Conditioned Reinforcing Effects of a Remifentanil-Paired Stimulus in the Rat

by

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DEDICATION

To all the rats, from PF-1 to RI-828 and counting.

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ABSTRACT

When environmental stimuli are paired with a primary reinforcer (e.g., food, certain drugs), these stimuli may become conditioned reinforcers capable of maintaining behavior in the absence of the primary reinforcer. Drug-associated conditioned reinforcers are thought to contribute significantly to human drug abuse and dependence; however, few studies have characterized specifically the conditioned reinforcing effects of drug-paired stimuli, controlling for other, confounding associative and nonassociative processes that can change behavior. The present experiments, therefore, assessed the conditioned reinforcing effects of a stimulus paired with the potent, short-acting *mu*-opioid agonist, remifentanil, using a behaviorally stringent new-response acquisition procedure. First, in Pavlovian conditioning (PAV) sessions, rats received response-independent IV injections of remifentanil and presentations of a light-noise stimulus. In paired PAV groups, injections and stimulus presentations always co-occurred. In random PAV control groups, injections and stimulus presentations occurred independently of each other. Next, in instrumental acquisition (ACQ) test sessions, rats could respond in an active nose-poke that produced the stimulus alone or an inactive nose-poke that had no scheduled consequences. Rats acquired nose-poking (i.e., active > inactive) after paired PAV, but not random PAV. These results show responding was (1) not due to association of the nose-poke with remifentail, (2) sensitive to the Pavlovian contingency between the stimulus and remifentanil, and (3) sensitive to the instrumental contingency between a nose-poke and the stimulus. After, thus, validating the behavioral procedure, the effects of the dopamine D3 receptor-preferring agonist, pramipexole, on responding with the remifentanil-paired stimulus was assessed. Dopamine D2-like receptor agonists can enhance new-response acquisition with food-paired conditioned

reinforcers, but this effect has not, to my knowledge, been previously demonstrated with drug-paired stimuli. When pretreatments of saline or pramipexole were given before ACQ sessions, pramipexole dose-dependently increased active responding without changing inactive responding. Control animals given pramixpole after random PAV did not acquire nose-poking. The response-enhancing effects of pramipexole were attenuated by the D2 receptor-preferring antagonist, L-741,626, but not the D3 receptor-preferring antagonist, SB-277011A. D2 activity may, therefore, be particularly important for responding with conditioned reinforcement. Together, these experiments demonstrate that new-response acquisition can provide a valid, practically useful measure of opioid-associated conditioned reinforcement.

CHAPTER I

General Introduction

Primary and conditioned reinforcement

Many instances of human and non-human animal behavior are separated significantly from the delivery of a primary reinforcer such as food, water, or certain drugs. These separations can involve temporal delays and physical distance to the delivery of the primary reinforcer, as well as the interpositioning of other behaviors of various kinds between a target response and the primary reinforcer (e.g., in multi-operant sequences or chains, Thompson and Pickens 1969, Figure 9). It is difficult, therefore, for primary reinforcement contingencies alone to account for many instances of learning and much of the behavioral repertoire. Rather, the environmental stimuli that have been associated with a primary reinforcer may play a significant role in structuring behavior until that primary reinforcer is ultimately obtained (Fantino 2008; Goldberg 1975; Goldberg and Gardner 1981; Hull 1943; Keller and Schoenfeld 1950; Pierce and Cheney 2004; Skinner 1953; BA Williams 1994). One important way in which these environmental stimuli may influence behavior is by serving as conditioned reinforcers. Whereas both primary and conditioned reinforcers increase the frequency of the responses that produce them, conditioned reinforcers may be distinguished from primary reinforcers in that conditioned reinforcers are effective only after certain histories (e.g., Kelleher and Gollub 1962, p 545).

Without these histories, stimulus presentation will not be an effective instrumental reinforcer and will not strengthen the behaviors upon which it is contingent. Primary reinforcers do not have the same dependency on previous experience. This is not to say that no learning is needed before a primary reinforcer will be effective (Balleine 2005) or that different histories cannot affect performance with primary reinforcement (Campbell and Carroll 2000; Robinson and Berridge 2001; Young et al. 1981), but that the types of histories or particular operations that make for effective primary and conditioned reinforcers are different.

Although alternatives have been proposed (e.g., Fantino 2008), the operations that establish conditioned reinforcers have most often been described as "pairing" the stimulus with a primary reinforcer or having the stimulus "accompany" the primary reinforcer (e.g., Hull 1943, Chapter 7; Hendry 1969; Hyde 1976; Keller and Schoenfeld 1950, Chapter 8; O'Brien and Gardner 2005; Schindler et al. 2002; Shahan 2010; Wike 1966, § 1; BA Williams 1994). It is important to recognize, however, that a number of different behavioral processes, other than conditioned reinforcement, can influence the rate of responding when an animal makes a response that produces a stimulus after that stimulus has been paired with a primary reinforcer. These processes may be associative or nonassociative, and they may be related to exposure to the stimulus itself, exposure to the primary reinforcer itself, or to some other effect or aspect of the stimulus-reinforcer pairing (Cunningham 1993). For example, depending on the particular situation, responding may be altered by the primary reinforcing effects of the stimulus (i.e., sensory reinforcement), discriminative effects of the stimulus, unconditioned effects of primary reinforcer or stimulus exposure (e.g., neurotoxic effects of certain drugs), nonassociative learning (e.g., habituation to the sensory aspects of the stimulus), and other influences. The need for more specific characterizations of the necessary and sufficient conditions for a stimulus to be

an effective conditioned reinforcer has been long recognized (e.g., Wike 1966, § 3.3.1). Mackintosh (1974, p 234) proposed three criteria for a sufficient demonstration of conditioned reinforcement: the rate of the response that produces the stimulus must (1) not depend on a current or historical association between the response and a primary reinforcer; rather, the rate must (2) depend on the Pavlovian association between the stimulus and a primary reinforcer and (3) depend on the instrumental association between the response and the stimulus. In brief, the first two criteria ensure that the putative conditioned reinforcer is, in fact, conditioned, whereas the third ensures that it is a reinforcer. These criteria will be used presently in evaluating the validity of the laboratory procedures that have been developed to study conditioned reinforcement.

Laboratory procedures used to study conditioned reinforcement

Since the initial studies of Grindley (1929) on chickens and KA Williams (1929) on rats running an alleyway or maze toward food-associated stimuli without receiving the food itself, several different types of procedures have been designed to study conditioned reinforcement in laboratory animals (reviewed by Fantino 1977; Kelleher and Gollub 1962; Mackintosh 1974; Wike 1966, § 1.4; BA Williams 1994). Because runway or maze procedures may demonstrate the ability of a stimulus to elicit Pavlovian conditioned approach, as well as or instead of instrumental reinforcement (Dickinson and Balleine 1994), the present review will focus on procedures involving free-operant responding (e.g., lever-pressing, nose-poking). Broadly, these procedures may be divided into two categories (cf., Wike 1966; BA Williams 1994): (1) methods in which both the primary reinforcer and (putative) conditioned reinforcer are

programmed concurrently on a response so that both types of reinforcer are delivered as a result of responding in the same experimental sessions (e.g., second-order schedules, chain schedules) and (2) methods in which responding produces the (putative) conditioned reinforcer in the absence of the primary reinforcer (e.g., resistance to extinction of an established response, new-response acquisition). In these two types of procedures, the primary reinforcement contingency and conditioned reinforcement contingency are positively and negatively correlated, respectively (BA Williams 1994).

Procedures in which a given response produces both the primary reinforcer and the stimulus under investigation (i.e., the putative conditioned reinforcer) have been favored by a number of authors who emphasize their practical utility (e.g., Hendry 1969): it is possible for stimulus presentation to generate high rates of responding and for these high rates to persist over time, providing stable baselines upon which the effects of different manipulations can be assessed within-subjects and with less concern for floor effects. However, the ongoing pairing of the response with the primary reinforcer makes it difficult to determine whether or not the stimuli have any reinforcing effects of their own (Mackintosh 1974). Presentation of the stimulus may result in high rates, but it is not necessarily acting as a conditioned reinforcer in doing so. Rather than having reinforcing effects, the stimulus may be acting strictly as discriminative stimulus for the primary reinforcement contingency (Schindler et al. 2002; BA Williams 1994). This discriminative stimulus function is sufficient to explain the differences observed when responding with a reinforcer-paired stimulus is compared to responding either without stimulus presentation or with control stimuli that have not been paired with the primary reinforcer (BA Williams 1994). Each of these control conditions includes differences in both the potential reinforcing effects and discriminative effects of the control stimulus compared to the

reinforcer-paired stimulus, and so they cannot resolve the mechanism(s) influencing responding. Separate analyses of different portions of the session (e.g., Di Ciano et al. 2003; Pilla et al. 1999) may help to account for changes in responding that result from the unconditioned effects of primary reinforcer presentation (e.g., drug-induced changes in locomotor behavior, Schindler et al. 2002), but they also cannot fully resolve the contributions of the conditioned and primary reinforcement contingencies. When delivery of the primary reinforcer is restricted to the end of the session or the end of an interval of analysis (e.g., a pre-drug interval and post-drug interval in the study of drug self-administration), the first portion of the session necessarily consists of presentation of the putative conditioned reinforcer alone. Reinforcer type is confounded with the passage of time, and other learning processes may control responding before vs. after primary reinforcer delivery (e.g., habituation and dishabituation to stimulus presentation, McSweeney et al. 2005). There are a number of compelling reasons to investigate the combined effects that primary reinforcers and their associated stimuli together have on behavior, or to determine how stimuli can influence a behavior that ultimately leads to a primary reinforcer (e.g., Goldberg and Gardner 1981; see also the two kinds of models of craving discussed by Markou et al. 1993). However, the present studies are concerned with establishing the ability of conditioned reinforcers to sustain behavior on their own and so will focus on situations in which responding produces the (putative) conditioned reinforcer in the absence of the primary reinforcer.

Among procedures with a negative correlation between the primary and conditioned reinforcement contingencies, a number of similar interpretational difficulties arise when assessing the effects of stimulus presentation on the extinction of an established response (Mackintosh 1974; Wike 1966; BA Williams 1994). As in the positive correlation procedures reviewed above, the response that produces the (putative) conditioned reinforcer is also

associated with the primary reinforcer. First, during the response-training phase of these experiments, responding produces both the primary reinforcer and the stimulus of interest. Then, during extinction, the primary reinforcer is withheld, and the stimulus alone is presented as a consequence of responding. In this case, the association of the response with the primary reinforcer is historical, rather than ongoing, but it may control behavior in similar ways. It is possible that, during response training, the stimulus is established as a discriminative stimulus for the primary reinforcer instead of or in addition to a conditioned reinforcer. Presentation of exteroceptive stimuli that were intentionally programmed as discriminative stimuli during response training can subsequently produce significant increases in extinction responding (e.g., Weiss et al. 2001). Stimuli "inadvertently" established as discriminative stimuli when attempting to generate conditioned reinforcers may also increase responding. Extinction responding may also be influenced by generalization decrements between the training and testing phases of the experiment: any manipulation that makes the extinction test sessions more similar to the response-training sessions could increase rates of responding as animals fail to discriminate between the two types of session (Kelleher and Gollub 1962; Mackintosh 1974; Wike 1966, § 1.4; BA Williams 1994). This similarity may depend on the elements of the experimental situation playing the same associative roles during training or testing or on the mere overlap of sensory elements. Therefore, control conditions or groups cannot resolve the particular importance of conditioned reinforcement, as these discriminative mechanisms could account for the differences when responding with the reinforcer-paired stimulus is compared to responding without stimulus presentation or with control stimuli. As above, there are a number of compelling reasons to study the effects of stimulus presentation on extinction responding. With drug self-administration, in particular, changes in extinction responding after drug self-

adminsitration training may provide useful models of human drug abusers' tendency to relapse after a period of abstinence (Epstein et al. 2006; Katz and Higgins 2003; Shaham et al. 2003). However, in considering conditioned reinforcement specifically, these procedures cannot provide statisfactory measures of the conditioned reinforcing effects of the stimuli (Mackintosh 1974).

New-response acquisition procedures can provide valid measures of conditioned reinforcement, in that they can produce responding that clearly meets Mackintosh's (1974) three criteria (e.g., Cador et al. 1991; Hyde 1976; Sosa et al. 2011; Taylor and Robbins 1984). Classical new-response acquisition procedures are divided into two phases: (1) an initial phase of Pavlovian conditioning, in which animals receive response-independent presentations of a primary reinforcer and an exteroceptive stimulus, and (2) a subsequent phase of instrumental acquisition, in which the stimulus alone is programmed as the consequence of a previously untrained response. Physically withholding the response manipulandum during the Pavlovian conditioning phase and the primary reinforcer during the instrumental acquisition phase prevents direct association of the response with the primary reinforcer. Appropriate control conditions can establish that responding during instrumental acquisition depends on both the Pavlovian contingency between the stimulus and the primary reinforcer and the instrumental contingency between the response and the stimulus. These controls do or do not program the stimulus as a consequence of responding after the stimulus has had different associative relationships with the primary reinforcer. For example, in a particularly extensive investigation of food-associated stimuli, Hyde (1976) compared rats that received pairings of an auditory stimulus and food delivery (CS+ condition) to control rats that received pairings of the stimulus and the absence of food delivery (CS– condition), random food and stimulus delivery (CSØ condition), or food delivery without stimulus presentation (US only condition). After establishing these different

associative histories, the rats were given access to a lever, and lever-pressing either did or did not produce the auditory stimulus. Rates of responding were higher when lever-pressing produced the food-paired stimulus than in all other conditions. The manipulations of the associative relationships during the first phase of the study established that responding was sensitive to the Pavlovian contingency between the stimulus and the food, and the comparison of the groups in which responding did vs. did not produce the stimulus established that responding was sensitive to the instrumental contingency between the response and the stimulus. These procedures, thus, provide a sufficient demonstration of the conditioned reinforcing effects of the stimulus (Mackintosh 1974).

Whereas the conceptual or interpretive advantages of new-response acquisition procedures have been long recognized, it has also been thought that negative correlation procedures produce "weak" responding (i.e., low rates) that is ultimately too unstable or transitory to be useful practically in studying conditioned reinforcement (e.g., Gollub 1977; Hendry 1969; Schindler et al. 2002; Shahan 2002; BA Williams 1994). The conditioned reinforcing effects of the stimulus are necessarily assessed after the pairing of the primary reinforcer and stimulus has stopped; therefore, in programming the stimulus alone as the consequence of responding, instrumental acquisition by design coincides with Pavlovian extinction. This Pavovian extinction may cause the stimulus to lose rapidly its conditioned reinforcing effects, limiting the number and/or length of the acquisition sessions that can be conducted before behavior is no longer maintained by stimulus presentation. As Mackintosh (1974, p 237) summarizes: "The very procedure used to provide an uncontaminated measure of conditioned reinforcement guarantees the effect will be evanescent." For instance, in the first operant new-response acquisition experiment reported, Skinner (1938, Figure 13) measured

lever-pressing in a single acquisition test session and found that responding slowed progressively over the course of the session. Several influential early reviews citing this experiment emphasized the apparent degradation of responding in the absence of the primary reinforcer (Hull 1943; Keller and Schoenfeld 1950; Miller 1951). Skinner (1938) himself characterized the pattern of performance obtained as a flattened extinction curve, emphasizing the ultimate decrease in behavior, but he also noted that "considerable conditioning can be effected before a state of more or less complete extinction is reached" (p. 82). Several more recent new-response acquisition studies have found persistent differences between the experimental and control groups over the course of multiple (up to 13) acquisition sessions (e.g., Hyde 1976; Di Ciano and Everitt 2004; Di Ciano et al. 2008). These more recent results suggest that at least some resiliency of responding is possible with new-response acquisition; however, more work is needed to determine when new-response acquisition procedures do or do not produce sustainable behavior (Pierce and Cheney 2004, Chapter 10). Whereas different authors may have different criteria for judging the durability of a conditioned reinforcer (Wike 1966, § 3.3.1), the present concern is that the conditioned reinforcing effects of the stimulus are detectable over the periods necessary for the evaluation of relevant environmental or pharmacological interventions. For example, when testing the effects of a drug on new-response acquisition, the conditioned reinforcing effects of the stimulus should be apparent in a vehicle control condition over the interval of testing required to establish the drug effect (see e.g., Beninger et al. 1981; Samaha et al. 2011; Slawecki et al. 1997).

Acquisition of responding with drug-associated conditioned reinforcement

Drug-associated conditioned reinforcers have long featured in theoretical accounts of human drug addiction (Berridge et al. 2009; Di Chiara 1999; Everitt and Robbins 2005; Everitt et al. 2008; Koob and Le Moal 2001, 2008; Milton and Everitt 2010; Robinson and Berridge 2008; Stewart et al. 1984; Wikler 1973; Wikler at al. 1971). Different authors may interpret the conditioned reinforcing effects of drug-paired stimuli differently or attribute particular importance to conditioned reinforcement in maintaining different phases or aspects of the addiction syndrome; however, refined techniques for measuring the conditioned reinforcing effects of drug-paired stimuli, as distinguished from other stimulus functions, should be useful to researchers with a wide variety of theoretical orientations.

Despite this potential usefulness, new-response acquisition studies with drug-paired stimuli have not been widely pursued in drug abuse research. Early work by Davis, SG Smith, and colleagues (reviewed by Davis and SG Smith 1976, 1987; see also Goddard and Leri 2006; Marcus et al. 1976) showed that rats would increase their responding on a lever that produced a buzzer noise after the noise was paired with response-independent IV injections of morphine or amphetamine, compared to a pre-conditioning baseline period when lever-presses produced the noise and IV saline injection. These results are consistent with the noise becoming a conditioned reinforcer by Pavlovian association with the drug, but several alternative explanations for these changes are equally viable, as these studies did not include a second, inactive lever or other control for nonspecific changes in behavior and/or a pharmacological control to account for potential effects of drug exposure regardless of the programmed drug-stimulus association. More recently, a variation on classical new-response acquisition procedures has been developed in which drug self-administration is trained using one type of manipulandum (e.g., a nose-poke), with each IV drug injection accompanied by a particular stimulus, and then responding on a

second type of manipulandum (e.g., a lever) is trained with the stimulus alone. These procedures have been used most commonly to study responding with cocaine-paired stimuli (Di Ciano 2008; Di Ciano and Everitt 2004; Di Ciano et al. 2007, 2008; Hutcheson et al. 2011; Panlilio et al. 2007; Samaha et al. 2011) or nicotine-paired stimuli (Palmatier et al. 2007, 2008). Crucially, among these studies, work with both cocaine (Di Ciano and Everitt 2004; Panlilio et al. 2007) and nicotine (Palmatier et al. 2007, 2008) has included both a control manipulandum and a pharmacological/associative control condition to assess the sensitivity of responding to the Pavlovian contingency between the stimulus and drug. In addition to studying cocaine-paired stimuli, Di Ciano and Everitt (2004) did measure rats' acquisition of responding with heroin-paired stimuli. However, whereas an unpaired stimulus control condition was included for the cocaine-trained animals, no control was included for the heroin-paired stimulus in the heroin-trained animals. Therefore, no study has yet, to my knowledge, established the conditioned reinforcing effects of opioid-paired stimuli under conditions that clearly meet Mackintosh's (1974) criteria for a sufficient demonstration of conditioned reinforcement.

Dopamine and conditioned reinforcement

If valid measures of the conditioned reinforcing effects of opioid-paired stimuli can be obtained, the ability of various environmental and neurobiological manipulations to alter these effects specifically can then be assessed. Dopaminergic agonism and antagonism are of particular interest because these manipulations have consistently been found to alter rats' performance with food- and water-conditioned reinforcers in new-response acquisition

procedures. It is important to determine if these effects depend on the class of primary reinforcer (i.e., non-drug vs. drug) with which the stimulus was paired.

Based on their effects on responding reinforced by electrical brain stimulation, Stein (1964, p 91) made an early suggestion that amphetamine and related drugs act on "a brain mechanism for reward" that includes the processing of reward-related and/or response-related environmental stimuli. Noting that these compounds can significantly increase or decrease behavior maintained by primary reinforcers depending on the schedule of reinforcement and other environmental variables, Hill (1970, p 783) proposed more specifically that amphetamine-like drugs increase the "effectiveness" of conditioned reinforcers. The first experiments addressing this hypothesis assessed the effects of pipradrol on the extinction of food- or water-trained responding. Systemic pipradrol administration dose-dependently increased lever-pressing that produced a sound and/or light that had been paired with food or water delivery during response training, whereas pipradrol did not increase responding when lever-pressing had no scheduled consequences or produced a stimulus that had not been previously paired with the primary reinforcer (Hill 1970; Robbins 1975). Pipradrol is structurally related to amphetamine and, like amphetamine, blocks reuptake and causes release of dopamine and norepinephrine (Coppola and Mondola 2012; Robbins et al. 1983). The response-enhancing effects of pipradrol appeared quite robust, as Hill (1970, Figure 2) noted that pipradrol could be repeatedly administered and withheld, with corresponding decreases and increases, respectively, in responding, and pipradrol administration could increase lever-pressing even after extensive extinction training (~20,000 responses made in ~100 sessions without food delivery).

Shortly thereafter, the response-enhancing effects of pipradrol were established in new-response acquisition procedures with food- or water-paired stimuli (Beninger et al. 1980;

Robbins 1976, 1978; see also Chu and Kelley 1992), and the effectiveness of several other indirect dopaminergic agonists was established. Methylphenidate produced numerical increases that resembled those produced by pipradrol, although the effects were highly variable across animals (Robbins 1978). Amphetamine, administered systemically (Beninger and Ranaldi 1992; de Borchgrave et al. 2002; Mazurski and Beninger 1986; Ranaldi and Beninger 1993; JK Smith et al. 1997; but see Robbins 1978; Beninger et al. 1981) or directly into the nucleus accumbens (NAcc) (Cador et al. 1991; Fletcher 1995; Kelley and Delfs 1991; Parkinson et al. 1999; Taylor and Robbins 1984; Wolterink et al. 1993) produced significant, dose-dependent increases in responding. It is important to note that, among these studies, the effects of amphetamine have been assessed under stringent conditions. Under the same circumstances where enhanced responding with a reinforcer-paired stimulus was observed, amphetamine (1) did not increase responding on a control manipulandum that did not produce the stimulus and (2) did not increase the response that produced the stimulus when the stimulus had not been consistently paired with the primary reinforcer (Cador et al. 1991; Taylor and Robbins 1984). In contrast to its effects in the NAcc, amphetamine did not increase responding when injected into the thalamus (Taylor and Robbins 1984) or into several other striatal regions (Kelley and Delfs 1991), but increases caused by injection into the caudate-putamen broadly (Taylor and Robbins 1984) or anterior dorsal striatum more specifically (Kelley and Delfs 1991) have been reported. Administered systemically, cocaine itself failed to increase responding (Beninger et al. 1981; Robbins et al. 1983), but the cocaine analogues WIN 35,428 and WIN 35,065-2 were effective (Robbins et al. 1983). Cocaine administered directly into the NAcc was shown to dose-dependently increase lever-pressing that produced a food-paired stimulus (Chu and Kelley 1992), but these results are

more difficult to interpret because neither a control response (i.e., an inactive lever) or associative control group (e.g., an unpaired-stimulus group) was included in this particular study.

Given that these indirect agonists can affect other neurotransmitter systems (e.g., norepinephrine), evidence for the involvement of dopamine, specifically, in the enhancement of respsonding with food- or water-conditioned reinforcers was provided initially by lesion studies, followed by studies with dopaminergic antagonists. Robbins and Everitt (1982, Figure 9) first reported that 6-hydroxydopamine (6-OHDA) lesions located preferentially in either the caudate-putamen or NAcc reduced the responding of animals treated systemically with pipradrol, compared to animals that received pipradrol after sham lesions or no surgery. Subsequently, it was shown that 6-OHDA lesions of the NAcc attenuated the effects of intra-NAcc amphetamine injection (Taylor and Robbins 1986), whereas lesions of the dorsal noradrenergic ascending bundle did not alter the effects of intra-NAcc amphetamine administration (Cador et al. 1991). In this latter study (Cador et al. 1991), acquisition of responding with a water-paired stimulus was also enhanced when dopamine itself, but not norepinephrine, was injected into the NAcc, and the effects of intra-NAcc dopamine administration were blocked by systemic administration of the nonselective dopamine receptor antagonist, α-flupenthixol. In a subsequent study of selective D1-like (D1, D5) or D2-like (D2, D3, D4) receptor antagonism, systemic administration of the D1-like antagonist, SCH 23390, or the D2-like antagonist, pimozide, produced complex patterns of increases or decreases in responding that, overall, suggested the amphetamine dose-effect function was shifted rightward (Ranaldi and Beninger 1993), although the descending limb was not clearly determined under the influence of the antagonists. Systemic administration of SCH 23390, or the D2-like antagonist, raclopride, reduced the responding of animals injected with pipradrol directly into the nucleus accumbens (Chu and Kelley 1992), and

intra-NAcc SCH 23390 or raclopride attenuated the response-enhancing effects of intra-NAcc amphetamine (Wolterink et al. 1993). When the effects of several antagonists were assessed on their own, however, systemic administration of the D2-like antagonists, haloperidol, pimozide, raclopride, and sulpiride, all produced significant increases in responding with a food-paired stimulus, whereas increases were not observed with SCH 23390 (JK Smith et al. 1997). These increases were interepreted as a result of the D2-like antagonists blocking autoreceptors, thus serving as indirect dopaminergic agonists (but see Wolterink et al. 1993).

The first studies with direct dopamine receptor agonists were conducted with the nonselective D1-like/D2-like agonist, apomorphine. Apomorphine did not enhance response acquisition with food- or water-conditioned reinforcers (Beninger and Ranaldi 1992; Mazurski and Beninger 1986; Robbins et al. 1983), producing instead changes in the rate of both the response that produced the stimulus and an inactive control response (Beninger and Ranaldi 1992; Robbins et al. 1983) or, over a smaller dose range, no significant change in either response (Mazurski and Beninger 1986). Subsequent studies with selective D1-like or D2-like agonists have shown that systemic administration of D2-like, but not D1-like, agonists can enhance response acquisition with food-paired stimuli. Systemtic administration of several D2-like agonists has been shown to increase responding with food-paired stimuli: bromocriptine (Beninger and Ranaldi 1992; Sutton et al. 2001), quinpirole (Beninger and Ranaldi 1992), or 7-OH-DPAT (Sutton et al. 2001). In contrast, systemic administration of a range of doses of a variety of D1-like agonists—SKF 38393, SKF 77434, SKF 81297, SKF 82958, and CY 208-243—either failed to increase or suppressed responding (Beninger and Ranaldi 1992; Beninger and Rolfe 1995). Different patterns of effects, however, have been reported with central agonist administration. Chu and Kelley (1992) reported that neither quinpirole (0.0-20.0 μg) nor CY

208-243 (0.0-10.0 μg) increased responding with a food-paired stimulus when injected into the NAcc, whereas Wolterink and colleagues (1993) reported that injection of either quinpirole (0.1-1.0 μg) or SKF 38393 (0.1-10.0 μg) into the NAcc increased responding.

Compared with these studies of food- or water-paired stimuli, the evidence that dopaminergic manipulations can alter new-response acquisition with drug-paired stimuli is limited. Several studies have examined the effects of amphetamine on new-response acquisition after drug self-administration training. Slawecki and colleagues (1997) assessed the effects of intra-NAcc amphetamine injection on responding with ethanol-paired stimuli; however, the increases in responding found are difficult to interpret in terms of drug-based conditioned reinforcement because the stimuli were also paired with sucrose during the course of ethanol self-administration training. Importantly, it was recently shown that systemic amphetamine can increase responding with cocaine-paired stimuli when rats acquired a response with the stimuli alone after IV cocaine self-administration training (Di Ciano 2008; Samaha et al. 2011). These results suggest that indirect dopaminergic agonism can increase responding with drug-paired stimuli, as it does with food- or water-paired stimuli. However, the behavioral selectivity of amphetamine's effects are unknown, as the previous studies did not examine the effects of amphetamine on responding with stimuli that were not consistently paired with cocaine (Di Ciano 2008; Samaha et al. 2011). Furthermore, no study has, to my knowledge, used new-response acquisition to characterize the effects of selective direct dopaminergic agonists or antagonsists on stimuli paired with IV drug. Characterizing the specific dopamine receptor subtypes involved in responding with drug-based conditioned reinforcement may be useful generally for understanding the neurobiological mechanisms of reinforcement and, more

specifically, for the development of medications designed to reduce the influence exerted by drug-paired stimuli over human drug abusers (e.g., Heidbreder and Newman 2010).

Dopamine D2-like agonists in clinical use

Among dopaminergic manipulations, the present study is concerned with the effects of systemic administration of the D2-like agonist, pramipexole. Pramipexole is of particular interest because of its widespread clinical use. Approved for human use internationally, pramipexole has become the most widely prescribed direct dopamine agonist treatment for Parkinson's disease (Antonini et al. 2010). Pramipexole has also been approved by regulators in both the United States and Europe to treat restless legs syndrome (Brindani et al. 2009), and pramipxole is commonly used "off label" to treat fibromyalgia (Roskell et al. 2011). Therefore, it may be important to characterize the behavioral effects of pramipexole and other, similar D2-like agonists that are presently approved for human use (e.g., ropinerole) using the routes of administration used clinically in dopamine agonist therapy: peroral, subcutaneous injection, and transdermal patch application (Perez-Lloret and Rascol 2010). Understanding possible changes in associative learning and/or motivational processes caused by systemic pramipexole administration may, thus, be particularly relevant to the behavioral effects of these drugs in humans.

