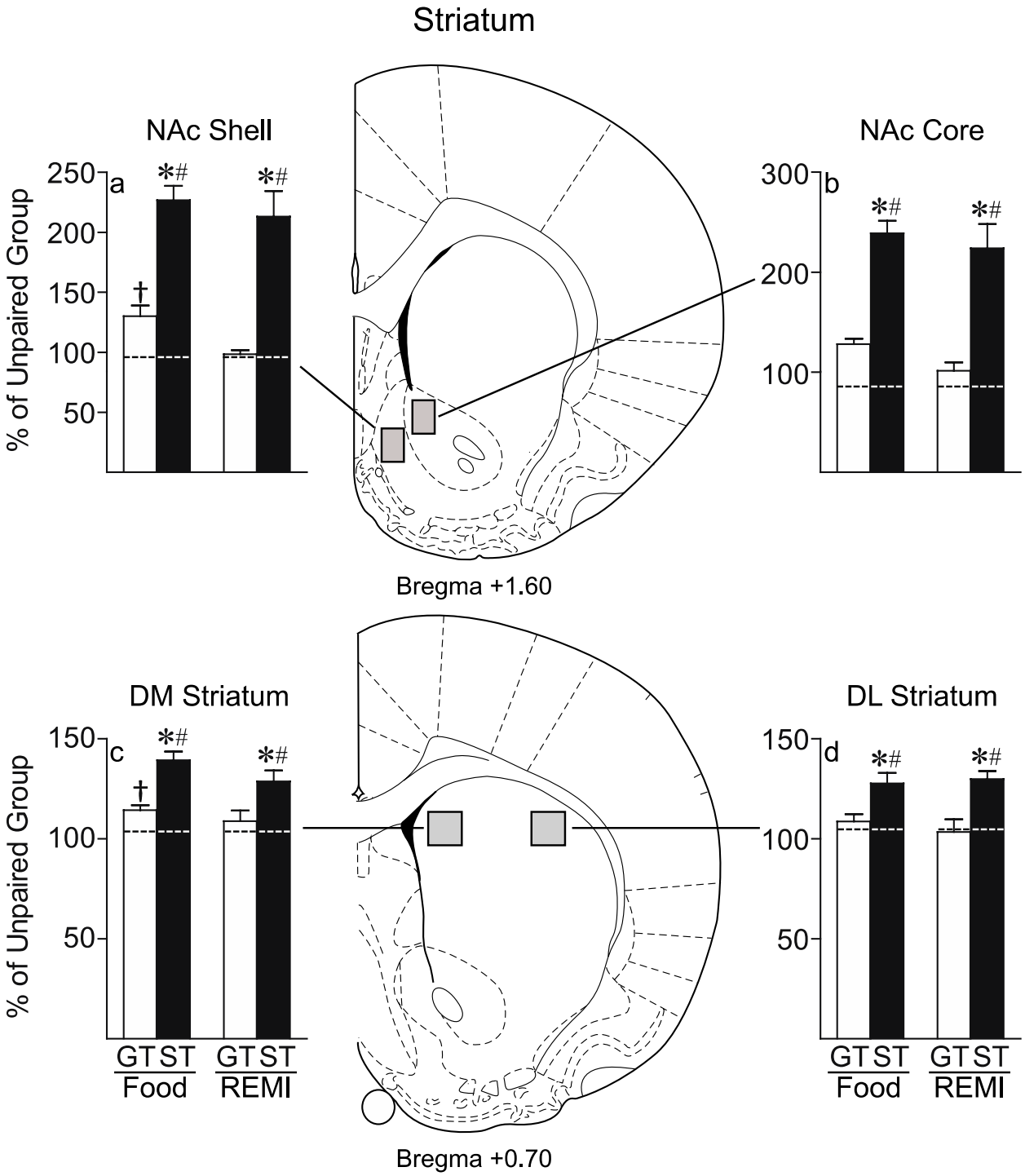


Figure 3.9. Mean ± SEM percent of Fos cells relative to the unpaired (UP) groups in the orbitofrontal cortex of rats presented with either the food cue (STs *n*= 6, GTs *n*= 5) or the REMI cue (STs *n*= 6, GTs *n*= 6) on test day. Representative atlas image from A-P level +3.2 mm anterior to bregma (Paxinos and Watson 1998). There were no significant group differences in Fos expression in the orbitofrontal cortex. Dashed lines indicate the percent of Fos cells in transport control rats relative to unpaired rats.

Figure 3.10. Mean ± SEM percent of Fos cells relative to the unpaired (UP) groups in the striatum of rats presented with either the food cue (STs *n*= 6, GTs *n*= 5) or the REMI cue (STs *n*= 6, GTs *n*= 6) on test day. Representative atlas images from A-P levels +1.6 and +0.70 mm anterior to bregma (Paxinos and Watson 1998). Abbreviations: NAc, nucleus accumbens; DM, dorsomedial; DL, dorsolateral. (a) Presentation of both the food and REMI cue elicited greater Fos expression in the NAc shell of STs relative to GTs and UP rats. Post hoc comparisons also revealed that presentation of the food cue elicited greater Fos expression in GTs relative to UP rats. (b) STs showed greater Fos expression in the NAc core relative to both GTs and UP rats, which did not differ from one another, after presentation of either the food and REMI cue. (c) Presentation of both the food and REMI cue elicited greater Fos expression in the DM striatum of STs relative to GTs and UP rats. Post hoc comparisons also revealed that presentation of the food cue elicited greater Fos expression in GTs relative to UP rats. (d) STs showed greater Fos expression in the DL striatum relative to both GTs and UP rats, which did not differ from one another, after presentation of either the food and REMI cue. Dashed lines indicate the percent of Fos cells in transport control rats relative to unpaired rats. *, indicates a significant difference between STs and GTs. #, indicates a significant difference between STs and UP rats. †, indicates a significant difference between GTs and UP rats. *p's*< 0.05.


Striatum
NAc Shell
NAc Core
% of Unpaired Group
250
200
150
100
50
a
b
300
200
100
*#
†
Bregma +1.60
DM Striatum
DL Striatum
150
100
50
c
d
GT ST
Food
GT ST
REMI
Bregma +0.70

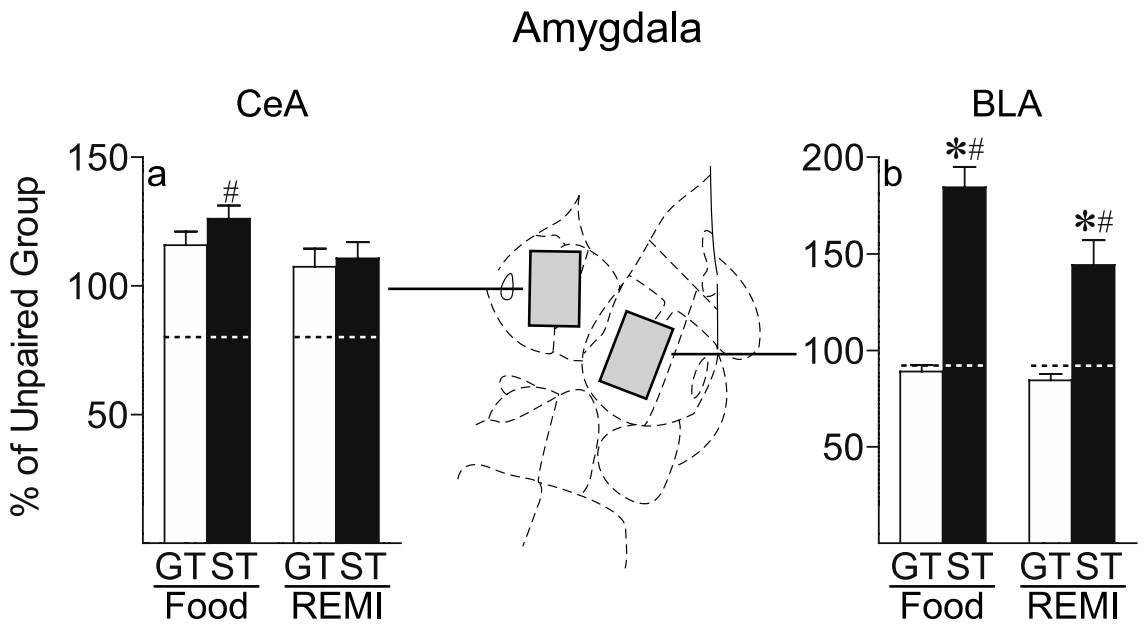


Figure 3.11. Mean ± SEM percent of Fos cells relative to the unpaired (UP) groups in the amygdala for rats presented with the food cue (STs *n*= 6, GTs *n*= 5) or the REMI cue (STs *n*= 6, GTs *n*= 6) on test day. Representative atlas image from A-P level -2.56 mm posterior to bregma (Paxinos and Watson 1998). Abbreviations: CeA, central nucleus of the amygdala; BLA, basolateral nucleus of the amygdala. (a) Presentation of the food cue elicited greater Fos expression in the CeA of STs relative to UP rats. There were no significant group differences in Fos expression in the CeA of rats presented with the REMI cue. (b) STs showed greater Fos expression in the BLA relative to both GTs and UP rats, which did not differ from one another, after presentation of either the food and REMI cue. Dashed lines indicate the percent of Fos cells in transport control rats relative to unpaired rats. *, indicates a significant difference between STs and GTs. #, indicates a significant difference between STs and UP rats. *p's*< 0.05.

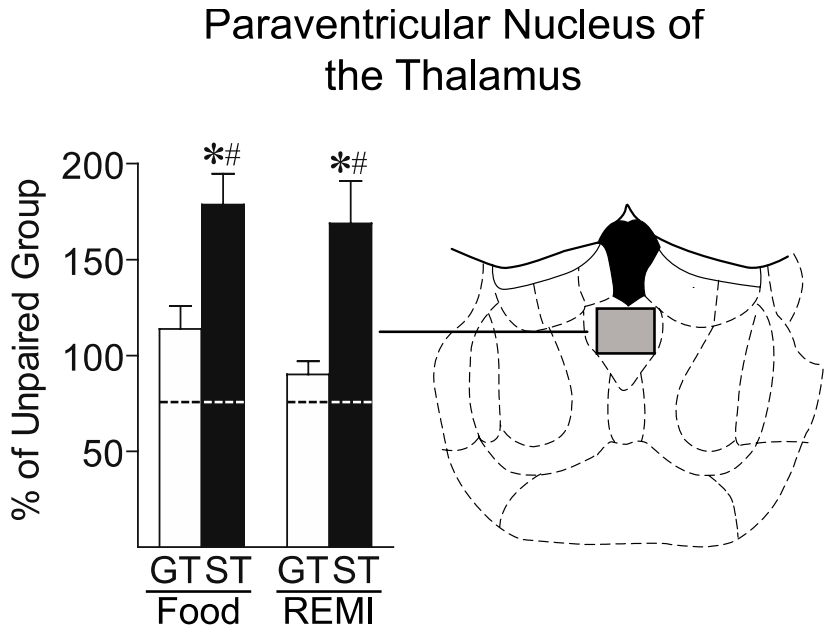


Figure 3.12. Mean ± SEM percent of Fos cells relative to the unpaired (UP) groups in the paraventricular nucleus of the thalamus (PVT) of rats presented with the food cue (STs *n*= 6, GTs *n*= 5) or the REMI cue (STs *n*= 6, GTs *n*= 6) on test day. Representative atlas image from A-P level -3.3 mm posterior to bregma (Paxinos and Watson 1998). Presentation of both the food and REMI cue elicited greater Fos expression in the PVT of STs relative to both GTs and UP rats, which did not differ from one another. Dashed lines indicate the percent of Fos cells in transport control rats relative to unpaired rats. *, indicates a significant difference between STs and GTs. #, indicates a significant difference between STs and UP rats. *p's*< 0.05.

Table 3.1. Mean ± SEM number of Fos-positive nuclei for each brain region examined in experiment 4.

		Brain Region[b]							
Cue	**Group[a] (*n*)**	**OFC**	**NAc Shell**	**NAc Core**	**DM Striatum**	**DL Striatum**	**CeA**	**BLA**	**PVT**
Food	ST (6)	728.0 ± 43.7	278.8 ± 14.9	257.3 ± 13.6	947.8 ± 30.0	961.3 ± 40.1	184.1 ± 7.5	164.3 ± 9.3	425.7 ± 38.1
	GT (5)	644.2 ± 29.3	160.3 ± 10.7	137.9 ± 6.0	778.2 ± 17.2	818.2 ± 26.9	169.1 ± 7.7	79.2 ± 2.9	271.2 ± 28.7
	UP (6)	629.0 ± 20.2	123.0 ± 6.7	107.7 ± 13.7	680.3 ± 35.7	752.5 ± 25.2	146.0 ± 8.6	89.0 ± 8.0	238.2 ±10.7
REMI	ST (6)	611.6 ± 37.3	271.1 ± 27.2	261.1 ± 28.4	851.5 ± 36.1	950.8 ± 28.9	155.2 ± 8.9	121.7 ± 10.8	437.8 ± 57.5
	GT (6)	684.8 ± 25.7	125.3 ± 4.0	118.4 ± 9.8	720.9 ± 34.7	757.5 ± 46.2	150.7 ± 9.8	71.2 ± 2.9	233.7 ± 17.8
	UP (6)	612.6 ± 26.5	127.2 ± 6.5	116.6 ± 13.7	661.3 ± 28.3	732 ± 25.8	140.2 ± 11.8	84.3 ± 9.8	259.3 ± 23.5
N/A	Transport	535.8 ± 13.8	119.3 ± 20.8	90.6 ± 6.4	690.8 ± 25.6	767.1 ± 22.2	111.3 ± 11.4	80.0 ± 3.8	182.5 ± 22.7

[a]Unpaired (UP) training
[b]Abbreviations are described in the Methods section

Chapter 4

A nicotine cue is equally attractive to sign-trackers and goal-trackers but differs in its conditioned reinforcing properties

Introduction

Cues in the environment that are associated with rewards can acquire motivational value (become incentive stimuli) and thus gain the ability to exert strong control over behavior. However, in a series of recent studies we have found that there is considerable individual variation in the extent to which reward cues are attributed with incentive salience (Fitzpatrick et al. 2013; Flagel et al. 2007; Meyer et al. 2012a; Robinson and Flagel 2009; Yager and Robinson 2010). When a discrete localizable cue (the conditioned stimulus, CS) is repeatedly paired with delivery of a food reward (the unconditioned stimulus, US), in some rats ('sign-trackers', STs; Hearst and Jenkins 1974), the food cue itself becomes attractive eliciting approach and engagement with it, and desired, in that rats will work to obtain it. For other rats ('goal-trackers', GTs; Boakes 1977), the food cue itself is less attractive, presentation of the cue elicits an initial glance followed by approach to the location of food delivery, and is less desired. Thus, while the cue is equally predictive in STs and GTs, as it reliably evokes a conditioned response in both, it becomes a more attractive and "wanted" incentive stimulus in STs than GTs (for review see Robinson et al. 2014; Saunders and Robinson 2013).

Not only can cues associated with natural rewards acquire incentive motivational properties, so can drug-associated cues. Indeed, the ability of cues to motivate behavior is

especially problematic in the context of addiction because drug-associated cues can goad continued drug-seeking behavior and relapse in addicts (Caggiula et al. 2001; de Wit and Stewart 1981; Milton and Everitt 2010; Robinson and Berridge 1993; Stewart et al. 1984). Thus, we have begun to investigate whether individual variation in the propensity to attribute incentive salience to a food cue predicts the extent to which drug cues can also motivate behavior. We have recently shown, in two independent studies, that a cocaine-associated cue is more attractive, eliciting more approach behavior, in STs than in GTs (Flagel et al. 2010; Yager and Robinson 2013). Importantly, we also established that even though GTs did not readily approach the cocaine cue they learned the CS-US association as shown by acquisition of a different conditioned response: conditioned orientation. We have also reported that a discrete cocaine cue produces greater reinstatement of drug-seeking behavior in STs than in GTs (Saunders and Robinson 2010; Saunders et al. 2013; Yager and Robinson 2013). Furthermore, this was true whether the cocaine cue acquired its motivational properties in an instrumental (i.e., traditional self-administration paradigm) setting, or using classical Pavlovian conditioning procedures. Thus, a cocaine-associated cue is both more attractive and desired in STs than GTs.

Although there is now considerable evidence that there is individual variation in the extent to which a drug associated cue can motivate behavior, all of these studies have been conducted using a cue associated with cocaine (Flagel et al. 2010; Meyer et al. 2012b; Saunders and Robinson 2010; Saunders et al. 2013; Yager and Robinson 2013) and it remains to be seen whether this effect generalizes across drug classes. This is an important question as there are several other classes of drugs that are readily abused by humans and which animals will also self-administer. Furthermore, work from several labs has shown that cues associated with both unsweetened alcohol and heroin can elicit approach behavior (Krank et al. 2008; Madsen and

Ahmed 2014; Peters and De Vries 2013; Tomie 2001). The purpose of the experiments reported here, therefore, was to extend these findings to another class of highly abused drugs, nicotine. We asked, 1) whether rats will approach and work for presentation of a cue associated with nicotine, and 2) whether this varies with the degree to which individuals attribute incentive salience to a food cue (i.e., in STs vs. GTs)?

Materials and Methods

Subjects

Male Sprague-Dawley rats (initial N= 200; Harlan, Haslett, Michigan) weighing 250-275g upon arrival were individually housed in a climate-controlled colony room on a 12-hr light/12-hr dark cycle (lights on at 0800 hr). All testing occurred during the light phase of the cycle. Food and water were available *ad libitum* (i.e., rats were not food restricted at any time). Rats were given one week to acclimate to the colony room before testing began, during which time the experimenter handled them several times. The University of Michigan Committee on the Use and Care of Animals approved all procedures.

Pavlovian training using food as the US

Apparatus. Behavioral testing was conducted in sixteen standard (22 x 18 x 13 cm) test chambers (Med Associates Inc., St. Albans, VT, USA) located in sound attenuating cabinets equipped with a ventilating fan to mask background noise. Each chamber was equipped with an illuminated retractable lever located 6 cm above a stainless steel grid floor either to the left or right of a centrally located food magazine, placed 3 cm above the floor. A red houselight was located on the wall opposite of the food cup and remained illuminated throughout the testing session.

Pavlovian training procedures. Rats were first trained using a Pavlovian conditioned approach (PCA) procedure as described previously (Flagel et al. 2007; Yager and Robinson 2013). For two

days prior to the start of training, 25 banana-flavored pellets (45 mg; BioServe) were placed into the home cage to familiarize the rats with this food. Approximately one week after arrival, rats underwent one magazine training session during which the lever remained retracted and 25 pellets were delivered into the food magazine according to a variable time (VT) 30 s (0-60 s) schedule. Subsequently, rats underwent 5 days of Pavlovian conditioning (one session/day). Each session consisted of 25 trials during which an illuminated lever (lever-CS) was inserted into the chamber for 8 s, after which a single 45-mg banana-flavored pellet (the US) was delivered into the food magazine. CS-US pairings occurred on a VT 90 s (30-150 s) schedule. Importantly, no instrumental response was required by the rat to initiate delivery of the food pellet. Lever deflections, magazine entries, latency to the first lever deflection, and latency to the first magazine entry during each CS presentation were recorded using Med Associates software.