Specific aims

Aim 1: The first set of experiments was designed to validate a new-response acquisition procedure for characterizing the conditioned reinforcing effects of a stimulus paired with the potent, short-acting μ-opioid agonist, remifentanil, in the rat. Pavlovian conditioning procedures alone were used to pair IV remifentanil injection with a light-noise compound stimulus, and animals had no operant training history before the start of the response acquisition test sessions, when the stimulus alone was programmed as the consequence of a novel nose-poke response. To verify that acquisition depended on the Pavlovian contingency between the stimulus and remifentanil, the responding of animals exposed to stimulus-remifentanil pairings was compared to the responding of control animals given remifentanil injections and stimulus presentations without consistent pairing (i.e., in a "truly random" arrangement, Rescorla 1967). Likewise, to verify that acquisition depended on the instrumental contingency between a particular response and the stimulus, responding in an active nose-poke, which produced the stimulus, was compared to responding in an inactive nose-poke, which had no scheduled consequences. Acquisition was, thus, assessed in terms of the effects of two types of associative history (paired vs. random) on two instrumental responses (active vs. inactive). Several parameters of the Pavlovian conditioning sessions and/or acquisition test sessions were also manipulated to determine their effects on responding with the remifentanil-paired sitmulus: the ability of responding to persist across multiple acquisition test sessions under different schedules of reinforcement was evaluated, and the influence of the number of remifentanil-stimulus pairings on response acquisition was assessed.

Aim 2: The second set of experiments was designed to characterize the effects of the dopamine D2-like receptor agonist, pramipexole, on responding with remifentanil-associated stimuli in two

different behavioral assays: the new-response acquisition procedure developed in Aim 1 and a resisistance-to-extinction procedure designed to study the response-maintaining effects of stimuli that had been paired with self-administered remifentanil. The resistance-to-extinction task was modeled on procedures recently used to assess the effects of pramipexole on responding with cocaine-associated stimuli (Collins et al. 2012); therefore, this procedure was used first in a preliminary study to guide the selection of behaviorally active doses of pramipexole. In the resistance-to-extinction experiment, after a pramipexole dose-effect function was determined, the role of different stimulus types (contextual stimuli, discriminative stimuli, discrete conditioned stimuli) in the effects of pramipexole was assessed. Next, a pramipexole dose-effect function was determined using the new-response acquisition procedure. The behavioral selectivity of pramipexole was established by giving the same course of pramipexole treatment to a "truly random" control group, as was used in Aim 1. Following the discovery that, in both behavioral assays, pramipexole increased rates of responding, but did so only in the final 40-50% of the session, a second new-response acquisition experiment was designed to separate the effects of the duration of exposure to pramipexole itself from the duration of exposure to the task while under the influence of pramipexole.

Aim 3: The final set of experiments was designed to clarify the receptor mechanisms involved in the response-enhancing effects of pramipexole observed in Aim 2. Whereas previous studies have indicated that pramipexole is D3-preferring *in vitro* and *in vivo*, pramipexole also has significant activity at D2 receptors (e.g., Collins et al. 2007). Therefore, pretreatments of the D3 -preferring antagonist, SB-277011A, or the D2-preferring antagonist, L-741,626, were given attempting to block the effects of pramipexole. After finding that SB-277011A did not

significantly modify the effects of pramipexole on new-response acquisition, an experiment was performed to demonstrate that SB-277011A could attenuate the yawning behavior and penile erection elicited by pramipexole. While not necessarily related to conditioned reinforcement, alterations in yawning and penile erection would verify that SB-277011A can modify responses in rats that have been previously linked specifically with D3 activity (Collins et al. 2005, 2007, 2009).

Works cited

Antonini A, Barone P, Ceravolo R, Fabbrini G, Tinazzi M, and Abbruzzese G (2010) Role of pramipexole in the management of Parkinson's disease. *CNS Drugs* **24:** 829–841. doi: 10.2165/11585090-000000000-00000

Balleine BW (2005) Incentive behavior, in *The Behavior of the Laboratory Rat: A Handbook with Tests* (Whishaw IQ and Kolb B eds) pp 436–446, Oxford University Press, New York.

Beninger RJ, Hanson DR, and Phillips AG (1980) The effects of pipradrol on the acquisition of responding with conditioned reinforcement: a role for sensory preconditioning. *Psychopharmacology (Berl)* **69:** 235–242. doi: 10.1007/BF00433088

Beninger RJ, Hanson DR, and Phillips AG (1981) The acquisition of responding with conditioned reinforcement: effects of cocaine, (+)-amphetamine and pipradrol. *Br J Pharmacol* **74:** 149–154. doi: 0.1111/j.1476-5381.1981.tb09967.x

Beninger RJ and Ranaldi R (1992) The effects of amphetamine, apomorphine, SKF 38393, quinpirole and bromocriptine on responding for conditioned reward in rats. *Behav Pharmacol* **3:** 155–163. doi: 10.1097/00008877-199204000-00009

Beninger RJ and Rolfe NG (1995) Dopamine D1-like receptor agonists impair responding for conditioned reward in rats. *Behav Pharmacol* **6:** 785–793. doi: 10.1097/00008877-199512000-00003

Berridge KC, Robinson TE, and Aldridge JW (2009) Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol* **9:** 65–73. doi: 10.1016/j.coph.2008.12.014

Brindani F, Vitetta F, and Gemignani F (2009) Restless legs syndrome: differential diagnosis and management with pramipexole. *Clin Interv Aging* **4:** 305–313. doi: 10.2147/CIA.S4143

Cador M, Taylor JR, and Robbins TW (1991) Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. *Psychopharmacology (Berl)* **104:** 377–385. doi: 10.1007/BF02246039

Campbell UC and Carroll ME (2000) Acquisition of drug self-administration: environmental and pharmacological interventions. *Exp Clin Psychopharmacol* **8:** 312–325. doi: 10.I037//1064-1297.8.3.312

Chu B and Kelley AE (1992) Potentiation of reward-related responding by psychostimulant infusion into the nucleus accumbens: role of dopamine receptor subtypes. *Psychobiology (Austin, Tex)* **20:** 153–162.

Collins GT, Witkin JM, Newman AH, Svensson KA, Grundt P, Cao J, and Woods JH (2005) Dopamine agonist-induced yawning in rats: a dopamine D3 receptor-mediated behavior. *J Pharmacol Exp Ther* **314:** 310–319. doi: 10.1124/jpet.105.085472

Collins GT, Newman AH, Grundt P, Rice KC, Husbands SM, Chauvignac C, Cheng J, Wang S, and Woods JH (2007) Yawning and hypothermia in rats: effects of dopamine D3 and D2 agonists and antagonists. *Psychopharmacology (Berl)* **193:** 159-170. doi: 10.1007/s00213-007-0766-3

Collins GT, Truccone A, Haji-Abdi F, Newman AH, Grundt P, Rice KC, Husbands SM, Greedy BM, Enguehard-Gueiffier C, Gueiffier A, Chen J, Wang S, Katz JL, Grandy DK, Sunahara RK, and Woods JH (2009) Proerectile effects of dopamine D2-like agonists are mediated by the D3 receptor in rats and mice. *J Pharmacol Exp Ther* **329:** 210–217. doi: 10.1124/jpet.108.144048

Collins GT, Cunningham AR, Chen J, Wang, S, Newman AH, and Woods JH (2012) Effects of pramipexole on the reinforcing effectiveness of stimuli that were previously paired with cocaine reinforcement in rats. *Psychopharmacology (Berl)* **219:** 123–135. doi: 10.1007/s00213-011-2382-5

Coppola M and Mondola R (2012) Research chemicals marketed as legal highs: the case of pipradrol derivatives. *Toxicol Lett* **212:** 57–60. doi: 10.1016/j.toxlet.2012.04.019

Cunningham CL (1993) Pavlovian drug conditioning, in *Methods in Behavioral Pharmacology* (van Haaren F ed) pp 349–381, Elsevier, New York.

Davis WM and Smith SG (1976) Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. *Pav J Biol Sci* **11:** 222–236. doi: 10.1007/BF03000316

Davis WM and Smith SG (1987) Conditioned reinforcement as a measure of the rewarding properties of drugs, in *Methods of Assessing the Reinforcing Properties of Abused Drugs* (Bozarth MA ed) pp 199–210, Springer-Verlag, New York.

de Borchgrave R, Rawlins JNP, Dickinson A, and Balleine BW (2002) Effects of cytotoxic nucleus accumbens lesions on instrumental conditioning in rats. *Exp Brain Res* **144:** 50–68. doi: 10.1007/s00221-002-1031-y

Di Chiara G (1999) Drug addiction as dopamine-dependent associative learning disorder. *Eur J Pharmacol* **375:** 13–30. doi: 10.1016/S0014-2999(99)00372-6

Di Ciano P, Underwood RJ, Hagan JJ, and Everitt BJ (2003) Attenuation of cue-controlled cocaine-seeking by a selective D3 dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology* **28:** 329–338. doi: 10.1038/sj.npp.1300148

Di Ciano P and 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. doi: 10.1016/j.neuropharm.2004.06.005

Di Ciano P, Benham-Hermetz J, Fogg AP, and Osborne GEC (2007) Role of the prelimbic cortex in the acquisition, re-acquisition or persistence of responding for a drug-paired conditioned reinforcer. *Neuroscience* **150:** 291–298. doi: 10.1016/j.neuroscience.2007.09.016

Di Ciano (2008) Facilitated acquisition but not persistence of responding for a cocaine-paired conditioned reinforcer following sensitization with cocaine. *Neuropsychopharmacology* **33:** 1426–1431. doi: 10.1038/sj.npp.1301542

Di Ciano P, Robbins TW, and Everitt BJ (2008) Differential effects of nucleus accumbens core, shell, or dorsal striatal inactivations on the persistence, reacquisition, or reinstatement of responding for a drug-paired conditioned reinforcer. *Neuropsychopharmacology* **33:** 1413–1425. doi: 10.1038/sj.npp.1301522

Dickinson A and Balleine B (1994) Motivational control of goal-directed action. *Anim Learn Behav* **22:** 1–18. doi: 10.3758/BF03199951

Epstein DH, Preston KL, Stewart J, and Shaham Y (2006) Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology (Berl)* **189:** 1–16. doi: 10.1007/s00213-006-0529-6

Everitt BJ and Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* **8:** 1481–1498. doi: 10.1038/nn1579

Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, and Robbins TW (2008) Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos Trans R Soc Lond B Biol Sci* **363:** 3125–3135. doi: 10.1098/rstb.2008.0089

Fantino E (1977) Conditioned reinforcement: choice and information, in *Handbook of Operant Behavior* (Honig WK and Staddon JER eds) pp 313–339, Prentice Hall, Englewood Cliffs, NJ.

Fantino E (2008) Choice, conditioned reinforcement and the Prius effect. *Behav Anal* **31:** 95–111.

Fletcher PJ (1995) Effects of *d*-fenfluramine and metergoline on responding for conditioned reward and the response potentiating effect of nucleus accumbens *d*-amphetamine. *Psychopharmacology (Berl)* **118:** 155–163. doi: 10.1007/BF02245834

Goddard B and Leri F (2006) Reinstatement of conditioned reinforcing properties of cocaine-conditioned stimuli. *Pharmacol Biochem Behav* **83:** 540–546. doi: 10.1016/j.pbb.2006.03.015

Goldberg SR (1975) Stimuli associated with drug injections as events that control behavior. *Pharmacol Rev* **27:** 325–340.

Goldberg SR and Gardner ML (1981) Second-order schedules: extended sequences of behavior controlled by brief environmental stimuli associated with drug self-administration. *NIDA Res Monogr* **37:** 241–270.

Gollub L (1977) Conditioned reinforcement: schedule effects, in *Handbook of Operant Behavior* (Honig WK and Staddon JER eds) pp 288–312, Prentice Hall, Englewood Cliffs, NJ.

Grindley GC (1929) Experiments on the influence of the amount of reward on learning in young chickens. *Br J Psychol Gen Sect* **20:** 173–180. doi: 10.1111/j.2044-8295.1929.tb01265.x

Heidbreder C and Newman AH (2010) Current perspectives on selective dopamine D3 receptor antagonists as pharmacotherapeutics for addictions and related disorders. *Ann N Y Acad Sci* **1187:** 4–34. doi: 10.1111/j.1749-6632.2009.05149.x.

Hendry DP (1969) *Conditioned Reinforcement*. Dorsey Press. Homewood, IL.

Hill RT (1970) Facilitation of conditioned reinforcement as a mechanism of psychomotor stimulation, in *Amphetamine and Related Compounds* (Costa E and Garatitini S eds) pp 781–795, Raven Press, New York.

Hull CL (1943) *Principles of Behavior: An Introduction to Behavior Theory*. Appleton-Century-Crofts, New York.

Hutcheson DM, Quarta D, Halbout B, Rigal A, Valerio E, and Heidbreder C (2011) Orexin-1 receptor antagonist SB-334867 reduces the acquisition and expression of cocaine-conditioned reinforcement and the expression of amphetamine-conditioned reward. *Behav Pharmacol* **22:** 173-181. doi: 10.1097/FBP.0b013e328343d761

Hyde TS (1976) The effect of Pavlovian stimuli on the acquisition of a new response. *Learn Motiv* **7:** 223–239. doi: 10.1016/0023-9690(76)90030-8

Katz JL and Higgins ST (2003) The validity of the reinstatement model of craving and relapse to drug use. *Psychopharmacology (Berl)* **168:** 21–30. doi: 10.1007/s00213-003-1441-y

Kelleher RT and Gollub LR (1962) A review of positive conditioned reinforcement. *J Exp Anal Behav* **5:** 543–597. doi: 10.1901/jeab.1962.5-s543

Keller FS and Schoenfeld WN (1950) *Principles of Psychology: A Systematic Text in the Science of Behavior*. Century-Appleton-Crofts, New York.

Kelley AE and Delfs JM (1991) Dopamine and conditioned reinforcement. I. differential effects of amphetamine microinjections into striatal subregions. *Psychopharmacology (Berl)* **103:** 187–196. doi: 10.1007/BF02244202

Koob GF and Le Moal M (2001) Drug addiction, dysregulation of reward and allostasis. *Neuropsychopharmacology* **24:** 97–129. doi: 10.1016/S0893-133X(00)00195-0

Koob GF and Le Moal M (2008) Neurobiological mechanisms for opponent motivational processes in addiction. *Philos Trans R Soc Lond B Biol Sci* **363:** 3113–3123. doi: 10.1098/rstb.2008.0094

Mackintosh NJ (1974) *The Psychology of Animal Learning*. Academic Press, New York.

Marcus R, Carnathan G, Meyer RE, and Cochin J (1976) Morphine-based secondary reinforcement: effects of different doses of naloxone. *Psychopharmacology (Berl)* **48:** 247–250. doi: 10.1007/BF00496856

Markou A, Weiss F, Gold LH, Caine B, Schulteis G, and Koob GF (1993) Animal models of drug craving. *Psychopharmacology (Berl)* **112:** 163–182. doi: 10.1007/BF02244907

Mazurski EJ and Beninger RJ (1986) The effects of (+)-amphetamine and apomorphine on responding for a conditioned reinforcer. *Psychopharmacology (Berl)* **90:** 239–243. doi: 10.1007/BF00181249

McSweeney FK, Murphy ES, and Kowal BP (2005) Regulation of drug taking by sensitization and habituation. *Exp Clin Psychopharmacol* **13:** 163–84. doi: 10.1037/1064-1297.13.3.163

Miller NE (1951) Learnable drives and rewards, in *Handbook of Experimental Psychology* (Stevens SS ed) pp 435–472, John Wiley & Sons, New York.

Milton AL and Everitt BJ (2010) The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *Eur J Neurosci* **31:** 2308–2319. doi: 10.1111/j.1460-9568.2010.07249.x

O'Brien CP and Gardner EL (2005) Critical assessment of how to study addiction and its treatment: human and non-human animal models. *Pharmacol Ther* **108:** 18–58. doi: 10.1016/j.pharmthera.2005.06.018

Palmatier MI, Liu X, Matteson GL, Donny EC, Caggiula AR, and Sved AF (2007) Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. *Psychopharmacology (Berl)* **195:** 235–243. doi: 10.1007/s00213-007-0897-6

Palmatier MI, Coddington SB, Liu X, Donny EC, Caggiula AR, and Sved AF (2008) The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose. *Neuropharmacology* **55:** 1425–1430. doi: 10.1016/j.neuropharm.2008.09.002

Panlilio LV, Thorndike EB, and Schindler CW (2007) Blocking of conditioning to a cocaine-paired stimulus: testing the hypothesis that cocaine perpetually produces a signal of larger-than-expected reward. *Pharmacol Biochem Behav* **86:** 774–777. doi: 10.1016/j.pbb.2007.03.005

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, and Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. *J Neurosci* **19:** 2401–2411.

Perez-Lloret S and Rascol O (2010) Dopamine receptor agonists for the treatment of early or advanced Parkinson's disease. *CNS Drugs* **24:** 941–968. doi: 10.2165/11537810-000000000-00000

Pierce WD and Cheney CD (2004) *Behavior Analysis and Learning*, 3rd ed. Lawrence Erlbaum Associates, Mahwah, NJ.

Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz J-C, Everitt BJ, and Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* **400:** 371–375. doi: 10.1038/22560

Ranaldi R and Beninger RJ (1993) Dopamine D1 and D2 antagonists attenuate amphetamine-produced enhancement of responding for conditioned reward in rats. *Psychopharmacology (Berl)* **113:** 110–118. doi: 10.1007/BF02244342

Rescorla RA (1967) Pavlovian conditioning and its proper control procedures. *Psychol Rev* **74:** 71–80. doi: 10.1037/h0024109

Robbins TW (1975) The potentiation of conditioned reinforcement by psychomotor stimulant drugs. a test of Hill's hypothesis. *Psychopharmacologia* **45:** 103–114. doi: 10.1007/BF00426218

Robbins TW (1976) Relationship between reward-enhancing and stereotypical effects of psychomotor stimulant drugs. *Nature* **264:** 57–59. doi: 10.1038/264057a0

Robbins TW (1978) The acquisition of responding with conditioned reinforcement: effects of pipradrol, methylphenidate, *d*-amphetamine, and nomifensine. *Psychopharmacology (Berl)* **58:** 79–87. doi: 10.1007/BF00426794

Robbins TW and Everitt BJ (1982) Functional studies of the central catecholamines. *Int Rev Neurobiol* **23:** 303–365. doi: 10.1016/S0074-7742(08)60628-5

Robbins TW, Watson BA, Gaskin M, and Ennis C (1983) Contrasting interactions of pipradrol, *d*-amphetamine, cocaine, cocaine analogues, apomorphine and other drugs with conditioned reinforcement. *Psychopharmacology (Berl)* **80:** 113–119. doi: 10.1007/BF00427952

Robinson TE and Berridge KC (2001) Incentive-sensitization and addiction. *Addiction* **96:** 103–114. doi: 10.1046/j.1360-0443.2001.9611038.x

Robinson TE and Berridge KC (2008) The incentive sensitization theory of addiction: some current issues. *Philos Trans R Soc Lond B Biol Sci* **363:** 3137–3146. doi: 10.1098/rstb.2008.0093

Roskell NS, Beard SM, Zhao Y, and Le TK (2011) A meta-analysis of pain response in the treatment of fibromyalgia. *Pain Pract* **11:** 516–527. doi: 10.1111/j.1533-2500.2010.00441.x

Samaha A-N, Minogianis E-A, and Nachar W (2011) Cues paired with either rapid or slower self-administered cocaine injections acquire similar conditioned rewarding properties. *PLoS One* **6:** e26481. doi: 10.1371/journal.pone.0026481

Schindler CW, Panlilio LV, and Goldberg SR (2002) Second-order schedules of drug self-administration in animals. *Psychopharmacology (Berl)* **163:** 327–344. doi: 10.1007/s00213-002-1157-4

Shaham Y, Shalev U, Lu L, de Wit H, and Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl)* **168:** 3–20. doi: 10.1007/s00213-002-1224-x

Shahan TA (2002) The observing-response procedure: a novel method to study drug-associated conditioned reinforcement. *Exp Clin Psychopharmacol* **10:** 3–9. doi: 10.1037//1064-1297.10.1.3

Shahan TA (2010) Conditioned reinforcement and response strength. *J Exp Anal Behav* **93:** 269–289. doi: 10.1901/jeab.2010.93-269

Skinner BF (1938) *The Behavior of Organisms: An Experimental Analysis*. Century-Appleton-Crofts, New York.

Skinner BF (1953) *Science and Human Behavior*. Macmillan, New York.

Slawecki CJ, Samson HH and Chappell A (1997) Intranucleus accumbens amphetamine infusions enhance responding maintained by a stimulus complex paired with oral ethanol self-administration. *Pharmacol Biochem Behav* **58:** 1065–1073. doi: 10.1016/S0091-3057(97)00310-9

Smith JK, Neill JC, and Costall B (1997) Bidirectional effects of dopamine D2 receptor antagonists on responding for a conditioned reinforcer. *Pharmacol Biochem Behav* **57:** 843–849. doi: 10.1016/S0091-3057(96)00399-1

Sosa R, dos Santos CV, and Flores C (2011) Training a new response using conditioned reinforcement. *Behav Processes* **87:** 231–236. doi: 10.1016/j.beproc.2011.03.001

Stein L (1964) Amphetamine and neural reward mechanisms, in *Animal Behaviour and Drug Action* (Steinberg H ed) pp 91–118, J & A Churchill, London.

Stewart J, de Wit H, and Eikelboom R (1984) Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychol Rev* **91:** 251–268. doi: 10.1037/0033-295X.91.2.251

Sutton MA, Rolfe NG, and Beninger RJ (2001) Biphasic effects of 7-OH-DPAT on the acquisition of responding for conditioned reward in rats. *Pharmacol Biochem Behav* **69:** 195–200. doi: 10.1016/S0091-3057(01)00540-8

Taylor JR and Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. *Psychopharmacology (Berl)* **84:** 405–412. doi: 10.1007/BF00555222

Taylor JR and Robbins TW (1986) 6-Hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens *d*-amphetamine. *Psychopharmacology (Berl)* **90:** 390–397. doi: 10.1007/BF00179197

Thompson T and Pickens R (1969) Drug self-administration and conditioning, in *Scientific Basis of Drug Dependence* (Steinberg H ed) pp 177–198, J & A Churchill, London.

Weiss F, Martin-Fardon R, Ciccocioppo R, Kerr TM, Smith DL, and Ben-Shahar O (2001). Enduring resistance to extinction of cocaine-seeking behavior induced by drug-related cues. *Neuropsychopharmacology* **25:** 361–372. doi: 10.1016/S0893-133X(01)00238-X

Wike EL (1966) *Secondary Reinforcement: Selected Experiments*. Harper & Row, New York.

Wikler A, Pescor FT, Miller D, and Norrell H (1971) Persistent potency of a secondary (conditioned) reinforcer following withdrawal of morphine from physically dependent rats. *Psychopharmacologia* **20:** 103–117. doi: 10.1007/BF00404365

Wikler A (1973) Dynamics of drug dependence: implications of a conditioning theory for research and treatment. *Arch Gen Psychiatry* **28:** 611–616. doi: 10.1001/archpsyc.1973.01750350005001.

Williams BA (1994) Conditioned reinforcement: experimental and theoretical issues. *Behav Anal* **17:** 261–285.

Williams KA (1929) The reward value of a conditioned stimulus. *Publ Psychol Berkeley* **4:** 31–55.

Wolterink G, Phillips G, Cador M, Donselaar-Wolterink I, Robbins TW, and Everitt BJ (1993) Relative roles of ventral striatal D1 and D2 dopamine receptors in responding with conditioned reinforcement. *Psychopharmacology (Berl)* **110:** 355–364. doi: 10.1007/BF02251293

Young AM, Herling S, and Woods JH (1981) History of drug exposures as a determinant of drug self-administration. *NIDA Res Monogr* **37:** 73–89.

CHAPTER II

Acquisition of a New Response with a Remifentanil-Paired Conditioned Reinforcer

Introduction

Exposure to drug-associated environmental stimuli can significantly enhance drug self-administration behaviors in both humans and laboratory animals (Everitt and Robbins 2000; Olive and Kalivas 2011; Le Foll and Goldberg 2005; See 2005). Many of these effects are consistent with the drug-associated stimuli functioning as conditioned reinforcers to increase the frequency of drug-taking and/or drug-seeking responses; however, it can be difficult experimentally to distinguish conditioned reinforcement from the other associative and nonassociative effects of drug exposure and stimulus presentation (Cunningham 1993; Kelleher and Gollub 1962; Mackintosh 1974; Williams 1994). Treatments for drug abuse and dependence are increasingly focused on techniques to reduce human drug takers' reactions to drug-associated stimuli (e.g., Milton and Everitt 2010; Myers and Carlezon 2010; Taylor et al. 2009). To decrease drug-taking and related maladaptive behaviors while minimizing the risk of disruptions to other, more adaptive behaviors, these treatments should target precisely the specific learning mechanisms responsible for drug-stimulus associations and stimulus-maintained behaviors (cf., Hogarth and Duka 2006). To help address the specific contributions that conditioned

reinforcement can make to drug abuse and dependence (as distinguished, even, from other Pavlovian conditioned effects; Milton and Everitt 2010), thorough behavioral assessments are needed to characterize the conditioned reinforcing effects of drug-associated stimuli and to determine the necessary and sufficient conditions for such stimuli to act as conditioned reinforcers.

Three criteria must be satisfied to establish that a stimulus is, indeed, acting as a conditioned reinforcer (Mackintosh 1974, p 234). Changes in the rate of the response that produces the stimulus must (1) not depend on a current or historical association between the response and a primary reinforcer; rather, rates must depend (2) on the Pavlovian association between a primary reinforcer and the stimulus and (3) on the instrumental association between the response and the stimulus. Among the experimental procedures developed to study conditioned reinforcement (reviewed by Williams 1994), new-response acquisition is considered particularly rigorous because it can generate behavior that clearly satisfies all three of these criteria (e.g., Hyde 1976; Sosa et al. 2011). In classical new-response acquisition procedures, animals are first given response-independent pairings of a primary reinforcer and exteroceptive stimulus. Subsequently, the stimulus alone is programmed as the consequence of a previously untrained instrumental response, and the ability of animals to learn to make that response is assessed. In this case, the animals do not have the opportunity to associate directly the instrumental response with the primary reinforcer, as the response that produces the stimulus does not and did not produce the primary reinforcer, and if adequate controls are included, the effects of the specified Pavlovian and instrumental associations can also be established.

New-response acquisition procedures have been used widely to study the conditioned reinforcing effects of food- or water-paired stimuli, and the basic behavioral procedures have

been adapted for more complex studies of the associative and neurobiological determinants of performance with conditioned reinforcement (e.g., Beninger and Ranaldi 1994; Beninger and Rolfe 1995; Burke et al. 2007; de Borchgrave et al. 2002; Olausson et al. 2004; Parkinson et al. 1999, 2005; Snycerski et al. 2005). Despite these advances with non-drug reinforcers, new-response acquisition has not been extensively used to study stimuli paired with drugs of abuse. Early work by Davis, Smith and colleagues (reviewed by Davis and Smith 1987; see also Goddard and Leri 2006; Marcus et al. 1976) showed that rats would increase their responding on a lever that produced a buzzer noise after the noise was paired with response-independent IV injections of morphine or amphetamine, compared to a pre-conditioning baseline period when lever-presses produced the noise and IV saline injection. These results are consistent with the noise becoming a conditioned reinforcer by Pavlovian association with the drug. However, several alternative explanations for these increases are equally viable, as these studies did not include a second, inactive lever or other control for nonspecific changes in behavior and/or a pharmacological/associative control to account for potential effects of drug exposure regardless of the programmed drug-stimulus association (see Cunningham 1993 for more on interpreting such pre- vs. post-conditioning designs).

More recently, new-response acquisition procedures have been developed in which self-administration of a drug is trained using one type of manipulandum (e.g., a nose-poke), with each IV drug injection accompanied by a particular stimulus, and then responding on a second type of manipulandum (e.g., a lever) is trained with the stimulus alone (e.g., Di Ciano and Everitt 2004; Palmatier et al. 2007; Panlilio et al. 2007). Importantly, the use of self-administered drug gives these procedures at least face validity with human drug abuse; however, the fact that the drug-stimulus pairing takes place in the context of operant reinforcement can make it more

difficult to resolve the associative structures controlling performance. Compared to explicitly Pavlovian procedures, experimenters have less control over the precise specification of the pairings, and the animals' operant training history could significantly influence subsequent response acquisition, as generalization occurs between response types (i.e., as the distributions of the form, force, duration, location, etc., of an animal's movements are or are not modified to differentiate a new response from a previously trained response).