Quantification of behavior using an index of Pavlovian conditioned approach (PCA). Following completion of Pavlovian training, animals were classed into three groups: (1) Those that preferentially interacted with the lever-CS ('sign-trackers', STs), (2) those that preferentially interacted with the food magazine during the lever-CS presentation ('goal-trackers', GTs), and (3) those that had no strong preference for the lever-CS or food magazine ('intermediate group', IG). The extent to which behavior was directed towards the lever-CS or the food magazine was quantified using a composite Pavlovian conditioned approach (PCA) index, based on performance during days 4 and 5 of training, as described previously (Lomanowska et al. 2011; Meyer et al. 2012a). The PCA Index score incorporated three measures of conditioned approach behavior: (1) the probability of contacting either the lever-CS or food magazine during a trial [P(lever)-P(food magazine)]; (2) the response bias for contacting the lever-CS or food magazine during a trial [(#lever deflections - #food magazine entries)/ (#lever deflections + #food

magazine entries)]; and (3) the mean latency to contact the lever or enter the food magazine during a trial [(magazine contract latency - lever deflection latency)/8]. This produces values ranging from -1.0 to +1.0, where a score of +1 indicates an animal made a ST CR on every trial, a score of -1 that an animal made a GT CR on every trial, and a score of 0 that an animal distributed ST and GT responses equally. For purposes of classification, rats with scores of -1.0 to -0.3 were operationally defined as GTs and rats with scores of +0.3 to +1.0 were defined as STs. Rats that were within the range of -0.29 to +0.29, whose behavior vacillated between the lever-CS and food magazine, were classified as intermediates (IGs) and were not used further because we were interested in comparing rats that differed strongly in their propensity to attribute incentive salience to food cues (Meyer et al. 2012a).

Pavlovian approach using nicotine as the US

Surgery. Following Pavlovian training using food as the US, chronic indwelling catheters were implanted into the jugular vein of STs and GTs as described previously (Crombag et al. 2000). Catheter patency was tested before the first training session and again after the last training session by intravenous injection of 0.2 ml of methohexital sodium (10 mg/ml in sterile water; JHP Pharmaceuticals). Rats were removed from the analysis if they failed to become ataxic within 5 s of injection (STs n= 1, GTs n= 4).

Apparatus. Behavioral testing was conducted in chambers identical to those used to screen animals for ST and GT, except the food magazine and lever were removed from the chamber and two stimulus lights were placed on the left and right sides of the wall opposite the white houselight, 13.5 cm above the stainless steel grid floor. The side of the stimulus light designated to serve as a CS (i.e., to be paired with nicotine infusion) was counterbalanced between rats. A syringe pump, located outside the sound attenuating chamber and connected to rats' catheter

back ports, delivered nicotine infusions. The infusion tubing was suspended into the chamber via a swivel mechanism, which allowed rats' free movement in the chamber.

Pavlovian training procedures. Pavlovian training procedure were similar to those described previously (Yager and Robinson 2013). Prior to training rats were assigned to either the Paired (CS and US presented together) or Unpaired groups (US explicitly not paired with presentation of the CS). Before Pavlovian training began, rats were first habituated to the presentation of the stimulus light (light-CS) and infusion procedure to decrease otherwise high levels of responding to what were novel stimuli (Uslaner et al. 2006). The habituation session consisted of 25 trials (VT 90 s schedule) during which both stimulus lights were simultaneously illuminated for 10 s and coincided with activation of the infusion pump and an intravenous (IV) infusion of saline (50 µL delivered over 2.8 s). Starting the next day, rats underwent 15 days of Pavlovian conditioning using nicotine as the US. Each session consisted of 8 trials (CS-US presentations) occurring on a VT schedule with a mean of 900 s (840-960 s). This long inter-trial interval (ITI) was chosen since nicotine, as opposed to a food pellet, has relatively long-lasting neurobiological and interoceptive effects. Thus, in order to have more discrete CS-US pairings, a long ITI is necessary (see Uslaner et al. 2006 for discussion). For rats in the Paired groups, each light-CS presentation was paired with an intravenous infusion of 7.5, 15, or 25 µg/kg of a nicotine solution (bitartrate salt, calculated on the weight of the base and dissolved in 0.9% saline, pH adjusted to 7.2-7.4, delivered in 50 µl over 2.8 s). Independent groups of rats were used for each dose of nicotine tested. Each trial consisted of illumination of the CS for 10 s and nicotine delivery coincided with the onset of the CS. No action was required by the rat to initiate either illumination of the light or the nicotine injection. These testing parameters were selected as we have previously shown, using identical methods, that when cocaine is used as the US rats will

approach the CS (Flagel et al. 2010; Uslaner et al. 2006; Yager and Robinson 2013). Rats in the Unpaired group received non-contingent infusions of 25 µg/kg nicotine that were explicitly not paired with illumination of the CS (nicotine was administered on a VT schedule with a mean of 180 s after the CS was extinguished). We opted to only use the highest dose of nicotine for the Unpaired animals as this was the dose that produced the most behavior in the Paired rats.

Video analysis. Video was scored offline by an observer blind to the experimental condition for two different conditioned responses (CRs) as described previously (Yager and Robinson 2013). (1) *Conditioned orientation*: an orientation response was scored if the rat made a head and/or body movement in the direction of the CS during the CS period, regardless of whether the rat approached the CS. (2) *Conditioned approach*: an approach response was scored if during the CS period a rat moved towards the CS, bringing its nose to within 1cm of the light. Due to the location of the cue light within the chamber, a rat had to rear, lifting both paws off the floor, in order to bring its nose within 1 cm of the light. It is important to note that if an approach response was scored on a given trial an orientation response would also be scored, as orientation always preceded approach. However, an orientation response could occur in the absence of an approach response.

Test for conditioned reinforcement

One week following the last training session with nicotine as the US all rats underwent a single 40 min test for conditioned reinforcement. During this test, the cue light was relocated to the middle of the front wall and was flanked by two nose-poke ports. Responses into one of the ports (Active) resulted in illumination of the nicotine cue (light-CS) for 2 s. Responses into the other port (Inactive) had no consequence. No nicotine was delivered during this test.

Statistical analysis

Linear mixed-models (LMM) analysis was used for all repeated measures data (Verbeke and Molenberghs 2000). The covariance structure was explored and modeled for each dependent variable. Analysis of variance was used to analyze dose-response data for conditioned orientation, conditioned approach, and to compare responding during conditioned reinforcement. When main effects were found post hoc comparisons were made using Fisher's LSD test. Statistical significance was set at $p< 0.05$.

Results

Individual variation in Pavlovian conditioned approach behavior to a food cue

As expected from previous studies (Flagel et al. 2007; Meyer et al. 2012a; Robinson and Flagel 2009; Saunders and Robinson 2012), two distinct phenotypes emerged as a result of Pavlovian training using food as the US. Figure 4.1 shows the performance of rats classified as STs or GTs based on the PCA index as described in the "Methods" section. Across training, STs came to reliably and rapidly approach the lever-CS (Fig. 4.1a, c) and they vigorously engaged it (Fig. 4.1b). In contrast, GTs rarely approached the lever-CS, but upon its presentation they instead reliably and rapidly approached the food cup (Fig. 4.1d, f), which they vigorously engaged (Fig. 4.1e).

A nicotine cue is equally attractive to STs and GTs

When a drug is used as the US, rats rarely physically engage the CS. Instead, a sign-tracking CR consists of approach to the vicinity of the CS, and sniffing and investigation of it (Flagel et al. 2010; Uslaner et al. 2006; Yager and Robinson 2013). Thus, when using nicotine as the US, we scored a CS-directed approach response (a ST CR) if a rat brought its nose to within 1 cm of the light-CS during the CS period, which required it to rear. In contrast, conditioned orientation was

defined as a head and/or body movement in the direction of the light-CS upon CS presentation, regardless of whether an animal approached it.

Conditioned orientation (7.5 µg/kg). Figure 4.2a illustrates the probability of conditioned orientation across training sessions when using 7.5 µg/kg nicotine as the US. With this dose neither Paired STs nor GTs acquired a conditioned orientation response as the probability of making an orientation CR did not increase across sessions [$F(2, 40.94)= 2.5$, $p= 0.09$]. However, both STs and GTs oriented significantly more relative to their respective Unpaired control groups [effect of pairing; STs: $F(1, 50.86)= 45.75$, $p< 0.001$; GTs: $F(1, 51.73)= 20.78$, $p< 0.001$].

Conditioned approach (7.5 µg/kg). Figure 4.3a shows that when using 7.5 µg/kg nicotine as the US neither Paired STs nor GTs acquired a conditioned approach response, as indicated by a non-significant effect of session [$F(2, 51.83)= 1.6$, $p=$ n.s.]. This result is consistent with the fact that neither STs nor GTs acquired a conditioned orientation response, as an orientation must precede an approach response. Furthermore, approach behavior did not differ between Paired and Unpaired groups.

Conditioned orientation (15 µg/kg). Figure 4.2b illustrates that when using 15 µg/kg nicotine as the US both Paired STs and GTs acquired a conditioned orientation response, as indicated by a significant increase in the probability of orientation behavior across sessions [$F(2, 27)= 14.76$, $p< 0.001$], and the two groups did not differ. In addition, both STs and GTs showed a significant increase in probability of orienting to the nicotine cue across sessions, relative to their respective Unpaired control groups [pairing x session interaction; STs: $F(2, 20)= 2.67$, $p= 0.03$; GTs: $F(2, 21)= 7.56$, $p= 0.003$].

Conditioned approach (15 µg/kg). Fig. 4.3b shows that both Paired STs and GTs acquired a conditioned approach response across sessions when using 15 µg/kg nicotine as the US [$F(2, 50.65)= 6.04$, $p= 0.004$], and the two groups did not differ. Furthermore, both STs and GTs approached the nicotine cue more than their respective Unpaired control groups [effect of pairing; STs: $F(1, 44.45)= 4.77$, $p= 0.03$; GTs: $F(1, 23.39)= 7.44$, $p= 0.01$].

Conditioned orientation (25 µg/kg). Figure 4.2c shows that when using 25 µg/kg nicotine as the US both Paired STs and GTs acquired a conditioned orientation response, as indicated by a significant increase in the probability of orienting behavior across sessions [$F(2, 64.54)= 42.39$, $p< 0.001$], and the two groups did not differ. In addition, both STs and GTs showed a significant increase in conditioned orientation to the nicotine cue across sessions, relative to their respective Unpaired control groups [pairing x session interaction; STs: $F(2, 48.75)= 14.9$, $p< 0.001$; GTs: $F(2, 46.65)= 9.17$, $p< 0.001$].

Conditioned approach (25 µg/kg). Figure 4.3c illustrates the probability of conditioned approach across training sessions when using 25 µg/kg nicotine as the US. Fig. 4.3c shows that both STs and GTs acquired a conditioned approach response [effect of session, $F(2, 59.95)= 15.81$, $p< 0.001$] and the two groups did not differ in this response. In addition, both STs and GTs approached the nicotine cue more than their respective Unpaired control groups [effect of pairing; STs: $F(1, 21.31)= 8.1$, $p= 0.01$; GTs: $F(1, 25.62)= 7.2$; $p= 0.01$]. Importantly, neither STs nor GTs in the Unpaired groups developed an orienting or an approach CR.

Latency to approach (25 µg/kg). When using 25 µg/kg nicotine as the US, we saw the most consistent change in approach behavior across sessions. Thus, we also analyzed the latency to approach the nicotine cue. As can be seen in Figure 4.4, the latency to approach the nicotine cue decreased across sessions [$F(2, 31.03)= 13.95$, $p< 0.001$], and this did not differ between groups.

Dose-response analysis. Figures 4.2d and 4.3d summarize the dose-response functions for the probability of conditioned orientation and conditioned approach on the final day of training. For conditioned orientation a two-way analysis of variance (ANOVA) revealed that there were no differences between STs and GTs, and the probability of this CR increased as a function of dose in both groups [$F(2, 78)= 16.49$, $p< 0.001$]. Similarly, as shown in Fig. 4.3d, the nicotine cue elicited similar approach behavior in STs and GTs and the probability of an approach CR increased as a function of dose in both groups [$F(2, 78)= 13.62$, $p< 0.001$]. We separately analyzed conditioned approach dose-response data for STs and GTs and included Unpaired control animals in this analysis. A one-way ANOVA showed a significant effect of treatment group for both STs and GTs [STs, $F(3, 45)= 6.15$, $p= 0.001$; GTs, $F(3, 47)= 6$, $p= 0.002$]. However, post hoc analysis (Fisher's LSD) revealed that, on the final day of testing, Paired STs differed from Unpaired STs at both 15 and 25 µg/kg (p's < 0.05) but not at the lowest dose ($p= 0.87$). However, Paired GTs only differed from Unpaired GTs at the highest dose tested [7.5 µg/kg, $p= 0.4$; 15 µg/kg, $p= 0.15$; 25 µg/kg, $p= 0.01$].

A nicotine cue is a more effective conditioned reinforcer in STs than GTs

Following one week of forced abstinence, all rats underwent a single test for conditioned reinforcement. During this test, during which no nicotine was delivered, the nicotine cue (light-CS) was relocated to the middle of the wall and was flanked by two nose-poke ports. Responses into the Active port produced presentation of the light-CS previously either paired or unpaired with nicotine infusions while responses into the Inactive port had no consequence. Figure 4.5 shows the mean difference in nose pokes into the Active minus Inactive port during the conditioned reinforcement test. As can be seen in Figures 4.5a and 4.5b, when 7.5 or 15 µg/kg nicotine was used as the US during Pavlovian conditioning, Paired STs and GTs did not differ in

the extent to which they would work for the nicotine cue [group x port interaction, 7.5 µg/kg: $F(1, 22)= 1.09$, $p= 0.31$; 15 µg/kg: $F(1, 27)= 0.26$, $p= 0.62$]. However, when 25 µg/kg nicotine was used during training, STs responded more for presentation of the nicotine cue than GTs, as indicated by a significant group x port interaction [$F(1, 29)= 4.606$, $p= 0.04$]. For rats in the Unpaired condition, there were no significant differences between groups.

Figure 4.5d summarizes the dose-response function for the conditioned reinforcement test. We separately analyzed conditioned reinforcement dose-response data for STs and GTs. Across doses, for GTs, there were no significant differences between the number of active minus inactive nose pokes [$F(2, 42)= 1.11$, $p= 0.34$]. However, the degree to which STs worked for presentation of the nicotine cue varied as a function of dose [$F(2, 40)= 3.35$, $p= 0.046$]. Post-hoc analysis (Fisher's LSD) revealed that STs that were trained with 25 µg/kg nicotine significantly more nose pokes into the active than the inactive port than STs trained with 7.5 µg/kg ($p= 0.015$). STs that were trained with 15 µg/kg did not differ for STs trained with either 7.5 or 25 µg/kg (p's > 0.05).

Discussion

We previously reported that individual variation in the propensity to attribute incentive salience to a food cue predicts the extent to which a cocaine cue acquires incentive motivational properties (Flagel et al. 2010; Meyer et al. 2012b; Saunders and Robinson 2010; Saunders et al. 2013; Yager and Robinson 2013). Here, we asked whether there is similar individual variation in the extent to which a cue associated with intravenous nicotine delivery acquires motivational properties. First, we found that when associated with an intravenous injection of nicotine, a light cue became attractive, eliciting both orientation towards it and approach into close proximity with it. However, in contrast to our findings with cocaine, we found that the nicotine cue was

equally attractive in STs and GTs, eliciting dose-dependent approach behavior in both. Therefore, by this measure it would seem that the nicotine cue was attributed with incentive salience to the same extent in STs and GTs. However, we also assessed the incentive stimulus properties of the nicotine cue using a different test – the ability to act as a conditioned reinforcer. On this test there was a difference in the performance of STs and GTs. The nicotine cue was a more effective conditioned reinforcer in STs than in GTs, at least at the highest dose tested, which is consistent with previous findings that used cocaine as the US (Meyer et al. 2012b; Saunders and Robinson 2010; Yager and Robinson 2013). Thus, the extent to which a food cue was attractive predicted some, but not other, incentive properties of a nicotine cue.

There is considerable evidence that classically conditioned *food* cues can be imbued with incentive salience, eliciting approach behavior (Brown and Jenkins 1968; Davey and Cleland 1982; Hearst and Jenkins 1974), but it has been shown only recently that classically conditioned *drug* cues can also elicit approach behavior (Everitt and Robbins 2005; Uslaner et al. 2006). The first demonstration of this was by Tomie and colleagues (Tomie 2001; Tomie et al. 2003) who reported that rats would approach a cue associated with a sweetened ethanol solution. Although Tomie included a number of controls suggesting otherwise, there was some concern as to whether rats approached the cue because it was associated with drug (ethanol) or because it was associated with a sweet solution. Supporting Tomie's original reports, Krank et al. (2008) later reported that rats also learned to approach an unsweetened ethanol solution. Initial attempts to determine if rats would learn to approach a cue associated with intravenous (IV) cocaine delivery were unsuccessful (Kearns and Weiss 2004). There are a number of reasons why this may have been the case (see Uslaner et al. 2006 for discussion), as there have now been several studies reporting that rats will approach a cue associated with an IV injection of cocaine (Aragona et al.

2009; Flagel et al. 2010; Uslaner et al. 2006; Yager and Robinson 2013) or heroin (Madsen and Ahmed 2014; Peters and De Vries 2013). The results reported here add nicotine to that list, indicating that animals will approach not only cues associated with food reward but also cues associated with drugs from a number of different classes.

Another goal of the present experiment was to determine if there is individual variation in the extent to which a classically conditioned nicotine cue acquires incentive salience. We found that the nicotine cue was equally attractive to STs and GTs but differed in its ability to serve as a conditioned reinforcer. What could account for this difference between conditioned approach and conditioned reinforcement? Caggiula and others have argued that the ability of nicotine to influence behavior involves three dissociable factors: 1) the ability of nicotine to act as a primary reinforcer, 2) the ability of nicotine to transform a neutral stimulus into a conditioned stimulus capable of acting as a conditioned reinforcer, and 3) the ability of nicotine to act as a "reinforcement enhancer" or an "incentive amplifier" (Bevins and Palmatier 2004; Caggiula et al. 2009; Chaudhri et al. 2006; Liu et al. 2007; Palmatier et al. 2007). We will address these points in relation to our findings. First, while nicotine is a primary reinforcer, it is a relatively weak one. For example, nicotine supports very low levels of self-administration behavior in the absence of any discrete cues (Chaudhri et al. 2007; Donny et al. 2003; Le Foll and Goldberg 2006; Sorge et al. 2009). In fact, several self-administration studies have shown that when a cue is paired with nicotine delivery rats will readily self-administer nicotine but removal of the nicotine-paired cue dramatically diminishes self-administration behavior (Caggiula et al. 2001; 2002; Sorge et al. 2009). This suggests that cues associated with nicotine delivery are at least as important as nicotine itself in maintaining self-administration behavior. Thus, it is possible that because nicotine is a weak primary reinforcer the nicotine cue becomes especially salient in all

animals, eliciting approach behavior. However, this explanation does not account for the difference that we saw in the ability of the nicotine cue to serve as a conditioned reinforcer.