The present experiments, therefore, characterized rats' acquisition of a novel instrumental response (nose-poking) that produced a light-noise stimulus that had been paired with the potent, short-acting μ-opioid agonist, remifentanil. Pavlovian conditioning procedures alone were used to establish the drug-stimulus pairing, and animals had no operant training history before the start of nose-poke acquisition. To establish that acquisition depended on, or was sensitive to, the Pavlovian contingency between the stimulus and remifentanil, animals exposed to stimulus-remifentanil pairings were compared to animals given remifentanil injections and stimulus presentations without consistent pairing (a "truly random" control, Rescorla 1967). Likewise, to establish that acquisition depended on the instrumental contingency between a particular response and the stimulus, animals were allowed to choose between an active nose-poke manipulandum, which produced the stimulus, and an inactive nose-poke manipulandum, which had no scheduled consequences. Three experiments were conducted. Experiment 1 characterized rats' responding in 2 instrumental acquisition sessions after 5 Pavlovian conditioning sessions. In Experiment 2, animals were tested in 7 instrumental acquisition sessions after 5 Pavlovian conditioning sessions. These additional acquisition sessions were conducted to assess the persistence of responding with the stimulus. Experiment 3 assessed the influence of the number of drug-stimulus pairings, giving animals 7 acquisition sessions after

only 1 Pavlovian conditioning session. Finally, to investigate the influence of the schedule of reinforcement on new-response acquisition, the active response produced the stimulus under either a random ratio (RR) 2 or fixed ratio (FR) 1 schedule in Experiments 2 and 3.

Methods

Subjects: Male Sprague-Dawley rats weighing at least 250 g were obtained from Harlan (Indianapolis, IN) to serve as subjects in all experiments. Experimental groups contained 8-12 rats. Animals were housed individually in a temperature (21-23 °C) and humidity controlled facility on a 12 h light/dark cycle (lights on at 7:00 am). Experimental sessions were conducted 6-7 days/week during the light phase of the cycle. All animals had unrestricted access to tap water and standard pellet chow in the home cage for the duration of their experiment. All studies were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Research 1996), as adopted and promulgated by the National Institutes of Health, and all experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Surgery: After at least 7 days of acclimation to the facility, each rat was implanted with a chronic indwelling femoral vein catheter to allow for IV drug administration. Catheterization surgery was performed under ketamine/xylazine (90:10 mg/kg, IP) anesthesia. Catheters, custom made from polyurethane tubing (MRE 040, Braintree Scientific, Braintree, MA) and Tygon tubing (S-54-HL, Norton Performance Plastics, Akron, OH), were inserted into the left femoral vein and routed subcutaneously to the area between the scapulae for externalization. At the

scapulae, the catheter was attached to 22 ga stainless steel tubing that was passed through and secured to a Dacron mesh back-plate (DC95BS, Instech Laboratories, Plymouth Meeting, PA). Rats were allowed at least 5 days to recover from surgery before starting experimental sessions. Catheters were flushed with 0.25 ml of saline with heparin (50 U/ml) each day during recovery, as well as before and after experimental sessions to ensure patency.

Apparatus: Experimental sessions were conducted in two experimental chambers (ENV-008, Med Associates, St. Albans, VT) contained inside light- and sound-attenuating cubicles. Each experimental chamber was located in a separate room of the laboratory. The right wall of each experimental chamber contained a white incandescent houselight (ENV-215M, Med Associates) and a sound generator and speaker (ENV-230 and ENV-224AM, Med Associates). Two nose-poke manipulanda with built-in LED stimulus lights (ENV-114BM, Med Associates) could also be inserted into the right wall. When present, the nose-pokes were located 2.5 cm above the grid floor. The right nose-poke was located 4 cm from the front wall of the experimental chamber, whereas the left nose-poke was located 4 cm from the rear wall. The houselight was centered horizontally between the nose-pokes and located 9 cm above the grid floor. The speaker was located above the right nose-poke, 7.5 cm above the grid floor. Blank aluminum panels were inserted when the nose-pokes were removed, but all other elements of the experimental chamber remained in place.

IV drug injections were delivered by motorized syringe drivers (PHM-107, Med Associates) through Tygon tubing (S-54-HL, Norton Performance Plastics) connected to a fluid swivel (375/22PS, Instech Laboratories or QCS-D, Strategic Applications Inc., Lake Villa, IL)

and spring tether, which were mounted to a counterbalanced arm. The syringe drivers were located outside of the light- and sound-attenuating cubicles.

Pavlovian conditioning: After recovery from catheterization surgery, rats received either "paired" or "random" Pavlovian conditioning (PAV) sessions. During all PAV sessions, the nose-pokes were removed from the experimental chambers, and all animals received response-independent IV injections of remifentanil (3.2 μg/kg/injection delivered in a volume of 100 μl/kg) and response-independent deliveries of a light-noise compound stimulus. The dose of remifentanil was chosen based on previous work in the laboratory on remifentanil self-administration (Cooper et al. 2008). The light-noise stimulus consisted of houselight illumination and white noise (80±5 db as measured at the center of the chamber). Injections and stimuli lasted 2.0±0.5 s, depending on the weight of the individual animal. In the paired PAV groups, a single variable time (VT) 3 min schedule controlled both remifentanil injection and stimulus delivery, and injections and stimuli always co-occurred. In the random PAV control groups, remifentanil injection and stimulus delivery were each controlled by independent VT3 min schedules. Injections and stimuli were not explicitly unpaired. For both paired PAV and random PAV, inter-injection/inter-stimulus intervals ranged from 0.0 to 6.0 min. The 3 min average inter-injection interval was chosen based on the half-life of remifentanil (Crespo et al. 2005) to allow for extensive drug metabolism between injections. PAV sessions lasted until 20 injections and 20 stimulus deliveries occurred, approximately 60 min. In Experiments 1 and 2, separate groups of animals received paired PAV or random PAV for 5 consecutive sessions (100 total injections/stimulus deliveries). In Experiment 3, all groups of animals received 1 session of paired PAV (20 total injections/stimulus deliveries).

Instrumental acquisition: Instrumental acquisition (ACQ) test sessions began the day after the conclusion of PAV. ACQ sessions were conducted the same way following paired PAV and random PAV. During ACQ sessions, the two nose-pokes were present in the experimental chambers. The start of each ACQ session was indicated by the illumination of the stimulus lights inside both nose-pokes, and both nose-pokes remained illuminated for the duration of the session. In each group, the right nose-poke was active for one half of the animals, whereas the left nose-poke was active for the other half of the animals. Responses in the active nose-poke produced the light-noise stimulus alone. No remifentanil injections were given: animals were attached to the tether, but saline replaced remifentanil on the syringe driver, and the syringe driver did not operate at any point. In Experiment 1, responses in the active nose-poke produced the stimulus under a modified RR2 schedule. Under the RR2 schedule, the first response in the active nose-poke in each session produced the stimulus with a probability of 1.0, whereas each subsequent response in the session produced the stimulus with a probability of 0.5. In Experiments 2 and 3, in separate paired PAV and random PAV groups, responses in the active nose-poke produced the stimulus under the RR2 schedule or under a FR1 schedule. In all groups, responses in the inactive nose-poke were recorded but had no scheduled consequences. Active and inactive responses made during stimulus presentation itself were not recorded. All ACQ sessions lasted for 60 min. In Experiment 1, ACQ was conducted for 2 consecutive sessions for all animals. In Experiments 2 and 3, ACQ was conducted for 7 consecutive sessions for all animals.

Data analysis: Based on the acquisition criteria of Cunningham (1993, p 375), two hypotheses were tested: (1) a remifentanil-associated conditioned reinforcer will produce differential responding, i.e., animals will make more active responses than inactive responses after paired PAV but not after random PAV, and (2) a remifentanil-associated conditioned reinforcer will increase responding compared to the control animals, i.e., animals will make more active responses after paired PAV than after random PAV. In Experiments 1 and 2, for each schedule of reinforcement, the mean active and inactive nose-pokes made in each ACQ session were analyzed using three-way ANOVA with the within-subjects factors of manipulandum (active vs. inactive) and session (ACQ1-2 in Experiment 1, ACQ1-7 in Experiment 2) and the between-subjects factor of PAV history (paired vs. random). Paired *t*-tests were then used to compare the active and inactive responses of each group in each ACQ session. Following a significant PAV history X manipulandum interaction and nonsignificant interactions involving PAV history and session, responding was averaged across sessions, and unpaired *t*-tests were used to compare the mean active responses of the paired PAV vs. random PAV groups and the mean inactive responses of the paired PAV vs. random PAV groups. The Holm-Bonferroni method was used to correct for multiple pairwise comparisons. In Experiment 3, for each schedule of reinforcement, the mean active and inactive nose-pokes made in each ACQ session were analyzed using two-way ANOVA with the within-subjects factors of manipulandum and session. Because no main effects or interactions were significant in these ANOVAs, no pairwise tests were performed. Analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, CA) or SPSS Statistics 20.0 (IBM, Armonk, NY). Differences were considered significant when $p < .05$, two-tailed.

Drugs: Remifentanil was obtained from the hospital pharmacy of the University of Michigan Health System (Ultiva brand, GlaxoSmithKline, Uxbridge, Middlesex, UK) and dissolved in sterile physiological saline.

Results

Experiment 1: Responding in 2 ACQ sessions after 5 PAV sessions

Figure 2.1 presents the nose-poke responses of rats in 2 ACQ sessions after 5 sessions of either paired PAV (Figure 2.1a) or random PAV (Figure 2.1b). Animals responded differently in the active vs. inactive nose-poke [main effect of manipulandum; $F(1,18) = 6.04$, $p = .024$; session X manipulandum: $F(1,18) = 4.45$, $p = .049$]. By pairwise comparison, animals that received paired PAV made significantly more active responses than inactive responses in ACQ2 [$t(9) = 3.55$, $p = .012$], whereas the active and inactive responses of animals that received random PAV were not different in either ACQ session [$0.12 < t(9) < 1.61$, all p's $> .10$]. Between groups, however, the effects of PAV history were not significant [main effect and all interactions: $0.24 < F(1,18) < 2.82$, all p's $> .10$].

Experiment 2: Responding in 7 ACQ sessions after 5 PAV sessions

Figure 2.2 presents the nose-poke responses of rats in 7 ACQ sessions after 5 sessions of either paired or random PAV. Animals responded under either the RR2 (Figures 2.2a-2.2c) or FR1 (Figures 2.2d-2.2f) schedule of reinforcement.

Under the RR2 schedule, animals responded differently in the active vs. inactive nose-poke [main effect of manipulandum: $F(1,20) = 16.48$, $p < .001$; session X manipulandum: $F(6,120) = 2.47$, $p = .027$]. By pairwise comparison, animals that received paired PAV made significantly more active responses than inactive responses in each session from ACQ2-7 [Figure 2.2a; $3.20 < t(11) < 4.64$, all p's $< .05$]. After random PAV, animals' active and inactive responses were not different in any ACQ session [Figure 2.2b; $0.20 < t(9) < 1.99$, all p's $> .10$]. Between groups, animals responded differently after paired PAV vs. random PAV [main effect of PAV history: $F(1,20) = 6.69$, $p = .018$], and the effects of PAV history differed for active vs. inactive responding [PAV history X manipulandum: $F(1,20) = 9.63$, $p = .006$]. Responding changed across ACQ sessions [main effect of session: $F(6,120) = 3.48$, $p = .003$], but the effects of PAV history did not depend on the session [session X PAV history: $F(6,120) = 1.08$, $p = .37$; session X PAV history X manipulandum: $F(6,120) = 1.56$, $p = .16$]. Collapsing across sessions to characterize the PAV history X manipulandum interaction (Figure 2.2c), animals made more active responses after paired PAV than after random PAV [$t(20) = 2.91$, $p = .017$), whereas inactive responding was not different after paired PAV vs. random PAV [$t(20) = 1.40$, $p = .17$].

Under the FR1 schedule, numerically, animals made more active responses than inactive responses after paired PAV and more inactive responses than active responses after random PAV. The main effect of manipulandum was not significant [$F(1,18) = 2.97$, $p = .10$], but responding differed significantly within the paired PAV group. By pairwise comparison, animals that received paired PAV made significantly more active responses than inactive responses in ACQ2 and from ACQ4-7 [Figure 2.2d; $3.41 < t(11) < 4.75$, all p's $< .05$]. After random PAV, animals' active and inactive responses were not different in any ACQ session [Figure 2.2e; $0.0 < t(7) < 2.93$, all p's $> .10$]. Animals' responding under the FR1 schedule was

affected by their PAV history as it was under the RR2 schedule: under the FR1 schedule, as well, animals responded differently after paired PAV vs. random PAV [main effect of PAV history: $F(1,18) = 7.17$, $p = .015$], and the effects of PAV history differed for active vs. inactive responding [PAV history X manipulandum: $F(1,18) = 15.48$, $p < .001$]. Responding changed across ACQ sessions [main effect of session: $F(6,108) = 16.23$, $p < .001$], but the effects of PAV history did not depend on the session [session X PAV history: $F(6,108) = 1.03$, $p = .40$; session X PAV history X manipulandum: $F(6,108) = 1.22$, $p = .30$]. Collapsing across sessions to characterize the PAV history X manipulandum interaction (Figure 2.2f), animals made more active responses after paired PAV than after random PAV [$t(18) = 3.60$, $p = .004$], whereas inactive responding did not differ by PAV history [$t(18) = 0.37$, $p = .71$]

Experiment 3: Responding in 7 ACQ sessions after 1 PAV sessions

Figure 2.3 presents the active and inactive responses of rats in 7 ACQ sessions after 1 session of paired PAV. Rats responded under either the RR2 (Figure 2.3a) or FR1 (Figure 2.3b) schedule of reinforcement. Under the RR2 schedule, responding did not differ by nose-poke [main effect of manipulandum: $F(1,7) = 2.07$, $p = .19$] or across sessions [main effect of session: $F(6,42) = 1.74$, $p = .13$; session X manipulandum: $F(6,42) = 1.14$, $p = .35$]. Under the FR1 schedule, likewise, responding did not differ by nose-poke [main effect of manipulandum: $F(1,9) = 3.96$, $p = .078$] or across sessions [main effect of session: $F(6,54) = 0.90$, $p = .49$; session X manipulandum: $F(6,54) = 0.99$, $p = .43$]. The trend toward a difference between the nose-pokes under the FR1 schedule was caused by a slight, but persistent, preference for the

inactive response over the active response. Because paired PAV did not produce any significant changes in ACQ responding, control groups with 1 session of random PAV were not tested.

Discussion

Various behavioral processes can change rates of responding when animals are exposed to a drug-paired environmental stimulus. These processes may be related to exposure to the drug itself, exposure to the stimulus itself, and/or the drug-stimulus pairing. In addition to the conditioned reinforcing effects of the stimulus, responding may be altered by the primary reinforcing effects of the drug, primary reinforcing effects of the stimulus (i.e., sensory reinforcement), discriminative effects of the stimulus, unconditioned effects of drug exposure, nonassociative learning (e.g., habituation to the sensory aspects of the stimulus), and other influences. These alternatives can confound a number of experimental preparations intended to measure conditioned reinforcement (Cunningham 1993; Kelleher and Gollub 1962; Mackintosh 1974; Shahan 2010; Williams 1994). The present study, therefore, used a behaviorally stringent new-response acquisition procedure to characterize the conditioned reinforcing effects of a light-noise stimulus that was paired with the μ-opioid agonist, remifentanil.

After 5 sessions of paired PAV, rats acquired a novel nose-poke response that produced the light-noise stimulus alone. Under either the RR2 or FR1 schedule of reinforcement, significant preferences for the active response developed rapidly (by ACQ2, Experiments 1 and 2) and persisted across multiple testing sessions (active > inactive even in ACQ7, Experiment 2). Control rats did not acquire nose-poking when the stimulus and remifentail were not consistently paired: after 5 sessions of random PAV, no significant preference for the active response was

observed in any ACQ session. With the 7 ACQ sessions in Experiment 2, furthermore, rats made more active responses after paired PAV than after random PAV. Pairing the stimulus with remifentanil selectively affected active responding, as inactive responding did not differ by PAV history under either schedule. Thus, the remifentanil-paired stimulus maintained both differential responding (active > inactive within-subjects) and increased responding (active > active between-subjects). Different criteria may be used to determine when a response has been successfully acquired with either conditioned or primary reinforcement; however, in experimental designs that include two manipulanda, testing for both within-group and between-group differences in active responding may provide a more comprehensive account of the response strength obtained, even if it is not always used as the minimum requirement for an adequate demonstration of reinforcement (Cunningham 1993; Snycerski et al. 2005). In contrast to the effects of 5 sessions of paired PAV, rats did not acquire responding under either schedule of reinforcement after 1 session of paired PAV. These results are consistent with earlier studies of the effects of pairing number on the conditioned reinforcing effects of food-associated stimuli, as well as more general notions of "associative strength" or the degree of association underlying other behaviors that depend on Pavlovian learning (reviewed by Kelleher and Gollub 1962; Mackintosh 1974).

Responding with the remifentanil-paired stimulus, therefore, satisfies the three criteria for conditioned reinforcement reviewed above (Mackintosh 1974, p 234). First, the absence of the nose-poke manipulanda during PAV and the absence of remifentanil during ACQ prevented direct association of the nose-poke response with remifentanil as a primary reinforcer. Rather, the differences between the paired PAV and random PAV groups show that acquisition depended on the Pavlovian pairing of the stimulus with remifentanil. Prior exposure to

remifentanil and stimulus presentation without consistent pairing did not produce differential responding during ACQ or as much active responding as paired PAV. Finally, the differences between active and inactive nose-poke responding during ACQ indicate that acquisition depended on the instrumental association between the active response and the stimulus. The side of the active nose-poke (left vs. right) was counterbalanced across animals in each group, and the houselight and speaker were not consistently located above the active nose-poke. It is, therefore, unlikely that either a spatial bias or Pavlovian conditioned approach to the remifentanil-paired stimuli was the sole basis for differential responding. Likewise, both nose-pokes simply remained illuminated for the duration of the session, and so the differences in responding are unlikely to have emerged from a difference in the sensory aspects of the active vs. inactive manipulanda themselves. The patterns of performance observed appear to depend on the different consequences of the active and inactive nose-poke responses.

This is not to say that independently programmed or randomized presentations of drugs and environmental stimuli have no effect on behavior, or that the random control groups learned nothing during their PAV sessions. Even with the significant differences between the paired PAV and random PAV groups reviewed above, animals in the random PAV groups still made ~5-10 active and inactive responses per session during ACQ. This responding may be due to associative processes (e.g., from pairing the operant chamber context generally with remifentanil) and/or nonassociative processes (e.g., reactions to the nose-poke manipulanda as novel objects inside the operant chamber). Some of these same processes may have also influenced the responding of the paired PAV groups, in addition to the effects of the remifentanil-stimulus pairing. It is beyond the scope of the present discussion to consider fully the different contingency-dependent and contingency-independent theories of Pavlovian

conditioning that have been proposed (see, e.g., Kirkpatrick and Church 2004; Miller and Matzel 1989; Papini and Bitterman 1990), but it is important to recognize that there continues to be debate about the procedures that comprise adequate controls in Pavlovian conditioning experiments. A random control procedure was chosen for the present study to ensure that the experimental and control groups were matched for their exposure to the individual experimental elements—both total remifentanil exposure and exposure to the light-noise stimulus—during PAV (Cunningham 1993). The present study cannot address the details of the learning of the animals in the random PAV groups, except to note that this learning (whatever it was) did not produce the same effect on nose-poke responding that paired PAV did, and so the differences between the groups in this target behavior are still relevant to understanding how a specific drug-paired stimulus can control a specific behavioral response.

In human drug abuse and dependence generally, Pavlovian drug-associated stimuli are thought to play a number of distinct, but interacting, roles in maintaining drug self-administration behaviors and provoking relapse (reviewed by Milton and Everitt 2010). As conditioned reinforcers, specifically, drug-paired stimuli may help to sustain (1) prolonged sequences or chains of behavior that ultimately lead to drug consumption and (2) drug-seeking responses in extinction, when the drug itself is unavailable (Milton and Everitt 2010). Human drug abusers are often required to engage in long, complex sequences of behavior to obtain and prepare drugs prior to consuming them, and laboratory animals can also be trained to produce extended multioperant chains with self-administered drug (e.g., Thompson and Pickens 1969, Figure 9). Reducing the conditioned reinforcing of drug-paired stimuli may disrupt the performance of such chains, reducing access to and drug-taking in their terminal links. Next, by maintaining existing responses and training new responses in the absence of the drug itself,

conditioned reinforcers may both complicate the detoxification process, as individuals attempt to break ongoing patterns of drug self-administration, and contribute to relapse after extended abstinence. The sustained preferences for the active response observed in the present study are noteworthy in this regard. Historically, researchers have questioned whether new-response acquisition behavior is too transient to be of practical use in studying conditioned reinforcement: because responses during instrumental acquisition necessarily present the stimulus in the absence of the primary reinforcer, Pavlovian extinction may rapidly reduce or eliminate the conditioned reinforcing effects of the stimulus (Mackintosh 1974; Williams 1994; Sosa et al. 2011). Many of the details of the interactions between Pavlovian and instrumental learning remain to be elucidated (e.g., Palmatier et al. 2008), but it is becoming increasingly clear that sustained response-acquisition performance can be obtained with drug-based conditioned reinforcement (see also Di Ciano and Everitt 2004). Altogether, therefore, interventions aimed at reducing the conditioned reinforcing effects of drug-paired stimuli may help make drug-seeking and drug-taking behaviors less flexible and less sustainable. New-response acquisition procedures may provide useful models for studying the enduring control drug-paired stimuli can exert over behavior and for testing interventions designed to reduce that control.

Works cited

Beninger RJ and Ranaldi R (1994) Dopaminergic agents with different mechanisms of action differentially affect responding for conditioned reward, in *Strategies for Studying Brain Disorders, Volume 1, Depressive, Anxiety and Drug Abuse Disorders* (Palomo T and Archer T eds) pp 411–428, Farrand Press, London.

Beninger RJ and Rolfe NG (1995) Dopamine D1-like receptor agonists impair responding for conditioned reward in rats. *Behav Pharmacol* **6:** 785–793. doi: 10.1097/00008877-199512000-00003

Burke KA, Franz TM, Miller DN, and Schoenbaum G (2007) Conditioned reinforcement can be mediated by either outcome-specific or general affective representations. *Front Integr Neurosci.* **1:** 2. doi: 10.3389/neuro.07/002.2007

Cooper ZD, Truong YN-T, Shi Y-G, and Woods JH (2008) Morphine deprivation increases self-administration of the fast- and short-acting μ-opioid receptor agonist remifentanil in the rat. *J Pharmacol Exp Ther* **326:** 920–929. doi: 10.1124/jpet.108.139196

Crespo JA, Sturm K, Saria A, and Zernig G (2005) Simultaneous intra-accumbens remifentanil and dopamine kinetics suggest that neither determines within-session operant responding. *Psychopharmacology (Berl)* **183:** 201–209. doi: 10.1007/s00213-005-0180-7

Cunningham CL (1993) Pavlovian drug conditioning, in *Methods in Behavioral Pharmacology* (van Haaren F ed) pp 349–381, Elsevier, New York.

Davis WM and Smith SG (1987) Conditioned reinforcement as a measure of the rewarding properties of drugs, in *Methods of Assessing the Reinforcing Properties of Abused Drugs* (Bozarth MA ed) pp 199–210, Springer-Verlag, New York.

de Borchgrave R, Rawlins JNP, Dickinson A, and Balleine BW (2002) Effects of cytotoxic nucleus accumbens lesions on instrumental conditioning in rats. *Exp Brain Res* **144:** 50–68. doi: 10.1007/s00221-002-1031-y

Di Ciano P and 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. doi: 10.1016/j.neuropharm.2004.06.005

Everitt BJ and 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. doi: 10.1007/s002130000566

Goddard B and Leri F (2006) Reinstatement of conditioned reinforcing properties of cocaine-conditioned stimuli. *Pharmacol Biochem Behav* **83:** 540–546. doi: 10.1016/j.pbb.2006.03.015

Hogarth L and Duka T (2006) Human nicotine conditioning requires explicit contingency knowledge: is addictive behavior cognitively mediated? *Psychopharmacology (Berl)* **184:** 553–566. doi: 10.1007/s00213-005-0150-0

Hyde TS (1976) The effect of Pavlovian stimuli on the acquisition of a new response. *Learn Motiv* **7:** 223–239. doi: 10.1016/0023-9690(76)90030-8

Institute of Laboratory Animal Research CoLS, National Research Council (1996) *Guide for the Care and Use of Laboratory Animals*, 7th ed. National Academies Press, Washington DC.

Kelleher RT and Gollub LR (1962) A review of positive conditioned reinforcement. *J Exp Anal Behav* **5:** 543–597. doi: 10.1901/jeab.1962.5-s543

Kirkpatrick K and Church RM (2004) Temporal learning in random control procedures. *J Exp Psychol Anim Behav Process* **30:** 213–228. doi: 10.1037/0097-7403.30.3.213

Le Foll B and Goldberg SR (2005) Control of the reinforcing effects of nicotine by associated environmental stimuli in animals and humans. *Trends Pharmacol Sci* **26:** 287–293. doi: 10.1016/j.tips.2005.04.005

Mackintosh NJ (1974) *The Psychology of Animal Learning*. Academic Press, New York.

Marcus R, Carnathan G, Meyer RE, and Cochin J (1976) Morphine-based secondary reinforcement: effects of different doses of naloxone. *Psychopharmacology (Berl)* **48:** 247–250. doi: 10.1007/BF00496856

Miller RR and Matzel LD (1989) Contingency and relative associative strength, in *Contemporary Learning Theories: Pavlovian Conditioning and the Status of Traditional Learning Theory*. (Klein SB and Mowrer RR eds) pp 61–84, Lawrence Erlbaum Associates, Hillsdale, NJ.

Milton AL and Everitt BJ (2010) The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *Eur J Neurosci* **31:** 2308–2319. doi: 10.1111/j.1460-9568.2010.07249.x

Myers KM and Carlezon WA (2010) Extinction of drug- and withdrawal-paired cues in animal models: relevance to the treatment of addiction. *Neurosci Biobehav Rev* **35:** 285–302. doi: 10.1016/j.neubiorev.2010.01.011

Olausson P, Jentsch JD, and Taylor JR (2004) Repeated nicotine exposure enhances responding with conditioned reinforcement. *Psychopharmacology (Berl)* **173:** 98–104. doi: 10.1007/s00213-003-1702-9

Olive MF and Kalivas PW (2011) Conditioning of addiction, in *Addiction Medicine* (Johnson BA ed) pp 159–178, Springer, New York. doi: 10.1007/978-1-4419-0338-9_8

Palmatier MI, Liu X, Matteson GL, Donny EC, Caggiula AR, and Sved AF (2007) Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. *Psychopharmacology (Berl)* **195:** 235–243. doi: 10.1007/s00213-007-0897-6

Palmatier MI, Coddington SB, Liu X, Donny EC, Caggiula AR, and Sved AF (2008) The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose. *Neuropharmacology* **55:** 1425–1430. doi: 10.1016/j.neuropharm.2008.09.002

Panlilio LV, Thorndike EB, and Schindler CW (2007). Blocking of conditioning to a cocaine-paired stimulus: testing the hypothesis that cocaine perpetually produces a signal of larger-than-expected reward. *Pharmacol Biochem Behav* **86:** 774–777. doi: 10.1016/j.pbb.2007.03.005

Papini MR and Bitterman ME (1990) The role of contingency in classical conditioning. *Psychol Rev* **97:** 396–403. doi: 10.1037/0033-295X.97.3.396

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, and Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. *J Neurosci* **19:** 2401–2411.

Parkinson JA, Roberts AC, Everitt BJ, and Di Ciano P (2005) Acquisition of instrumental conditioned reinforcement is resistant to the devaluation of the unconditioned stimulus. *Q J Exp Psychol B* **58:** 19–30. doi: 0.1080/02724990444000023

Rescorla RA (1967) Pavlovian conditioning and its proper control procedures. *Psychol Rev* **74:** 71–80. doi: 10.1037/h0024109

See RE (2005) Neural substrates of cocaine-cue associations that trigger relapse. *Eur J Pharmacol* **526:** 140–146. doi: 10.1016/j.ejphar.2005.09.034

Shahan TA (2010) Conditioned reinforcement and response strength. *J Exp Anal Behav* **93:** 269–289. doi: 10.1901/jeab.2010.93-269

Snycerski S, Laraway S, and Poling A (2005) Response acquisition with immediate and delayed conditioned reinforcement. *Behav Processes* **68:** 1–11. doi: 10.1016/j.beproc.2004.08.004

Sosa, R, dos Santos CV, and Flores C (2011) Training a new response using conditioned reinforcement. *Behav Processes* **87:** 231–236. doi: 10.1016/j.beproc.2011.03.001

Taylor JR, Olausson P, Quinn JJ, and Torregrossa MM (2009) Targeting extinction and reconsolidation mechanisms to combat the impact of drug cues on addiction. *Neuropharmacology* **56:** 186–195. doi: 10.1016/j.neuropharm.2008.07.027

Thompson T and Pickens R (1969) Drug self-administration and conditioning, in *Scientific Basis of Drug Dependence* (Steinberg H ed) pp 177–198, J & A Churchill, London.

Williams BA (1994) Conditioned reinforcement: experimental and theoretical issues. *Behav Anal* **17:** 261–285.

Figure 2.1. Acquisition of a novel nose-poke response when responses in the active nose-poke produced a stimulus that was previously paired with response-independent IV remifentanil injection

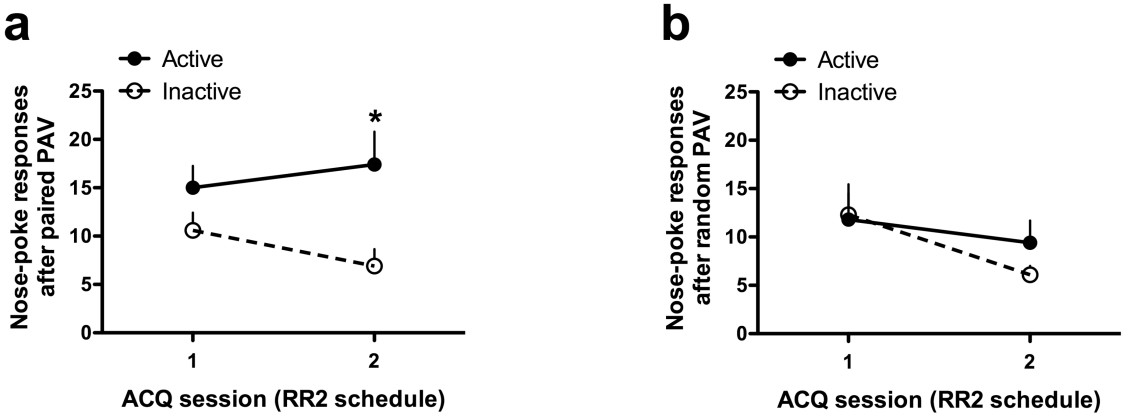

Figure 2.1. Acquisition of a novel nose-poke response when responses in the active nose-poke produce a stimulus that was previously paired with response-independent IV remifentanil injection. *a*: Active and inactive nose-poke responses made by rats (n = 10) after 5 sessions of paired PAV. *b*: Active and inactive nose-poke responses made by control rats (n = 10) after 5 sessions of random PAV. * $p < .05$. Significant difference between active and inactive responding in the given ACQ session as assessed by paired *t*-test. All data are presented as the mean±SEM.

Figure 2.2. Persistence of responding across ACQ sessions with the remifentanil-paired stimulus under both the RR2 and FR1 schedules of reinforcement

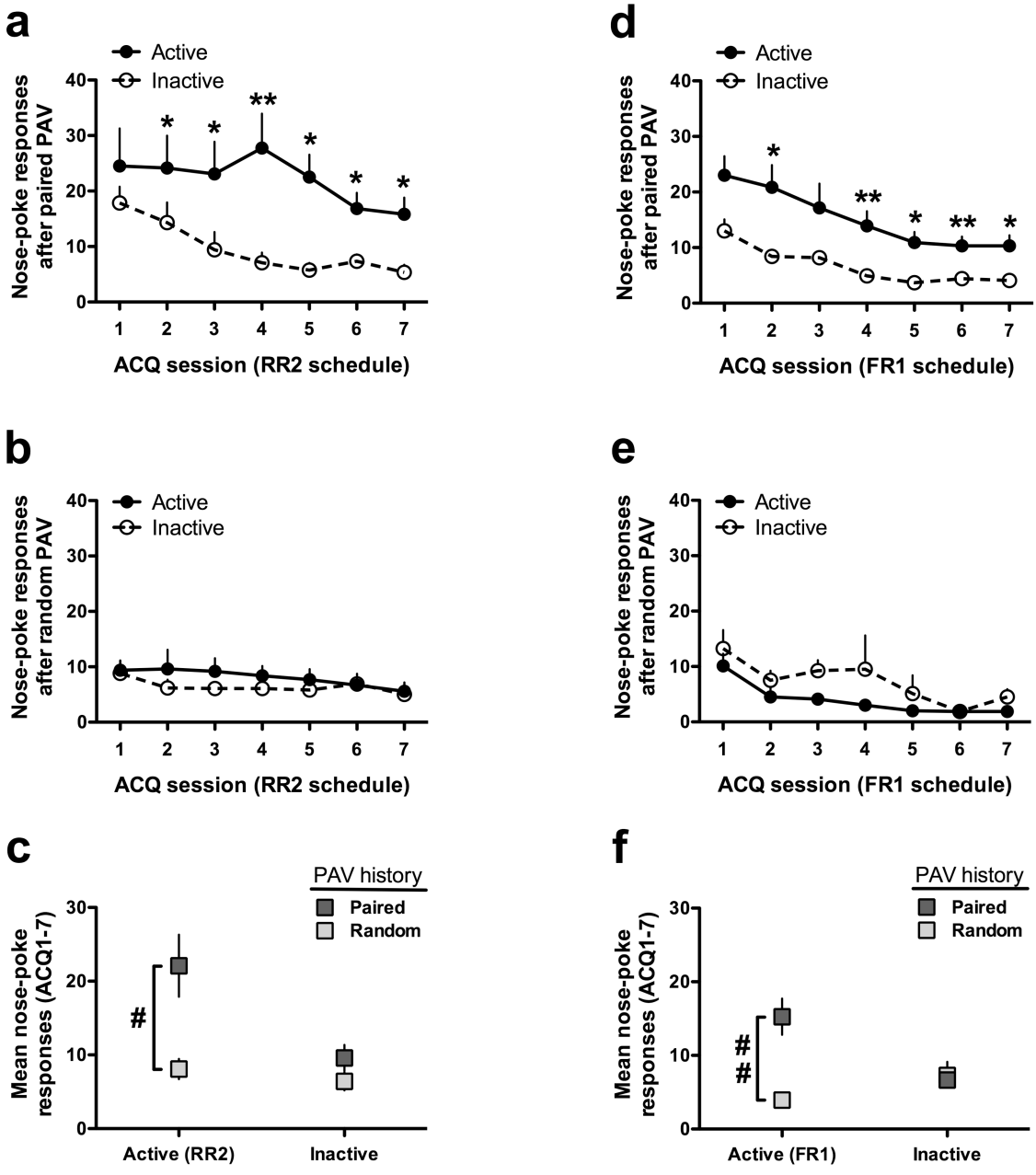

Figure 2.2. Persistence of responding across ACQ sessions with the remifentanil-paired stimulus under both the RR2 and FR1 schedules of reinforcement. *a*: Active and inactive nose-poke responses made by rats (n = 12) under the RR2 schedule after 5 sessions of paired PAV. *b*: Active and inactive nose-poke responses made by control rats (n = 10) under the RR2 schedule after 5 sessions of random PAV. *c*: Mean active and inactive responses made from ACQ1-7 under the RR2 schedule after paired or random PAV. *d*: Active and inactive nose-poke responses made by rats (n = 12) under the FR1 schedule after 5 sessions of paired PAV. *e*: Active and inactive nose-poke responses made by control rats (n = 8) under the FR1 schedule after 5 sessions of random PAV. *f*: Mean active and inactive responses made from ACQ1-7 under the FR1 schedule after paired or random PAV. * $p < .05$; ** $p < .01$. Significant difference between active and inactive responding in the given ACQ session as assessed by paired *t*-test. # $p < .05$; ## $p < .01$. Significant difference between paired and random PAV as assessed by unpaired *t*-test. All data are presented as the mean±SEM.

Figure 2.3. Lack of acquisition of nose-poke responding when the number of remifentanil-stimulus pairings is reduced

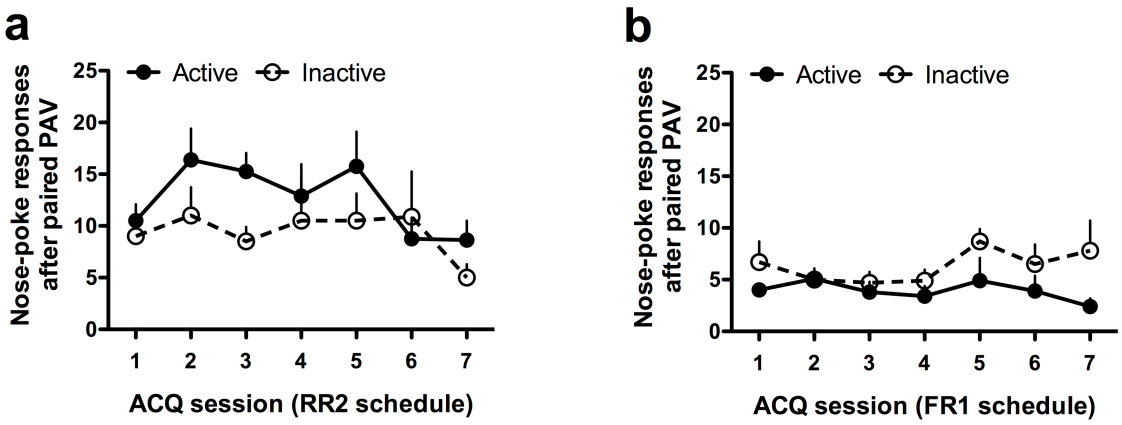

Figure 2.3. After 1 session of PAV, rats do not acquire nose-poke responding with the remifentanil-paired stimulus. *a*: Active and inactive nose-poke responses made by rats (n = 8) under the RR2 schedule after 1 session of paired PAV. *b*: Active and inactive nose-poke responses made by rats (n = 10) under the FR1 schedule after 1 session of paired PAV. All data are presented as the mean±SEM.

CHAPTER III

Effects of Pramipexole on Responding Maintained by Remifentanil-Paired Stimuli: Resistance to Extinction of Self-Administration and New-Response Acquisition

Introduction

Among the dopamine D1-like (D1, D5) and D2-like (D2, D3, D4) receptors, the D3 receptor shows a particularly constrained neuroanatomical distribution, with expression focused in limbic brain regions (Beaulieu and Gainetdinov 2011). In both rodents and primates, moderate-to-high densities of D3 receptor protein and/or mRNA are found in brain structures and systems associated with the reinforcing effects of drugs of abuse and responding with drug-associated stimuli, including the mesolimbic dopaminergic system, extended amygdala, and corticostriatal loops (reviewed by Heidbreder and Newman 2010; Le Foll et al. 2005; Shafer and Levant 1998; Sokoloff et al. 2006). Accordingly, D3 receptor activity is thought to be important for drug self-administration behaviors, and D3-preferring or D3/D2 ligands have received considerable attention as potential therapeutics for human drug abuse and dependence (Garcia-Ladona and Cox 2003; Heidbreder 2013; Heidbreder and Newman 2010; Le Foll et al. 2005; Newman et al. 2012; Sokoloff et al. 2006; Shafer and Levant 1998).

In both laboratory rodents and nonhuman primates, treatment with D3-preferring or D3/D2 agonists and antagonists has been shown to modify significantly both drug self-

administration responding and responding with drug-associated stimuli (reviewed by Self 2010). In rats, much of this work has focused on the ability of these compounds to alter responding with cocaine-associated stimuli in extinction after cocaine self-administration training (e.g., in reinstatement procedures) or with ongoing self-administration under second-order schedules of stimulus and cocaine availability. Tested in extinction after self-administration training, systemic pretreatments of a variety of D3-preferring or D3/D2 agonists can increase (Cervo et al. 2003; Collins and Woods 2009; Collins et al. 2012; De Vries et al. 1999, 2002; Dias et al. 2004; Edwards et al. 2007; Fuchs et al. 2002; Koeltzow and Vezina 2005; Self et al. 1996; Wise et al. 1990), and D3-preferring or D3/D2 antagonists can decrease (Cervo et al. 2003, 2007; Crombag et al. 2002; Gál and Gyertyán 2006; Gilbert et al. 2005; Weiss et al. 2001), cocaine-appropriate responding. In these studies, rats responded in the presence of cocaine-associated contextual and discriminative stimuli, and/or responding on the cocaine-associated manipulandum produced stimuli (e.g., cue lights, tones) previously paired with cocaine injection, but not cocaine itself. D3-preferring agonists also can enhance (Fuchs et al. 2002; Self et al. 1996), and D3-preferring or D3/D2 antagonists can attenuate (Peng et al. 2009; Vorel et al. 2002; Xi et al. 2006; see also Weissenborn et al. 1996), the ability of cocaine pretreatment to increase extinction responding under these circumstances. Likewise, pretreatments of either a D2-preferring or a D3-preferring antagonist can attenuate the ability of a D3-preferring agonist to increase cocaine-trained responding (Collins et al. 2012). Under second-order schedules of cocaine self-administration, D3-preferring antagonist administration has been shown to reduce rats' behavior before any cocaine has actually been delivered, i.e., in an initial phase of the session when responding has only produced the cocaine-associated stimuli (Di Ciano et al. 2003; see also Pilla et al. 1999).

Compared to this body of work with cocaine, fewer studies have examined the effects of D3-preferring or D3/D2 ligands on responding with stimuli associated with other drugs of abuse, particularly opioid-associated stimuli (Heidbreder 2013; Heidbreder and Newman 2010; Self 2010). Wise and colleagues (1990) first reported that response-independent IV injection of the D3/D2 agonist, bromocriptine, increased rats' extinction responding after either cocaine or heroin self-administration. Subsequently, De Vries and colleagues (2002) reported that SC injection of the D3-preferring agonist, quinpirole, increased extinction responding after heroin self-administration training, and this effect depended on the number of extinction sessions conducted before the quinpirole challenge sessions (see also De Vries et al. 1999). Among antagonists, the D3/D2 antagonist, raclopride, was shown (numerically) to attenuate the increase in extinction responding caused by experimenter-administered heroin pretreatment after heroin self-administration training (Shaham and Stewart 1996). These results demonstrate the importance of D3/D2 activity in opioid-related behaviors, but the role(s) played by particular opioid-associated stimuli remain unclear. For example, when testing the effects of bromocriptine, Wise and colleagues (1990) continued to present response-contingently the cue light that was paired with heroin injection, whereas De Vries and colleagues (2002) sought specifically to limit the effects of drug-paired cues in their quinpirole test sessions and withheld the stimuli that had previously accompanied heroin injection. There were a number of other procedural differences between these studies, and so the relative importance of delivering vs. withholding the drug-paired stimuli during agonist testing is unclear. Therefore, additional work is needed to characterize (1) the effects of D3-preferring ligands on opioid-trained responding and (2) the behavioral mechanisms by which these compounds act when they change responding.

In particular, several authors have suggested that D2-like receptor ligands can alter the conditioned reinforcing effects of drug-paired stimuli (Cervo et al. 2003, 2007; Collins and Woods 2009; Collins et al. 2012; Di Ciano et al. 2003; Gál and Gyertyán 2006; Le Foll et al. 2005; Pilla et al. 1999). The exteroceptive stimuli that accompany drug injections may, because of this pairing, become conditioned reinforcers. If so, increases or decreases in responding when these stimuli are presented in the absence of the drug could be due to increases or decreases, respectively, in the effectiveness of these conditioned reinforcers. However, the behavioral procedures so far used cannot isolate the conditioned reinforcing effects of the stimuli: changes in the extinction of a previously trained response or in responding under second-order schedules of reinforcement can be caused by a number of behavioral mechanisms other than conditioned reinforcement, including the primary reinforcing effects of the training drug and the discriminative stimulus functions of the training drug, the testing drug, and the stimuli (Collins et al. 2012; Kelleher and Gollub 1962; Mackintosh 1974; Wike 1966; Williams 1994). Measuring the ability of animals to acquire a new response that produces a drug-paired stimulus (i.e., a response that does not or did not also produce the drug itself) can provide a more valid assessment of the conditioned reinforcing effects of that stimulus (Mackintosh 1974; Williams 1994). Consistent with a role for D2-like receptor activity in conditioned reinforcement, specifically, systemic administration of D2-like, but not D1-like, receptor agonists has been shown to enhance new-response acquisition with food-paired stimuli (Beninger and Ranaldi 1992; Beninger and Rolfe 1995; Sutton et al. 2001). However, there have not been corresponding studies reported, to my knowledge, of the effects of D2-like agonists on new-response acquisition with drug-paired conditioned reinforcers.

To clarify the potential importance of changes in conditioned reinforcement to the effects of D2-like agonists on opioid-related behaviors, the present study characterized the effects of the D3-preferring agonist, pramipexole (PRAM), on responding with opioid-associated stimuli in two different behavioral tasks: (1) resistance-to-extinction/reinstatement of remifentanil self-administration and (2) new-response acquisition after Pavlovian remifentanil-stimulus pairing. Three experiments were conducted. Experiment 1 tested a series of doses of PRAM in extinction after rats were trained to self-administer remifentanil under a progressive ratio (PR) schedule. Finding that PRAM significantly increased responding, and that these increases depended on the presentation of the stimuli that had been paired with remifentanil injection, the effects of PRAM on new-response acquisition were evaluated in Experiment 2. Finally, Experiment 3 addressed the time-course of the effects of PRAM on new-response acquisition.

Methods

General methods for Experiments 1-3

Animals: Male Sprague-Dawley rats weighing at least 250 g were obtained from Harlan (Indianapolis, IN) to serve as subjects in all experiments. Experimental groups contained 8 rats except where noted in Experiment 3. Animals were housed in a climate controlled facility under a 12 h light-dark cycle (lights on at 7:00 am). All animals were allowed to acclimate to the facility for at least 7 days before the start of any experimental procedures. Experimental sessions were conducted 5-7 days/week during the light phase of the cycle. All animals had unrestricted access to standard pellet chow and tap water in the home cage for the duration of their

experiment. All studies were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Research 1996), as adopted and promulgated by the National Institutes of Health, and all experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Surgery: After acclimating to the facility, each animal received a chronic, indwelling venous catheter to allow for IV drug administration. Catheters were custom made from polyurethane tubing (MRE 040, Braintree Scientific, Braintree, MA) and Tygon tubing (S-54-HL, Norton Performance Plastics, Akron, OH). Catheterization surgery was performed under ketamine/xylazine (90:10 mg/kg, IP) anesthesia. The catheter was inserted into the left femoral vein and routed subcutaneously to the area between the scapulae for externalization. At the scapulae, the catheter was attached to 22 ga stainless steel tubing which was passed through and secured to a Dacron mesh back-plate (DC95BS, Instech Laboratories, Plymouth Meeting, PA). Rats were allowed at least 5 days to recover from surgery before starting experimental sessions. Catheters were flushed with 0.25 ml of heparinized saline (50 U/ml) each day during recovery, as well as before and after experimental sessions to ensure patency. In Experiment 1, animals continued to be tested if catheter patency was lost after the conclusion of self-administration. In Experiments 2 and 3, animals had patent catheters throughout the experiment.

Drugs: Remifentanil was obtained from the hospital pharmacy of the University of Michigan Health System (Ultiva brand, GlaxoSmithKline, Uxbridge, Middlesex, UK). Pramipexole was obtained from APAC Pharmaceutical (Columbia, MD). Both drugs were dissolved in sterile physiological saline.

Experiment 1: Resistance to extinction of remifentanil self-administration responding

The methods of Experiment 1 were adapted from those used by Collins and colleagues (2012) to study the effects of PRAM in cocaine-trained rats.

Apparatus: Experimental sessions were conducted in four experimental chambers (ENV-008, Med Associates Inc., St. Albans, VT) located inside light- and sound-attenuating cubicles. The right wall of each experimental chamber contained a nose-poke manipulandum (ENV-114BM, Med Associates), located 5 cm above the grid floor and 4 cm from the front wall, and a lever manipulandum (H21-03R, Coulbourn Instruments, Whitehall, PA), located 5 cm above the grid floor and 4 cm from the rear wall. The nose-poke aperture contained a yellow LED stimulus light, and a set of green, yellow, and red LED stimulus lights (ENV-222M, Med Associates) was located directly above the nose-poke. A white incandescent houselight (ENV-215M, Med Associates) was located in the left wall of each experimental chamber, centered horizontally 9 cm above the grid floor.

Drug solutions were delivered by a motorized syringe driver (PHM-107, Med Associates) through Tygon tubing (S-54-HL, Norton Performance Plastics) connected to a fluid swivel (375/22, Instech Laboratories) and spring tether that were mounted to a counterbalanced arm. The syringe drivers were located outside of the light- and sound-attenuating cubicles.

Remifentanil self-administration training: Following recovery from surgery, animals were trained to self-administer 3.2 μg/kg/injection remifentanil (delivered in a volume of 100 μl/kg) in

90 min sessions. Responding was initially trained under a fixed ratio (FR) 1 schedule of reinforcement. The ratio requirement was gradually increased to FR5 before training under the PR schedule began. Under the PR schedule, ratio requirements increased within the session (1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1,102, etc.) according to the equation of Richardson and Roberts (1996): ratio value = $[5e^{(\text{reinforcer number} * 0.2)}]-5$. PR sessions lasted for 240 min or until a ratio requirement was not completed in 45 min, whichever occurred first. The value of the final ratio completed before the end of the session was recorded as the animal's break point.

Under both the FR and PR schedules, the start of the session was signaled by the illumination of the nose-poke aperture light. Upon completion of a ratio requirement in the nose-poke, a remifentanil injection was delivered accompanied by the illumination of the three LED stimulus lights above the nose-poke aperture for the duration of the injection (2.0±0.5 s, depending on the weight of individual animal). A 5 s time out (TO) followed the conclusion of each injection. During the TO, the nose-poke aperture light was extinguished, and the houselight was illuminated. The houselight was extinguished, and the nose-poke aperture light was re-illuminated at the conclusion of the TO. Responses on the lever were recorded but had no scheduled consequences.

Extinction testing I, PRAM dose-effect determination: Testing began when remifentanil self-administration under the PR schedule stabilized (at least 3 sessions with less than 20% difference and no increasing or decreasing trend in the number of ratios completed across sessions). Animals were tested in extinction under the PR schedule. Sessions began with the illumination of the nose-poke aperture light, and nose-poke responses produced the LED stimulus light

illumination and TO but no injection. Animals were attached to the tether, but saline replaced remifentanil on the syringe driver, and the syringe driver did not operate at any point. Lever responses were counted but had no scheduled consequences. Pretreatment injections were administered SC immediately before the start of each test session. All animals received all PRAM doses: vehicle (0.0 mg/kg), 0.032-1.0 mg/kg. PRAM doses were administered in either ascending or descending order, counterbalanced across animals, and each dose was administered for three consecutive sessions.

Extinction testing II, stimulus manipulation: After the determination of the dose-effect function, the effects of 0.0 mg/kg and 0.32 mg/kg PRAM were reassessed within-subjects when different remifentanil-associated stimuli were presented/withheld in the session. Stimulus manipulation sessions began directly after completion of the PRAM dose-effect determination: no additional remifentanil self-administration training or exposure to remifentanil was given. Under all stimulus manipulation conditions, the PR schedule was in effect, and responses were counted as they were in the previous phases of the study. Responding was measured in four stimulus conditions, differentiated by whether or not the remifentail-associated discriminiative stimulus (DS, nose-poke aperture illumination) and/or the remifentanil-paired conditioned stimuli (CS, LED and houselight illumination) were presented. (1) In the context condition, the nose-poke aperture light was not illuminated, and responses in the nose-poke did not produce LED or houselight illumination. (2) In the DS only condition, the nose-poke aperture light was illuminated for the duration of the session, and responses in the nose-poke did not produce LED or houselight illumination. (3) In the CS only condition, the nose-poke aperture light was not illuminated at any point, but responses in the nose-poke produced the ~2 s LED and 5 s

houselight illumination. (4) In the DS+CS condition, the nose-poke aperture light was illuminated for the duration of the session (except during TO), and responses in the nose-poke produced the ~2 s LED and 5 s house-light illumination. Each stimulus condition was tested for two sequential sessions: in a randomly determined order for each animal, 0.0 mg/kg was administered immediately prior to one session, whereas 0.32 mg/kg PRAM was administered immediately before the other session. After all four stimulus conditions were tested once, this process was repeated, and a second set of sessions was conducted with the order of stimulus conditions and order of pretreatments re-determined randomly for each animal.

Data analysis: The following endpoints of PR performance were measured: active responses, break point, number of ratios completed, total session length (min), and inactive responses. The three sessions conducted with each pretreatment dose, as well as the final three remifentanil self-administration training sessions, were averaged for analysis. As a preliminary test to check for effects of pretreatment dose order, the number of ratios completed in extinction was analyzed using a two-way ANOVA with the within-subjects factor of pretreatment dose and the between-subjects factor of dose order (ascending vs. descending). Not finding significant effects of dose order, the data were combined between orders for all subsequent analyses. To determine whether PRAM altered extinction responding, each endpoint was analyzed using repeated-measures ANOVA with the within-subjects factor of pretreatment dose. Following a significant omnibus effect, each dose of PRAM was compared to vehicle (0.0 mg/kg) using post hoc Bonferroni tests. To determine if extinction responding differed from self-administration responding, each pretreatment condition was compared to the final self-administration training sessions using Bonferroni-corrected paired *t*-tests.

To characterize the effects of PRAM on the intrasession allocation of responding, the active responses made in extinction were reanalyzed. Because the total session length varied across sessions, each session was divided into 5 blocks (BLOCK1-5), each encompassing a sequential 20% of the total session length. The rate of responding (responses/min) in each block was calculated. As above, the three sessions conducted with each pretreatment dose were averaged for analysis. The average rate of responding in each block was analyzed using two-way ANOVA with the within-subjects factors of block and dose. Following a significant block X dose interaction, post hoc Bonferroni tests were used to compare each dose of PRAM to vehicle (0.0 mg/kg) in each block.

To assess the influence of stimulus manipulation on the response-increasing effects of PRAM, the two determinations of each pretreatment condition under each stimulus condition were averaged. Animals' active responses and inactive responses were analyzed separately using two-way ANOVA with the within-subjects factors of pretreatment (0.0 mg/kg vs. 0.32 mg/kg PRAM) and stimulus condition (context, DS only, CS only, DS+CS). Following a significant pretreatment X stimulus condition interaction, post hoc Bonferroni tests were used to compare 0.0 mg/kg and 0.32 mg/kg PRAM under each stimulus condition.

Analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, CA) or SPSS Statistics 20.0 (IBM, Armonk, NY). Differences were considered significant when $p < .05$, two-tailed.

Experiments 2 and 3: New-response acquisition with a remifentanil-paired stimulus

Apparatus: Experimental sessions were conducted in two experimental chambers (ENV-008, Med Associates) contained inside light- and sound-attenuating cubicles. Each experimental chamber was located in a separate room of the laboratory. The right wall of each experimental chamber contained a white incandescent houselight (ENV-215M, Med Associates) and a sound generator and speaker (ENV-230 and ENV-224AM, Med Associates). Two nose-poke manipulanda with built-in LED stimulus lights (ENV-114BM, Med Associates) could also be inserted into the right wall. When present, the nose-pokes were located 2.5 cm above the grid floor. The right nose-poke was located 4 cm from the front wall of the experimental chamber, whereas the left nose-poke was located 4 cm from the rear wall. The houselight was centered horizontally between the nose-pokes and located 9 cm above the grid floor. The speaker was located above the right nose-poke, 7.5 cm above the grid floor. Blank aluminum panels were inserted when the nose-pokes were removed, but all other elements of the experimental chamber remained in place.

IV drug injections were delivered by motorized syringe drivers (PHM-107, Med Associates) through Tygon tubing (S-54-HL, Norton Performance Plastics) connected to a fluid swivel (375/22PS, Instech Laboratories or QCS-D, Strategic Applications Inc.) and spring tether, which were mounted to a counterbalanced arm. The syringe drivers were located outside of the light- and sound-attenuating cubicles.

Pavlovian conditioning: After recovery from catheterization surgery, rats received either "paired" or "random" Pavlovian conditioning (PAV) for five consecutive sessions. During all PAV sessions, the nose-pokes were removed from the experimental chambers, and all animals received response-independent IV injections of remifentanil (3.2 μg/kg delivered in a volume of

100 µl/kg) and response-independent deliveries of a light-noise compound stimulus. The dose of remifentanil was chosen based on previous work in the laboratory on remifentanil self-administration (Cooper et al. 2008). The light-noise stimulus consisted of houselight illumination and white noise (80±5 db as measured at the center of the chamber). Injections and stimuli lasted 2.0±0.5 s, depending on the weight of the individual animal. In the paired PAV groups, a single variable time (VT) 3 min schedule controlled both remifentanil injection and stimulus delivery, and injections and stimuli always co-occurred. In the random PAV control groups, remifentanil injection and stimulus delivery were each controlled by independent VT3 min schedules. Injections and stimuli were not explicitly unpaired. For both paired PAV and random PAV, inter-injection/inter-stimulus intervals ranged from 0.0 to 6.0 min. The 3 min average inter-injection interval was chosen based on the half-life of remifentanil (Crespo et al. 2005) to allow for extensive drug metabolism between injections. Each PAV session lasted until 20 injections and 20 stimuli were delivered, approximately 60 min.

Instrumental acquisition: Instrumental acquisition (ACQ) test sessions began the day after the conclusion of PAV. In all ACQ sessions, the two nose-pokes were present in the chamber, and animals could respond in the active nose-poke, which produced the light-noise stimulus alone, or the inactive nose-poke, which had no scheduled consequences. Illumination of the stimulus lights inside both nose-pokes signaled the start of each ACQ session, and both nose-pokes remained illuminated for the duration of the session. In each group, the side of the active nose-poke (left vs. right) was counterbalanced across animals. Responses in the active nose-poke produced the light-noise stimulus under a modified random ratio (RR) 2 schedule of reinforcement: the first response in the active nose-poke in each session produced the stimulus

with a probability of 1.0, whereas each subsequent response in the session produced the stimulus with a probability of 0.5. No remifentanil injections were given at any point during ACQ. Animals were attached to the tether, but saline replaced remifentanil on the syringe driver, and the driver never operated. Responses in the inactive nose-poke were recorded but had no scheduled consequences. Active and inactive responses made during stimulus presentation itself were not recorded. In each experiment, pretreatment group assignments were made randomly before the start of ACQ. The number and duration of ACQ sessions varied between experiments, as described below.