In addition to nicotine acting as a primary reinforcer and establishing cues as conditioned reinforcers (Palmatier et al. 2008; Palmatier et al. 2007), nicotine can also directly amplify the incentive properties of cues, and thus has been termed an "incentive amplifier" (Bevins and Palmatier 2004; Caggiula et al. 2009; Palmatier et al. 2012). For example, systemic injections of nicotine can enhance the ability of a conditioned stimulus to attract (Guy and Fletcher 2013; Palmatier et al. 2013) and to serve as a conditioned reinforcer (Guy and Fletcher 2013; Olausson et al. 2004; Palmatier et al. 2007). Nicotine can even enhance the incentive properties of unconditioned stimuli (Chaudhri et al. 2007; Donny et al. 2003). Importantly, nicotine amplifies the incentive value of cues "on-the-fly" as discontinuation of nicotine treatment reverses the enhancement of approach behavior (Guy and Fletcher 2013). This property of nicotine, the ability to enhance the incentive motivational properties of cues, may help to interpret our results. During Pavlovian conditioning, when nicotine was on board, nicotine may have acted as an incentive amplifier, enhancing the motivational properties of the cue. This would have the effect of making the cue an attractive stimulus and therefore eliciting approach in both STs and GTs. However, during the conditioned reinforcement test, when no nicotine was on board, it was revealed that STs attributed more incentive salience to the nicotine cue than GTs, as STs worked more avidly for presentation of the cue. Thus, the ability of nicotine to act as an incentive amplifier may have masked any differences between STs and GTs in the extent to which the nicotine cue acquired incentive salience as measured by conditioned approach. Consistent with this hypothesis, other incentive amplifiers, such as amphetamine and yohimbine (Feltenstein and

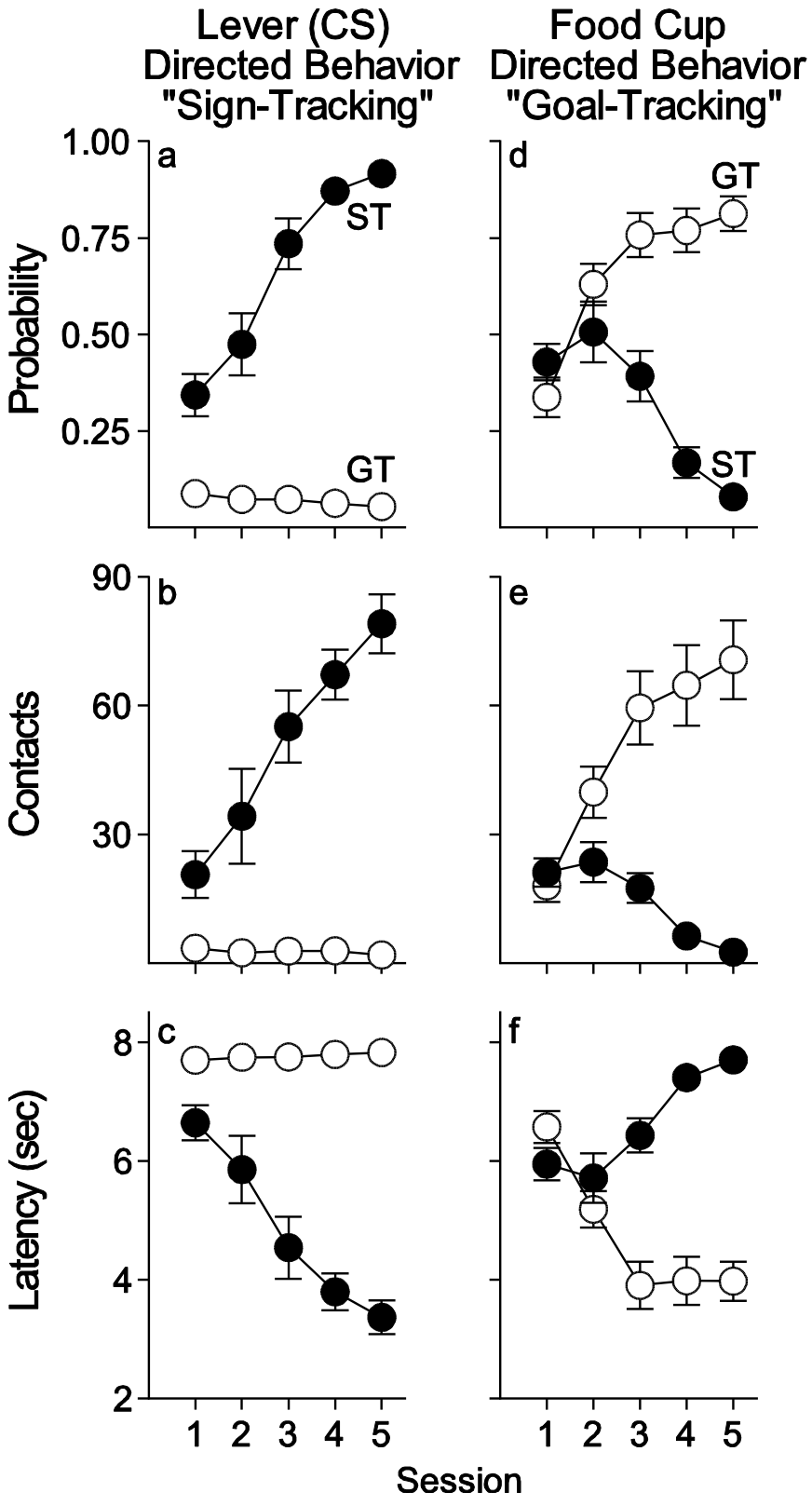
See 2006; Robbins 1978), have been found to increase the incentive value of reward-associated cues to the same extent in STs and GTs (Meyer et al. 2014).

It is important to point out that rats in the Unpaired group, who received non-contingent IV infusions of nicotine that were explicitly not paired with presentation of the cue light, did not acquire a conditioned approach CR. At first this may seem to be in contract to recent findings where non-contingent nicotine delivery increased responding for a visual stimulus that was not associated with any other reward besides illumination of the cue light (Donny et al. 2003). Based on these data, it might be assumed that in our study rats that received unpaired CS-US pairings during Pavlovian training would also approach the cue light if nicotine generally amplifies the incentive value of cues. However, in the study conducted by Donny et al. (2003), rats had to actively work for presentation of the visual stimulus which is quite different than the situation here. Additionally, previous work has shown that rats find light stimuli inherently reinforcing and will sustain instrumental responding for the light stimulus even in the absence of any other reinforcer (Olsen and Winder 2009; Stewart 1960). Thus, in the Donny et al. (2003) study, nicotine may be acting to enhance the unconditioned reinforcing properties of the visual stimulus.

In conclusion, we report that the propensity of an individual to attribute incentive salience to a food cue predicts the extent to which a nicotine cue can serve as a conditioned reinforcer but not the extent to which a nicotine cue becomes attractive. This dissociation in the ability of a nicotine cue to motivate behavior may be due to the ability of nicotine to directly amplify the incentive value of cues. Importantly, these data highlight the necessity of studying multiple properties of an incentive stimulus as these properties are psychologically and neurobiologically dissociable (Cardinal et al. 2002; Everitt and Robbins 2005; Milton and Everitt 2010). The

present data are also interesting to think about in terms of a recent study in human smokers. Mahler and de Wit (2010) found that smokers who reported the highest craving when presented with food cues when food deprived also reported the highest craving when presented with smoking cues during abstinence (see also Styn et al. 2013). Thus, some human individuals may also be more susceptible to the motivating effects of nicotine-associated cues during abstinence (i.e., no nicotine on board), making them more likely to relapse. The results reported here extend our previous studies with STs and GTs and suggest that the ability of drug cues to acquire properties of an incentive stimulus varies by the type of drug (i.e., cocaine or nicotine). It will be important to further understand the psychological and neurobiological mechanisms that underlie individual variation in susceptibility to addiction as well as differences between drug classes in order to develop more targeted treatments.

Figure 4.1. Behavior directed towards the lever-CS (sign-tracking, ST) vs. behavior directed towards the food cup during the CS presentation (goal-tracking, GT). Data are presented as means ± SEM for: (a) probability of approaching the lever-CS during the 8 s CS period [#trials with a lever-CS contact/#trials per session], (b) number of lever contacts, (c) latency to first lever contact after CS presentation, (d) probability of approaching the food magazine during the 8 s CS period [#trials with a food cup entry/#trials per session], (e) number of food magazine entries during the 8 s CS period, and (f) latency to the first food cup entry after CS presentation. For all measures there was a significant effect of group (ST or GT), session, and a group x session interaction (*p's* < 0.001).


Lever (CS)
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Food Cup
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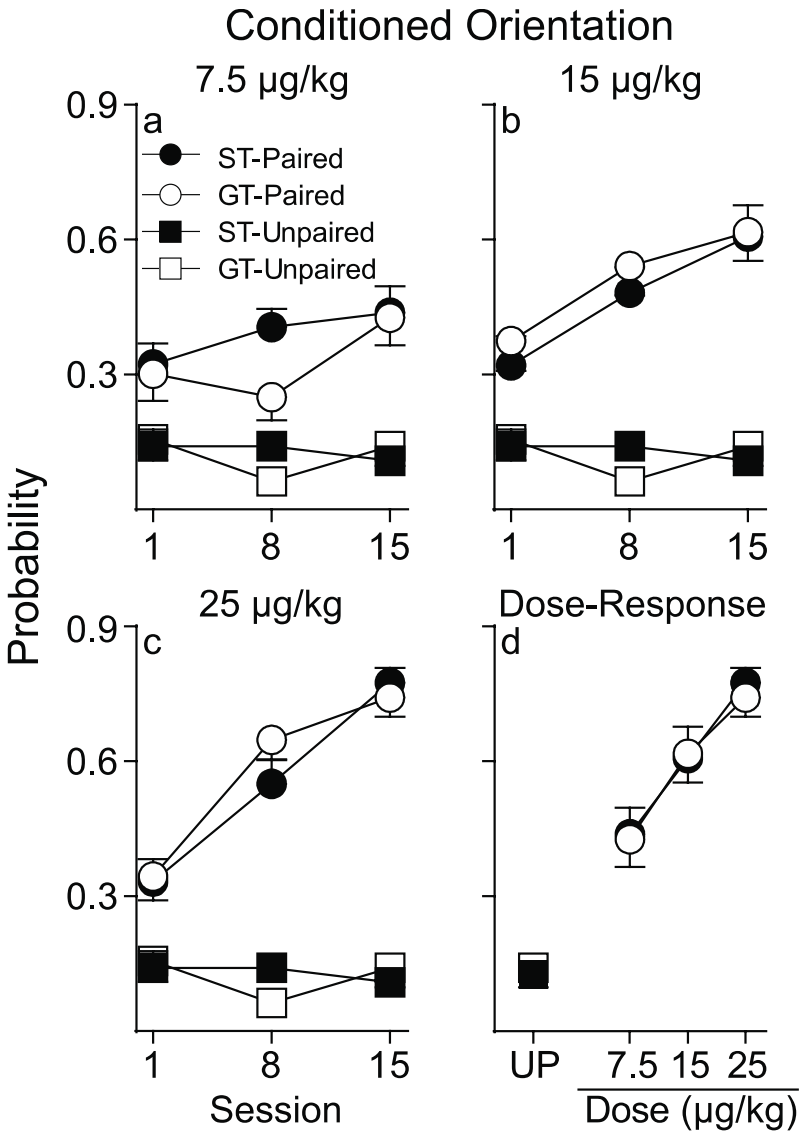

Figure 4.2. Probability of conditioned orientation to a cue associated with a non-contingent intravenous injection of nicotine. Data are illustrated as the mean ± SEM. All unpaired rats were trained with 25 µg/kg nicotine (STs *n*= 8, GTs *n*= 8). (a) Conditioned orientation in rats that received 7.5 µg/kg nicotine (Paired STs *n*= 12, GTs *n*= 12). (b) Conditioned orientation in rats that received 15 µg/kg nicotine (Paired STs *n*= 15, GTs *n*= 16) (c) Conditioned orientation in rats that received 25 µg/kg nicotine (Paired STs *n*= 14, GTs *n*= 15). (d) Dose-response function for the probability of conditioned orientation on the final day of training where each data point represents an independent group of rats. UP= unpaired.

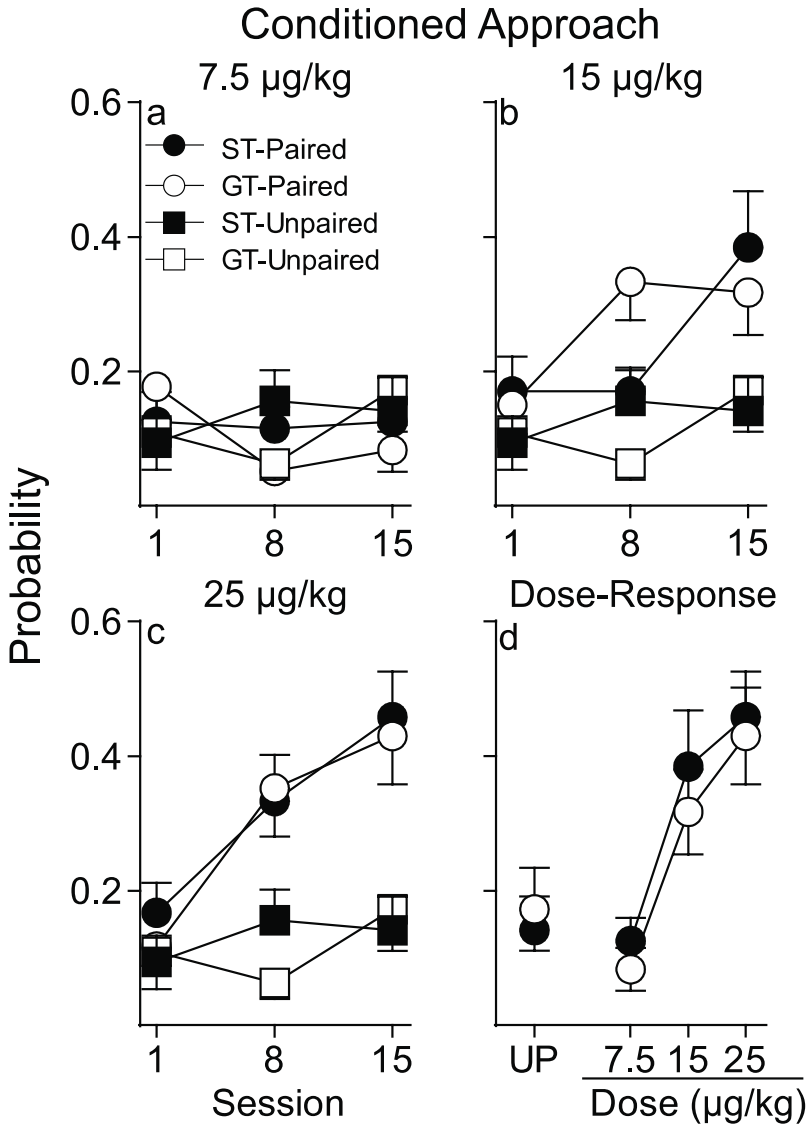

Figure 4.3. Probability of conditioned approach to a cue associated with a non-contingent intravenous injection of nicotine. Data are illustrated as the mean ± SEM. All unpaired rats were trained with 25 µg/kg nicotine (STs *n*= 8, GTs *n*= 8). (a) Conditioned approach in rats that received 7.5 µg/kg nicotine (Paired STs *n*= 12, GTs *n*= 12). (b) Conditioned approach in rats that received 15 µg/kg nicotine (Paired STs *n*= 15, GTs *n*= 16). (c) Conditioned approach in rats that received 25 µg/kg nicotine (Paired STs *n*= 14, GTs *n*= 15). (d) Dose-response function for the probability of conditioned approach on the final day of training where each data point represents an independent group of rats. UP= unpaired.

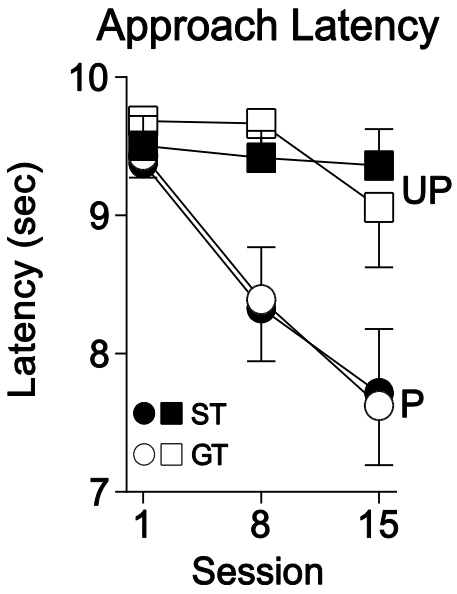

Figure 4.4. Latency to approach a cue associated with an intravenous injection of 25 μg/kg nicotine (Paired: STs *n*= 14, GTs *n*= 15; Unpaired: STs *n*= 8, GTs *n*= 8). P= paired, UP= unpaired.

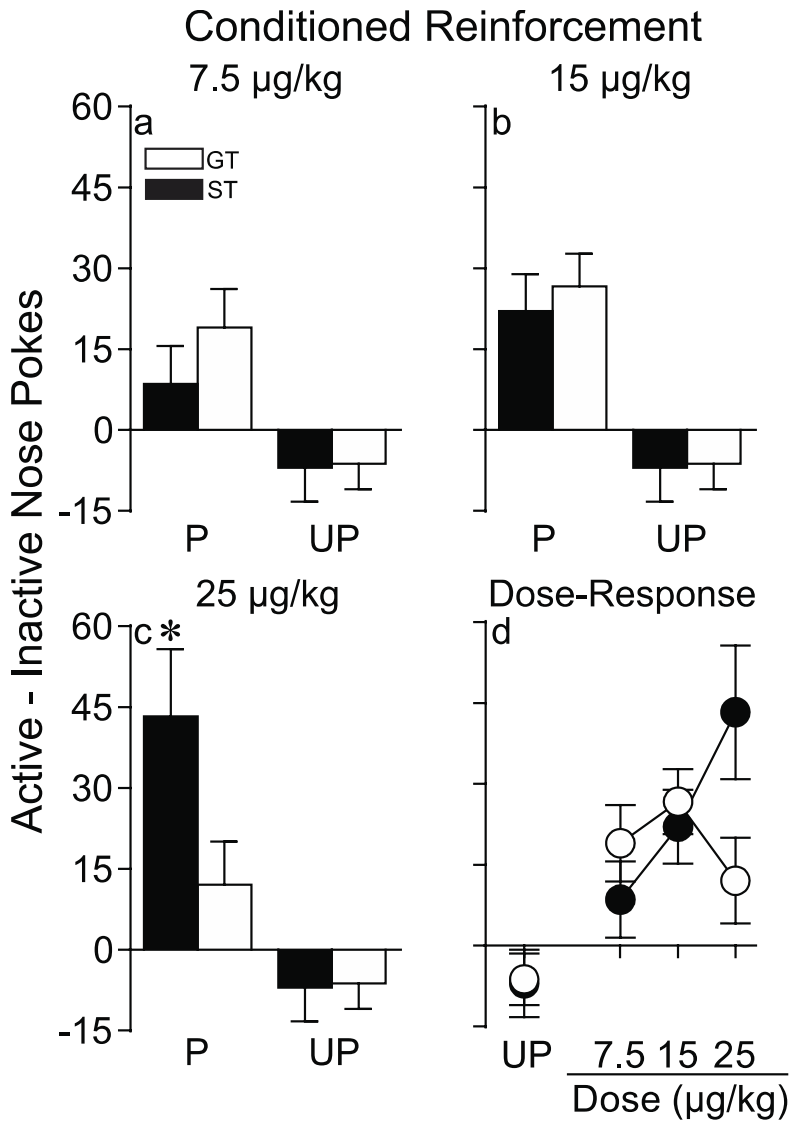

Figure 4.5. Performance during the test for conditioned reinforcement. During this test a nose poke into the active port resulted in presentation of the cue either previously paired or unpaired with non-contingent nicotine delivery for 2 sec. Data represent the mean ± SEM difference in nose pokes into the Active minus Inactive port for rats that were trained with (a) 7.5 µg/kg nicotine (Paired STs *n*= 12, GTs *n*= 12), (b) 15 µg/kg nicotine (Paired STs *n*= 15, GTs *n*= 16), or (c) 25 µg/kg nicotine (Paired STs *n*= 14, GTs *n*= 15). (d) Dose-response function for the difference in Active minus Inactive responses during the conditioned reinforcement test where each data point represents an independent group of rats. All unpaired rats were trained with 25 µg/kg nicotine (STs *n*= 8, GTs *n*= 8). P= paired, UP= unpaired. Asterisk indicates a significant group difference between STs and GTs, $p < 0.05$.

Chapter 5

General Discussion

The experiments described in this dissertation explored individual variation in the motivational properties of classically conditioned drug cues and the neurobiological correlates underlying this individual variation.

Individual variation in the attribution of incentive salience to classically (Pavlovian) conditioned drug cues

In a series of recent studies we have shown that a food cue becomes more attractive, eliciting approach, and is a more effective conditioned reinforcer in some rats (sign-trackers, STs) than in other rats (goal-trackers, GTs; Flagel et al. 2007; Meyer et al. 2012a; Robinson and Flagel 2009; Yager and Robinson 2010). We have suggested that these behavioral differences are due to differences in the extent to which individuals attribute incentive salience to reward cues. Furthermore, we have also shown that a discrete, localizable cocaine cue acquires greater control over self-administration behavior, is a more effective conditioned reinforcer, and spurs drug-seeking behavior to a greater degree in STs than in GTs (Saunders and Robinson 2010; Saunders et al. 2013). However, in both of these latter studies, the cocaine cue acquired its motivational properties in an instrumental setting (i.e., animals made responses which resulted in cocaine delivery and presentation of the cocaine associated cue). There is, however, evidence to suggest that different psychological and neurobiological processes underlie the attribution of motivational value to cues in an instrumental setting vs. in a classic Pavlovian conditioning

setting (Cardinal et al. 2002; Dickinson et al. 2000; Thomas et al. 2003; Thomas and Everitt 2001). Furthermore, in the "real world" of addicts, drug-associated cues often precede drug-taking actions, rather than following them, and, consequently, Pavlovian drug cues may be potent instigators of relapse in addicts. Therefore, we thought it important to explore whether STs and GTs also differed in the extent to which a cue associated with drug (cocaine, remifentanil, or nicotine) using classical Pavlovian conditioning procedures acquires incentive motivational properties.

Individual variation in the extent to which a classically conditioned cocaine cue acquires incentive salience

In chapter 2 (Yager and Robinson 2013), we assessed whether the propensity of animals to attribute incentive salience to a food cue predicted the extent to which a classically conditioned cocaine cue acquired incentive motivational properties. To do this we asked whether STs and GTs differed in the extent to which a classically conditioned cocaine cue came to (1) elicit approach towards it and (2) the degree to which it became itself desired, in that it reinforced actions to get it, using an extinction-reinstatement procedure. It is important to study several properties of an incentive stimulus as these properties are dissociable and attractiveness to a food cue may only predict some properties of an incentive stimulus (Cardinal et al. 2002; Everitt and Robbins 2005; Milton and Everitt 2010).

To assess the extent to which a cocaine cue became attractive, STs and GTs were trained on a Pavlovian conditioned approach task where the presentation of a cue light (the conditioned stimulus, CS) was paired with a non-contingent intravenous (IV) injection of cocaine (the unconditioned stimulus, US). Rats' behavior was scored during these training sessions for two different conditioned responses (CRs): (1) orientation (head and/or body movements) in the

direction of the light-CS and (2) approach to the light-CS (rearing to within 1 cm of the cue). Next, to assess the degree to which the cocaine cue became desired, an independent cohort of rats were trained to self-administer IV cocaine in the absence of any explicit cues. Following acquisition of stable self-administration, rats received two days of Pavlovian conditioning where a cue (illumination of a light) was paired with a non-contingent IV injection of cocaine. Next, rats underwent extinction training and then, on the reinstatement test day, rats were placed back into the test chamber but could now respond for contingent presentation of the Pavlovian cocaine cue under extinction conditions (i.e., no cocaine was delivered). We found that STs were more attracted to the classically conditioned cocaine cue (they approached it) and they also found it more desirable (they worked for presentation of it) than GTs.

We have now shown, in two independent studies, that STs are more attracted to a classically conditioned cocaine cue than GTs (Flagel et al. 2010; Yager and Robinson 2013). However, it remained unclear whether GTs did not readily approach the cocaine cue because they did not attribute sufficient incentive salience to the cue or whether they did not learn the CS-US association, as there is no "goal" to approach in this situation. To address this issue we used the acquisition of a different CR- orientation to the cue, as a measure of learning the CS-US association (Grastyan and Vereczkei 1974; Sokolov 1963). First, we quantified conditioned orientation behavior when using food as the US to verify whether conditioned orientation and conditioned approach were dissociable responses because it has been argued that sign-tracking behavior is merely an elaboration of a conditioned orienting response (Buzsaki 1982; Grastyan and Buzsaki 1979; Grastyan and Vereczkei 1974; Holland 1980). We found that these CRs were dissociable because both STs and GTs oriented towards the lever-CS before making their respective approach responses (approach to the lever-CS vs. approach to the food cup,

respectively). Furthermore, we found that even if GTs were in the food cup when the lever was presented they would remove their head from the food cup, look at the lever, and then enter the food magazine again. These data argue against a sign-tracking response simply being an extension of a conditioned orientation response because GTs will disengage from on-going behavior and orient to the cue. Therefore, we used the acquisition of an orientation CR as a measure of whether GTs learned the CS-US association when using IV delivery of cocaine as the US. We found that both STs and GTs (but not unpaired control animals) learned a conditioned orienting response to the cocaine cue. Furthermore, STs and GTs did not differ in this behavior even though GTs were less likely to approach the cocaine cue. Therefore, these data suggest that the reason GTs did not approach the cue was not because they failed to learn the CS-US association, because they acquired an orientation CR, but because they had not attributed sufficient incentive salience towards the cue to make it attractive.

These findings are consistent with several other studies from our lab. First, the finding that a cocaine cue was more attractive to STs than GTs is consistent with previous findings from our lab using the selectively bred bHR/ST and bLR/GT rat lines (Flagel et al. 2010). Flagel et al. (2010) reported that bHR/STs learned to approach a cocaine-associated cue whereas bLR/GTs did not. Second, Saunders and Robinson (2010) previously reported that a cocaine cue which gained its incentive value in an instrumental setting (i.e., during self-administration) was a more effective conditioned reinforcer in STs than GTs. Here, we expand on this work and show that a cocaine cue that gains its motivational properties in a Pavlovian conditioning procedure is also a more effective conditioned reinforcer in STs than GTs. It is worth noting that in the self-administration procedure we used, animals never learned to make a response that resulted in presentation of the cocaine cue. Therefore, presentation of the cocaine cue on the reinstatement

test day could not have elicited a habitual S-R response pattern. Furthermore, this self-administration paradigm not only provides a more rigorous assessment of the motivating properties of the cocaine cue, but it also offers the potential to determine the neural mechanisms involved in the attribution of incentive salience to discrete drug cues (See 2005). For example, work form the lab has recently shown that blockade of dopamine D1 receptors within the nucleus accumbens (NAc) core in STs just prior to the classical conditioning session attenuated subsequent reinstatement on test day (V. Lovic, unpublished observation). An interesting avenue for future work would be to look at whether increasing dopamine signaling within the NAc core during the classical conditioning session can potentiate subsequent reinstatement in GTs.

It is important to note that these results cannot be explained by differences in cocaine exposure or the number of cue-cocaine pairings, as these variables were held constant across groups. Furthermore, these results are also unlikely to be due to differences in the inherent reinforcing properties of cocaine during conditioning because we found no differences in acquisition of self-administration behavior, which is consistent with previous results (Saunders and Robinson 2010; 2011; Saunders et al. 2013). However, while we found that GTs did not acquire a conditioned approach CR with a low dose of cocaine (0.2 mg/kg), they did begin to approach the cocaine cue with a higher dose of cocaine (0.4 mg/kg). Therefore, it is possible that a cue associated with an even higher dose of cocaine might come to elicit robust approach behavior even in GTs, but this remains to be seen.

Individual variation in the extent to which an opioid (remifentanil) cue becomes attractive and desired

While it has now been established that the propensity of an individual to attribute incentive salience to a food cue predicts the extent to which a *cocaine* cue acquires all three

properties of an incentive stimulus (Flagel et al. 2010; Saunders and Robinson 2010; Saunders et al. 2013; Yager and Robinson 2013), it remained to be seen whether this effect generalizes across drug classes. Therefore, in chapter 3, we explored whether the extent to which an opioid cue is attractive and desired is predicted by the propensity to attribute incentive salience to a food cue (i.e., in STs vs. GTs).

To address these questions, STs and GTs were first trained on a Pavlovian conditioned approach task where the presentation of a cue light (the CS) was paired with a non-contingent IV injection of the μ-opioid agonist remifentanil (the US). The rats' behavior was again measured for the acquisition of two different conditioned responses: conditioned orientation and conditioned approach. To assess whether the remifentanil cue became desired, we measured the degree to which rats acquired a novel response (nose-poking) that resulted in presentation of the remifentanil cue. Similar to our findings with cocaine, we found that the remifentanil cue was both more attractive and desired in STs than GTs. Furthermore, as shown by the acquisition of a condition orientation response, both STs and GTs learned the CS-US association. These data suggest that the differences we saw in conditioned approach and conditioned reinforcement between STs and GTs were not due to differences in learning, but due to differences in the attribution of incentive salience to the remifentanil cue. These data are also consistent with recent work from the lab using the self-administration paradigm described above, where the cue was only paired with remifentanil delivery during a single Pavlovian conditioning session. Using this self-administration paradigm, it was shown that STs reinstated extinguished drug-seeking behavior to a greater degree than GTs (V. Lovic, unpublished observation). Thus, using two different paradigms, we have now shown that a remifentanil cue is a more effective conditioned reinforcer in STs than GTs.

Dissociation in the extent to which a nicotine cue becomes attractive and desired

There is now considerable evidence that cues associated with cocaine, alcohol, and heroin acquire the ability to become attractive and elicit approach behavior (Krank et al. 2008; Peters and De Vries 2013; Tomie 2001; Uslaner et al. 2006). However, it remained to be seen whether the same was true of another drug: nicotine. Therefore, in chapter 4, we first asked whether a cue associated with nicotine delivery could also become attractive and desired. Next, we asked whether these properties of an incentive stimulus varied by the degree to which an individual attributed incentive salience to a food cue.

STs and GTs were first trained on a Pavlovian conditioned approach procedure where a light cue was paired with a non-contingent IV injection of nicotine (7.5, 15, or 25 μg/kg). First, we asked whether the nicotine cue became attractive by measuring conditioned orientation and conditioned approach behavior. Then, following a one-week period of forced abstinence, we assessed whether the nicotine cue was desired, by measuring whether animals would learn an instrumental response (nose-poke) for presentation of the nicotine cue alone. It is reported here, for the first time, that a cue associated with IV nicotine delivery can become attractive and elicit approach. However, in contrast to our findings with both cocaine and remifentanil, we found that the nicotine cue was equally attractive in STs and GTs, eliciting dose-dependent approach behavior in both, but differed in the extent to which it served as a conditioned reinforcer. We found, at least with the highest dose tested, that the nicotine cue was a significantly more effective conditioned reinforcer in STs than in GTs.

This is the first time where we have observed a dissociation between the extent to which a drug cue becomes attractive and the extent to which it becomes desired. There are two potential explanations for our data. First, nicotine itself is a relatively weak reinforcer and thus the cue

may be especially salient in all animals. However, this explanation does not account for the differences seen during the conditioned reinforcement test. In addition to being a primary reinforcer, nicotine has also been shown to act as an "incentive amplifier" (Bevins and Palmatier 2004; Caggiula et al. 2009; Palmatier et al. 2012), meaning that nicotine is capable of enhancing the incentive value of both conditioned and unconditioned cues. It is possible that during conditioned approach training, when nicotine was on board, nicotine may have amplified the incentive value of the cue making it attractive to both STs and GTs. However, during the conditioned reinforcement test, when no nicotine was on board, the degree to which the nicotine cue had been attributed with incentive salience was revealed as STs worked more avidly for presentation of the nicotine cue than GTs. Therefore, the ability of nicotine to amplify the incentive value of the cue masked any differences between STs and GTs in attribution of incentive salience to the cue as measured by conditioned approach. An interesting avenue for future research may be to look at whether pretreatment with nicotine increases the conditioned reinforcing properties of a nicotine cue in GTs. I would predict, based on the available information, that pretreatment with nicotine would amplify the incentive value of the cue and that GTs would work for presentation of the cue just as much as STs.

These data bring up an interesting question: how is nicotine acting in the brain to amplify the incentive value of cues? We know that nicotine, like both cocaine and remifentanil, acts on the dopaminergic system to increase dopamine release, specifically from dopamine neurons projecting from the ventral tegmental area to the nucleus accumbens (Luscher and Ungless 2006; Nestler 2005; Pierce and Kumaresan 2006; Vander Weele et al. 2011). However, while all drugs of abuse act to increase dopamine release, they all have different mechanisms of doing so. For example, opiates act to disinhibit dopamine neurons (Johnson and North 1992) while cocaine

blocks dopamine reuptake via the dopamine transporter (DAT) (Chen et al. 2006). Nicotine affects dopamine release in multiple ways. Nicotine can increase dopamine signaling by acting at nicotinic acetylcholine receptors (nAChRs) located on dopamine neurons, which acts to depolarize the cell. Nicotine can also affect dopamine release by acting on nAChRs located on GABA neurons and on glutamatergic inputs to dopamine neurons (Fagen et al. 2003; Luscher and Ungless 2006). However, nicotine, at levels experienced by smokers, actually desensitizes nAChRs which results in decreased dopamine release (Pidoplichko et al. 1997). Therefore, at times nicotine can potentiate dopamine release and at other times attenuate dopamine release. Zhang and Sulzer (2004) (see also Rice and Cragg 2004) recently explored how nicotine can sometimes elevate extracellular dopamine and at other times depress dopamine release. They found that nicotine's effects on dopamine release depended on the firing pattern of the dopamine neurons- if dopamine neurons were tonically firing then nicotine inhibited dopamine release whereas if dopamine neurons were phasically firing then nicotine increased dopamine release (Rice and Cragg 2004; Zhang and Sulzer 2004). The switch in activity of dopamine neurons, from tonic to phasic firing, can be caused by salient conditioned stimuli (Rice and Cragg 2004). These findings led Chaudhri et al. (2007) to speculate that nicotine can create "a dopamine system that is hyper-excited and potentially more responsive to stimulation from incoming pharmacological and non-pharmacological reinforcers" (p. 360). This is one possible mechanism by which nicotine can act to increase the incentive value of cues. However, there is much more work that needs to be done to determine the mechanism by which nicotine increases the incentive value of cues, including the composition of the nAChRs that are involved and whether this effect is specifically related to dopamine release within the nucleus accumbens.

Neural circuitry underlying sign- and goal-tracking behavior

In addition to exploring individual variation in the extent to which an opioid cue acquires control over motivated behavior, the work presented in chapter 3 also investigated the neural underpinnings of these behavioral differences.

The role of dopamine in incentive motivation

In chapter 3, the role of dopamine signaling within the NAc core on expression of conditioned approach to a cue associated with the opioid remifentanil was explored.

We decided to look at the role of dopamine signaling in the attribution of incentive motivation to an opioid cue for two reasons. First, the primary reinforcing effects of opiates may not be dopamine dependent but the secondary reinforcing effects might be (Badiani et al. 2011). Second, there are now several lines of evidence from our lab that dopamine plays an important role in the attribution of incentive motivation to reward cues. The focus of this work has been on the NAc core as it has been shown that one property of an incentive stimulus, the ability to elicit approach, is dependent on intact neural transmission within the NAc (Blaiss and Janak 2009; Chang et al. 2012; Dalley et al. 2002; Di Ciano et al. 2001; Parkinson et al. 1999a; Parkinson et al. 2002; Parkinson et al. 2000). Furthermore, dopamine signaling within the NAc core shows a prediction-error signal (Day et al. 2007). However, while Chang et al. (2012) reported that lesions of the entire NAc impaired acquisition of a ST CR, they recently reported that lesions of only the NAc core or NAc shell had no effect on sign-tracking behavior (Chang and Holland 2013). There are several reasons why this may be the case, including differences in the extent to which the NAc core was damaged between their studies as well as differences between the Pavlovian conditioning procedure they used and the one we typically use (i.e., they used liquid

sucrose as the US, they used a discriminative autoshaping procedure). Further work needs to be done to parse apart these differences.

To begin to address the role of dopamine in stimulus-reward learning and incentive motivation, Flagel et al. (2011b) recorded dopamine transmission within the NAc core over the course of Pavlovian conditioning with food as the US and found that there is a transfer of phasic dopamine signaling from the food-US to the lever-CS in STs but not in GTs. Consistent with this finding, Parker et al. (2010) reported that in mice that only learn a GT CR there is no transfer of the dopamine signal from the US to the CS. Flagel et al. (2011b) next went on to test whether dopamine is necessary for learning (acquiring) a ST or GT CR. To do this, they gave animals a systemic injection of the dopamine receptor antagonist flupenthixol prior to each training session. They found that administration of flupenthixol blocked learning a ST CR but had no effect on learning a GT CR (see also Danna and Elmer 2010). Saunders and Robinson (2012) recently expanded on this work and examined the effect of dopamine receptor blockade, via flupenthixol, within the NAc core on performance of an already learned ST or GT CR elicited by a food cue. They found that flupenthixol dose-dependently decreased performance of a ST CR but had no effect on performance of a GT CR. Together these data suggest that dopamine transmission, at least within the accumbens core, is important in regulating the attribution of incentive salience to reward cues

While we have now shown that dopamine transmission within the NAc core is involved in both the acquisition and expression of a ST CR to a food cue, one question that remained was whether approach to a drug cue is also dependent on dopamine transmission within the NAc core. Interestingly, a recent study by Aragona et al. (2009) reported that dopamine release within the NAc core was correlated with the extent to which a cocaine cue became attractive, eliciting

approach. In line with this observation, we found that blocking dopamine receptors within the NAc core dose-dependently attenuated approach to an opioid cue. Furthermore, administration of flupenthixol impaired approach to the opioid cue on the very first trial before any new learning could occur. Therefore, this effect could not be due to an updated prediction-error signal. Importantly, flupenthixol did not effect conditioned orientation, which suggests that the CS-US association remained intact. These data are consistent with the work by Saunders and Robinson (2012) and their conclusions that "dopamine antagonism attenuates the learning and performance 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" (p 2529).