In Experiment 2, each group was tested in 14 ACQ sessions (ACQ1-14). ACQ1 lasted for 60 min, and no pretreatment injection was given to any group before ACQ1. ACQ1 provided a "baseline" measurement of responding with the stimulus to check that the groups did not differ significantly before the start of PRAM administration. From ACQ2-14, the session length was increased to 240 min, and a pretreatment injection was administered 10 min before each session. After paired PAV, PRAM (0.1 mg/kg, 0.32 mg/kg, or 1.0 mg/kg) or vehicle (0.0 mg/kg) was administered to separate groups of animals on ACQ2-8 and ACQ10-14. A control group was pretreated with 0.32 mg/kg PRAM after random PAV. On ACQ 9, all groups were pretreated with vehicle to examine possible carry-over or history effects from prior PRAM exposure. All animals were returned to their homecages for the 10 min between injection and the start of the session.

In Experiment 3, each group was tested in 8 ACQ sessions (ACQ1-8). All ACQ sessions lasted 60 min. As in Experiment 2, no pretreatment injection was given before ACQ1. From ACQ2-8, animals were injected with either vehicle or 0.32 mg/kg PRAM either 10 min or 190 min before each session, giving four different pretreatment conditions: 10 min vehicle (n = 4),

190 min vehicle (n = 4), 10 min PRAM (n = 6), and 190 min PRAM (n = 8). As in Experiment 2, animals were returned to their homecages for the interval between injection and the start of the session.

Data analysis: In Experiment 2, ACQ responding was analyzed in four phases, corresponding to when the animals did or did not receive PRAM: (1) ACQ1, when no pretreatment injection was given; (2) ACQ2-8, when PRAM was given before each session; (3) ACQ 9, when all groups received vehicle pretreatment; and (4) ACQ10-14, when PRAM was again given before each session. For ACQ1 and ACQ9, the mean active and inactive nose-pokes made by each group were analyzed using two-way ANOVA with the within-subjects factor of manipulandum (active vs. inactive) and the between-subjects factor of group (vehicle, 0.1 mg/kg PRAM, 0.32 mg/kg PRAM, 1.0 mg/kg PRAM). For ACQ1, group assignments represent the animals' pretreatment fate; for ACQ9, group assignments represent the animals' pretreatment history. For ACQ2-8 and ACQ10-14, the mean active and inactive responses made by each group in each session were analyzed using three-way ANOVA with the within-subjects factors of manipulandum and session and the between-subjects factor of group. If significant effects of manipulandum and group were found, responding was averaged across sessions to perform two sets of pairwise comparisons. To determine if stimulus presentation reinforced the active response, paired t-tests were used to compare the active and inactive nose-pokes of each group. To determine if PRAM increased responding, unpaired t-tests were used to compare the active and inactive responses of each PRAM-treated group to the active and inactive responses, respectively, of the vehicle-treated group. To characterize the effects of PRAM on the intrasession allocation of responding, the active responses made by the vehicle and 0.32 mg/kg

PRAM groups during ACQ2-8 were re-analyzed. The rate of responding (responses/min) in each of the four hours (HOUR1-4) of each of these sessions was calculated. The mean rates in each hour of each session were analyzed using three-way ANOVA with the within-subjects factors of session and hour and the between-subjects factor of group. Following significant effects of hour and group, the data were averaged across sessions for pairwise comparison (unpaired *t*-tests) of the vehicle and 0.32 mg/kg PRAM groups at each hour. The Holm-Bonferonni method was used to correct for multiple pairwise comparisons.

In Experiment 3, as a preliminary analysis, the responses of the vehicle-treated animals were evaluated using three-way ANOVA with the within-subjects factors of session (ACQ1-8) and manipulandum and the between-subjects factor of pretreatment interval (10 min vs. 190 min). Because the effects of pretreatment interval were not significant, the two groups were combined into a single vehicle control condition for all subsequent analyses. As in Experiment 2, responding in ACQ1 and ACQ2-8 were analyzed separately. For ACQ1, the mean active and inactive nose-pokes made by each group were analyzed using two-way ANOVA with the within-subjects factor of manipulandum and the between-subjects factor of group (vehicle, 10 min PRAM, 190 min PRAM). For ACQ2-8, the mean active and inactive responses made by each group in each session were analyzed using three-way ANOVA with the within-subjects factors of manipulandum and session and the between-subjects factor of group. Following significant effects involving manipulandum and group, responding was averaged across sessions, and two sets of pairwise comparisons were made. To determine if stimulus presentation reinforced the active response, paired *t*-tests were used to compare the active vs. inactive responses of each group. To determine if PRAM increased responding, unpaired *t*-tests were used to compare the

active and inactive responses of each PRAM-treated group to the active and inactive responses, respectively, of the vehicle-treated group.

All analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, CA) or SPSS Statistics 20.0 (IBM, Armonk, NY). Differences were considered significant when $p < .05$, two-tailed.

Results

Experiment 1: Resistance-to-extinction of remifentanil self-administration responding

Based on the number of ratios completed, the effects of PRAM on extinction responding [main effect of pretreatment dose: $F(4,24) = 12.61$, $p < .001$] did not differ when the doses were given in ascending vs. descending order [main effect of dose order: $F(1,6) = 0.93$, $p = .37$; pretreatment dose X dose order: $F(4,24) = 1.95$, $p = .13$]. For all subsequent analyses, therefore, the data were combined across dose orders. Figure 3.1 uses the combined data to present the PR responding of rats when stably self-administering remifentanil and when tested with PRAM in extinction. Rats' extinction responding was affected by the PRAM pretreatment dose, with significant changes in active responses [Figure 3.1a; $F(4,28) = 8.61$, $p < .001$], break point [Figure 3.1b; $F(4,28) = 8.92$, $p < .001$], the number of ratios completed [Figure 3.1c; $F(4,28) = 11.11$, $p < .001$], and session length [Figure 3.1d; $F(4,28) = 11.66$, $p < .001$]. Compared to vehicle, 0.32 mg/kg PRAM significantly increased each of these endpoints ($3.77 < t(28) < 5.29$, all p's $< .01$), whereas the effects of the other PRAM doses were not significantly different from vehicle ($0.19 < t(28) < 2.25$, all p's $> .05$). Compared to remifentanil self-administration,

extinction significantly reduced responding when animals were pretreated with vehicle or the smaller doses of PRAM: active responses [0.0-0.032 mg/kg PRAM: $5.09 < t(7) < 6.99$, all p's < .05], break point [0.0-0.1 mg/kg PRAM: $3.95 < t(7) < 7.07$, all p's < .05], and the number of ratios completed [0.0-0.1 mg/kg PRAM: $5.04 < t(7) < 9.23$, all p's < .05]. For each of these endpoints, when animals were pretreated with higher PRAM doses, extinction responding was not significantly different from remifentanil self-administration [$0.054 < t(7) < 3.87$, all p's > .05]. Session length did not differ between self-administration and extinction [$0.21 < t(7) < 3.92$, all p's > .05], except for a significant increase in session length with 0.32 mg/kg PRAM pretreatment [$t(7) = 4.59$, $p = .022$]. Inactive responding was infrequent throughout (mean < 3 in all conditions) and did not change with PRAM preatement dose [Figure 3.1e; $F(4,28) = 1.20$, $p = .31$] or between self-administration and extinction [$0.30 < t(7) < 0.95$, all p's > .05].

To characterize the effects of PRAM on the intrasession allocation of responding, each extinction session was divided into 5 blocks, each encompassing a sequential 20% of the total session length. The rate of active responding in each block is presented in Figure 3.2. As noted above, responding was significantly affected by PRAM pretreatment [main effect of dose: $F(4,35) = 6.88$, $p < .001$]. The rate of active responding changed significantly over the course of the session [main effect of block: $F(4,140) = 7.17$, $p < .001$], and the effect of PRAM pretreatment depended on the portion of the session [pretreatment X block: $F(16,140) = 15.32$, $p < .001$]. Numerically, when pretreated with vehicle, 0.032 mg/kg PRAM, or 0.1 mg/kg PRAM, animals responded most rapidly in BLOCK1, with decreases across the subsequent blocks. A different pattern was observed in animals treated with 0.32 mg/kg or 1.0 mg/kg PRAM: the lowest rate of responding occurred in BLOCK1, with increases across the subsequent blocks. By pairwise comparison to vehicle, PRAM significantly decreased response rate in BLOCK1: 0.1

mg/kg [$t(140) = 4.28$, $p < .001$], 0.32 mg/kg [$t(140) = 5.44$, $p < .001$], and 1.0 mg/kg [$t(140) = 5.64$, $p < .001$]. Only 0.32 mg/kg PRAM changed responding later in the session: compared to vehicle, rates were increased in BLOCK3 [$t(140) = 5.55$, $p < .001$], BLOCK4 [$t(140) = 5.93$, $p < .001$], and BLOCK5 [$t(140) = 4.71$, $p < .001$]. All other comparisons to vehicle were not significant [$0.055 < t(140) < 2.77$, all p's $> .05$].

Figure 3.3 presents the effects of PRAM on animals' active responding when responding either did or did not produce the remifentanil-associated stimuli. Responding differed significantly when the stimuli present in the session were changed [main effect of stimulus condition: $F(3,21) = 6.76$, $p = .002$]. Responding also differed when animals were administered PRAM vs. vehicle [main effect of pretreatment: $F(1,7) = 6.58$, $p = .037$]; however, the effects of pretreatment differed depending on the stimuli present in the session [pretreatment X stimulus condition: $F(3,21) = 5.12$, $p = .008$]. By pairwise comparison to vehicle, 0.32 mg/kg PRAM increased responding only in the CS alone condition [difference: 438.25; 95% confidence interval: 45.47, 831.02]. Inactive responding (data not shown) did not differ by stimulus condition or pretreatment [main effects and interaction: $0.91 < F < 1.05$, all p's $> .10$].

Experiment 2: New-response acquisition with remifentanil-paired stimuli

Figure 3.4 presents the active and inactive nose-poke responses of animals treated with PRAM after paired PAV. Figures 3.4a-3.4d present the responses of the four groups in each of the 14 ACQ sessions. Responding in each of the four phases of ACQ is summarized in Figures 3.4e-3.4h.

In ACQ1 (Figure 3.4e), animals responded differently in the active vs. inactive nose-pokes [main effect of manipulandum: $F(1,28) = 5.73$, $p = .023$], but responding did not differ among the groups [main effect of group: $F(3,28) = 0.14$, $p = .93$; group X manipulandum: $F(3,28) = 0.42$, $p = .73$]. Collapsing across groups, animals made significantly more active responses than inactive responses [$t(31) = 2.46$, $p = .019$].

In ACQ2-8, animals continued to respond differently in the active vs. inactive nose-pokes [main effect of manipulandum: $F(1,28) = 44.79$, $p < .001$]. Neither the main effect of session [$F(6,168) = 0.37$, $p = .89$] nor the session X manipulandum interaction [$F(6,168) = 1.63$, $p = .14$] was significant. Averaged across sessions (Figure 3.4f), animals pretreated with vehicle [$t(7) = 3.55$, $p = .027$], 0.1 mg/kg PRAM [$t(7) = 3.17$, $p = .031$], and 0.32 mg/kg PRAM [$t(7) = 5.77$, $p = .002$] made significantly more active responses than inactive responses. Numerically, animals pretreated with 1.0 mg/kg PRAM made more active responses than inactive responses, but the difference was not statistically significant [$t(7) = 1.90$, $p = .098$]. PRAM pretreatment dose significantly affected responding [main effect of group: $F(3,28) = 11.43$, $p < .001$; group X session: $F(18,168) = 2.25$, $p = .004$], and the effects of PRAM differed for active vs. inactive responding [group X manipulandum: $F(3,28) = 14.75$, $p < .001$; group X session X manipulandum: $F(18,168) = 2.83$, $p < .001$]. By pairwise comparison, 0.32 mg/kg PRAM significantly increased active responding compared to vehicle [$t(14) = 4.97$, $p = .0012$], whereas neither 0.1 mg/kg PRAM [$t(14) = 0.23$, $p = .81$] nor 1.0 mg/kg PRAM [$t(14) = 1.21$, $p = .48$] changed active responding. No dose of PRAM significantly changed inactive responding compared to vehicle [$0.007 < t(14) < 0.58$, all p's $> .10$].

In ACQ9 (Figure 3.4g), when all groups received a vehicle pretreatment, animals responded differently in the active vs. inactive nose-pokes [main effect of manipulandum:

$F(1,28) = 23.49$, $p < .001$], but responding did not differ among the groups [main effect of group: $F(3,28) = 1.79$, $p = .17$; group X manipulandum: $F(3,28) = 0.91$, $p = .44$]. Collapsed across groups, animals made more active responses than inactive responses [$t(31) = 4.86$, $p < .001$]. Numerically, animals that had been pretreated with 0.32 mg/kg PRAM made the most active responses in ACQ9, but their responding was not significantly different from that of the animals that had been pretreated with vehicle [$t(14) = 1.66$, $p = .11$].

During ACQ10-14, animals continued to respond differently in the active vs. inactive nose-pokes [main effect of manipulandum: $F(1,28) = 32.45$, $p < .001$], whereas responding did not differ significantly across sessions [main effect of session and all interactions: $0.50 < F < 1.15$, $p > .10$]. Collapsed across sessions (Figure 3.4h), animals in all groups made significantly more active responses than inactive responses [vehicle: $t(7) = 4.24$, $p = .011$; 0.1 mg/kg PRAM: $t(7) = 4.32$, $p = .014$; 0.32 mg/kg PRAM: $t(7) = 3.69$, $p = .007$; 1.0 mg/kg PRAM: $t(7) = 3.93$, $p = .011$]. PRAM pretreatment significantly affected responding [main effect of group: $F(3,28) = 6.91$, $p < .001$], and the effects of PRAM differed for active vs. inactive responding [group X manipulandum: $F(3,28) = 7.87$, $p < .001$]. Animals treated with 0.32 mg/kg PRAM [$t(14) = 3.33$, $p = .024$] or 1.0 mg/kg PRAM [$t(14) = 3.49$, $p = .021$], but not 0.1 mg/kg PRAM [$t(14) = 2.26$, $p = .16$], made significantly more active responses than vehicle-treated animals. PRAM pretreatment did not alter inactive responding compared to vehicle [$0.18 < t(14) < 1.26$, all p's $> .10$].

Figure 3.5 presents the active and inactive nose-poke responses of the control group that was treated with 0.32 mg/kg PRAM after random PAV. Responding in this group did not differ by manipulandum or session in any phase of ACQ [ANOVA main effects and interactions: $0.65 < F < 4.79$, all p's $> .05$; pairwise comparisons: $0.32 < t(7) < 0.75$, all p's $> .05$].

Figure 3.6 presents the rate of active responding in each hour of ACQ2-8 of the groups pretreated with vehicle or 0.32 mg/kg PRAM. These two groups were chosen because only 0.32 mg/kg significantly increased the overall quantity of active responding during this first phase of PRAM administration. The rate of active responding in ACQ1 is also presented in Figure 3.6 for reference. In ACQ2-8, the rate of responding differed across the hours of the session [main effect of hour: $F(3,42) = 8.94$, $p < .001$; hour X session: $F(18,252) = 1.72$, $p = .036$] and by pretreatment [main effect of group: $F(1,14) = 24.30$, $p < .001$; group X session: $F(6,84) = 3.52$, $p = .004$]. However, the effect of PRAM pretreatment depended on the hour [group X hour: $F(3,42) = 13.08$, $p < .001$]. The main effect of session was not significant [$F(6,84) = 1.14$, $p = .34$], and the pretreatment X hour interaction did not depend on the session [session X pretreatment X hour: $F(18,252) = 0.88$, $p = .60$]; therefore, responding was averaged across sessions for pairwise comparison of the groups in each hour. PRAM significantly increased responding in HOUR3 [$t(14) = 6.06$, $p < .001$] and HOUR4 [$t(14) = 4.06$, $p = .003$], but not in HOUR1 [$t(14) = 1.35$, $p = .19$] or HOUR2 [$t(14) = 2.19$, $p = .091$].

Experiment 3: Comparison of PRAM pretreatment intervals in new-response acquisition

In the vehicle-treated animals, the pretreatment interval (10 min vs. 190 min) did not affect responding [all main effects and interactions: $0.009 < F < 1.57$, all p's $> .10$]. Therefore, these data were combined to form a single vehicle control group for all subsequent analyses. Figure 3.7 presents the active (Figure 3.7a) and inactive (Figure 3.7b) nose-poke responses of the vehicle-treated animals and animals treated with 0.32 mg/kg PRAM either 10 min or 190 min before the start of the session. In ACQ1 (Figure 3.7c), before the start of pretreatments,

responding did not differ among the groups [main effect of group: $F(2,19) = 0.38$, $p = .69$; group X manipulandum: $F(2,19) = 0.06$, $p = .94$]. The main effect of manipulandum was not significant [$F(1,19) = 4.08$, $p = .057$]; however, combined across groups, animals made significantly more active responses than inactive responses by pairwise comparison [$t(21) = 2.09$, $p = .048$]. In ACQ2-8, when pretreatments were given, the animals responded differently in the active vs. inactive nose-pokes [main effect of manipulandum: $F(1,19) = 19.92$, $p < .001$; session X manipulandum: $F(6,114) = 0.44$, $p = .84$; group X manipulandum: $F(2,19) = 1.06$, $p = .36$]. Averaged across sessions (Figure 3.7d), the animals in each group made significantly more active responses than inactive responses in ACQ2-8 [vehicle: $t(7) = 2.94$, $p = .043$; 10 min PRAM: $t(5) = 4.12$, $p = .027$; 190 min PRAM: $t(7) = 2.37$, $p = .049$]. Responding in ACQ2-8 also differed by pretreatment condition [main effect of group: $F(2,19) = 3.63$, $p = .046$; session X group: $F(12,114) = 2.86$, $p = .002$; session X group X manipulandum: $F(12,114) = 2.20$, $p = .016$]. Numerically, both of the PRAM-treated groups responded less than the vehicle-treated group. Collapsed across sessions, however, the mean active responses of the PRAM-treated animals did not differ from the mean active responses of the vehicle-treated animals, and the mean inactive responses of the PRAM-treated animals did not differ from the mean inactive responses of the vehicle-treated animals [$1.34 < t < 2.73$, all p's $> .05$].

Discussion

Drug-associated environmental stimuli are thought to contribute significantly to human drug abuse and dependence (e.g., Di Chiara 1999; Everitt et al. 2008; Koob and Le Moal 2001; Robinson and Berridge 2008). The conditioned reinforcing effects of drug-paired stimuli,

specifically, may be particularly important for (1) preserving drug-seeking behaviors over extended delays to drug delivery (i.e., in extended chains of responses that ultimately lead to drug or under extinction conditions) and (2) maintaining the flexibility and diversity of drug-seeking responses by influencing behavior in a consequence-dependent manner (i.e., as contrasted with stimulus-response "habit" mechanisms) and by training new types of responses that had not necessarily led previously to drug delivery (reviewed by Milton and Everitt 2010). The present studies assessed the effects of the D3-preferring agonist, PRAM, on responding maintained by stimuli associated with the potent, short-acting μ-opioid agoinst, remifentanil. These stimuli were studied in two different behavioral preparations: Experiment 1 examined the resistance to extinction of remifentanil self-administration, a situation in which responding may be influenced by the discriminative and/or conditioned reinforcing effects of the stimuli, whereas Experiments 2 and 3 focused specifically on the conditioned reinforcing effects of the stimuli in a behaviorally stringent new-response acquisition procedure. Previous studies of the effects of selective dopaminergic agonists on rats' acquisition of responding with conditioned reinforcement have focused on stimuli that were paired with non-drug primary reinforcers. Systemic administration of several selective D2-like, but not D1-like, agonists has been shown to enhance rats' new-response acquisition with food-paired stimuli (Beninger and Ranaldi 1992; Beninger and Rolfe 1995; Sutton et al. 2001), whereas both the D2-like agonist, quinpirole, and the D1-like partial agonist, SKF 38393, enhanced acquisition with water-paired stimuli when injected directly into the nucleus accumbens (Wolterink et al. 1993). The ability of selective, direct dopamine receptor agonists to enhance the acquisition of a new response with drug-conditioned reinforcement has not, to my knowledge, been previously reported.

In Experiment 1, pretreatment with PRAM dose-dependently increased responding in extinction after remifentanil self-administration training. These results are consistent with previous reports of the response-enhancing effects of the D3/D2 agonist, bromocriptine (Wise et al. 1990), and the D3-preferring agonist, quinpirole (De Vries at al. 2002), in rats trained to self-administer heroin, confirming the importance of D2-like receptor activity generally in opioid-trained animals. The present study expands on these previous results in several noteable ways.

First, in complement to the FR1 training procedures used previously, the use of a PR schedule for training and testing in the present study provides an alternative assessment of the motivational effects of the drug-associated stimuli. In the work of Wise and colleagues (1990) and De Vries and colleagues (2002) animals made 30-50 responses per session when stimulated by the D2-like agonist. Presently, rats made more than 600 total responses and more than 100 responses with a single delivery of the stimulus (i.e., break point > 100) when treated with PRAM. Interpretation of PR breakpoints as a pure index of motivation remains controversial (Bradshaw and Killeen 2012), but it is clear from the present results that, under the influence of PRAM, rats will respond with the stimuli alone as much as or more than they responded with self-administered remifentanil during the training phase.

Second, the present study systematically assessed the interaction of PRAM pretreatment with the different types of exteroceptive stimuli that were programmed during the session. When the remifentanil-associated DS and/or remifentanil injection-paired CS were or were not presented, PRAM pretreatment increased responding only when the injection-paired CS were presented. These results are consistent with a mechanism involving the conditioned reinforcing effects of the remifentanil-paired stimuli. For example, if generalization decrements alone were responsible for changes in extinction responding, the most responding should have been

observed in the DS+CS condition because the stimuli present in this condition were most similar to the stimuli that were present during self-administration training (Wike 1966; Williams 1994). However, because the same response produced the CS and remifentanil during self-administration training, these results can still not determine conclusively whether the injection-paired stimuli were functioning as conditioned reinforcers on their own or as discriminative stimuli for the primary reinforcement contingency between responding and remifentanil.

In addition to this exteroceptive discriminative stimulus function, the rats' extinction responding may also have been influenced by the interoceptive discriminative stimulus functions of the training and testing drugs. The interoceptive stimulus produced by the self-administered drug during training can provide a signal that responding will be reinforced: drug in the body indicates that drug is available. If the interoceptive stimulus properties of the pretreatment test drug resemble those of the self-administration training drug, animals may respond as though the manipuladum were still active (i.e., producing drug injections), increasing rates of responding (Collins et al. 2012; Stewart and de Wit 1987). This interoceptive similarity may be especially important when testing dopaminergic agonists in cocaine-trained animals. Both D2-like and D1-like agonists can substitute partially or fully for cocaine in cocaine-saline drug discrimination studies in rats (reviewed by Callahan et al. 1997; see also Caine et al. 2000; Chausmer and Katz, 2002). When co-administered with the training drug, D2-like ligands can alter the discriminative stimulus effects of μ-opioid agonists in rats, although these effects are often observed only under conditions of significant rate suppression (Colpaert et al. 1977; Cook and Beardsley 2004a, 2004b; Corrigall and Coen 1990; McCarten and Lal 1979). In substitution tests, a variety of D3-preferring agonists have failed to substitute for heroin in rats trained to discriminate heroin from water (Cook and Beardsley 2004b), and morphine did not substitute for the D3-preferring

agonist, quinpirole, in rats trained to discriminate quinpirole from saline (Baladi et al. 2010). This bidirectional lack of substitution suggests that the interoceptive stimulus properties of μ-opioid agonists and D2-like dopamine agonists are significantly dissimilar, and so changes in extinction responding after opioid self-administration may be less likely to be caused by the animals' difficulty discriminating the activity of the manipulanda. Nonetheless, even if the content of the interoceptive stimulus is different, as revealed in drug discrimination experiments, any drugged state may increase responding by being more like the interoceptive state experienced during self-administration training compared to drug-free extinction. The training and test drugs may also share internal effects that are not detected by or relevant to drug discrimination assays (Stewart and de Wit 1987).

The new-response acquisition procedures used presently minimize the influence of both exteroceptive and interoceptive discriminative stimuli: because the animals never self-administered remifentanil, the light-tone stimulus could not have been established as a discriminative stimulus for an association between the nose-pokes and remifentanil, and the interoceptive stimulus properties of remifentnail could, likewise, not serve as a discriminative stimulus indicating the activity of the nose-pokes. In Experiment 2, animals pretreated with vehicle acquired responding with the remifentanil-paired stimulus, as indicated by their significant preference for the active nose-poke, which produced the stimulus alone, over the inactive nose-poke, which had no scheduled consequences, during both ACQ2-8 and ACQ10-14. Compared to vehicle, PRAM pretreatment significantly increased active responding without changing inactive responding. In the first period of PRAM pretreatment (ACQ2-8, Figure 3.4f), PRAM pretreatment produced a biphasic dose-effect function, with only 0.32 mg/kg PRAM significantly increasing active responding. In the second period of PRAM pretreatment

(ACQ10-14, Figure 3.4h), animals treated with either 0.32 mg/kg PRAM or 1.0 mg/kg PRAM made significantly more active responses than vehicle-treated animals. Such biphasic dose-response curves have been previously observed with amphetamine-enhanced acquisition of food-associated responding (e.g., Mazurski and Beninger 1986). In contrast to these effects with the remifentanil-paired stimulus, systematic changes in active responding were not observed when the active nose-poke produced the stimulus after the stimulus had been randomly presented with remifentanil during PAV. After random PAV, animals did not differentiate between the nose-pokes in any phase of ACQ. PRAM's behavioral selectivity based on the Pavlovian contingency between the stimulus and remifentanil is consistent with the effects of pipradrol (Robbins 1976) and amphetamine (Taylor and Robbins 1984) on the acquisition of responding with stimuli that either were or were not consistently paired with water. As required for a change in conditioned reinforcement, therefore, the difference in responding between the active and inactive nose-pokes depends on the difference between paired and random PAV.

These results provide direct evidence that D2-like activation enhances responding with drug-conditioned reinforcement specifically, apart from the other associative and nonassociative behavioral processes that can change rates of responding and that could, likewise, be influenced by dopaminergic manipulations. Even so, responding with conditioned reinforcement has a complex associative basis, depending by definition on both Pavlovian and instrumental learning (Mackintosh 1974; O'Brein and Gardner 2005; Williams 1994). Therefore, the changes in nose-poking observed presently could have resulted from PRAM changing (1) the Pavlovian associaton between the stimulus and remifentanil, (2) the instrumental contingency between the response and the stimulus, and/or (3) discrimination between the active and inactive manipulanda (Gerdjikov et al. 2011; Palmatier et al. 2008; cf., Berridge et al. 2009). For

example, the present results cannot separate the ability of PRAM to increase the relative reinforcing effectiveness of the stimulus in the instrumental contingency from the ability of PRAM to prevent the extinction of the Pavlovian stimulus-remifentanil association that would otherwise have occurred as the stimulus was presented by itself during ACQ. It is important to note that limitations of this type are not more or less of an issue with any particular method of studying conditioned reinforcement, but they are in the nature of conditioned reinforcement as a stimulus function. Additional studies with dedicated experimental preparations designed to study instrumental vs. Pavlovian learning (e.g., reinforcer devaluation, Pavlovian-to-instrumental transfer) may provide complementary insight into the ability of PRAM to alter individual learning processes, as opposed to its ability to alter one specific stimulus function vs. another, which was the focus of the present experiments on conditioned reinforcement.

In both Experiment 1 and Experiment 2, the effects of PRAM varied significantly over the course of the session, with increases in responding observed only in the final 50-60% of the session. This pattern of performance may be mediated by extended exposure to PRAM, extended exposure to the task, or extended exposure to the task under the influence of PRAM. The results of Experiment 3 emphasize the importance to new-response acquisition of exposure to the task under the influence of PRAM. Compared to vehicle-treated animals, animals given 0.32 mg/kg PRAM 190 min before 60 min sessions did not increase their responding, as animals given PRAM 10 min before 240 min sessions did. Crucially, in these latter animals, increases were observed specifically in the last 60 min of the 240 min session. Thus, if 190 min of exposure to PRAM itself were sufficient to increase responding, regardless of whether that exposure occurred in the home cage or experimental chamber, then differences between the vehicle and 190 min PRAM groups should have been observed in Experiment 3. Instead, daily

exposure to PRAM, administered either 10 min or 190 min before 60 min test sessions, did not significantly increase responding. These results suggest that rats must perform the task for an extended period under the influence of PRAM for PRAM to increase responding, although the importance of extended exposure to the operant context itself, independent of actual task performance, cannot be resolved from the present results.