While our work has focused on the role of dopamine signaling in the acquisition and expression of ST and GT CRs, there are a variety of other neurotransmitters including glutamate, serotonin, endogenous opioids, and norepinephrine that may be involved in mediating the incentive motivational properties of reward associated cues (Cardinal et al. 2002; Di Ciano et al. 2001; DiFeliceantonio and Berridge 2012; Mahler and Berridge 2009; Nonkes et al. 2014; O'Connor et al. 2010; Puglisi-Allegra and Ventura 2012a; b; Tomie et al. 2000; Tomie et al. 2004). For example, Mahler and Berridge (2009) recently reported that stimulation of μ-opioid receptors within the central nucleus of the amygdala (CeA) increased approach and engagement with a food cue in STs while it increased approach and engagement with the food cup in GTs (see also DiFeliceantonio and Berridge 2012). Furthermore, when Mahler and Berridge (2012) stimulated μ-opioid receptors in the CeA during PIT testing they found that this increased instrumental responding during presentation of the Pavlovian cue, indicating a heightened state of conditioned motivation. Additionally, other physiological correlates, such as stress-induced

corticosterone release, are also related to individual variation in attribution of incentive value to reward cues (Flagel et al. 2009; Tomie et al. 2000; Tomie et al. 2004). Future work will need to be conducted to parse out the role of these neurotransmitter systems in individual variation in response to reward cues, especially in regions outside of the nucleus accumbens.

Engagement of brain reward circuitry by incentive stimuli

In addition to assessing the role of dopamine in conditioned approach to an opioid cue, the work presented in chapter 3 also explored the extent to which both food and opioid cues engaged brain reward circuitry.

There is now an abundance of evidence that food-associated cues (both discrete cues and contextual cues associated with food availability) can engage brain reward circuitry (Kest et al. 2012; Pelchat et al. 2004; Schiltz et al. 2007; Volkow et al. 2002). However, as previously discussed, reward cues acquire predictive value and, in some cases, can also acquire motivational value. To parse out the extent to which either the predictive value or the motivational value of a food cue is sufficient to engage reward circuitry, Flagel et al. (2011a) used *in situ* hybridization to quantify the ability of a food cue to elicit c-fos mRNA in STs and GTs throughout the brain. Measurements of both c-fos mRNA and Fos protein expression have been extensively used as metabolic markers of neuronal activity and serve as an indirect measure of neuronal activation (Dragunow and Faull 1989; Kovacs 1998; Sagar et al. 1988). Flagel et al. (2011a) measured c-fos mRNA expression throughout the so-called "motive circuit", which has been shown to be engaged by reward cues. This "motive circuit" includes the prefrontal cortex, dorsal and ventral striatum, thalamus, habenula, and amygdala (Cardinal et al. 2002; Kalivas and Volkow 2005; Weiss 2005). Flagel et al. (2011a) reported that presentation of a food cue produced significant increases in c-fos mRNA expression throughout the "motive circuit" in STs, but not in GTs,

relative to unpaired control rats. This finding suggests that the predictive value of reward cues is not sufficient to engage traditional brain reward systems- the cue must be imbued with incentive salience to do so.

Not only can food cues engage the "motive circuit", but so can drug cues (Childress et al. 1999; Grant et al. 1996; Kelley et al. 2005; Schroeder et al. 2001; Tang et al. 2012; Volkow et al. 2008b; Zavala et al. 2008). While we have now shown that a *food* cue must be imbued with incentive salience in order to engage brain reward circuitry, it remained to be seen whether the same was true of *drug* cues. Here, we used Fos protein expression, rather than c-fos mRNA, as a marker of neural activity and quantified the extent to which presentation of either a food or an opioid cue elicited Fos expression throughout the brains of STs, GTs, and an Unpaired control group. We found that presentation of both the food and opioid cue elicited greater Fos expression in STs, but not in GTs, relative to unpaired control animals in almost every region examined. For example, presentation of both the food and opioid cue elicited greater Fos expression throughout the striatum in STs relative to GTs. These data are consistent with the ability of both food and drug cues to engage the striatum (Kufahl et al. 2009; Schiltz et al. 2007; Volkow et al. 2002; Volkow et al. 2006). Furthermore, both the dorsal and ventral striatum are critically involved in cue-induced reinstatement of drug-seeking behavior (Fuchs et al. 2006; Fuchs et al. 2004). These data suggest that like a food cue, a drug cue must be imbued with incentive salience in order to engage the "motive circuit". Importantly, Fos expression was not correlated with any of our behavioral measures (e.g., approach to the food or opioid cue). This suggests that Fos expression was not merely an artifact of increased locomotor behavior in STs in response to cue presentation.

While these findings nearly replicate the findings of Flagel et al. (2011a), there are two major differences in our results. First, Flagel et al. (2011a) reported group differences in c-fos mRNA expression in the orbitofrontal cortex (OFC) while we did not find any group differences in Fos protein expression in the OFC, although there was a non-significant trend for greater Fos expression in STs in response to the food cue. This finding was surprising given that the OFC is engaged by drug cues and has been implicated in cue-induced drug craving (McClernon et al. 2009; Schoenbaum and Shaham 2008; Zavala et al. 2008). However, it is important to point out that Fos expression was only assessed at one Anterior-Posterior (A-P) level. In fact, Flagel et al. (2011a) reported that there were no significant group differences at more rostral levels of the OFC. Therefore, a more detailed analysis of the OFC across A-P levels may be necessary. Second, we found that presentation of both the food and opioid cue elicited greater Fos expression in STs in the basolateral nucleus of the amygdala (BLA) while Flagel et al. (2011a) did not find any group differences. The lack of effect in the BLA in the Flagel et al. (2011a) study was unexpected given that there is considerable evidence that both food and drug cues can engage the amygdala (Brown et al. 1992; Childress et al. 1999; Grant et al. 1996; Schiltz et al. 2007). Our findings, however, are in agreement with several preclinical studies that have demonstrated that the BLA plays an important role in drug-seeking behavior and relapse (Fuchs and See 2002; Kantak et al. 2002; Meil and See 1997).

In addition to reporting differences in the extent to which a food cue elicited c-fos mRNA expression within a given brain region, Flagel et al. (2011a) also conducted correlational analyses between brain regions to look at inter-regional "connectivity". They found several differences between STs and GTs in the degree to which c-fos mRNA expression was correlated between regions. For example, c-fos mRNA expression was positively correlated between the

orbitofrontal cortex and pavaventricular nucleus of the thalamus in GTs but not in STs. We also conducted correlational analyses to assess inter-regional "connectivity", but failed to find any significant correlations. There are a few potential reasons for this. First, our group sizes were smaller than those in the Flagel et al. (2011a) study. Second, there was not a lot of variation in the levels of Fos expression within groups (i.e., within STs). Together, these factors would make it difficult to find any correlation in Fos expression between regions. Another factor to consider is that Fos expression has not been quantified in several of the regions that Flagel et al. (2011a) reported correlations among (e.g., intermediodorsal and central medial nuclei of the thalamus), and I plan to do this in the future.

Together, these studies add to a growing literature that different neural systems are engaged by cues that only act as predictors of reward vs. cues that are also imbued with incentive salience. One commonality amongst the regions that we explored in the Fos study is that all of these regions receive input from dopamine neurons. An interesting avenue for future research may be to look at whether dopamine projections from the VTA to other regions of the "motive circuit" are selectively activated in response to food and drug cues.

Individual variation in liability to addiction

We have now shown using a variety of different measures and procedures that there is considerable individual variation in the propensity to attribute incentive salience to a food cue and that this predicts the extent to which discrete drug cues acquire control over motivated behavior. For example, both discrete drug cues and internal drug cues (the interoceptive effects of the drug) acquire greater incentive motivational value in STs than in GTs (Flagel et al. 2010; Meyer et al. 2012b; Saunders and Robinson 2010; 2011; Saunders et al. 2013; Yager and Robinson 2013). Additionally, Beckmann et al. (2011) recently reported that in a free access

cocaine self-administration paradigm STs acquired self-administration faster than GTs and we have also shown that STs are more susceptible to psychomotor sensitization than GTs (Flagel et al. 2008). This had led us to hypothesize that STs may be more susceptible to addiction than GTs, as they are more motivated by discrete drug cues and also show a higher tendency to relapse.

There are, however, several other traits, including impulsivity, novelty-seeking, sensation-seeking, and decreased attentional control, that are associated with addiction liability in both humans and animals (Belin et al. 2011; Belin and Deroche-Gamonet 2012; Belin et al. 2008; Cain et al. 2005; Ersche et al. 2010; Jentsch and Taylor 1999; Molander et al. 2011; Wills et al. 1994; Winstanley et al. 2010). Thus, we have begun to explore the extent to which STs and GTs also differ in these traits. Tomie and colleagues (Tomie 1996; Tomie et al. 1998) were the first to suggest that poor inhibitory control, which is one characteristic of impulsivity, may be associated with sign-tracking behavior. We have recently shown that STs are in fact more action impulsive than GTs, in that they have difficulty withholding a response when doing so is required to receive a reward, as indicated by STs making more premature responses on both the DRL (differential reinforcement of low rates) task and a 2-choice serial reaction time task (2-CSRTT) (Lovic et al. 2011). Flagel et al. (2010) also showed a similar pattern of results using the bHR/ST and bLR/GT rat lines and found that bHR/STs were also more impulsive than bLR/GTs on the DRL task. In addition to being more impulsive, STs also show more novelty-seeking behavior than GTs (Beckmann et al. 2011). Finally, STs, relative to GTs, also have poor attentional control over behavior which was shown to be associated with decreased cholinergic function in the prefrontal cortex (Paolone et al. 2013). It is important to mention that while STs tend to be more action impulsive than GTs, there was a large amount of variation within both

groups. In fact, premature responses during the 2-CSRTT only accounted for about 15% of the variance in conditioned approach behavior to the food cue (Lovic et al. 2011). Therefore, we have speculated that individuals that have a compilation of these traits- have the propensity to attribute incentive salience to reward cues, are impulsive, have poor top-down executive control, and are novelty-seekers- may be especially vulnerable to addiction (Christiansen et al. 2012).

While we have now explored the extent to which STs and GTs vary on traits associated with addiction liability and also vary in the extent to which discrete drug cues motivate behavior, it remained to be seen whether another class of cues, contextual cues, motivates behavior differently in STs and GTs. Contextual cues (i.e., places were drugs are acquired and/or taken) are another class of cues known to contribute to relapse in both rats and humans (Crombag et al. 2008; Crombag and Shaham 2002; Fuchs et al. 2005; Mayo et al. 2013; McFarland and Ettenberg 1997; O'Brien et al. 1992). Thus, Saunders et al. (2012) asked whether STs and GTs differed in the extent to which contextual cues acquire control over motived behavior by using two different measures. First, they examined the ability of a drug-paired context to produce a state of conditioned motivation as measured by conditioned hyperactivity (Beninger et al. 1981; Jones and Robbins 1992). Second, they measured the ability of a drug-paired context to reinstate extinguished drug-seeking behavior (Crombag et al. 2008). As opposed to our previous findings with a discrete drug cue, they found that GTs showed greater context-conditioned hyperactivity and greater context-induced reinstatement of drug-seeking behavior than STs (Saunders et al. 2012). Interestingly, these data are consistent with a recent finding using a fear conditioning paradigm. Morrow et al. (2011) reported that STs, relative to GTs, display greater discrete cue (tone)-evoked fear (freezing behavior) while GTs, relative to STs, display greater contextual conditioned fear. Furthermore, Ahrens and Robinson (2013) recently conducted a study where

contextual information served as an occasion setter (different contexts indicated whether presentation of the CS would be followed by reward or not). They found that GTs behavior quickly came under control of the occasion setter where as STs failed to modify their behavior based on the contextual information. Together these data suggest that STs preferentially assign incentive motivational value to discrete cues while GTs preferentially assign incentive motivational value to contextual cues, regardless of the emotional valence of the outcome (i.e. appetitive or aversive). These data require that we modify our hypothesis that STs are more vulnerable to addiction than GTs. Instead, it appears that individuals may be more sensitive to different "triggers" (i.e. discrete vs. contextual cues) that are capable of motivating behavior and instigating relapse. Therefore, STs are not more vulnerable to addiction than GTs but there may be different pathways to addiction, which has important implication for clinical treatments.

Psychological processes mediating sign- and goal-tracking behavior

We have now shown in a series of studies, including the studies presented in this dissertation, that both food and drug-associated cues motivate behavior differently across individuals. We have also shown that this is true across a variety of different behavioral paradigms, including tasks that differ in emotional valence. Importantly, we have also reported that sign-tracking and goal-tracking behaviors are subserved by different neural systems. For example, as mentioned previously, both the learning and expression of a ST CR, but not a GT CR, is dependent on endogenous dopamine signaling (Danna and Elmer 2010; Flagel et al. 2011b; Saunders and Robinson 2012). Together, these data suggest that sign-tracking and goal-tracking behavior are not only mediated by different neural systems but also by different psychological processes.

We have recently begun to speculate on what psychological process may be regulating sign- and goal-tracking behavior. We have suggested that while a bottom-up Pavlovian incentive process motivates sign-tracking behavior a more top-down cognitive process underlies goal-tracking behavior (Flagel et al. 2011a; Meyer et al. 2012a; Saunders and Robinson 2012). This idea is consistent with several lines of evidence from our lab. For example, STs are more impulsive than GTs (Flagel et al. 2010; Lovic et al. 2011). This difference in impulsivity could reflect differences in top-down inhibitory control. Indeed, we have recently shown that STs, relative to GTs, have poor cognitive control over attention (Paolone et al. 2013). These data are also consistent with differences in inter-regional connectivity between STs and GTs. Flagel et al. (2011a) found that c-fos mRNA expression in response to a food cue was correlated within cortico-striatal and thalamocortical circuitry in GTs but not in STs. STs, however, showed strong correlations in c-fos mRNA expression among subcortical regions. While this inter-regional connectivity data is speculative, it does suggest that GTs may engage more top-down cortical circuitry resulting in greater executive control over behavior. Furthermore, these data also support the idea that in GTs presentation of the reward cue elicits a cognitive representation of the reward, which would result in approach to the location of reward delivery. This behavior is not dopamine-dependent and is thought to be dependent on corticostriatal circuitry (Balleine and Dickinson 1998; Dickinson and Balleine 2002; Dickinson et al. 2000; Wassum et al. 2011; Yin et al. 2008).

Clinical relevance

The focus of my dissertation work has been on identifying individual variation in the extent to which drug cues can motivate behavior in a preclinical rodent model. However, there is considerable evidence supporting the ability of reward cues to become incentive stimuli and

motivate maladaptive behavior in humans. For example, if moderately sated humans, who minutes earlier expressed no desire to eat, are presented with a highly palatable food the desire to eat is reinstated (Cornell et al. 1989). Food-associated cues, such as signs for fast food restaurants, can also elicit desire for food and are thought to contribute to overeating and obesity (Jansen 1998). Furthermore, drug associated cues can also motivate behavior in humans.

Individual variation in the extent to which reward cues acquire properties of an incentive stimulus in humans: behavioral evidence

In the human literature the ability of reward cues to acquire properties of an incentive stimulus has been demonstrated using tasks such as the Stroop task (Cox et al. 2006) and the dot probe task (Bradley et al. 2003). These tasks measure attentional bias for reward cues and can be used as a proxy for how "attractive" cues are. It has now been shown, using both the Stroop task and dot probe task, that humans find both food and drug-associated cues attractive (Duka and Townshend 2004; Hickey et al. 2010a; Hickey and van Zoest 2012; Pool et al. 2014; Wiers et al. 2009). While reward cues can bias attention, this is especially problematic for addicts. Drug-cues not only attract attention in addicts (Field and Cox 2008; Franken 2003; Hester et al. 2006; Schoenmakers et al. 2008), but addicts will choose to view drug-associated pictures (Moeller et al. 2009), drug cues can support responding on a second-order schedule of reinforcement in human addicts (Panlilio et al. 2005), and drug cues can evoke a state of conditioned motivation that elicits craving and/or relapse (Ehrman et al. 1992; O'Brien et al. 1992). There have also been several studies showing that drug cues not only acquire the ability to bias attention but they can also elicit approach behavior (Christiansen et al. 2012; Cousijn et al. 2011; Field et al. 2008; Field et al. 2005; Palfai 2006; Thewissen et al. 2007; Van Gucht et al. 2008; Wiers et al. 2009). While the ability of drug cues to attract humans is difficult to directly measure, researchers have

come up with a way to circumvent this problem by measuring the speed to which participants move a character on a computer screen or use a joystick to "approach" images presented on a screen (Field et al. 2005; Franken 2003; Wiers et al. 2009). Therefore, like in our preclinical rodent model, drug cues acquire all three properties of an incentive stimulus in human addicts.

There is, however, considerable individual variation in the extent to which cues acquire control over motivated behavior in humans as measured by the ability of cues to bias attention, evoke craving, and to instigate relapse (Beaver et al. 2006; Carpenter et al. 2012; Carpenter et al. 2009; Carter and Tiffany 1999; de Wit et al. 1987; Hickey et al. 2010b; Janes et al. 2010; Kambouropoulos and Staiger 2001; Kilts et al. 2014; Niaura et al. 1998; Tetley et al. 2010). For example, scores on the Behavioral Activation Scale (BAS), a measure of "reward-seeking" personality characteristics which are thought to measure the propensity for appetitive motivation, are correlated with both the propensity to overeat and the ability of alcohol-related cues to elicit craving (Beaver et al. 2006; Kambouropoulos and Staiger 2001). Furthermore, Mahler and de Wit (2010) recently reported that individuals that reported the highest craving in response to a food cue when food deprived also reported the highest craving to a smoking cue during a period of abstinence. This effect has also now been replicated in a larger sample size (Styn et al. 2013). Additionally, variation in the extent to which humans find drug cues attractive predicts craving for drugs, future drug use, and also the probability that an individual will relapse (Carpenter et al. 2006; Copersino et al. 2004; Cox et al. 2002; Field and Cox 2008; Franken et al. 2000; Marissen et al. 2006; Vadhan et al. 2007). These studies parallel much of the recent work from our lab as well as the studies I presented in this dissertation looking at a preclinical model of individual variation in response to reward cues (Flagel et al. 2010; Meyer et al. 2012b; Saunders and Robinson 2010; 2011; Saunders et al. 2013; Yager and Robinson 2013). Together these data

suggest that there is considerable individual variation in the extent to which drug cues can motivate behavior in humans as well as in non-human animals.