Systemic PRAM administration can significantly alter rats' locomotor activity in open-field tests: either increases or decreases in locomotion can be observed depending on the dose (Maj et al. 1997) and time from PRAM administration (Chang et al. 2011; Lagos et al. 1998). For example, Lagos and colleagues (1998) measured the effects of 0.5 mg/kg PRAM in a 30 min session beginning either 5 min or 125 min after drug administration. Compared to saline, activity was reduced by PRAM after a 5 min pretreatment but increased by PRAM after a 125 min pretreatment. Similarly, measuring locomotor activity for 90 min immediately after PRAM injection, Chang and colleagues (2011) found that 0.3 mg/kg PRAM significantly suppressed activity in the first 30 min of the session and significantly increased activity in the final 40 min of the session. This pattern of initial decreases in behavior followed by subsequent increases in behavior parallel the intrasession changes in response rate observed in the present experiments (Figure 3.2, Figure 3.6). It is, however, unlikely that the increases in nose-poking are the result only of general locomotor activation by PRAM. In Experiment 1, PRAM increased responding only when the CS were presented. If PRAM were simply eliciting nose-poking, responding should not have been significantly modulated by the presence/absence of the CS. Likewise, in Experiment 2, PRAM increased responding only in the active nose-poke, with responding in the inactive nose-poke never differing significantly from vehicle pretreatment, and the difference

between active and inactive nose-poking was observed only when remifentnail and the stimulus had been consistently paired during PAV.

The ability of PRAM, specifically, to increase responding with conditioned reinforcement may be especially noteworthy considering PRAM's widespread clinical use. Approved for human use internationally, PRAM has become the most widely prescribed direct dopamine agonist treatment for Parkinson's disease (Antonini et al. 2010). PRAM has also been approved by regulators in both the United States and Europe to treat restless legs syndrome (Brindani et al. 2009) and is commonly used "off label" to treat fibromyalgia (Roskell et al. 2011). In all three of these patient populations, PRAM administration is associated with a set of behavioral side effects known collectively as impulse control disorders (ICD) (e.g., Cornelius et al. 2010; Holman 2009; Pourcher et al. 2010; Weintraub et al., 2010). ICD are observed at similar rates in Parkinson's disease and restless legs syndrome patients, with ~15% of those receiving PRAM showing one of more ICD (Cornelius et al. 2010; Pourcher et al. 2010; Weintraub et al. 2010). Common ICD involve excessive or compulsive gambling, eating, shopping, sexual behavior, hobby activities, and punding. Whereas ICD may be influenced by changes in the processing of primary reinforcers, many of these behaviors occur in stimulus-dense environments and may be influenced, at least in part, by increased control over behavior by conditioned reinforcement (e.g., the lights and sounds of slot machine gambling and money itself as a conditioned reinforcer of human behavior). Whereas a number of ICD are unrelated to drugs of abuse (e.g., compulsive shopping for clothing items), recent evidence suggests that PRAM treatment can also induce increased drug use (Bienfait et al. 2010). The case reported by Bienfait and colleagues (2010) concerns PRAM-induced changes in cigarette smoking, and the effects of PRAM on this particular behavior may be especially relevant, given accumulating evidence for the importance

of nicotine-associated stimuli for both tobacco smoking in humans and nicotine self-administration in laboratory animals (Chaudhri et al. 2006; Le Foll and Goldberg 2005). Moreover, ICD are agonist-induced, as either discontinuation of PRAM or dose reduction resolves the behavior, and reintroduction of agonist treatment is associated with ICD return (e.g., Bienfait et al. 2010). A similar pattern was observed in the present study in the animals treated with 0.32 mg/kg PRAM. When the vehicle pretreatment probe session (ACQ9) was inserted into the course of PRAM treatment, the responses of animals that had been receiving PRAM were not different from animals that had never received PRAM; however, when PRAM pretreatments were resumed, animals' responding was again increased.

The similarity of the ICD induced in humans treated for Parkinson's disease vs. restless leg syndrome and fibromyalgia suggests that large-scale dopaminergic depletion is not necessary for PRAM to produce these behaviors. Nonetheless, future studies should investigate the ability of PRAM to increase responding with conditioned reinforcement in animals with dopaminergic lesions to model the interaction of changes experienced by human Parkinson's disease patients undergoing agonist therapy. Subsequent experiments should also focus on the neuroanatomical locus of PRAM's effects. Previous studies have specifically implicated D2-like receptors in the nucleus accumbens in the enhanced acquisition responding with water-conditioned reinforcement (Wolterink et al. 1993), and D3 receptors in the amygdala may be particularly important for responding with drug-associated stimuli under second-order schedules of cocaine-self administration (Di Ciano 2008). To the extent that D2-like receptor activity appears to be important for responding with stimuli paired with both drug and non-drug primary reinforcers, it will be important to determine if similar brain structures and systems are involved.

Works cited

Antonini A, Barone P, Ceravolo R, Fabbrini G, Tinazzi M, and Abbruzzese G (2010) Role of pramipexole in the management of Parkinson's disease. *CNS Drugs* **24:** 829–841. doi: 10.2165/11585090-000000000-00000.

Baladi MG, Newman AH, and France CP (2010) Dopamine D3 receptors mediate the discriminative stimulus effects of quinpirole in free-feeding rats. *J Pharmacol Exp Ther* **332:** 308–315. doi 10.1124/jpet.109.158394

Beaulieu J-M and Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* **63:** 182–217. doi: 10.1124/pr.110.002642

Beninger RJ and Ranaldi R (1992) The effects of amphetamine, apomorphine, SKF 38393, quinpirole and bromocriptine on responding for conditioned reward in rats. *Behav Pharmacol* **3:** 155–163. doi: 10.1097/00008877-199204000-00009

Beninger RJ and Rolfe NG (1995) Dopamine D1-like receptor agonists impair responding for conditioned reward in rats. *Behav Pharmacol* **6:** 785–793. doi: 10.1097/00008877-199512000-00003

Berridge KC, Robinson TE, and Aldridge JW (2009) Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol* **9:** 65–73. doi: 10.1016/j.coph.2008.12.014

Bienfait KL, Menza M, Mark MH, and Dobkin RD (2010) Impulsive smoking in a patient with Parkinson's disease treated with dopamine agonists. *J Clin Neurosci* **17:** 539–540. doi: 10.1016/j.jocn.2009.09.001

Bradshaw CM and Killeen PR (2012) A theory of behaviour on progressive ratio schedules, with applications in behavioural pharmacology. *Psychopharmacology (Berl)* **222:** 549–564. doi: 10.1007/s00213-012-2771-4

Brindani F, Vitetta F, and Gemignani F (2009) Restless legs syndrome: differential diagnosis and management with pramipexole. *Clin Interv Aging* **4:** 305–313. doi: 10.2147/CIA.S4143

Caine SB, Negus SS, Mello NK, and Bergman J (2000) Effects of dopamine D1-like and D2-like agonists in rats trained to discriminate cocaine from saline: influence of experimental history. *Exp Clin Psychopharmacol* **8:** 404–414. doi: 10.1037/1064-1297.8.3.404

Callahan PM, De La Garza, and Cunningham KA (1997) Mediation of the discriminative stimulus properties of cocaine by mesocorticolimbic dopamine systems. *Pharmacol Biochem Behav* **57:** 601–607. doi: 10.1016/S0091-3057(96)00434-0

Cervo L, Carnovali F, Stark JA, and Mennini T (2003) Cocaine-seeking behavior in response to drug-associated stimuli in rats: involvement of D3 and D2 dopamine receptors. *Neuropsychopharmacology* **28:** 1150–1159. doi: 10.1038/sj.npp.1300169

Cervo L, Cocco A, Petrella C, and Heidbreder CA (2007) Selective antagonism at dopamine D3 receptors attenuates cocaine-seeking behaviour in the rat. *Int J Neuropsychopharmacol* **10:** 167–181. doi: 10.1017/S1461145705006449

Chang W-L, Breier MR, Yang A, and Swerdlow NR (2011) Disparate effects of pramipexole on locomotor activity and sensorimotor gating in Sprague–Dawley rats. *Pharmacol Biochem Behav* **99:** 634–638. doi: 10.1016/j.pbb.2011.06.002

Chaudhri N, Caggiula AR, Donny EC, Palmatier MI, Liu X, and Sved AF (2006) Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. *Psychopharmacology (Berl)* **184:** 353–366. doi: 10.1007/s00213-005-0178-1

Chausmer AL and Katz JL (2002) Comparison of interactions of D1-like agonists, SKF 81297, SKF 82958 and A-77636, with cocaine: locomotor activity and drug discrimination studies in rodents. *Psychopharmacology (Berl)* **159:** 145–153. doi: 10.1007/s002130100896

Collins GT and Woods JH (2009) Influence of conditioned reinforcement on the response-maintaining effects of quinpirole in rats. *Behav Pharmacol* **20:** 492–504. doi: 10.1097/FBP.0b013e328330ad9b

Collins GT, Cunningham AR, Chen J, Wang, S, Newman AH, and Woods JH (2012) Effects of pramipexole on the reinforcing effectiveness of stimuli that were previously paired with cocaine reinforcement in rats. *Psychopharmacology (Berl)* **219:** 123–135. doi: 10.1007/s00213-011-2382-5

Colpaert FC, Niemegeers CJE, and Janssen PAJ (1977) Differential haloperidol effect on two indices of fentanyl-saline discrimination. *Psychopharmacology (Berl)* **53:** 169–173. doi: 10.1007/BF00426488

Cook CD and Beardsley PM (2004a) Modulation of the discriminative stimulus effects of mu opioid agonists in rats: I. effects of dopamine D2/3 antagonists. *Behav Pharmacol* **15:** 65–74.

Cook CD and Beardsley PM (2004b) Modulation of the discriminative stimulus effects of mu opioid agonists in rats: II. effects of dopamine D2/3 agonists. *Behav Pharmacol* **15:** 75–83.

Cooper ZD, Truong YN-T, Shi Y-G, and Woods JH (2008) Morphine deprivation increases self-administration of the fast- and short-acting μ-opioid receptor agonist remifentanil in the rat. *J Pharmacol Exp Ther* **326:** 920–929. doi: 10.1124/jpet.108.139196

Cornelius JR, Tippmann-Peikert M, Slocumb NL, Frerichs CF, and Silber MH (2010) Impulse control disorders with the use of dopaminergic agents in restless legs syndrome: a case-control study. *Sleep* **33:** 81–87.

Corrigall WA and Coen KM (1990) Selective D1 and D2 dopamine antagonists decrease response rates of food-maintained behavior and reduce the discriminative stimulus produced by heroin. *Pharmacol Biochem Behav* **35:** 351–355. doi: 10.1016/0091-3057(90)90168-H

Crespo JA, Sturm K, Saria A, and Zernig G (2005) Simultaneous intra-accumbens remifentanil and dopamine kinetics suggest that neither determines within-session operant responding. *Psychopharmacology (Berl)* **183:** 201–209. doi: 10.1007/s00213-005-0180-7

Crombag HS, Grimm JW, and Shaham Y (2002) Effect of dopamine receptor antagonists on renewal of cocaine seeking by reexposure to drug-associated contextual cues. *Neuropsychopharmacology* **27:** 1006–1015. doi: 10.1016/S0893-133X(02)00356-1

De Vries TJ, Schoffelmeer ANM, Binnekade R, and Vanderschuren LJMJ (1999) Dopaminergic mechanisms mediating the incentive to seek cocaine and heroin following long-term withdrawal of IV drug self-administration. *Psychopharmacology (Berl)* **143:** 254–260. doi: 10.1007/s002130050944

De Vries TJ, Schoffelmeer ANM, Vinnekade R, Raasø H, and Vanderschuren LJMJ (2002) Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. *Neuropsychopharmacology* **26:** 18–26. doi: 10.1016/S0893-133X(01)00293-7

Di Chiara G (1999) Drug addiction as dopamine-dependent associative learning disorder. *Eur J Pharmacol* **375:** 13–30. doi: 10.1016/S0014-2999(99)00372-6

Di Ciano P (2008) Drug seeking under a second-order schedule of reinforcement depends on dopamine D3 receptors in the basolateral amygdala. *Behav Neurosci*. **122:** 129–139. doi: 10.1037/0735-7044.122.1.129.

Di Ciano P, Underwood RJ, Hagan JJ, and Everitt BJ (2003) Attenuation of cue-controlled cocaine-seeking by a selective D3 dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology* **28:** 329–338. doi: 10.1038/sj.npp.1300148

Dias C, Lachize S, Boilet V, Huitelec, and Cador M (2004) Differential effects of dopaminergic agents on locomotor sensitization and on the reinstatement of cocaine-seeking and food-seeking behaviour. *Psychopharmacology (Berl)* **175:** 414–427. doi: 10.1007/s00213-004-1839-1

Edwards S, Whisler KN, Fuller DC, Orsulak PJ, and Self DW (2007) Addiction-related alterations in D1 and D2 dopamine receptor behavioral responses following chronic cocaine self-administration. *Neuropsychopharmacology* **32:** 354–366. doi: 10.1038/sj.npp.1301062

Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, and Robbins TW (2008) Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos Trans R Soc Lond B Biol Sci* **363:** 3125–3135. doi: 10.1098/rstb.2008.0089

Fuchs RA, Tran-Nguyen LTL, Weber SM, Khroyan TV, and Neisewander JL (2002) Effects of 7-OH-DPAT on cocaine-seeking behavior and on re-establishment of cocaine self-administration. *Pharmacol Biochem Behav* **72:** 623–632. doi: 10.1016/S0091-3057(02)00731-1

Gál K and Gyertyán I **(**2006) Dopamine D3 as well as D2 receptor ligands attenuate the cue-induced cocaine-seeking in a relapse model in rats. *Drug Alcohol Depend* **81:** 63–70. doi: 10.1016/j.drugalcdep.2005.05.011

Garcia-Ladona FJ and Cox BF (2003) BP 897, a selective dopamine D3 receptor ligand with therapeutic potential for the treatment of cocaine-addiction. *CNS Drug Rev* **9:** 141–158. doi: 10.1111/j.1527-3458.2003.tb00246.x

Gerdjikov TV, Baker TW, and Beninger RJ (2011) Amphetamine-induced enhancement of responding for conditioned reward in rats: interactions with repeated testing. *Psychopharmacology (Berl)* **214:** 891–899. doi: 10.1007/s00213-010-2099-x

Gilbert JG, Newman AH, Gardner EL, Ashby Jr CR, Heidbreder CA, Pak AC, Peng X-Q, and Xi Z-X (2005). Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: role of dopamine D3 receptors. *Synapse* **57:** 17–28. doi: 10.1002/syn.20152

Heidbreder C (2013) Rationale in support of the use of selective dopamine D3 receptor antagonists for the pharmacotherapeutic management of substance use disorders. *Naunyn Schmiedebergs Arch Pharmacol* **386:** 167–176. doi: 10.1007/s00210-012-0803-6

Heidbreder C and Newman AH (2010) Current perspectives on selective dopamine D3 receptor antagonists as pharmacotherapeutics for addictions and related disorders. *Ann N Y Acad Sci* **1187:** 4–34. doi: 10.1111/j.1749-6632.2009.05149.x.

Holman AJ (2009) Impulse control disorder behaviors associated with pramipexole used to treat fibromyalgia. *J Gambl Stud* **25:** 425–431. doi: 10.1007/s10899-009-9123-2

Institute of Laboratory Animal Research CoLS, National Research Council (1996) *Guide for the Care and Use of Laboratory Animal*s, 7th ed. National Academies Press, Washington DC.

Kelleher RT and Gollub LR (1962) A review of positive conditioned reinforcement. *J Exp Anal Behav* **5:** 543–597. doi: 10.1901/jeab.1962.5-s543

Koeltzow TE and Vezina P (2005) Locomotor activity and cocaine-seeking behavior during acquisition and reinstatement of operant self-administration behavior in rats. *Behav Brain Res* **160:** 250–259. doi: 10.1016/j.bbr.2004.12.005

Koob GF and Le Moal M (2001) Drug addiction, dysregulation of reward and allostasis. *Neuropsychopharmacology* **24:** 97–129. doi: 10.1016/S0893-133X(00)00195-0

Lagos P, Scorza C, Monti JM, Jantos H, Reyes-Parada M, Silveira R, and Ponzoni A (1998) Effects of the D3 preferring dopamine agonist pramipexole on sleep and waking, locomotor activity and striatal dopamine release in rats. *Eur Neuropsychopharmacol* **8:** 113–120. doi: 10.1016/S0924-977X(97)00054-0

Le Foll B and Goldberg SR (2005) Control of the reinforcing effects of nicotine by associated environmental stimuli in animals and humans. *Trends Pharmacol Sci* **26:** 287–293. doi: 10.1016/j.tips.2005.04.005

Le Foll B, Goldberg SR, and Sokoloff P (2005) The dopamine D3 receptor and drug dependence: effects on reward or beyond? *Neuropharmacology* **49:** 525–41. doi: 10.1016/j.neuropharm.2005.04.022

Mackintosh NJ (1974) *The Psychology of Animal Learning*. Academic Press, New York.

Maj J, Rogóż Z, Skuza G, and Kołodziejczyk K (1997) The behavioural effects of pramipexole, a novel dopamine receptor agonist. *Eur J Pharmacol* **324:** 31–37. doi: 10.1016/S0014-2999(97)00066-6

Mazurski EJ and Beninger RJ (1986) The effects of (+)-amphetamine and apomorphine on responding for a conditioned reinforcer. *Psychopharmacology (Berl)* **90:** 239–243. doi: 10.1007/BF00181249

McCarten MD and Lal H (1979) Attenuation of the discriminative stimulus strength of morphine by haloperidol. *Neuropharmacology* **18:** 465–467. doi: 10.1016/0028-3908(79)90071-6

Milton AL and Everitt BJ (2010) The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *Eur J Neurosci* **31:** 2308–2319. doi: 10.1111/j.1460-9568.2010.07249.x

Newman AH, Blaylock BL, Nader MA, Bergman J, Sibley DR, and Skolnick P (2012) Medication discovery for addiction: translating the dopamine D3 receptor hypothesis. *Biochem Pharmacol* **84:** 882–890. doi: 10.1016/j.bcp.2012.06.023

O'Brien CP and Gardner EL (2005) Critical assessment of how to study addiction and its treatment: human and non-human animal models. *Pharmacol Ther* **108:** 18–58. doi: 10.1016/j.pharmthera.2005.06.018

Palmatier MI, Coddington SB, Liu X, Donny EC, Caggiula AR, and Sved AF (2008) The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose. *Neuropharmacology* **55:** 1425–1430. doi: 10.1016/j.neuropharm.2008.09.002

Peng X-Q, Ashby Jr CR, Spiller K, Li X, Li J, Thomasson N, Millan MJ, Mocaër E, Muńoz C, Gardner EL, and Xi Z-X (2009) The preferential dopamine D3 receptor antagonist S33138 inhibits cocaine reward and cocaine-triggered relapse to drug-seeking behavior in rats. *Neuropharmacology* **56:** 752–760. doi: 10.1016/j.neuropharm.20 08.12.007

Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz J-C, Everitt BJ, and Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* **400:** 371–375. doi: 10.1038/22560

Pourcher E, Rémillard S, and Cohen H (2010) Compulsive habits in restless legs syndrome patients under dopaminergic treatment. *J Neurol Sci* **290:** 52–56. doi: 10.1016/j.jns.2009.11.010

Rescorla RA (1967) Pavlovian conditioning and its proper control procedures. *Psychol Rev* **74:** 71–80. doi: 10.1037/h0024109

Richardson NR and Roberts DC (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Meth* **66:** 1–11. doi: 10.1016/0165-0270(95)00153-0

Robbins TW (1976) Relationship between reward-enhancing and stereotypical effects of psychomotor stimulant drugs. *Nature* **264:** 57–59. doi: 10.1038/264057a0

Robinson TE and Berridge KC (2008) The incentive sensitization theory of addiction: some current issues. *Philos Trans R Soc Lond B Biol Sci* **363:** 3137–3146. doi: 10.1098/rstb.2008.0093

Roskell NS, Beard SM, Zhao Y, Le TK (2011) A meta-analysis of pain response in the treatment of fibromyalgia. *Pain Pract* **11:** 516–527. doi: 10.1111/j.1533-2500.2010.00441.x

Self DW (2010) Dopamine receptor subtypes in reward and relapse, in *The Dopamine Receptors,* 2nd Ed. (Neve KA ed) pp 479–524, Humana Press, New York.

Self DW, Barnhart WJ, Lehman DA, and Nestler EJ (1996) Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. *Science* **271:** 1586–1589. doi: 10.1126/science.271.5255.1586

Shafer RA and Levant B (1998) The D3 dopamine receptor in cellular and organismal function. *Psychopharmacology (Berl)* **135:** 1–16. doi: 10.1007/s002130050479

Shaham Y and Stewart J (1996) Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats. *Psychopharmacology (Berl)* **125:** 385–391. doi: 10.1007/BF02246022

Sokoloff P, Diaz J, Le Foll B, Guillin O, Leriche L, Bezard E, and Gross C (2006) The dopamine D3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders. *CNS Neurol Disord Drug Targets* **5:** 25–43. doi: 10.2174/187152706784111551

Stewart J and de Wit H (1987) Reinstatement of drug-taking behavior as a method of assessing incentive motivational properties of drugs, in *Methods of Assessing the Reinforcing Properties of Abused Drugs* (Bozarth MA ed) pp 211–227, Springer-Verlag, New York.

Sutton MA, Rolfe NG, and Beninger RJ (2001) Biphasic effects of 7-OH-DPAT on the acquisition of responding for conditioned reward in rats. *Pharmacol Biochem Behav* **69:** 195–200. doi: 10.1016/S0091-3057(01)00540-8

Taylor JR and Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. *Psychopharmacology (Berl)* **84:** 405–412. doi: 10.1007/BF00555222

Vorel SR, Ashby Jr CR, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, and Gardner EL (2002) Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci* **22:** 9595–9603.

Weintraub D, Koester J, Potenza MN, Siderowf AD, Stacy M, Voon V, Whetteckey J, Wunderlich GR, and Lang AE (2010) Impulse control disorders in Parkinson disease: a cross-sectional study of 3090 patients. *Arch Neurol* **67:** 589–595. doi: 10.1001/archneurol.2010.65.

Weiss F, Martin-Fardon R, Ciccocioppo R, Kerr TM, Smith DL, and Ben-Shahar O (2001). Enduring resistance to extinction of cocaine-seeking behavior induced by drug-related cues. *Neuropsychopharmacology* **25:** 361–372. doi: 10.1016/S0893-133X(01)00238-X

Weissenborn R, Deroche V, Koob GF, and Weiss F (1996) Effects of dopamine agonists and antagonists on cocaine-induced operant responding for a cocaine-associated stimulus. *Psychopharmacology (Berl)* **126:** 9595–9603. doi: 10.1007/BF02247382

Wike EL (1966) *Secondary Reinforcement: Selected Experiments*. Harper & Row, New York.

Williams BA (1994) Conditioned reinforcement: experimental and theoretical issues. *Behav Anal* **17:** 261–285.

Wise RA, Murray A, and Bozarth MA (1990) Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing in rats. *Psychopharmacology (Berl)* **100:** 355–360. doi: 10.1007/BF02244606

Wolterink G, Phillips G, Cador M, Donselaar-Wolterink I, Robbins TW, and Everitt BJ (1993) Relative roles of ventral striatal D1 and D2 dopamine receptors in responding with conditioned reinforcement. *Psychopharmacology (Berl)* **110:** 355–364. doi: 10.1007/BF02251293

Xi Z-X, Newman AH, Gilbert JG, Pak AC, Peng X-Q, Ashby Jr CR, Gitajn L, and Gardner EL (2006) The novel dopamine D3 receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. *Neuropsychopharmacology* **31:** 1393–1405. doi: 10.1038/sj.npp.1300912

Figure 3.1. Progressive ratio responding of rats when self-administering remifentanil or treated with pramipexole in extinction

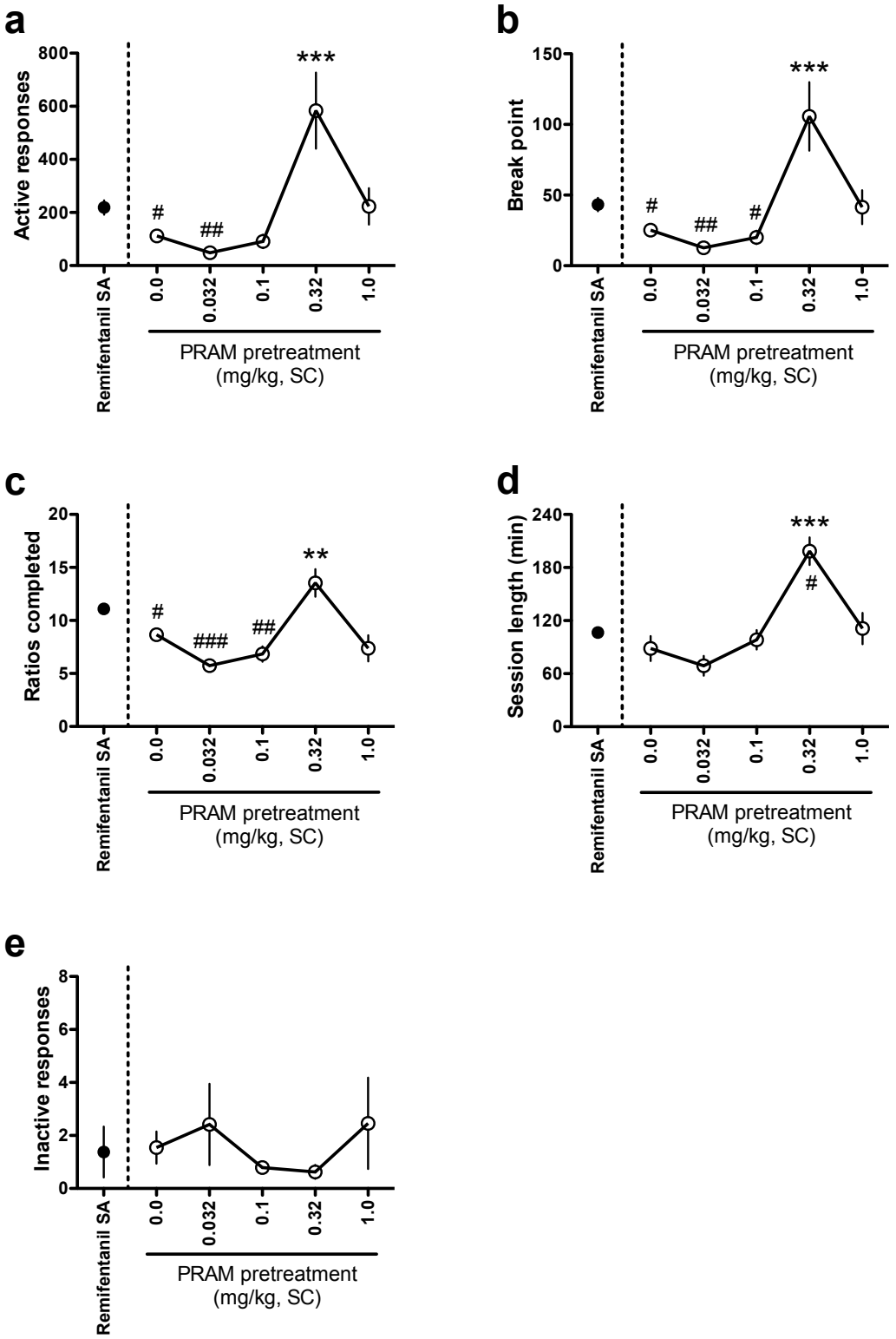

Figure 3.1. Effects of pretreatment with pramipexole (PRAM) prior to sessions in which rats' responding was reinforced under a progressive ratio schedule by presentation of stimuli previously associated with self-administered remifentanil. The remifentanil self-administration (SA) point represents the mean±SEM of the final 3 self-administration training sessions. All pretreatment points represent the mean±SEM of the 3 extinction test sessions conducted with each pretreatment dose. *a:* Active (nose-poke) responses resulted in the presentation of stimulus light illumination previously paired with remifentanil injection. *b:* Break point was defined as the value of the final ratio completed in the session. *c:* For remifentanil SA, the number of ratios completed corresponds to the number of remifentanil injections earned. For pretreatment sessions, the number of ratios completed corresponds to the number of stimulus presentations earned. *d:* Sessions lasted 240 min or until a ratio was not completed in 45 min, whichever occurred first. *e:* Inactive (lever) responses were counted but had no programmed consequences in any session. ***, $p < .001$. Significant difference in effect of the pretreatment dose vs. vehicle (0.0 mg/kg) determined by one-way repeated measures ANOVA with post hoc Bonferroni tests. #, $p < .05$; ##, $p < .01$; ###, $p < .001$. Significant difference from remifentanil SA determined by Bonferroni-corrected paired *t*-test.