Individual variation in the extent to which reward cues acquire properties of an incentive stimulus in humans: genetic and neurophysiological correlates

We have now demonstrated in several studies with rodents that the dopamine system is involved in the attribution and expression of incentive motivation towards both food- and drug-associated cues (Flagel et al. 2011b; Flagel et al. 2007; Saunders and Robinson 2012; Saunders et al. 2013). There is also considerable evidence from the human literature that both drugs and drug cues can engage the mesocorticolimbic dopamine system (Boileau et al. 2007; Ersche et al. 2010; Leyton 2007; Leyton et al. 2002; Volkow et al. 2004; Volkow et al. 2008a; Volkow et al. 2006; Wong et al. 2006; Zijlstra et al. 2009). Even reward cues presented outside conscious awareness can activate mesocorticolimbic circuitry (Childress et al. 2008; Wetherill et al. 2014a). Furthermore, simply presenting words related to drug use can activate this system in addicts (Goldstein et al. 2009). Behaviorally, dopamine release within the dorsal striatum elicited by presentation of a drug cue is correlated with self-reports of craving (Volkow et al. 2006; Wong et al. 2006). Additionally, dopamine signaling is both necessary and sufficient for drug cues to bias attention in human drug users (for review see Franken et al. 2005). For example, blockade of dopamine receptors by the dopamine receptor antagonist haloperidol reduced attentional bias in heroin users (Franken et al. 2004) while treatment with the dopamine receptor agonist pramipexole enhanced attentional bias toward drug cues (Ersche et al. 2010).

While food and drug cues can engage the mesolimbic system in humans, there is considerable individual variation in brain responses to reward-related cues (Beaver et al. 2006; Bogdan et al. 2012; Janes et al. 2010; Kilts et al. 2014; Simon et al. 2010). There are many

possible factors that may underlie this individual variation in brain responses to reward cues, including differences in personality traits (Beaver et al. 2006; Simon et al. 2010) and in impulsivity (Buckholtz et al. 2010). Individual variation in the extent to which reward-associated cues engage the mesolimbic system may also be explained, in part, by genetic variation (Bogdan et al. 2012; Kreek et al. 2005; Wiers et al. 2009). A large focus of this work has been on polymorphisms within the genes coding for the dopamine transporter (DAT) (Aarts et al. 2010; McClernon et al. 2007). For example, a polymorphism within the DAT SLC6A3 gene is related to the extent to which a smoking cue can engage the ventral striatum, ventral pallidum, and orbitofrontal cortex in smokers (Franklin et al. 2009; Franklin et al. 2011) and the degree to which smoking cues can bias attention and influence brain activity (Wetherill et al. 2014b). Given that our preclinical findings closely reflect the findings in the human literature, this preclinical model allows us the opportunity to explore the genetic, epigenetic, environmental, and neurophysiological correlates that confer susceptibility to disorders such as addiction.

Conclusions

The work presented in this dissertation explored the extent to which individual variation in the propensity to attribute incentive salience to a food cue predicts the extent to which classically conditioned drug cues acquire control over motivated behavior. Through this work I have shown that some rats are more susceptible than others in the extent to which classically conditioned drug cues motivate behavior and that this effect varies across drug classes. Furthermore, I have shown that the underlying neural circuitry contributing to the attribution of incentive salience to food and drug cues is similar. These results have important clinical implications, as some individuals may be more reactive to discrete cues than others. In the future both preclinical and clinical research should continue to focus on understanding individual

variation in the psychological and neural mechanism underlying attribution of aberrant incentive motivation to reward cues to develop better target treatment for disorders such as addiction, obesity, and gambling.

References

Aarts E, Roelofs A, Franke B, Rijpkema M, Fernandez G, Helmich RC, Cools R (2010) Striatal dopamine mediates the interface between motivational and cognitive control in humans: evidence from genetic imaging. Neuropsychopharmacol 35: 1943-51.

Ahrens AM, Robinson TE (2013) An occasion-setter exters much greater control over conditioned responding in goal-trackers than in sign-trackers 2013 Neuroscience Meeting Planner. Society for Neuroscience

Anselme P, Robinson MJ, Berridge KC (2013) Reward uncertainty enhances incentive salience attribution as sign-tracking. Behav Brain Res 238: 53-61.

Anthony JC, Warner LA, Kessler RC (1994) Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: Basic findings from the National Comorbidity Survey. Exp Clin Psychopharmacol 2: 244-268.

Aragona BJ, Day JJ, Roitman MF, Cleaveland NA, Wightman RM, Carelli RM (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: 1889-99.

Arroyo M, Markou A, Robbins TW, Everitt BJ (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 (Berl) 140: 331-44.

Badiani A, Belin D, Epstein D, Calu D, Shaham Y (2011) Opiate versus psychostimulant addiction: the differences do matter. Nat Rev Neurosci 12: 685-700.

Balleine BW, Dickinson A (1998) Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37: 407-19.

Barker JM, Torregrossa MM, Taylor JR (2012) Low prefrontal PSA-NCAM confers risk for alcoholism-related behavior. Nat Neurosci 15: 1356-8.

Baylon GJ, Kaplan HL, Somer G, Busto UE, Sellers EM (2000) Comparative abuse liability of intravenously administered remifentanil and fentanyl. J Clin Psychopharm 20: 597-606.

Beaver JD, Lawrence AD, van Ditzhuijzen J, Davis MH, Woods A, Calder AJ (2006) Individual differences in reward drive predict neural responses to images of food. J Neurosci 26: 5160-5166.

Beckmann JS, Bardo MT (2012) Environmental enrichment reduces attribution of incentive salience to a food-associated stimulus. Behav Brain Res 226: 331-4.

Beckmann JS, Marusich JA, Gipson CD, Bardo MT (2011) Novelty seeking, incentive salience and acquisition of cocaine self-administration in the rat. Behav Brain Res 216: 159-65.

Belin D, Berson N, Balado E, Piazza PV, Deroche-Gamonet V (2011) High-novelty-preference rats are predisposed to compulsive cocaine self-administration. Neuropsychopharmacol 36: 569-79.

Belin D, Deroche-Gamonet V (2012) Responses to novelty and vulnerability to cocaine addiction: contribution of a multi-symptomatic animal model. Cold Spring Harb Perspect Med 2: 1-20.
Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008) High impulsivity predicts the switch to compulsive cocaine-taking. Science 320: 1352-5.
Beninger RJ, Hanson DR, Phillips AG (1981) The acquisition of responding with conditioned reinforcement: effects of cocaine, (+)-amphetamine and pipradrol. Br J Pharmacol 74: 149-54.
Berridge KC (2001) Reward learning: Reinforcement, incentives, and expectations. Psychol Learn Motiv 40: 223-278.
Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology (Berl) 191: 391-431.
Berridge KC (2012) From prediction error to incentive salience: Mesolimbic computation of reward motivation. Eur J Neurosci 35: 1124-43.
Berridge KC, Ho CY, Richard JM, DiFeliceantonio AG (2010) The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. Brain Res 1350: 43-64.
Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 28: 309-69.
Berridge KC, Robinson TE (2003) Parsing reward. Trends Neurosci 26: 507-513.
Bertz JW, Woods JH (2013) Acquisition of responding with a remifentanil-associated conditioned reinforcer in the rat. Psychopharmacology (Berl) 229: 235-43.
Bevins RA, Palmatier MI (2004) Extending the role of associative learning processes in nicotine addiction. Behav Cogn Neurosci Rev 3: 143-58.
Bindra D (1968) Neuropsychological interpretation of the effects of drive and incentive-motivation on general activity and instrumental behavior. Psychol Rev 75: 1-22.
Bindra D (1978) How adaptive behavior is produced: a perceptual-motivation alternative to response reinforcement. Behav Brain Sci 1: 41-52.
Blaiss CA, Janak PH (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 (1977) Performance on learning to associate a stimulus with positive reinforcement. In: Davis H, Hurwits H (eds) Operant-Pavlovian Interactions. Lawrence Erlbaum Associates, Hillsdale, pp 67-97
Bogdan R, Carre JM, Hariri AR (2012) Toward a mechanistic understanding of how variability in neurobiology shapes individual differences in behavior. Curr Top Behav Neurosci 12: 361-93.
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.
Bradley BP, Mogg K, Wright T, Field M (2003) Attentional bias in drug dependence: vigilance for cigarette-related cues in smokers. Psychol Addict Behav 17: 66-72.
Breland K, Breland M (1961) The misbehavior of organisms. Am Psychol 16: 681.
Brown EE, Robertson GS, Fibiger HC (1992) Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. J Neurosci 12: 4112-21.

Brown PL, Jenkins HM (1968) Auto-shaping of the pigeon's key-peck. J Exp Anal Behav 11: 1-8.
Buckholtz JW, Treadway MT, Cowan RL, Woodward ND, Li R, Ansari MS, Baldwin RM, Schwartzman AN, Shelby ES, Smith CE, Kessler RM, Zald DH (2010) Dopaminergic network differences in human impulsivity. Science 329: 532.
Burns M, Domjan M (1996) Sign tracking versus goal tracking in the sexual conditioning of male Japanese quail (Coturnix japonica). J Exp Psychol Anim B 22: 297-306.
Buzsaki G (1982) The "Where is it?" reflex: Autoshaping the orienting response. J Exp Anal Behav 37: 461-484.
Caggiula AR, Donny EC, Palmatier MI, Liu X, Chaudhri N, Sved AF (2009) The role of nicotine in smoking: a dual-reinforcement model. Nebr Symp Motiv 55: 91-109.
Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, Hoffman A, Perkins KA, Sved AF (2001) Cue dependency of nicotine self-administration and smoking. Pharmacol Biochem Behav 70: 515-530.
Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, Hoffman A, Perkins KA, Sved AF (2002) Environmental stimuli promote the acquisition of nicotine self-administration in rats. Psychopharmacology (Berl) 163: 230-7.
Cain ME, Saucier DA, Bardo MT (2005) Novelty seeking and drug use: contribution of an animal model. Exp Clin Psychopharmacol 13: 367-75.
Cardinal RN, Everitt BJ (2004) Neural and psychological mechanisms underlying appetitive learning: links to drug addiction. Curr Opin Neurobiol 14: 156-62.
Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav R 26: 321-352.
Carpenter KM, Martinez D, Vadhan NP, Barnes-Holmes D, Nunes EV (2012) Measures of attentional bias and relational responding are associated with behavioral treatment outcome for cocaine dependence. Am J Drug Alcohol Abuse 38: 146-54.
Carpenter KM, Schreiber E, Church S, McDowell D (2006) Drug Stroop performance: relationships with primary substance of use and treatment outcome in a drug-dependent outpatient sample. Addict Behav 31: 174-81.
Carpenter MJ, Saladin ME, DeSantis S, Gray KM, LaRowe SD, Upadhyaya HP (2009) Laboratory-based, cue-elicited craving and cue reactivity as predictors of naturally occurring smoking behavior. Addict Behav 34: 536-41.
Carter BL, Tiffany ST (1999) Meta-analysis of cue-reactivity in addiction research. Addiction 94: 327-40.
Chang SE, Holland PC (2013) Effects of nucleus accumbens core and shell lesions on autoshaped lever-pressing. Behav Brain Res 256: 36-42.
Chang SE, Wheeler DS, Holland PC (2012) Roles of nucleus accumbens and basolateral amygdala in autoshaped lever pressing. Neurobiol Learn Mem 97: 441-51.
Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib M, Craven L, Palmatier MI, Liu X, Sved AF (2006) Operant responding for conditioned and unconditioned reinforcers in rats is differentially enhanced by the primary reinforcing and reinforcement-enhancing effects of nicotine. Psychopharmacology (Berl) 189: 27-36.
Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib M, Craven L, Palmatier MI, Liu X, Sved AF (2007) Self-administered and noncontingent nicotine enhance reinforced operant responding in rats: impact of nicotine dose and reinforcement schedule. Psychopharmacology (Berl) 190: 353-62.

Chen R, Tilley MR, Wei H, Zhou F, Zhou FM, Ching S, Quan N, Stephens RL, Hill ER, Nottoli T, Han DD, Gu HH (2006) Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. Proc Natl Acad Sci U S A 103: 9333-8.
Childress AR, Ehrman RN, Wang Z, Li Y, Sciortino N, Hakun J, Jens W, Suh J, Listerud J, Marquez K, Franklin T, Langleben D, Detre J, O'Brien CP (2008) Prelude to passion: limbic activation by "unseen" drug and sexual cues. PLoS One 3: e1506.
Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156: 11-18.
Christiansen P, Cole JC, Goudie AJ, Field M (2012) Components of behavioural impulsivity and automatic cue approach predict unique variance in hazardous drinking. Psychopharmacology (Berl) 219: 501-10.
Ciccocioppo R, Martin-Fardon R, Weiss F (2004) Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. Nat Neurosci 7: 495-6.
Cleland GG, Davey GCL (1983) Autoshaping in the rat: The effects of localizable visual and auditory signals for food. J Exp Anal Behav 40: 47-56.
Cole PD, Adamo SA (2005) Cuttlefish (Sepia officinalis: Cephalopoda) hunting behavior and associative learning. Anim Cogn 8: 27-30.
Cooper A, Barnea-Ygael N, Levy D, Shaham Y, Zangen A (2007) A conflict rat model of cue-induced relapse to cocaine seeking. Psychopharmacology (Berl) 194: 117-125.
Copersino ML, Serper MR, Vadhan N, Goldberg BR, Richarme D, Chou JC, Stitzer M, Cancro R (2004) Cocaine craving and attentional bias in cocaine-dependent schizophrenic patients. Psychiatry Res 128: 209-18.
Corbit LH, Janak PH (2007) Ethanol-associated cues produce general pavlovian-instrumental transfer. Alcohol Clin Exp Res 31: 766-74.
Cornell CE, Rodin J, Weingarten H (1989) Stimulus-induced eating when satiated. Physiol Behav 45: 695-704.
Cousijn J, Goudriaan AE, Wiers RW (2011) Reaching out towards cannabis: approach-bias in heavy cannabis users predicts changes in cannabis use. Addiction 106: 1667-74.
Cox WM, Fadardi JS, Pothos EM (2006) The addiction-stroop test: Theoretical considerations and procedural recommendations. Psychol Bull 132: 443-76.
Cox WM, Hogan LM, Kristian MR, Race JH (2002) Alcohol attentional bias as a predictor of alcohol abusers' treatment outcome. Drug Alcohol Depend 68: 237-43.
Crombag HS, Badiani A, Maren S, Robinson TE (2000) The role of contextual versus discrete drug-associated cues in promoting the induction of psychomotor sensitization to intravenous amphetamine. Behav Brain Res 116: 1-22.
Crombag HS, Bossert JM, Koya E, Shaham Y (2008) Review. Context-induced relapse to drug seeking: a review. Philos Trans R Soc Lond B Biol Sci 363: 3233-43.
Crombag HS, Shaham Y (2002) Renewal of drug seeking by contextual cues after prolonged extinction in rats. Behav Neurosci 116: 169-73.
Cunningham CL, Niehus JS, Noble D (1993) Species difference in sensitivity to ethanol's hedonic effects. Alcohol 10: 97-102.
Dalley JW, Chudasama Y, Theobald DE, Pettifer CL, Fletcher CM, Robbins TW (2002) Nucleus accumbens dopamine and discriminated approach learning: interactive effects of 6-hydroxydopamine lesions and systemic apomorphine administration. Psychopharmacology (Berl) 161: 425-33.

Danna CL, Elmer GI (2010) Disruption of conditioned reward association by typical and atypical antipsychotics. Pharmacol Biochem Behav 96: 40-7.
Davey GC, Cleland GG (1982) Topography of signal-centered behavior in the rat: Effects of deprivation state and reinforcer type. J Exp Anal Behav 38: 291-304.
Davis WM, Smith SG (1976) Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. Pavlovian J Biol Sci 11: 222-36.
Day JJ, Roitman MF, Wightman RM, Carelli RM (2007) Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci 10: 1020-8.
de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl) 75: 134-143.
de Wit H, Uhlenhuth EH, Pierri J, Johanson CE (1987) Individual differences in behavioral and subjective responses to alcohol. Alcohol Clin Exp Res 11: 52-9.
Deroche-Gamonet V, Piat F, Le Moal M, Piazza PV (2002) Influence of cue-conditioning on acquisition, maintenance and relapse of cocaine intravenous self-administration. Eur J Neurosci 15: 1363-70.
Di Chiara G (1998) A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. J Psychopharmacol 12: 54-67.
Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (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: 9471-7.
Di Ciano P, Everitt BJ (2004) Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: Implications for the persistence of addictive behaviour. Neuropharmacology 47: 202-213.
Dickinson A, Balleine B (2002) The role of learning in the operation of motivational systems. In: Pashler H, Gallistel R (eds) Stevens' handbook of experimental psychology: Learning, motivation, and emotion. John Wiley & Sons, New York, pp 497-533
Dickinson A, Smith J, Mirenowicz J (2000) Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. Behav Neurosci 114: 468.
DiFeliceantonio AG, Berridge KC (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.
DiFeliceantonio AG, Mabrouk OS, Kennedy RT, Berridge KC (2012) Enkephalin surges in dorsal neostriatum as a signal to eat. Curr Biol 22: 1918-24.
Donny EC, Chaudhri N, Caggiula AR, Evans-Martin FF, Booth S, Gharib MA, Clements LA, Sved AF (2003) Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. Psychopharmacology (Berl) 169: 68-76.
Dragunow M, Faull R (1989) The use of c-fos as a metabolic marker in neuronal pathway tracing. J Neurosci Methods 29: 261-5.
Duka T, Townshend JM (2004) The priming effect of alcohol pre-load on attentional bias to alcohol-related stimuli. Psychopharmacology (Berl) 176: 353-61.
Ehrman RN, Robbins SJ, Childress AR, O'Brien CP (1992) Conditioned responses to cocaine-related stimuli in cocaine abuse patients. Psychopharmacology (Berl) 107: 523-9.
Ersche KD, Turton AJ, Pradhan S, Bullmore ET, Robbins TW (2010) Drug addiction endophenotypes: impulsive versus sensation-seeking personality traits. Biol Psychiatry 68: 770-3.

Estes WK (1943) Discriminative conditioning. I. A discriminative property of conditioned anticipation. J Exp Psychol 32: 150.
Estes WK (1948) Discriminative conditioning. II. Effects of a Pavlovian conditioned stimulus upon a subsequently established operant response. J Exp Psychol 38: 173.
Ettenberg A, Pettit HO, Bloom FE, Koob GF (1982) Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology (Berl) 78: 204-9.
Everitt BJ, Dickinson A, Robbins TW (2001) The neuropsychological basis of addictive behaviour. Brain Res Rev 36: 129-138.
Everitt BJ, Robbins TW (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 BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8: 1481-1489.
Fagen ZM, Mansvelder HD, Keath JR, McGehee DS (2003) Short- and long-term modulation of synaptic inputs to brain reward areas by nicotine. Ann N Y Acad Sci 1003: 185-95.
Fantino E (1977) Conditioned reinforcement: Choice and information. In: Honig WK, Staddon JER (eds) Handbook of operant behavior. Prentice-Hall, Englewood Cliffs, NJ, pp 313-339
Fantino E (2008) Choice, conditioned reinforcement, and the prius effect. Behav Anal 31: 95-111.
Feltenstein MW, See RE (2006) Potentiation of cue-induced reinstatement of cocaine-seeking in rats by the anxiogenic drug yohimbine. Behav Brain Res 174: 1-8.
Field M, Cox WM (2008) Attentional bias in addictive behaviors: a review of its development, causes, and consequences. Drug Alcohol Depend 97: 1-20.
Field M, Kiernan A, Eastwood B, Child R (2008) Rapid approach responses to alcohol cues in heavy drinkers. J Behav Ther Exp Psychiatry 39: 209-18.
Field M, Mogg K, Bradley BP (2005) Craving and cognitive biases for alcohol cues in social drinkers. Alcohol Alcoholism 40: 504-10.
Fitzpatrick CJ, Gopalakrishnan S, Cogan ES, Yager LM, Meyer PJ, Lovic V, Saunders BT, Parker CC, Gonzales NM, Aryee E, Flagel SB, Palmer AA, Robinson TE, Morrow JD (2013) Variation in the form of Pavlovian conditioned approach behavior among outbred male Sprague-Dawley rats from different vendors and colonies: sign-tracking vs. goal-tracking. PLoS One 8: e75042.
Flagel SB, Akil H, Robinson TE (2009) Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. Neuropharmacology 56: 139-148.
Flagel SB, Cameron CM, Pickup KN, Watson SJ, Akil H, Robinson TE (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 SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA, Clinton SM, Phillips PEM, Akil H (2011b) A selective role for dopamine in stimulus-reward learning. Nature 469: 53-57.
Flagel SB, Robinson TE, Clark JJ, Clinton SM, Watson SJ, Seeman P, Phillips PEM, Akil H (2010) An animal model of genetic vulnerability to behavioral disinhibition and

responsiveness to reward-related cues: Implications for addiction. Neuropsychopharmacol 35: 388-400.
Flagel SB, Watson SJ, Akil H, Robinson TE (2008) Individual differences in the attribution of incentive salience to a reward-related cue: Influence on cocaine sensitization. Behav Brain Res 186: 48-56.
Flagel SB, Watson SJ, Robinson TE, 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.
Franken IH (2003) Drug craving and addiction: integrating psychological and neuropsychopharmacological approaches. Prog Neuropsychopharmacol Biol Psychiatry 27: 563-79.
Franken IH, 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 IH, Hendriks VM, Stam CJ, Van den Brink W (2004) A role for dopamine in the processing of drug cues in heroin dependent patients. Eur Neuropsychopharmacol 14: 503-8.
Franken IH, Kroon LY, Hendriks VM (2000) Influence of individual differences in craving and obsessive cocaine thoughts on attentional processes in cocaine abuse patients. Addict Behav 25: 99-102.
Franklin TR, Lohoff FW, Wang Z, Sciortino N, Harper D, Li Y, Jens W, Cruz J, Kampman K, Ehrman R, Berrettini W, Detre JA, O'Brien CP, Childress AR (2009) DAT genotype modulates brain and behavioral responses elicited by cigarette cues. Neuropsychopharmacol 34: 717-728.
Franklin TR, Wang Z, Li Y, Suh JJ, Goldman M, Lohoff FW, Cruz J, Hazan R, Jens W, Detre JA, Berrettini W, O'Brien CP, Childress AR (2011) Dopamine transporter genotype modulation of neural responses to smoking cues: confirmation in a new cohort. Addict Biol 16: 308-22.
Fuchs RA, Branham RK, See RE (2006) Different neural substrates mediate cocaine seeking after abstinence versus extinction training: a critical role for the dorsolateral caudate-putamen. J Neurosci 26: 3584-8.
Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE (2005) The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacol 30: 296-309.
Fuchs RA, Evans KA, Parker MC, See RE (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: 459-65.
Fuchs RA, See RE (2002) Basolateral amygdala inactivation abolishes conditioned stimulus- and heroin-induced reinstatement of extinguished heroin-seeking behavior in rats. Psychopharmacology (Berl) 160: 425-33.
Gallagher M, Graham PW, Holland PC (1990) The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior. J Neurosci 10: 1906-11.
Gamzu E, Schwam E (1974) Autoshaping and automaintenance of a key-press response in squirrel monkeys. J Exp Anal Behav 21: 361-71.
Glasner SV, Overmier JB, Balleine BW (2005) The role of Pavlovian cues in alcohol seeking in dependent and nondependent rats. J Stud Alcohol 66: 53-61.

Goldberg SR (1973) Comparable behavior maintained under fixed-ratio and second-order schedules of food presentation, cocaine injection or d-amphetamine injection in the squirrel monkey. J Pharmacol Exp Ther 186: 18-30.
Goldberg SR, Tang AH (1977) Behavior maintained under second-order schedules of intravenous morphine injection in squirrel and rhesus monkeys. Psychopharmacology (Berl) 51: 235-42.
Goldstein RZ, Tomasi D, Alia-Klein N, Honorio Carrillo J, Maloney T, Woicik PA, Wang R, Telang F, Volkow ND (2009) Dopaminergic response to drug words in cocaine addiction. J Neurosci 29: 6001-6.
Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, Phillips RL, Kimes AS, Margolin A (1996) Activation of memory circuits during cue-elicited cocaine craving. Proc Natl Acad Sci U S A 93: 12040-5.
Grastyan E, Buzsaki G (1979) The orienting-exploratory response hypothesis of discriminative conditioning. Acta Neurobiol Exp (Wars) 39: 491-501.
Grastyan E, Vereczkei L (1974) Effects of spatial separation of the conditioned signal from the reinforcement: A demonstration of the conditioned character of the orienting response or the orientational character of conditioning. Behav Biol 10: 121-146.
Guy EG, Fletcher PJ (2013) The effects of nicotine exposure during Pavlovian conditioning in rats on several measures of incentive motivation for a conditioned stimulus paired with water. Psychopharmacology (Berl): In Press.
Haidar SH, Moreton JE, Liang Z, Hoke JF, Muir KT, Eddington ND (1997) Evaluating a possible pharmacokinetic interaction between remifentanil and esmolol in the rat. J Pharm Sci 86: 1278-82.
Han JS, McMahan RW, Holland P, Gallagher M (1997) The role of an amygdalo-nigrostriatal pathway in associative learning. J Neurosci 17: 3913-9.
Harris JA, Andrew BJ, Kwok DW (2013) Magazine approach during a signal for food depends on Pavlovian, not instrumental, conditioning. J Exp Psychol Anim B 39: 107-16.
Hasselmo ME, Sarter M (2010) Modes and Models of Forebrain Cholinergic Neuromodulation of Cognition. Neuropsychopharmacol 36: 52-73.
Hearst E, Jenkins HM (1974) Sign Tracking: the stimulus-reinforcer relation and directed action. Monograph of the Psychonomic Society, Austin
Hester R, Dixon V, Garavan H (2006) A consistent attentional bias for drug-related material in active cocaine users across word and picture versions of the emotional Stroop task. Drug Alcohol Depend 81: 251-7.
Hickey C, Chelazzi L, Theeuwes J (2010a) Reward changes salience in human vision via the anterior cingulate. J Neurosci 30: 11096-103.
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. Curr Biol 22: R219-20.
Holland PC (1977) Conditioned stimulus as a determinant of form of Pavlovian conditioned response. J Exp Psychol Anim B 3: 77-104.
Holland PC (1980) Infuence of visual conditioned stimulus characteristics on the form of Pavlovian appetitive conditioned responding in rats. J Exp Psychol Anim B 6: 81-97.
Hollis KL (1982) Pavlovian conditioning of signal-centered action patterns and autonomic behavior: A biological analysis of function. In: Rosenblatt JS (ed) Advances in the Study of Behavior. Academic Press, New York, pp 1-63

Holmes NM, Marchand AR, Coutureau E (2010) Pavlovian to instrumental transfer: a neurobehavioural perspective. Neurosci Biobehav R 34: 1277-95.
Hull CL (1943) Principles of behavior: An introduction to behavior theory. Appleton-Century, Oxford, England
James MK, Feldman PL, Schuster SV, Bilotta JM, Brackeen MF, Leighton HJ (1991) Opioid receptor activity of GI 87084B, a novel ultra-short acting analgesic, in isolated tissues. J Pharmacol Exp Ther 259: 712-8.
Janes AC, Pizzagalli DA, Richardt S, Frederick Bde B, Holmes AJ, Sousa J, Fava M, Evins AE, Kaufman MJ (2010) Neural substrates of attentional bias for smoking-related cues: an FMRI study. Neuropsychopharmacol 35: 2339-45.
Jansen A (1998) A learning model of binge eating: Cue reactivity and cue exposure. Behav Res Ther 36: 257-272.
Jentsch JD, Taylor JR (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology 146: 373-390.
Johnson SW, North RA (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 12: 483-8.
Johnson WG (1974) Effect of cue prominence and subject weight on human food-directed performance. J Pers Soc Psychol 29: 843-848.
Jones GH, Robbins TW (1992) Differential effects of mesocortical, mesolimbic, and mesostriatal dopamine depletion on spontaneous, conditioned, and drug-induced locomotor activity. Pharmacol Biochem Behav 43: 887-95.
Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162: 1403-13.
Kambouropoulos N, Staiger PK (2001) The influence of sensitivity to reward on reactivity to alcohol-related cues. Addiction 96: 1175-85.
Kantak KM, Black Y, Valencia E, Green-Jordan K, Eichenbaum HB (2002) Dissociable effects of lidocaine inactivation of the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. J Neurosci 22: 1126-1136.
Katz JL (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: 419-31.
Kearns DN, Weiss SJ (2004) Sign-tracking (autoshaping) in rats: A comparison of cocaine and food as unconditioned stimuli. Learn Behav 32: 463-476.
Kelleher RT, Gollub LR (1962) A review of positive conditioned reinforcement. J Exp Anal Behav 5: 543-597.
Kelley AE (2004) Memory and addiction: Shared neural circuitry and molecular mechanisms. Neuron 44: 161-179.
Kelley AE, Schiltz CA, Landry CF (2005) Neural systems recruited by drug- and food-related cues: Studies of gene activation in corticolimbic regions. Physiol Behav 86: 11-14.
Kest K, Cruz I, Chen DH, Galaj E, Ranaldi R (2012) A food-associated CS activates c-Fos in VTA DA neurons and elicits conditioned approach. Behav Brain Res 235: 150-7.
Killeen PR (2003) Complex dynamic processes in sign tracking with an omission contingency (negative automaintenance). J Exp Psychol Anim B 29: 49-61.

Kilts CD, Kennedy A, Elton AL, Tripathi SP, Young J, Cisler JM, James GA (2014) Individual Differences in Attentional Bias Associated with Cocaine Dependence Are Related to Varying Engagement of Neural Processing Networks. Neuropsychopharmacol 39: 1135-1147.
Ko MC, Terner J, Hursh S, Woods JH, Winger G (2002) Relative reinforcing effects of three opioids with different durations of action. J Pharmacol Exp Ther 301: 698-704.
Konorski J (1967) Integrative activity of the brain. An interdisciplinary approach. University of Chicago Press, Chicago
Kovacs KJ (1998) c-Fos as a transcription factor: a stressful (re)view from a functional map. Neurochem Int 33: 287-97.
Krank MD (2003) Pavlovian conditioning with ethanol: Sign-tracking (autoshaping), conditioned incentive, and ethanol self-administration. Alcohol Clin Exp Res 27: 1592-1598.
Krank MD, 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.
Kreek MJ, Nielsen DA, Butelman ER, LaForge KS (2005) Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nat Neurosci 8: 1450-7.
Kruzich PJ, Congleton KM, See RE (2001) Conditioned reinstatement of drug-seeking behavior with a discrete compound stimulus classically conditioned with intravenous cocaine. Behav Neurosci 115: 1086-1092.
Kufahl PR, Zavala AR, Singh A, Thiel KJ, Dickey ED, Joyce JN, Neisewander JL (2009) c-Fos expression associated with reinstatement of cocaine-seeking behavior by response-contingent conditioned cues. Synapse 63: 823-835.
Kuhn S, Gallinat J (2011) A quantitative meta-analysis on cue-induced male sexual arousal. J Sex Med 8: 2269-75.
Lajoie J, Bindra D (1976) An interpretation of autoshaping and related phenomena in terms of stimulus-incentive contingencies alone. Can J Psychology 30: 157-173.
Le Foll B, Goldberg SR (2006) Nicotine as a typical drug of abuse in experimental animals and humans. Psychopharmacology (Berl) 184: 367-81.
LeBlanc KH, Ostlund SB, Maidment NT (2012) Pavlovian-to-instrumental transfer in cocaine seeking rats. Behav Neurosci 126: 681-9.
Levine AI, Bryson EO (2010) Intranasal self-administration of remifentanil as the foray into opioid abuse by an anesthesia resident. Anesth Analg 110: 524-5.
Leyton M (2007) Conditioned and sensitized responses to stimulant drugs in humans. Prog Neuropsychopharmacol Biol Psychiatry 31: 1601-13.
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. Neuropsychopharmacol 27: 1027-35.
Liu X, Palmatier MI, Caggiula AR, Donny EC, Sved AF (2007) Reinforcement enhancing effect of nicotine and its attenuation by nicotinic antagonists in rats. Psychopharmacology (Berl) 194: 463-73.
Locurto C, Terrace HS, Gibbon J (1976) Autoshaping, random control, and omission training in the rat. J Exp Anal Behav 26: 451-62.

Lomanowska AM, Lovic V, Rankine MJ, Mooney SJ, Robinson TE, Kraemer GW (2011) Inadequate early social experience increases the incentive salience of reward-related cues in adulthood. Behav Brain Res 220: 91-99.
Lovibond PF (1983) Facilitation of instrumental behavior by a pavlovian appetitive conditioned - stimulus. J Exp Psychol Anim B 9: 225-247.
Lovic V, Saunders BT, Yager LM, Robinson TE (2011) Rats prone to attribute incentive salience to reward cues are also prone to impulsive action. Behav Brain Res 223: 255-261.
Luscher C, Ungless MA (2006) The mechanistic classification of addictive drugs. PLoS Med 3: e437.
Mackintosh NJ (1974) The Psychology of Animal Learning. Academic Press, London
Madsen HB, Ahmed SH (2014) Drug versus sweet reward: greater attraction to and preference for sweet versus drug cues. Addict Biol: In Press.
Mahler SV, Berridge KC (2009) Which cue to "want?" Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue. J Neurosci 29: 6500-13.
Mahler SV, Berridge KC (2012) What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex. Psychopharmacology (Berl) 221: 407-26.
Mahler SV, de Wit H (2010) Cue-reactors: Individual differences in cue-induced craving after food or smoking abstinence. PLoS One 5: e15475.
Marissen MA, Franken IH, Waters AJ, Blanken P, van den Brink W, Hendriks VM (2006) Attentional bias predicts heroin relapse following treatment. Addiction 101: 1306-12.
Mayo LM, Fraser D, Childs E, Momenan R, Hommer DW, de Wit H, Heilig M (2013) Conditioned preference to a methamphetamine-associated contextual cue in humans. Neuropsychopharmacol 38: 921-9.
McClernon FJ, Hutchison KE, Rose JE, Kozink RV (2007) DRD4 VNTR polymorphism is associated with transient fMRI-BOLD responses to smoking cues. Psychopharmacology (Berl) 194: 433-41.
McClernon FJ, Kozink RV, Lutz AM, Rose JE (2009) 24-h smoking abstinence potentiates fMRI-BOLD activation to smoking cues in cerebral cortex and dorsal striatum. Psychopharmacology (Berl) 204: 25-35.
McDannald M, Kerfoot E, Gallagher M, Holland PC (2004) Amygdala central nucleus function is necessary for learning but not expression of conditioned visual orienting. Eur J Neurosci 20: 240-8.
McFarland K, Ettenberg A (1997) Reinstatement of drug-seeking behavior produced by heroin-predictive environmental stimuli. Psychopharmacology (Berl) 131: 86-92.
Meil WM, See RE (1997) Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. Behav Brain Res 87: 139-148.
Meyer PJ, Cogan ES, Robinson TE (2014) The conditioned stiumulus as a determinant of the incentive properties of reward cues. PLoS One: Under Revision.
Meyer PJ, Lovic V, Saunders BT, Yager LM, Flagel SB, Morrow JD, Robinson TE (2012a) Quantifying individual variation in the propensity to attribute incentive salience to reward cues. PLoS One 7: e38987.
Meyer PJ, Ma ST, Robinson TE (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.

Michelsen LG, Hug CC, Jr. (1996) The pharmacokinetics of remifentanil. J Clin Anesth 8: 679-82.
Milton AL (2012) Drink, drugs and disruption: memory manipulation for the treatment of addiction. Curr Opin Neurobiol 23: 706-12.
Milton AL, Everitt BJ (2010) The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. Eur J Neurosci 31: 2308-2319.
Moeller SJ, Maloney T, Parvaz MA, Dunning JP, Alia-Klein N, Woicik PA, Hajcak G, Telang F, Wang GJ, Volkow ND, Goldstein RZ (2009) Enhanced choice for viewing cocaine pictures in cocaine addiction. Biol Psychiatry 66: 169-76.
Molander AC, Mar A, Norbury A, Steventon S, Moreno M, Caprioli D, Theobald DE, Belin D, Everitt BJ, Robbins TW, Dalley JW (2011) High impulsivity predicting vulnerability to cocaine addiction in rats: some relationship with novelty preference but not novelty reactivity, anxiety or stress. Psychopharmacology (Berl) 215: 721-31.
Montague PR, Dayan P, Sejnowski TJ (1996) A framework for mesencephalic dopamine systems based on predictive Hebbian learning. J Neurosci 16: 1936-47.
Morrow JD, Maren S, Robinson TE (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: 238-43.
Nair SG, Adams-Deutsch T, Epstein DH, 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.
Nestler EJ (2005) Is there a common molecular pathway for addiction? Nat Neurosci 8: 1445-9.
Niaura R, Shadel WG, Abrams DB, Monti PM, Rohsenow DJ, Sirota A (1998) Individual differences in cue reactivity among smokers trying to quit: effects of gender and cue type. Addict Behav 23: 209-24.
Nie H, Janak PH (2003) Comparison of reinstatement of ethanol- and sucrose-seeking by conditioned stimuli and priming injections of allopregnanolone after extinction in rats. Psychopharmacology (Berl) 168: 222-8.
Nilsson J, Kristiansen TS, Fosseidengen JE, Ferno A, van den Bos R (2008) Sign- and goal-tracking in Atlantic cod (Gadus morhua). Anim Cogn 11: 651-9.
Nonkes LJ, van de V, II, Homberg JR (2014) The attribution of incentive salience to an appetitive conditioned cue is not affected by knockout of the serotonin transporter in rats. Behav Brain Res 259: 268-73.
O'Brien CP, Childress AR, McLellan AT, Ehrman R (1992) Classical conditioning in drug-dependent humans. Ann N Y Acad Sci 654: 400-15.
O'Connor EC, Crombag HS, Mead AN, Stephens DN (2010) The mGluR5 antagonist MTEP dissociates the acquisition of predictive and incentive motivational properties of reward-paired stimuli in mice. Neuropsychopharmacol 35: 1807-17.
Olausson P, Jentsch JD, Taylor JR (2004) Nicotine enhances responding with conditioned reinforcement. Psychopharmacology (Berl) 171: 173-8.
Olmstead MC, Parkinson JA, Miles FJ, Everitt BJ, Dickinson A (2000) Cocaine-seeking by rats: regulation, reinforcement and activation. Psychopharmacology (Berl) 152: 123-31.
Olsen CM, Winder DG (2009) Operant sensation seeking engages similar neural substrates to operant drug seeking in C57 mice. Neuropsychopharmacol 34: 1685-94.