Figure 3.2. Intrasession allocation of active responding in extinction under the progressive ratio schedule

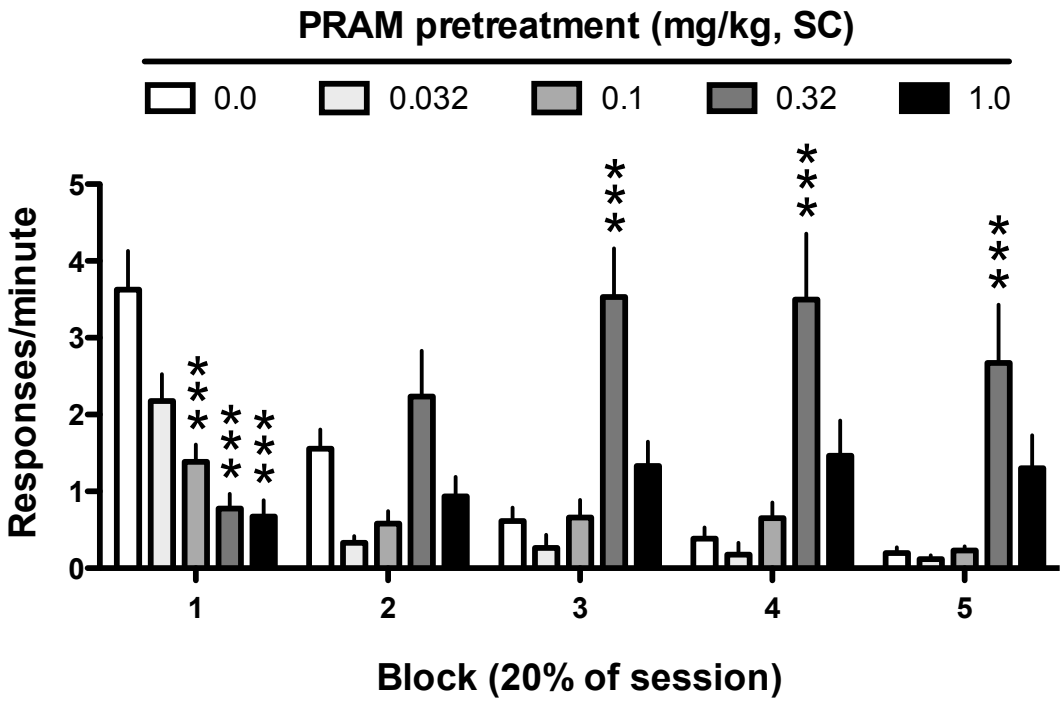

Figure 3.2. Intrasession allocation of active responding in extinction under the progressive ratio schedule. Each block represents 20% of the total session length, which varied across sessions. Each bar displays the mean±SEM number of responses made per minute in each block. ***, $p <$ .001. Significant difference within each block compared to vehicle (0.0 mg/kg) as determined by two-way ANOVA followed by post hoc Bonferroni tests.

Figure 3.3. Effects of pramipexole when active responding did or did not produce the illumination stimuli previously paired with remifentanil availability and/or remifentanil injection

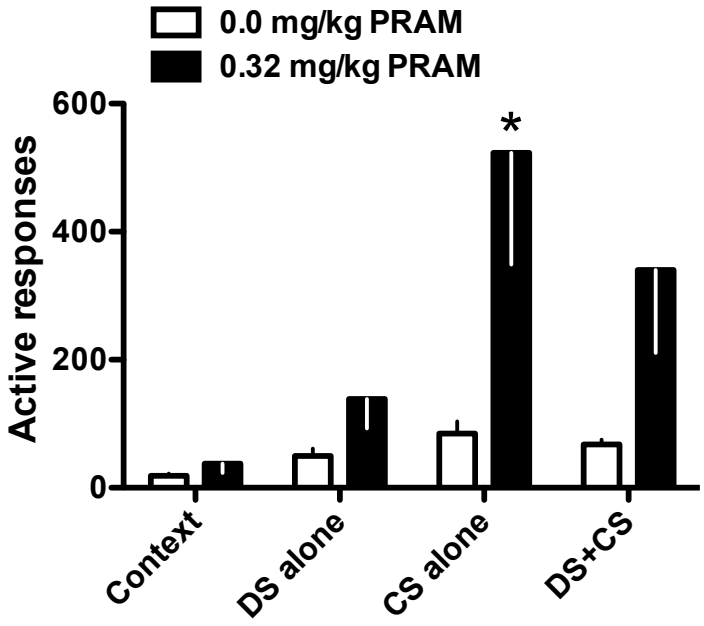

Figure 3.3. Effects of PRAM on active responding when responding did or did not produce the illumination stimuli previously paired with remifentanil availability and/or remifentanil injection. Illumination of the nose-poke aperture served as the discriminative stimulus (DS) indicating remifentanil availability. Illumination of LED the stimulus lights above the nose-poke aperture and the houselight served as injection-paired conditioned stimuli (CS). In the context condition, neither the DS nor the CS was presented. Under all stimulus conditions, the PR schedule was in effect for the active response, and inactive responses (data not shown) were counted but had no scheduled consequences. Each bar represents the mean±SEM. *, $p < .05$. Significant difference between 0.0 mg/kg PRAM and 0.32 mg/kg PRAM determined by two-way repeated measures ANOVA with post hoc Bonferroni tests.

Figure 3.4. Effects of PRAM pretreatment on the acquisition of a novel nose-poke response that produced a stimulus previously paired with response-independent IV remifentanil injection

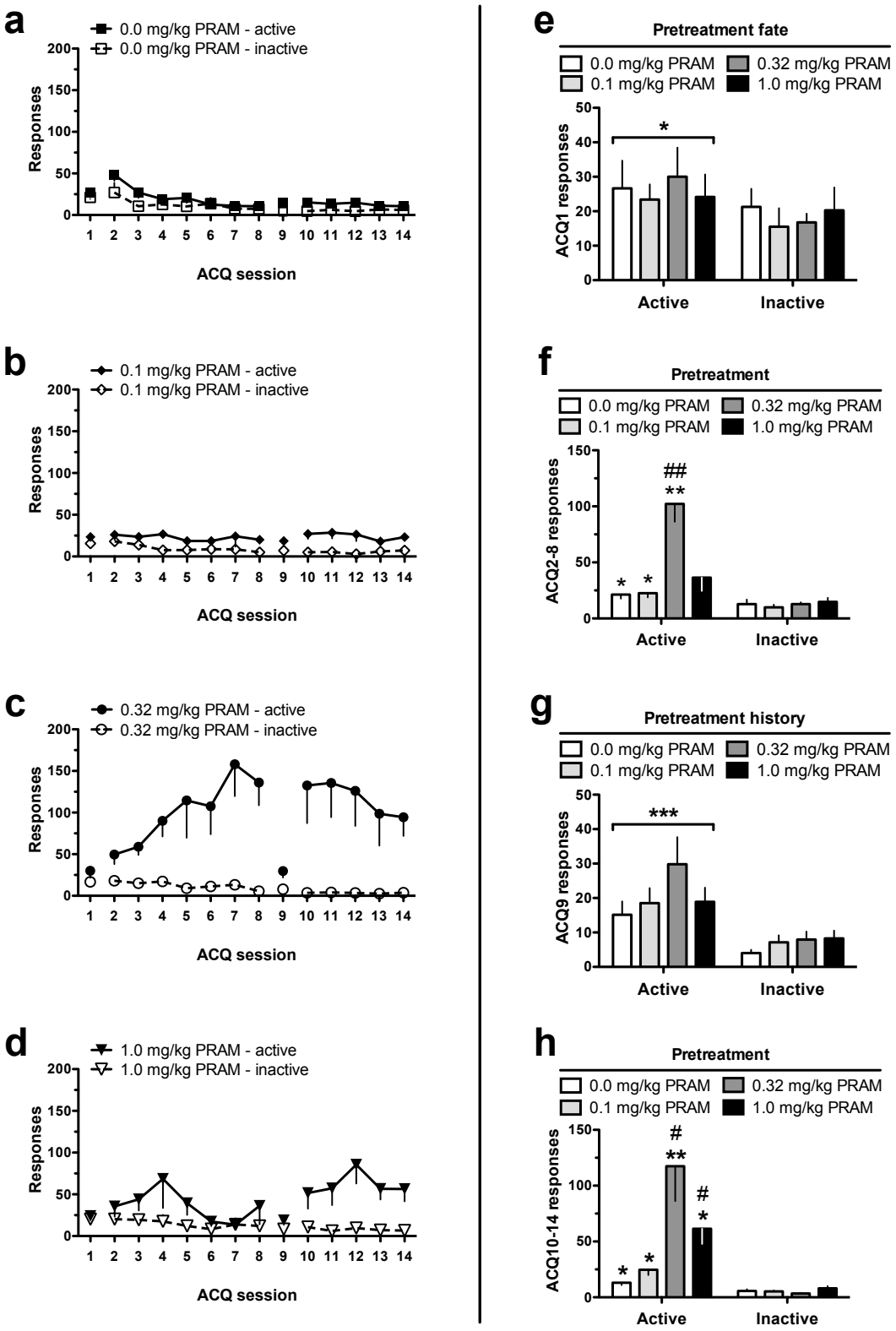

Figure 3.4. Effects of PRAM pretreatment on the acquisition of a novel nose-poke response that produced a stimulus previously paired with response-independent IV remifentanil injection. Responses in the active nose-poke produced the stimulus alone under the modified RR2 schedule. Responses in the inactive nose-poke had no scheduled consequences. ACQ1 lasted 60 min, whereas ACQ2-14 lasted 240 min. *a-d:* Session-by-session record of responding by separate groups of animals treated with vehicle (0.0 mg/kg PRAM), 0.1 mg/kg PRAM, 0.32 mg/kg PRAM, or 1.0 mg/kg PRAM, respectively. *e:* Responding in ACQ1, when no pretreatment was given. *f:* Mean responding in ACQ2-8, when a pretreatment injection of vehicle or PRAM was given before each session. *g:* Responding in ACQ9, when all groups received a vehicle pretreatment. *h:* Mean responding in ACQ10-14, when pretreatments of vehicle or PRAM were resumed. All data are presented as the mean±SEM. *, $p < .05$; **, $p < .01$; ***, $p < .001$. Significant difference between the active and inactive nose-pokes either within each group (for ACQ2-8 and ACQ10-14) or combined across groups (for ACQ1 and ACQ9) as determined by paired *t*-test with the Holm-Bonferroni correction. #, $p < .05$; ##, $p < .01$; ###, $p < .001$. Significant difference in responding on the same manipulandum compared to the vehicle treatment as determined by unpaired *t*-test with the Holm-Bonferroni correction.

Figure 3.5. Nose-poke responses made by rats treated with PRAM after random PAV, when the remifentanil and stimulus were paired only by chance

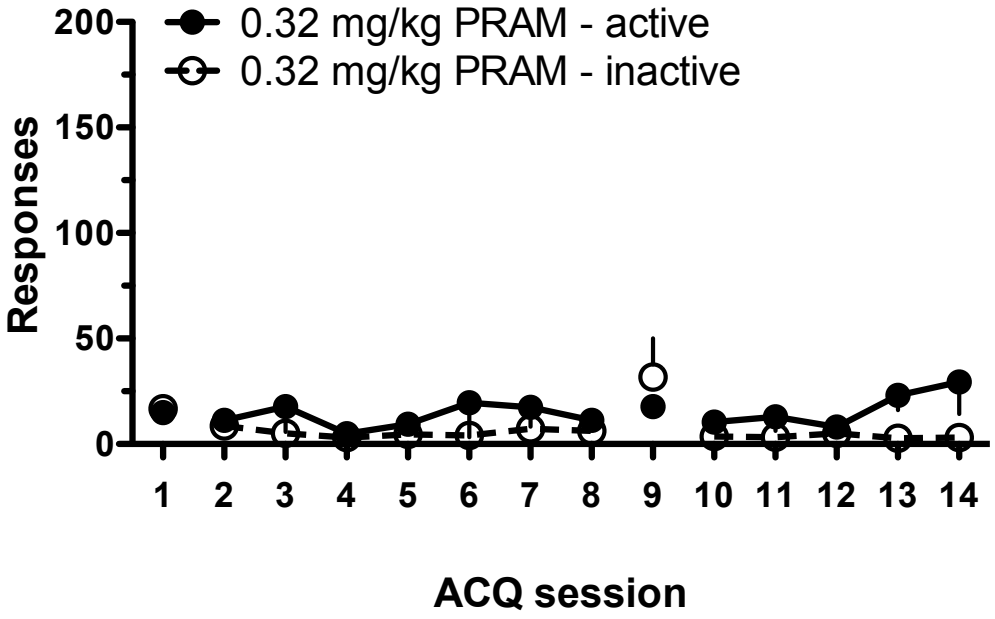

Figure 3.5. Nose-poke responses made by rats treated with PRAM after random PAV, when the remifentanil and stimulus were paired only by chance. PRAM pretreatments were given during ACQ2-8 and ACQ10-14, with a vehicle pretreatment given on ACQ9. Each point represents the mean±SEM. Rats did not acquire nosepoking: active vs. inactive responses were not significantly different in any phase of ACQ, as assessed by one-way ANOVA (ACQ2-8, ACQ10-14) or paired *t*-test (ACQ1, ACQ9).

Figure 3.6. Intrasession allocation of active responding during ACQ2-8 by animals treated with either vehicle (0.0 mg/kg) or 0.32 mg/kg PRAM

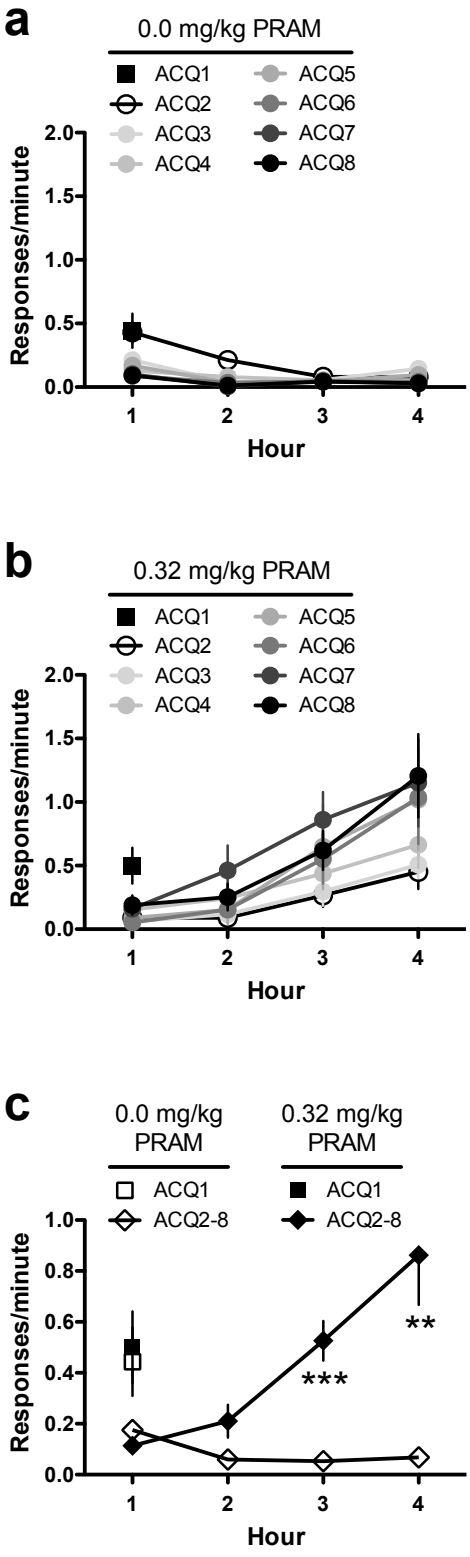

Figure 3.6. Intrasession allocation of active responding during ACQ2-8 by animals treated with either vehicle (0.0 mg/kg) or 0.32 mg/kg PRAM. Rates in ACQ1 are presented for reference. *a*: Rate in each hour of each ACQ session by animals treated with vehicle. b) Rate in each hour of each ACQ session by animals treated with 0.32 mg/kg PRAM. c) Rate in each hour averaged across ACQ2-8. All data are presented as the mean±SEM. **, $p < .01$; ***, $p < .001$. Significant difference between groups as determined by unpaired *t*-test with the Holm-Bonferroni correction.

Figure 3.7. Effects of different PRAM pretreatment intervals (10 min vs. 190 min) on the acquisition of nose-poking with the remifentanil-paired stimulus

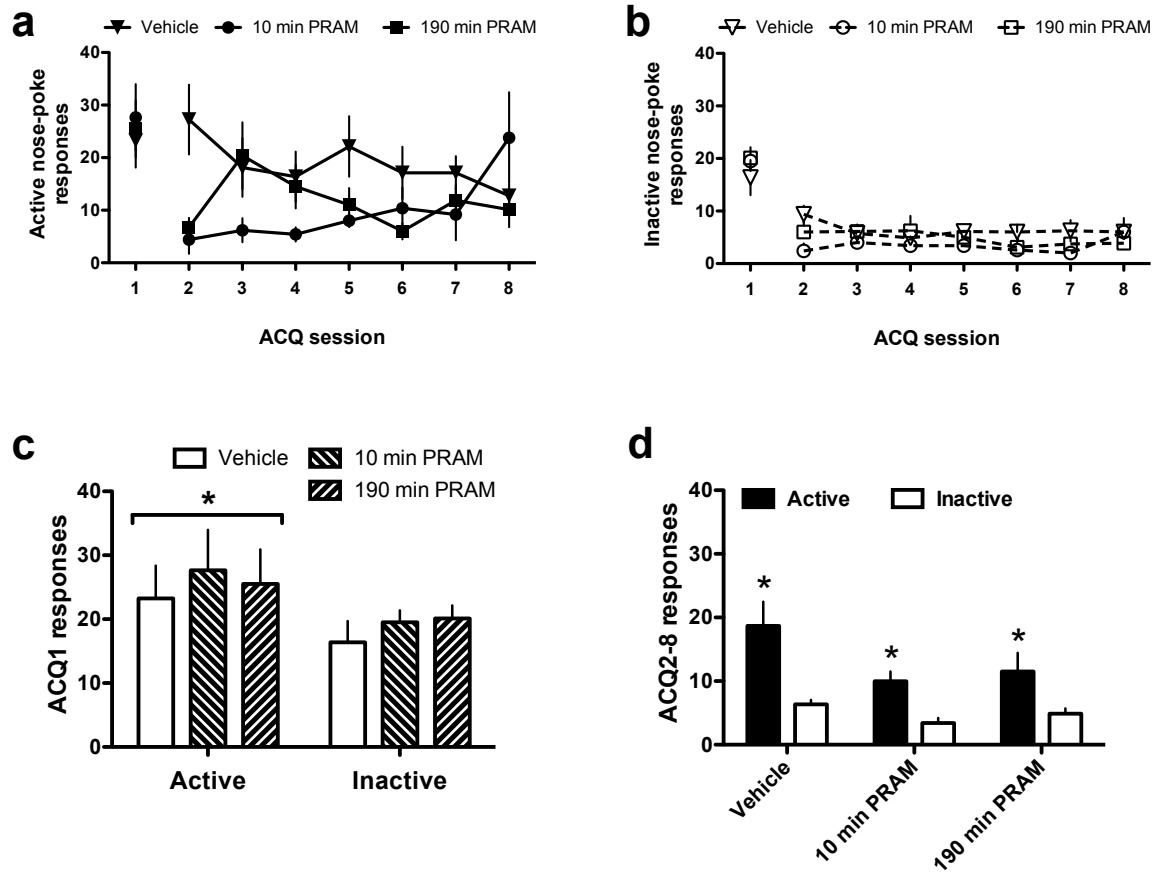

Figure 3.7. Effects of different PRAM pretreatment intervals (10 min vs. 190 min) on the acquisition of nose-poking with the remifentanil-paired stimulus. Responses in the active nose-poke produced the stimulus alone under the modified RR2 schedule. Responses in the inactive nose-poke had no scheduled consequences. All ACQ sessions lasted 60 min. Pretreatments of vehicle or PRAM were given either 10 min or 190 min before ACQ2-8. The responses of the 10 min vehicle and 190 min vehicle groups were collapsed to form the single vehicle group presented. *a:* Session-by-session record of active responding by the three groups. *b:* Session-by-session record of inactive responding by the three groups. *c:* Responding in ACQ1, when no pretreatment injection was given. *d:* Mean responding in ACQ2-8, when a pretreatment injection was given before each session. All data are presented as the mean±SEM. *, $p < .05$. Significant difference between the active and inactive responses, as determined by paired *t*-test with the Holm-Bonferonni correction.

CHAPTER IV

Effects of Pramipexole on New-Response Acquisition with Remifentanil-Conditioned Reinforcement: Involvement of Dopamine D3 vs. D2 Receptors

Introduction

Following the localization of dopamine D3 receptor protein and/or mRNA in brain structures and systems associated in laboratory animals with the reinforcing effects of drugs of abuse and responding with drug-associated stimuli (e.g., the mesolimbic dopaminergic system, extended amygdala, corticostriatal loops), D3-preferring antagonists have received considerable attention as potential pharmacotherapies for human drug abuse and dependence (Garcia-Ladona and Cox 2003; Heidbreder 2013; Heidbreder and Newman 2010; Le Foll et al. 2005; Shafer and Levant 1998; Sokoloff et al. 2006). In rats, much of this work has focused on the ability of D3-preferring compounds (both agonists and antagonists) to alter responding with cocaine-associated stimuli in reinstatement procedures or under second-order schedules of cocaine self-administration. Administered systemically, a variety of D3-preferring agonists can increase (Cervo et al. 2003; Collins and Woods 2009; Collins et al. 2012; De Vries et al. 1999, 2002; Dias et al. 2004; Edwards et al. 2007; Fuchs et al. 2002; Koeltzow and Vezina 2005; Self et al. 1996) and D3-preferring antagonists can decrease (Cervo et al. 2007; Gál and Gyertyán 2006; Gilbert et al. 2005) cocaine-appropriate responding in extinction after cocaine self-administration

training. These changes are observed when rats respond in the presence of cocaine-associated contextual and discriminative stimuli, and/or their responding on the cocaine-appropriate manipulandum produces exteroceptive stimuli (e.g., cue lights, tones) previously paired with cocaine injection, but not cocaine itself. D3-preferring antagonists can also attenuate the ability of cocaine itself (Peng et al. 2009; Vorel et al. 2002; Xi et al. 2006) or a D3-preferring agonist (Collins et al. 2012) to increase extinction responding under these conditions, whereas D3-preferring agonists can enhance cocaine's response-increasing effects (Fuchs et al. 2002; Self et al. 1996). Finally, D3-preferring antagonist administration can reduce rats' cocaine self-administration under second-order schedules of reinforcement before any cocaine has actually been delivered, i.e., in the first phase of the session when responding has produced only the cocaine-associated stimuli (Di Ciano et al. 2003; see also Pilla et al. 1999).

Compared to this body of work with cocaine, relatively few studies have examined the effects of D3-preferring or D3/D2 ligands on responding with stimuli paired with other drugs of abuse, particularly opioid-paired stimuli (Beninger and Banasikowski 2008; Heidbreder 2013; Heidbreder and Newman 2010). Wise and colleagues (1990) first reported that response-independent IV injection of the D3/D2 agonist, bromocriptine, could increase rats' drug-appropriate responding in extinction after either cocaine or heroin self-administration. Subsequently, De Vries and colleagues (2002) reported that SC injection the D3-preferring agonist, quinpirole, could increase heroin-appropriate responding in extinction after heroin self-administration (see also De Vries et al. 1999). Among antagonists, the D3/D2 antagonist, raclopride, was shown to attenuate (numerically) the response-increasing effects of experimenter-administered heroin in extinction after heroin self-administration (Shaham and Stewart 1996). These results implicate D2-like receptor activity in opioid-trained responding,

but both the associative learning mechanisms and D2-like receptor subtype(s) involved in these effects remain unclear.

First, considering associative mechanisms, several authors have suggested that D3-preferring ligands can alter the conditioned reinforcing effects of drug-paired stimuli (Cervo et al. 2003, 2007; Collins and Woods 2009; Collins et al. 2012; Di Ciano et al. 2003; Gál and Gyertyán 2006; Le Foll et al. 2005; Pilla et al. 1999). If the exteroceptive stimuli that are paired with drug injection during self-administration training become conditioned reinforcers as a result of this pairing, then responding in the absence of the drug could be maintained by these conditioned reinforcers. In turn, increases or decreases in responding when D3-preferring agonists or antagonists are given could be due to increases or decreases, respectively, in the effectiveness of these conditioned reinforcers. However, changes in the extinction of an established (self-administration) response or in responding under second-order schedules of reinforcement can be influenced by a number of behavioral mechanisms other than conditioned reinforcement, including the primary reinforcing effects of the training drug and the discriminative stimulus functions of the training drug, testing drug, and stimuli (Collins et al. 2012; Kelleher and Gollub 1962; Mackintosh 1974; Wike 1966; Williams 1994). Measuring the ability of animals to acquire a new response that produces a drug-paired stimulus (i.e., a response that does not and did not also produce the drug itself) can provide a more valid assessment of the conditioned reinforcing effects of that stimulus (Mackintosh 1974; Williams 1994). Systemic administration of several D2-like agonists, including 7-OH-DPAT, bromocriptine, and quinpirole, has been shown to enhance new-response acquisition with food-paired stimuli (Beninger and Ranaldi 1992; Sutton et al. 2001). However, additional work is needed to

determine the effects of D3-preferring ligands on new-response acquisition with drug-paired stimuli.

Second, considering receptor subtypes, it is unclear whether the changes in responding reported previously depend on D3 activity and/or D2 activity. In particular, the use of bromocriptine (Beninger and Ranaldi 1992; Sutton et al. 2001; Wise et al. 1990) and quinpirole (Beninger and Ranaldi 1992; De Vries et al. 2002) in previous studies of both heroin-trained responding and new-response acquisition with food-paired stimuli is noteworthy. Whereas quinpirole is D3-preferring *in vitro* and *in vivo* (Collins et al. 2005, 2007; Freedman et al. 1994; Millan et al. 2002; Sautel et al. 1995), bromocriptine has been shown to have either approximately equal affinity for D2 and D3 receptors or a modest preference for D2 receptors (Coldwell et al. 1999; Freedman et al. 1994; Millan et al. 2002; Sautel et al. 1995; Seeman et al. 2005). These results suggest that strong D3 preference is not necessary for a D2-like agonist to enhance responding; however, bromocriptine's effects as a dopaminergic agonist are difficult to interpret because of its high affinity for and efficacy at non-dopaminergic sites (e.g., serotonin receptors, Millan et al. 2002; Newman-Tancredi et al. 2002; see also Filip et al. 2010). Therefore, the specific importance of D3 receptor activity in responding with opioid-paired stimuli remains to be determined.

To focus specifically on opioid-associated conditioned reinforcement, the present study characterized the effects of the D3-preferring agonist, pramipexole (PRAM), on rats' acquisition of a new response with a remifentanil-paired stimulus. PRAM is D3-preferring both *in vitro* and *in vivo*, but there is considerable variability in the degree of D3 vs. D2 selectivity reported among *in vitro* studies, from a low of 2-fold selective (Seeman et al. 2005) to a high of 488-fold selective (Gerlach et al. 2003). These values also differ from the 32-fold *in vivo* D3 vs. D2

selectivity calculated by Collins and colleagues (2007) by comparing the smallest doses of PRAM capable of eliciting a D3-mediated response (yawning) or a D2-mediated response (hypothermia) in rats. Therefore, in Experiment 1, pretreatments of the D3-preferring antagonist, SB-277011A, or the D2-preferring antagonist, L-741,626, were used to clarify the necessity of D3 or D2 activation to the response-enhancing effects of PRAM. These antagonists were classified as D3- or D2-preferring based on previous *in vivo* work in rats (Collins et al. 2007). After finding that SB-277011A did not alter the effects of PRAM on new-response acquisition, Experiment 2 was conducted to verify the ability of SB-277011A to block two D3-mediated responses elicited by PRAM: yawning and penile erection (PE) in male rats (Collins et al. 2005, 2007, 2009). These elicited responses are not necessarily related to the effects of PRAM on conditioned reinforcement; rather, alterations in yawning and PE would indicate that SB-277011A can block behavioral effects of PRAM in rats that have previously been shown to depend specifically on D3 activity.

Methods

General methods

Animals: Male Sprague-Dawley rats weighing at least 250 g were obtained from Harlan (Indianapolis, IN) to serve as subjects in both experiments. In Experiment 1, all experimental groups contained 8 rats. In Experiment 2, the experimental group contained 6 rats. Animals were housed in a climate controlled facility under a 12 h light-dark cycle (lights on at 7:00 am). All animals were allowed to acclimate to the facility for at least 7 days before the start of any

experimental procedures. Experimental sessions were conducted 5-7 days/week (except where noted in Experiment 2) during the light phase of the cycle. All animals had unrestricted access to standard pellet chow and tap water in the home cage for the duration of their experiment. All studies were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Research 1996), as adopted and promulgated by the National Institutes of Health, and all experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Drugs: Remifentanil was obtained from the hospital pharmacy of the University of Michigan Health System (Ultiva brand, GlaxoSmithKline, Uxbridge, UK). Pramipexole was obtained from APAC Pharmaceutical (Columbia, MD). SB-277011A was synthesized by the laboratory of Dr. Shaomeng Wang (University of Michigan, Ann Arbor, MI). L-741,626 was obtained from Tocris Biosciences (Bristol, UK). Remifentanil and pramipexole were dissolved in sterile physiological saline. SB-277011A was dissolved in a 20% (w/v) solution of β-cyclodextrin and sterile water. L-741,626 was dissolved in a 5% (v/v) solution of ethanol and sterile water. Remifentail injections were delivered IV in a volume of 100 µl/kg. Pramipexole and L-741,626 were injected SC in a volume of 1.0 ml/kg. Due to solubility limitations, SB-277011A was injected SC in volumes of 1.0-3.2 ml/kg.