Ostrander MM, Badiani A, Day HEW, Norton CS, Watson SJ, Akil H, Robinson TE (2003) Environmental context and drug history modulate amphetamine-induced C-FOS mRNA expression in the basal ganglia, central extended amygdala and associated limbic forebrain. Neuroscience 120: 551-571.

Overton P, Dean P (1988) Detection of visual stimuli after lesions of the superior colliculus in the rat; deficit not confined to the far periphery. Behav Brain Res 31: 1-15.

Palfai TP (2006) Activating action tendencies: The influence of action priming on alcohol consumption among male hazardous drinkers. J Stud Alcohol 67: 926-33.

Palmatier MI, Coddington SB, Liu X, Donny EC, Caggiula AR, Sved AF (2008) The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose. Neuropharmacology 55: 1425-30.

Palmatier MI, Liu X, Matteson GL, Donny EC, Caggiula AR, Sved AF (2007) Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. Psychopharmacology (Berl) 195: 235-43.

Palmatier MI, Marks KR, Jones SA, Freeman KS, Wissman KM, Sheppard AB (2013) The effect of nicotine on sign-tracking and goal-tracking in a Pavlovian conditioned approach paradigm in rats. Psychopharmacology (Berl) 226: 247-59.

Palmatier MI, O'Brien LC, Hall MJ (2012) The role of conditioning history and reinforcer strength in the reinforcement enhancing effects of nicotine in rats. Psychopharmacology (Berl) 219: 1119-31.

Panlilio LV, Schindler CW (2000) Self-administration of remifentanil, an ultra-short acting opioid, under continuous and progressive-ratio schedules of reinforcement in rats. Psychopharmacology (Berl) 150: 61-6.

Panlilio LV, Yasar S, Nemeth-Coslett R, Katz JL, Henningfield JE, Solinas M, Heishman SJ, Schindler CW, Goldberg SR (2005) Human cocaine-seeking behavior and its control by drug-associated stimuli in the laboratory. Neuropsychopharmacol 30: 433-43.

Paolone G, Angelakos CC, Meyer PJ, Robinson TE, Sarter M (2013) Cholinergic control over attention in rats prone to attribute incentive salience to reward cues. J Neurosci 33: 8321-35.

Parikh V, Kozak R, Martinez V, Sarter M (2007) Prefrontal acetylcholine release controls cue detection on multiple timescales. Neuron 56: 141-54.

Parker JG, Zweifel LS, Clark JJ, Evans SB, Phillips PE, Palmiter RD (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: 13491-6.

Parkinson J, Robbins T, Everitt B (1999a) Selective excitotoxic lesions of the nucleus accumbens core and shell differentially affect aversive Pavlovian conditioning to discrete and contextual cues. Psychobiology 27: 256-266.

Parkinson JA, Dalley JW, Cardinal RN, Bamford A, Fehnert B, Lachenal G, Rudarakanchana N, Halkerston KM, Robbins TW, Everitt BJ (2002) Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behaviour: implications for mesoaccumbens dopamine function. Behav Brain Res 137: 149-63.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999b) 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. J Neurosci 19: 2401-2411.

Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ (2000) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. Behav Neurosci 114: 42-63.
Pavlov IP (1927) Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Oxford University Press, London
Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th edn. Academic Press, San Diego
Pelchat ML, Johnson A, Chan R, Valdez J, Ragland JD (2004) Images of desire: food-craving activation during fMRI. Neuroimage 23: 1486-93.
Peters J, De Vries TJ (2013) Pavlovian conditioned approach, extinction, and spontaneous recovery to an audiovisual cue paired with an intravenous heroin infusion. Psychopharmacology (Berl) 231: 447-53.
Peterson GB, Ackil JE, Frommer GP, Hearst ES (1972) Conditioned approach and contact behavior toward signals for food or brain-stimulation reinforcement. Science 177: 1009-1011.
Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berl) 84: 167-73.
Pidoplichko VI, DeBiasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. Nature 390: 401-4.
Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav R 30: 215-38.
Pithers R (1985) The roles of event contingencies and reinforcement in human autoshaping and omission responding. Learn Motiv 16: 210-237.
Pool E, Brosch T, Delplanque S, Sander D (2014) Where is the chocolate? Rapid spatial orienting toward stimuli associated with primary rewards. Cognition 130: 348-59.
Puglisi-Allegra S, Ventura R (2012a) Prefrontal/accumbal catecholamine system processes emotionally driven attribution of motivational salience. Rev Neurosci 23: 509-26.
Puglisi-Allegra S, Ventura R (2012b) Prefrontal/accumbal catecholamine system processes high motivational salience. Front Behav Neurosci 6: 31.
Rescorla RA (1988) Pavlovian conditioining- It's not what you think it is. Am Psychol 43: 151-160.
Rice ME, Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. Nat Neurosci 7: 583-4.
Robbins TW (1978) The acquisition of responding with conditioned reinforcement: effects of pipradrol, methylphenidate, d-amphetamine, and nomifensine. Psychopharmacology (Berl) 58: 79-87.
Roberts DC, Koob GF, Klonoff P, Fibiger HC (1980) Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol Biochem Behav 12: 781-7.
Robinson TE, Berridge KC (1993) The neural basis of drug craving- An incentive-sensitization theory of addiction. Brain Res Rev 18: 247-291.
Robinson TE, Flagel SB (2009) Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. Biol Psychiatry 65: 869-873.

Robinson TE, Yager LM, Cogan ES, Saunders BT (2014) On the motivational properties of reward cues: individual differences. Neuropharmacology 76 PtB: 450-9.
Rosenberg H (2009) Clinical and laboratory assessment of the subjective experience of drug craving. Clin Psychol Rev 29: 519-534.
Sagar SM, Sharp FR, Curran T (1988) Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 240: 1328-31.
Saunders BT, Aurbach EL, Robinson TE (2012) Individual variation in the influence of a cocaine-associated context on behavior 2012 Neuroscience Meeting Planner. Society for Neuroscience
Saunders BT, Robinson TE (2010) A cocaine cue acts as an incentive stimulus in some but not others: Implications for addiction. Biol Psychiatry 67: 730-736.
Saunders BT, Robinson TE (2011) Individual variation in the motivational properties of cocaine. Neuropsychopharmacol 36: 1668-1676.
Saunders BT, Robinson TE (2012) The role of dopamine in the accumbens core in the expression of Pavlovian-conditioned responses. Eur J Neurosci 36: 2521-32.
Saunders BT, Robinson TE (2013) Individual variation in resisting temptation: implications for addiction. Neurosci Biobehav R 37: 1955-75.
Saunders BT, Yager LM, Robinson TE (2013) Cue-evoked cocaine "craving": role of dopamine in the accumbens core. J Neurosci 33: 13989-4000.
Schachter S (1968) Obesity and eating- Internal and external cues differentially affect eating behavior of obese and normal subjects. Science 161: 751-756.
Schenk S, Partridge B (2001) Influence of a conditioned light stimulus on cocaine self-adiminstration in rats. Psychopharmacology (Berl) 154: 390-396.
Schiltz CA, Bremer QZ, Landry CF, Kelley AE (2007) Food-associated cues alter forebrain functional connectivity as assessed with immediate early gene and proenkephalin expression. BMC Biology 5.
Schindler CW, Panlilio LV, Goldberg SR (2002) Second-order schedules of drug self-administration in animals. Psychopharmacology (Berl) 163: 327-44.
Schoenbaum G, Shaham Y (2008) The role of orbitofrontal cortex in drug addiction: a review of preclinical studies. Biol Psychiatry 63: 256-62.
Schoenmakers T, Wiers R, Field M (2008) Effects of a low dose of alcohol on cognitive biases and craving in heavy drinkers. Psychopharmacology 197: 169-178.
Schroeder BE, Binzak JM, Kelley AE (2001) A common profile of prefrontal cortical activation following exposure to nicotine- or chocolate-associated contextual cues. Neuroscience 105: 535-45.
Schroeder BE, Holahan MR, Landry CF, Kelley AE (2000) Morphine-associated environmental cues elicit conditioned gene expression. Synapse 37: 146-58.
Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80: 1-27.
Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275: 1593-9.
See RE (2002) Neural substrates of conditioned-cued relapse to drug-seeking behavior. Pharmacol Biochem Behav 71: 517-529.
See RE (2005) Neural substrates of cocaine-cue associations that trigger relapse. Eur J Pharmacol 526: 140-146.
Shaham Y, Shalev U, Lu L, de Wit H, Stewart J (2003) The reinstatement model of drug relapse: History, methodology and major findings. Psychopharmacology (Berl) 168: 3-20.

Simon JJ, Walther S, Fiebach CJ, Friederich HC, Stippich C, Weisbrod M, Kaiser S (2010) Neural reward processing is modulated by approach- and avoidance-related personality traits. Neuroimage 49: 1868-74.
Sokolov EN (1963) Perception and the conditioned reflex. Pergamon Press, New York
Sorge RE, Pierre VJ, Clarke PB (2009) Facilitation of intravenous nicotine self-administration in rats by a motivationally neutral sensory stimulus. Psychopharmacology (Berl) 207: 191-200.
Spear DJ, Katz JL (1991) Cocaine and food as reinforcers: effects of reinforcer magnitude and response requirement under second-order fixed-ratio and progressive-ratio schedules. J Exp Anal Behav 56: 261-75.
Stewart J (1960) Reinforcing effects of light as a function of intensity and reinforcement schedule. J Comp Physiol Psychol 53: 187-193.
Stewart J, Dewit H, Eikelboom R (1984) Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 91: 251-268.
Styn MA, Bovbjerg DH, Lipsky S, Erblich J (2013) Cue-induced cigarette and food craving: A common effect? Addict Behav 38: 1840-1843.
Talmi D, Seymour B, Dayan P, Dolan RJ (2008) Human pavlovian-instrumental transfer. J Neurosci 28: 360-8.
Tang DW, Fellows LK, Small DM, Dagher A (2012) Food and drug cues activate similar brain regions: a meta-analysis of functional MRI studies. Physiol Behav 106: 317-24.
Tetley AC, Brunstrom JM, Griffiths PL (2010) The role of sensitivity to reward and impulsivity in food-cue reactivity. Eat Behav 11: 138-43.
Thewissen R, Havermans RC, Geschwind N, van den Hout M, Jansen A (2007) Pavlovian conditioning of an approach bias in low-dependent smokers. Psychopharmacology (Berl) 194: 33-9.
Thomas KL, Arroyo M, Everitt BJ (2003) Induction of the learning and plasticity-associated gene Zif268 following exposure to a discrete cocaine-associated stimulus. Eur J Neurosci 17: 1964-1972.
Thomas KL, Everitt BJ (2001) Limbic-cortical-ventral striatal activation during retrieval of a discrete cocaine-associated stimulus: A cellular imaging study with gamma protein kinase C expression. J Neurosci 21: 2526-35.
Thompson T, Pickens R (1969) Drug self-administration and conditioning. In: Steinberg H (ed) Scientific Basis of Drug Dependence. J&A Churchill, London, pp 177-198
Thompson T, Schuster CR (1964) Morphine self-administration, food-reinforced, and avoidance behaviors in rhesus monkeys. Psychopharmacologia 5: 87-94.
Timberlake W, Grant DL (1975) Auto-shaping in rats to presentation of another rat predicting food. Science 190: 690-692.
Timberlake W, Lucas GA (1985) The basis of superstitious behavior: chance contingency, stimulus substitution, or appetitive behavior? J Exp Anal Behav 44: 279-99.
Toates FM (1986) Motivational systems. Cambridge University Press, Cambridge, UK
Tomie A (1996) Locating reward cue at response manipulandum (CAM) induces symptoms of drug abuse. Neurosci Biobehav R 20: 505-535.
Tomie A (2001) Autoshaping and drug-taking. In: Mowrer RR, Klein SB (eds) Handbook of Contemporary Learning Theories. Lawrence Erlbaum Associates, Mahwah, pp 409-439

Tomie A, Aguado AS, Pohorecky LA, Benjamin D (1998) Ethanol induces impulsive-like responding in a delay-of-reward operant choice procedure: impulsivity predicts autoshaping. Psychopharmacology (Berl) 139: 376-82.
Tomie A, Aguado AS, Pohorecky LA, Benjamin D (2000) Individual differences in Pavlovian autoshaping of lever pressing in rats predict stress-induced corticosterone release and mesolimbic levels of monoamines. Pharmacol Biochem Behav 65: 509-517.
Tomie A, Festa ED, Sparta DR, Pohorecky LA (2003) Lever conditioned stimulus-directed autoshaping induced by saccharin-ethanol unconditioned stimulus solution: effects of ethanol concentration and trial spacing. Alcohol 30: 35-44.
Tomie A, Lincks M, Nadarajah SD, Pohorecky LA, 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: 571-8.
Tomie A, Tirado AD, Yu L, Pohorecky LA (2004) Pavlovian autoshaping procedures increase plasma corticosterone and levels of norepinephrine and serotonin in prefrontal cortex in rats. Behav Brain Res 153: 97-105.
Uslaner JM, Acerbo MJ, Jones SA, Robinson TE (2006) The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. Behav Brain Res 169: 320-324.
Vadhan NP, Carpenter KM, Copersino ML, Hart CL, Foltin RW, Nunes EV (2007) Attentional bias towards cocaine-related stimuli: relationship to treatment-seeking for cocaine dependence. Am J Drug Alcohol Abuse 33: 727-36.
Van Gucht D, Vansteenwegen D, Van den Bergh O, Beckers T (2008) Conditioned craving cues elicit an automatic approach tendency. Behav Res Ther 46: 1160-9.
Vander Weele CM, Lovic V, Aragona BJ (2011) Rapid dopamine signaling in response to a short-acting opiate, remifentanil 2011 Neuroscience Meeting Planner. Society for Neuroscience, Washington, D.C.
Verbeke G, Molenberghs G (2000) Linear Mixed Models for Longitudinal Data. Springer, New York
Volkow ND, Fowler JS, Wang GJ, Swanson JM (2004) Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Mol Psychiatry 9: 557-69.
Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Childress A-R, Jayne M, Ma Y, Wong C (2008a) Dopamine increases in striatum do not elicit craving in cocaine abusers unless they are coupled with cocaine cues. NeuroImage 39: 1266-1273.
Volkow ND, Wang GJ, Fowler JS, Logan J, Jayne M, Franceschi D, Wong C, Gatley SJ, Gifford AN, Ding YS, Pappas N (2002) "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. Synapse 44: 175-80.
Volkow ND, Wang GJ, Fowler JS, Telang F (2008b) Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. Philos Trans R Soc Lond B Biol Sci 363: 3191-200.
Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma YM, Wong C (2006) Cocaine cues and dopamine in dorsal striatum: Mechanism of craving in cocaine addiction. J Neurosci 26: 6583-6588.
Waelti P, Dickinson A, Schultz W (2001) Dopamine responses comply with basic assumptions of formal learning theory. Nature 412: 43-8.

Wassum KM, Ostlund SB, Balleine BW, Maidment NT (2011) Differential dependence of Pavlovian incentive motivation and instrumental incentive learning processes on dopamine signaling. Learn Mem 18: 475-83.
Wassum KM, Ostlund SB, Maidment NT, Balleine BW (2009) Distinct opioid circuits determine the palatability and the desirability of rewarding events. Proc Natl Acad Sci U S A 106: 12512-7.
Weiss F (2005) Neurobiology of craving, conditioned reward and relapse. Curr Opin Pharmacol 5: 9-19.
Wetherill RR, Childress AR, Jagannathan K, Bender J, Young KA, Suh JJ, O'Brien CP, Franklin TR (2014a) Neural responses to subliminally presented cannabis and other emotionally evocative cues in cannabis-dependent individuals. Psychopharmacology (Berl) 231: 1397-407.
Wetherill RR, Jagannathan K, Lohoff FW, Ehrman R, O'Brien CP, Childress AR, Franklin TR (2014b) Neural correlates of attentional bias for smoking cues: modulation by variance in the dopamine transporter gene. Addict Biol 19: 294-304.
Wiers RW, 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: 101-6.
Wilcove WG, Miller JC (1974) CS-USC presentations and a lever: human autoshaping. J Exp Psychol 103: 868-77.
Williams DR, Williams H (1969) Auto-maintenance in the pigeon: sustained pecking despite contingent non-reinforcement. J Exp Anal Behav 12: 511-20.
Wills TA, Vaccaro D, McNamara G (1994) Novelty seeking, risk taking, and related constructs as predictors of adolescent substance use: an application of Cloninger's theory. J Subst Abuse 6: 1-20.
Winstanley CA, Olausson P, Taylor JR, Jentsch JD (2010) Insight into the relationship between impulsivity and substance abuse from studies using animal models. Alcohol Clin Exp Res 34: 1306-18.
Wong DF, Kuwabara H, Schretlen DJ, Bonson KR, Zhou Y, Nandi A, Brasic JR, Kimes AS, Maris MA, Kumar A, Contoreggi C, Links J, Ernst M, Rousset O, Zukin S, Grace AA, Lee JS, Rohde C, Jasinski DR, Gjedde A, London ED (2006) Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. Neuropsychopharmacol 31: 2716-27.
Yager LM, Robinson TE (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 LM, Robinson TE (2013) A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue. Psychopharmacology (Berl) 226: 217-28.
Yin HH, Ostlund SB, Balleine BW (2008) Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. Eur J Neurosci 28: 1437-48.
Zavala AR, Osredkar T, Joyce JN, Neisewander JL (2008) Upregulation of Arc mRNA expression in the prefrontal cortex following cue-induced reinstatement of extinguished cocaine-seeking behavior. Synapse 62: 421-31.
Zener K (1937) The significance of behavior accompanying conditioned salivary secretion for theories of the conditioned response. Am J Psychol 50: 384-403.

Zhang H, Sulzer D (2004) Frequency-dependent modulation of dopamine release by nicotine. Nat Neurosci 7: 581-2.
Zhang J, Berridge KC, Tindell AJ, Smith KS, Aldridge JW (2009) A neural computational model of incentive salience. PLoS Comput Biol 5: e1000437.
Zhou L, Pruitt C, Shin CB, Garcia AD, Zavala AR, See RE (2013) Fos expression induced by cocaine-conditioned cues in male and female rats. Brain Struct Funct.
Zijlstra F, Veltman DJ, Booij J, van den Brink W, Franken IH (2009) Neurobiological substrates of cue-elicited craving and anhedonia in recently abstinent opioid-dependent males. Drug Alcohol Depend 99: 183-92.
Zombeck JA, Chen GT, Johnson ZV, Rosenberg DM, Craig AB, Rhodes JS (2008) Neuroanatomical specificity of conditioned responses to cocaine versus food in mice. Physiol Behav 93: 637-650.