Experiment 1: D3 vs. D2 receptor involvement in the enhancement of new-response acquisition with remifentanil-conditioned reinforcement

Surgery: After acclimating to the facility, each animal received a chronic, indwelling venous catheter to allow for IV drug administration. Catheters were custom made from polyurethane tubing (MRE 040, Braintree Scientific, Braintree, MA) and Tygon tubing (S-54-HL, Norton Performance Plastics, Akron, OH). Catheterization surgery was performed under ketamine/xylazine (90:10 mg/kg, IP) anesthesia. The catheter was inserted into the left femoral vein and routed subcutaneously to the area between the scapulae for externalization. At the scapulae, the catheter was attached to 22 ga stainless steel tubing which was passed through and secured to a Dacron mesh back-plate (DC95BS, Instech Laboratories, Plymouth Meeting, PA). Rats were allowed at least 5 days to recover from surgery before starting experimental sessions. Catheters were flushed with 0.25 ml of heparinized saline (50 U/ml) each day during recovery, as well as before and after experimental sessions to ensure patency.

Apparatus: Experimental sessions were conducted in two experimental chambers (ENV-008, Med Associates, St. Albans, VT) located inside light- and sound-attenuating cubicles. Each experimental chamber was located in a separate room of the laboratory. The right wall of each experimental chamber contained a white incandescent houselight (ENV-215M, Med Associates) and a sound generator and speaker (ENV-230 and ENV-224AM, Med Associates). Two nose-poke manipulanda with built-in LED stimulus lights (ENV-114BM, Med Associates) could also be inserted into the right wall. When present, the nose-pokes were located 2.5 cm above the grid floor and 4 cm from the front wall (right nose-poke) or 4 cm from the rear wall (left nose-poke). The houselight was located 9 cm above the grid floor, centered between the two nose-pokes. The speaker was located above the right nose-poke, 7.5 cm above the grid floor. Blank

aluminum panels were inserted when the nose-pokes were removed; all of the other elements of the experimental chamber remained in place.

Drug solutions were delivered by a motorized syringe driver (PHM-107, Med Associates) through Tygon tubing (S-54-HL, Norton Performance Plastics) connected to a fluid swivel (375/22PS, Instech Laboratories) and spring tether, which were mounted to a counterbalanced arm. The syringe drivers were located outside of the light- and sound-attenuating cubicles.

Pavlovian conditioning: After recovery from catheterization surgery, all rats received 5 consecutive sessions of Pavlovian conditioning (PAV). During each PAV session, the nose-pokes were removed from the experimental chambers, and animals received 20 response-independent IV injections of 3.2 μg/kg/injection remifentanil. A light-noise compound stimulus consisting of houselight illumination and white noise (80±5 db as measured at the center of the chamber) co-occurred with each remifentanil injection. Injections and stimuli lasted 2.0±0.5 s, depending on the weight of the individual animal. Injections and stimuli were controlled by a variable time (VT) 3 min schedule (range of intervals: 0.0-6.0 min). The dose of remifentanil was chosen based on previous work in the laboratory on remifentanil self-administration (Cooper et al. 2008), and the 3 min average interval was chosen based on the half-life of remifentanil (Crespo et al. 2005) to allow for extensive drug metabolism between injections. PAV sessions ended after 20 injections and stimuli were delivered, approximately 60 min.

Instrumental acquisition: Instrumental acquisition (ACQ) sessions began the day after the final PAV session. During ACQ sessions, the two nose-pokes were present in the chamber. Illumination of the stimulus lights inside both nose-pokes signaled the start of each ACQ session,

and both nose-pokes remained illuminated for the duration of the session. Responses in the active nose-poke produced the light-noise stimulus alone under a modified random ratio (RR) 2 schedule of reinforcement. The first response in the active nose-poke in each session produced the stimulus with a probability of 1.0, and each subsequent response in the session produced the stimulus with a probability of 0.5. No remifentanil injections were given at any point: animals were attached to the tether, but saline replaced remifentanil on the syringe driver, and the driver did not operate. Responses in the inactive nose-poke were recorded but had no scheduled consequences. Active and inactive responses made during stimulus presentation itself were not recorded. In each group, the side of the active nose-poke (left vs. right) was counterbalanced across animals.

Ten ACQ sessions (ACQ1-10) were conducted as follows. ACQ1 lasted for 60 min, and no pretreatment injection was given. ACQ2-10 lasted for 240 min. An injection of 0.32 mg/kg PRAM was given to all animals 10 min before ACQ2-6. ACQ7-10 were the antagonist test sessions, and all animals received two pretreatment injections before each of these sessions. The first pretreatment injection was given 40 min before the start of the session and contained SB-277011A, L-741,626, or their vehicles. The second pretreatment injection was given 10 min before the start of the session and contained 0.32 mg/kg PRAM or its vehicle. Two groups of animals were tested with L-741,626: a high-dose group (High L) received vehicle, 0.32 mg/kg, and 3.2 mg/kg, whereas a low-dose group (Low L) received vehicle, 0.1 mg/kg, and 1.0 mg/kg. One group of animals received SB-277011A (SB): vehicle, 5.6 mg/kg, and 56.0 mg/kg. Each antagonist dose was tested for a single session. In each group, antagonist doses were given in either ascending or descending order before 0.32 mg/kg PRAM. An antagonist vehicle + PRAM vehicle condition was tested first or last (i.e., on ACQ7 or ACQ10) in each group. This latter

condition was included to check that 0.32 mg/kg PRAM increased responding over vehicle pretreatment (i.e., that PRAM had a response-enhancing in each group). Antagonist pretreatment doses and times were based on previous work in the laboratory (Collins et al. 2007, 2012). Group assignments were made randomly before the start of ACQ.

Data Analysis: For ACQ1, the mean active and inactive nose-pokes made by each group were analyzed using two-way ANOVA with the within-subjects factor of manipulandum (active vs. inactive) and the between-subjects factor of group (SB, Low L, High L). Following a nonsignificant group X manipulandum interaction, responding was collapsed across groups, and a paired *t*-test was used to compare active vs. inactive responding. To check that PRAM did not have a different effect in the three groups before the start of the antagonist test sessions, the mean active and inactive nose-pokes made in each session from ACQ2-6 were analyzed using three-way ANOVA with the within-subjects factors of manipulandum and session and the between-subjects factor of group. Following a lack of significant effects involving group, responding was collapsed across groups for pairwise comparison. Paired *t*-tests were used to compare the active and inactive responses made in each session to each other and to compare the responses made in each subsequent session to the responses made in ACQ2. The Holm-Bonferroni method was used to correct for multiple comparisons. In the antagonist test sessions, the mean active and inactive nose-pokes of each group were analyzed using two-way ANOVA with the within-subjects factors of manipulandum and pretreatment condition. Based on a previous study in the laboratory of D3- vs. D2-preferring antagonists (Collins et al. 2012), it was hypothesized *a priori* that both SB-277011A and L-741,626 would attenuate the effects of PRAM, and so planned comparisons were used to determine if active or inactive responding in the other pretreatment

conditions differed from the vehicle antagonist + 0.32 mg/kg PRAM condition. To determine if antagonist pretreatments altered responding before the stimulus was delivered, the latency to the first active response in each antagonist test session was calculated in minutes. If an animal made no (0) active responses in a session, the latency was recorded as 240 min. Prior to analysis, the latencies were log-transformed to reduce the heterogeneity of variance. For each group, the transformed latencies were analyzed using one-way ANOVA with the within-subjects factor of pretreatment condition. If a significant effect of pretreatment was found, post hoc Dunnett's tests were used to compare the vehicle antagonist + 0.32 mg/kg PRAM condition to each of the other conditions. Analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, CA) or SPSS Statistics 20.0 (IBM, Armonk, NY). Differences were considered significant when $p < .05$, two-tailed.

Experiment 2: Antagonism of D3-mediated elicited behaviors by SB-277011A

Apparatus: PRAM-induced yawning and PE were measured in two transparent plastic observation chambers (48 x 23 x 20 cm). The observation chambers resembled the animals' home cages, except there was no food, water, or bedding in the observation chambers. Angled mirrors were placed behind the observation chambers to facilitate viewing the animal regardless of its location in the chamber.

Behavioral observation: One group of six rats was observed in two sessions to measure yawning behavior and PE elicited by PRAM. Two rats were observed at a time, one per chamber. In each session, yawns and PE were recorded by an experienced observer who was

blind to the antagonist treatment condition. Yawning was defined as a prolonged (~1 s), wide opening of the mouth followed by a rapid closure, whereas PE was defined as an emerging, engorged penis, typically followed by an upright posture and genital grooming (Collins et al., 2009).

Animals were allowed to acclimate to the observation chamber for 30 min at the start of the session. After the acclimation period, animals were injected with 56.0 mg/kg SB-277011A or its vehicle. Half of the animals were first injected with SB-277011A, whereas the other half were first injected with vehicle, and the pretreatments were counterbalanced across the two sessions. 30 min after antagonist injection, animals were injected with 0.1 mg/kg PRAM and observed for 1 h beginning immediately after PRAM injection. The second observation session was conducted 3 days after the first to allow for drug washout. The dose of 0.1 mg/kg PRAM was selected based on previous dose-effect studies of PRAM-induced yawning (Collins et al. 2005, 2007).

Data analysis: To analyze the effects of SB-277011A on yawning, the mean number of yawns made in each observation session was compared using a paired *t*-test. To analyze the effects of SB-277011A on PE, each observation session was divided into 15 min blocks, and PE incidence was calculated as the percentage of blocks that contained at least one PE. Mean PE incidence in each observation session was compared using a paired *t*-test. Analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, CA), and differences were considered significant when $p < .05$, two-tailed.

Results

Experiment 1: D3 vs. D2 receptor involvement in the enhancement of new-response acquisition with remifentanil-conditioned reinforcement

Figure 4.1 presents the effects of pretreatment with SB-277011A or L-741,626 on the response-enhancing effects of 0.32 mg/kg PRAM. Figure 4.1a presents the nose-poke responses of the three antagonist pretreatment groups (SB, Low L, High L) before the start of antagonist administration: in ACQ1 when no pretreatment was given and ACQ2-6 when all groups were treated with 0.32 mg/kg PRAM alone. In ACQ1, animals responded differently in the active vs. inactive nose-pokes [main effect of manipulandum: $F(1,21) = 5.22$, $p = .032$]. Overall responding varied among the groups [main effect of group: $F(2,21) = 5.36$, $p = .013$], but the difference between the nose-pokes did not depend on the group [group X manipulandum: $F(2,21) = 0.65$, $p = .53$]. Collapsed across groups, animals made more active responses than inactive responses [$t(23) = 2.32$, $p = .029$]. In ACQ2-6, animals responded differently in the active vs. inactive nose-pokes [main effect of manipulandum: $F(1,21) = 34.10$, $p < .001$] and across sessions [main effect of session: $F(4,84) = 5.07$, $p < .001$; session X manipulandum: $F(4,84) = 6.34$, $p < .001$]; however, responding did not differ by group [main effect of group and all interactions involving group: $0.10 < F < 0.75$, all p's $> .10$]. Collapsing across groups, animals made significantly more active responses than inactive responses in each session from ACQ2-ACQ6 [$2.99 < t(23) < 6.52$, all p's $< .05$]. Active responding also increased over the course of PRAM treatment: animals made more active responses in ACQ6 than ACQ2 [$t(23) = 4.03$, $p = .002$], whereas inactive responding did not differ in any subsequent session compared to ACQ2 [$0.74 < t(23) < 1.65$, all p's $> .10$].

Figure 4.1b presents the responses of animals pretreated with SB-277011A. Across pretreatment conditions, animals made more active responses than inactive responses [main effect of manipulandum: $F(1,7) = 32.78$, $p < .001$]. Antagonist pretreatment condition did not have a significant effect on responding overall [main effect of pretreatment: $F(3,21) = 0.93$, $p = .44$; pretreatment X manipulandum: $F(3,21) = 1.31$, $p = .29$]. However, by pairwise comparison, animals made significantly fewer active responses when pretreated with vehicle SB-277011A + vehicle PRAM than with vehicle SB-277011A + 0.32 mg/kg PRAM [$t(7) = 3.32$, $p = .012$]. Inactive responding did not differ among pretreatment conditions [$0.52 < t(7) < 1.92$, all p's $> .05$].

Figures 4.1c and 4.1d present the responses of the animals pretreated with L-741,626. Animals in the Low L group (Figure 4.1c) made more active responses than inactive responses across pretreatment conditions [main effect of manipulandum: $F(1,7) = 16.74$, $p = .005$]. Antagonist pretreatment condition affected active and inactive responding differently [main effect of pretreatment: $F(3,21) = 4.16$, $p = .018$; pretreatment X manipulandum: $F(3,21) = 4.62$, $p = .012$]. Pretreatment with 1.0 mg/kg L-741,626 + 0.32 mg/kg PRAM [$t(7) = 2.49$, $p = .041$] or vehicle L-741,626 + vehicle PRAM [$t(7) = 2.57$, $p = .036$] significantly reduced active responding compared to vehicle L-741,626 + 0.32 mg/kg PRAM, whereas inactive responding did not differ among pretreatment conditions [$0.31 < t(7) < 1.16$, all p's $> .10$]. Animals in the High L group (Figure 4.1d) also made more active responses than inactive responses across pretreatment conditions [main effect of manipulandum: $F(1,7) = 30.70$, $p < .001$]. Antagonist pretreatment condition affected active and inactive responding differently [main effect of pretreatment: $F(3,21) = 6.19$, $p = .004$; pretreatment X manipulandum: $F(3,21) = 5.52$, $p = .006$]. Pretreatment with 3.2 mg/kg L-741,626 + 0.32 mg/kg PRAM [$t(7) = 3.52$, $p = .009$] or

vehicle L-741,626 + vehicle PRAM [$t(7) = 2.64$, $p = .033$] significantly reduced active responding compared to vehicle L-741,626 + 0.32 mg/kg PRAM, whereas inactive responding did not differ among pretreatment conditions [$0.065 < t(7) < 2.27$, all p's $> .05$].

Figure 4.2 presents the mean latency (log transformed) between the start of each antagonist test session and the first active response made. In the SB group, the latency to initiate responding varied across pretreatment conditions [$F(3,21) = 3.49$, $p = .033$]; however, by pairwise comparison, no other pretreatment condition was different from vehicle SB-277011A + 0.32 mg/kg PRAM [$0.70 < q < 2.27$, all p's $> .05$]. In both the Low L group [$F(3,21) = 1.96$, $p = .15$] and High L group [$F(3,21) = 2.33$, $p = .10$], pretreatment condition did not affect the latency to initiate responding.

Experiment 2: Antagonism of D3-mediated elicited behaviors by SB-277011A

Figure 4.3 presents the effects of SB-277011A on PRAM-induced yawning and PE. Compared to vehicle, pretreatment with 56.0 mg/kg SB277011A significantly reduced both the number of yawns made [$t(5) = 5.02$, $p = .004$] and the incidence of PE [$t(5) = 3.50$, $p = .017$] after rats were administered 0.1 mg/kg PRAM.

Discussion

The dopamine D3 receptor has received considerable attention in recent years as a target for new medications for human drug abuse and dependence (e.g., Heidbreder 2013; Heidbreder and Newman 2010). In rats, D3-preferring antagonists have been shown to reduce responding in

extinction after cocaine self-administration training (Cervo et al. 2007; Gál and Gyertyán 2006; Gilbert et al. 2005; Peng et al. 2009; Vorel et al. 2002; Xi et al. 2006) and under second-order schedules of cocaine self-administration (Di Ciano et al. 2003; see also Pilla et al. 1999). These effects suggest that D3 activity is important for drug self-administration behaviors, with a particular focus on responding with drug-associated stimuli (e.g., Le Foll et al 2005). However, fewer studies have been performed with D2-preferring antagonists, and so it has been difficult to compare directly the relative importance of D3 vs. D2 activity or to draw conclusions about the necessity and sufficiency of D2 receptor activity for different drug self-administration behaviors. Specifically, only one previous study has, to my knowledge, directly compared the effects of a D2-preferring antagonist with a D3-preferring antagonist on responding with drug-associated stimuli: Collins and colleagues (2012) compared within-subjects the ability of L-741,626 and the D3-preferring antagonist, PG01037, to attenuate the response-enhancing effects of PRAM in extinction after cocaine self-administration training. In this study, 1.0 mg/kg L-741,626 produced a parallel rightward shift of the PRAM dose-effect function, whereas 32.0 mg/kg PG01037 produced a downward shift of the PRAM dose-effect function. These results suggest that D2 receptor activity, as well as D3 receptor activity, may be important for responding with drug-associated stimuli.

The present study used L-741,626 and the D3-preferring antagonist, SB-277011A, to clarify the roles of the D2 and D3 receptors in the effects of PRAM on responding with drug-conditioned reinforcement. Because several behavioral processes, other than conditioned reinforcement, can influence the extinction of a previously trained (self-administration) response and the maintenance of responding under second-order schedules (Kelleher and Gollub 1962; Mackinstosh 1974; Schindler et al. 2002; Wike 1966; Williams 1994), the present study assessed

the effects of PRAM on the acquisition of a new response with a remifentanil-paired stimulus. Among laboratory procedures, new-response acquisition provides a comparatively stringent test for the conditioned reinforcing effects of a stimulus (Mackintosh 1974; Williams 1994).

Presently, the remifentanil-paired stimulus served as a conditioned reinforcer, as indicated by the significant preference for the active nose-poke over the inactive nose-poke in ACQ1 before the start of PRAM treatment. Between ACQ2-6, when pretreatments of PRAM alone were given before each session, active responding increased systematically, whereas inactive responding did not differ among sessions. In the antagonist test sessions, substituting vehicle for PRAM significantly reduced active responding selectively (i.e., without changing inactive responding), indicating that PRAM treatment significantly enhanced the conditioned reinforcing effects of the remifentanil-paired stimulus. L-741,626 attenuated the effects of 0.32 mg/kg PRAM, with both 1.0 mg/kg L-741,626 and 3.2 mg/kg L-741,626 significantly decreasing active responding selectively compared to vehicle L-741,626 + 0.32 mg/kg PRAM. In a previous study of the effects of L-741,626 on elicited responses in rats, 1.0 mg/kg L-741,626 was D2-selective, attenuating the D2-mediated hypothermia, but not the D3-mediated yawning behavior, elicited by D2-like agonist administration (Collins et al. 2007). In the same study, 3.2 mg/kg L-741,626 was not selective. This higher dose of L-741,626 affected both behaviors, indicating that it exerts a significant blockade at both D2 and D3 receptors. In contrast, SB-277011A did not change the effect of PRAM pretreatment on new-response acquisition in the present study. The highest dose of SB-277011A tested presently, 56.0 mg/kg, has been shown to be D3-selective *in vivo* (Collins et al. 2007) and is equal to or larger than doses of SB-277011A that have been effective in several other behavioral tasks involving drug self-administration and/or responding with drug-paired stimuli (e.g., Cervo et al. 2007; Di Ciano et al. 2003; Higley

et al. 2011; Ross et al. 2007; Xi et al. 2005). However, under circumstances that were designed specifically to produce responses previously associated with D3 activity specifically (Collins et al. 2005; 2007; 2009), 56.0 mg/kg SB-277011A was active in the present study, significantly reducing both the yawning behavior and PE observed after PRAM administration.

In open-field tests, locomotor effects of PRAM (Chang et al. 2011; Lagos et al. 1998; Maj et al. 1997), SB-277011A (Song et al. 2011), and L-741,626 (Chang et al. 2011; Millan et al. 2000) have been observed, but it is unlikely that the changes in nose-poke responding observed presently were caused exclusively by nonspecific locomotor effects of the drugs. Changes in active responding, but not inactive responding, were observed both when PRAM was administered alone and in combination with the antagonsits. This difference between the nose-pokes may have been due to the difference between the rates of each response prior to the start of drug administration (active > inactive); however, there were also no significant differences in animals' latency to initiate active responding when treated with SB-277011A or L-741,626. In each group, giving the antagonist + 0.32 mg/kg PRAM did not increase latencies over the vehicle + 0.32 mg/kg PRAM condition. Consistent with a reinforcement mechanism, animals began responding at the beginning of the session, before the first stimulus presentation, but they did not then persist in responding after the stimulus was presented when treated with L-741,626 (or when PRAM was omitted and vehicle was given).

The present results, therefore, indicate that PRAM is not acting through the D3 receptor in enhancing new-response acquisition. Rather, D2 activity may be particularly important for responding with (opioid-based) conditioned reinforcement. Whereas considerable attention has focused on the D3 receptor, there are several lines of evidence suggesting that D2 receptor activity is also important for drug self-administration and responding with drug-associated

stimuli, beyond the work of Collins and colleagues (2012) with L-741,626 reviewed above. First, when D3-preferring agonists are used to increase extinction responding after either cocaine or heroin self-administration, the effective doses are consistently in the range of doses that produce significant D2-mediated elicited effects in rats (Collins et al. 2005, 2007): PRAM, ≥ 0.32 mg/kg (Collins et al. 2012); quinpirole, ≥ 0.1 mg/kg (Collins and Woods 2007; De Vries et al. 1999, 2002; Koetzlow and Vezina 2005; Self et al. 1996); 7-OH-DPAT, ≥ 1.0 mg/kg (Cervo et al. 2003; Fuchs et al. 2002; Self et al., 1996); quinelorane, 0.25 mg/kg (Dias et al. 2004). At lower doses, including those doses that produce D3-mediated elicited effects without producing D2-mediated elicited effects, these agonists cause either no change or a significant reduction in extinction responding. Second, D2 receptor knockout mice have been shown to fail to acquire both morphine-conditioned place preference (Maldonado et al. 1997; Smith et al. 2002; but see Dockstader et al. 2001) and morphine self-administration (Elmer et al. 2002). Some of these deficits may be preferentially expressed with opioids; for example, in these same studies, D2 knockout mice were shown to acquire responding with water reinforcement (Elmer et al. 2002) and to acquire cocaine-conditioned place preference (Smith et al. 2002; see also Bello et al. 2011). It has also been reported that D2 knockout mice successfully self-administer cocaine (Caine et al. 2002), and D2 knockout mice show a loss of morphine-potentiation, but not amphetamine-potentiation, of electrical brain stimulation reward (Elmer et al. 2005).

The lack of effect of SB-277011A in Experiment 1 may depend on the particular training drug (μ-opioid agonist) and/or the particular stimulus function maintaining behavior (conditioned reinforcement). The effects of SB-277011A on the acquisition of responding with cocaine-paired stimuli has not, to our knowledge, been reported, but SB-277011A has been shown to block the conditioned place preference produced by both cocaine (Vorel et al. 2002) and heroin

(Ashby et al. 2003). Therefore, SB-277011A is not categorically inactive with opioid-paired stimuli. However, like resistance-to-extinction and second-order schedule performance, conditioned place preference cannot isolate the conditioned reinforcing effects of the drug-paired stimuli from other behavioral processes, especially Pavlovian conditioned approach (Bardo and Bevins 2000; Tzschentke 2007). Therefore, additional work is needed to determine whether there is a particular role for D2 receptors (or a particular lack of a role for D3 receptors) in drug-conditioned reinforcement. It is also important to replicate the present findings with additional D3 and D2 preferring antagonists, to check that the present results are not an outcome of the particular compounds chosen, before drawing strong inferences about the functions of either receptor subtype. There are several other D3-preferring antagonists that have been evaluated in multiple behavioral tasks with drug-associated stimuli (reviewed by Heidbreder and Newman 2010). Less *in vivo* work has been done with D2-preferring antagonists, but several new compounds have recently been reported which may prove useful for future studies (Langlois et al. 2012; Luedtke et al. 2012). If their selectivity and lack of agonist activity can be verified *in vivo*, systematic comparison of novel D2-preferring compounds with L-741,626, as well as the various D3-preferring antagonists, should provide considerable additional insight into the roles of the D2 vs. D3 receptor in drug self-administration and responding with drug-associated stimuli.

Works cited

Ashby Jr CR, Paul M, Gardner EL, Heidbreder CA, and Hagan JJ (2003) Acute administration of the selective D3 receptor antagonist SB-277011A blocks the acquisition and expression of the conditioned place preference response to heroin in male rats. *Synapse* **48:** 154–156. doi: 10.1002/syn.10188

Bardo MT and Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)* **153:** 31–43. doi: 10.1007/s002130000569

Bello EP, Mateo Y, Gelman DM, Noaín D, Shin JH, Low MJ, Alvarez VA, Lovinger DM, and Rubinstein M (2011) Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nat Neurosci* **14:** 1033–1038. doi: 10.1038/nn.2862.

Beninger RJ and Ranaldi R (1992) The effects of amphetamine, apomorphine, SKF 38393, quinpirole and bromocriptine on responding for conditioned reward in rats. *Behav Pharmacol* **3:** 155–163. doi: 10.1097/00008877-199204000-00009

Beninger RJ and Banasikowski TJ (2008) Dopaminergic mechanism of reward-related incentive learning: focus on the dopamine D3 receptor. *Neurotox Res* **14:** 57–70. doi: 10.1007/BF03033575.

Caine SB, Negus SS, Mello NK, Patel S, Bristow L, Kulagowski J, Vallone D, Saiardi A, and Borrelli E (2002) Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. *J Neurosci* **22:** 2977–2988.

Cervo L, Carnovali F, Stark JA, and Mennini T (2003) Cocaine-seeking behavior in response to drug-associated stimuli in rats: involvement of D3 and D2 dopamine receptors. *Neuropsychopharmacology* **28:** 1150–1159. doi: 10.1038/sj.npp.1300169

Cervo L, Cocco A, Petrella C, and Heidbreder CA (2007) Selective antagonism at dopamine D3 receptors attenuates cocaine-seeking behaviour in the rat. *Int J Neuropsychopharmacol* **10:** 167–181. doi: 10.1017/S1461145705006449

Chang W-L, Breier MR, Yang A, and Swerdlow NR (2011) Disparate effects of pramipexole on locomotor activity and sensorimotor gating in Sprague–Dawley rats. *Pharmacol Biochem Behav* **99:** 634–638. doi: 10.1016/j.pbb.2011.06.002

Coldwell MC, Boyfield I, Brown T, Hagan JJ, and Middlemiss DN (1999) Comparison of the functional potencies of ropinirole and other dopamine receptor agonists at human D2(long), D3 and D4.4 receptors expressed in Chinese hamster ovary cells. *Br J Pharmacol* **127:** 1696–1702. doi: 10.1038/sj.bjp.0702673

Collins GT, Witkin JM, Newman AH, Svensson KA, Grundt P, Cao, J, and Woods JH (2005) Dopamine agonist-induced yawning in rats: a dopamine D3 receptor-mediated behavior. *J Pharmacol Exp Ther* **314:** 310–319. doi: 10.1124/jpet.105.085472

Collins GT, Newman AH, Grundt P, Rice KC, Husbands SM, Chauvignac C, Cheng J, Wang S, and Woods JH (2007) Yawning and hypothermia in rats: effects of dopamine D3 and D2 agonists and antagonists. *Psychopharmacology (Berl)* **193:** 159–170. doi: 10.1007/s00213-007-0766-3

Collins GT, Truccone A, Haji-Abdi F, Newman AH, Grundt P, Rice KC, Husbands SM, Greedy BM, Enguehard-Gueiffier C, Gueiffier A, Chen J, Wang S, Katz JL, Grandy DK, Sunahara RK, and Woods JH (2009) Proerectile effects of dopamine D2-like agonists are mediated by the D3 receptor in rats and mice. *J Pharmacol Exp Ther* **329:** 210–217. doi: 10.1124/jpet.108.144048

Collins GT and Woods JH (2009) Influence of conditioned reinforcement on the response-maintaining effects of quinpirole in rats. *Behav Pharmacol* **20:** 492–504. doi: 10.1097/FBP.0b013e328330ad9b

Collins GT, Cunningham AR, Chen J, Wang, S, Newman AH, and Woods JH (2012) Effects of pramipexole on the reinforcing effectiveness of stimuli that were previously paired with cocaine reinforcement in rats. *Psychopharmacology (Berl)* **219:** 123–135. doi: 10.1007/s00213-011-2382-5

Cooper ZD, Truong YN-T, Shi Y-G, Woods JH (2008) Morphine deprivation increases self-administration of the fast- and short-acting μ-opioid receptor agonist remifentanil in the rat. *J Pharmacol Exp Ther* **326:** 920–929. doi: 10.1124/jpet.108.139196

Crespo JA, Sturm K, Saria A, Zernig G (2005) Simultaneous intra-accumbens remifentanil and dopamine kinetics suggest that neither determines within-session operant responding. *Psychopharmacology (Berl)* **183:** 201–209. doi: 10.1007/s00213-005-0180-7

De Vries TJ, Schoffelmeer ANM, Binnekade R, and Vanderschuren LJMJ (1999) Dopaminergic mechanisms mediating the incentive to seek cocaine and heroin following long-term withdrawal of IV drug self-administration. *Psychopharmacology (Berl)* **143:** 254–260. doi: 10.1007/s002130050944

De Vries TJ, Schoffelmeer ANM, Vinnekade R, Raasø H, and Vanderschuren LJMJ (2002) Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. *Neuropsychopharmacology* **26:** 18–26. doi: 10.1016/S0893-133X(01)00293-7

Di Ciano P, Underwood RJ, Hagan JJ, and Everitt BJ (2003) Attenuation of cue-controlled cocaine-seeking by a selective D3 dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology* **28:** 329–338. doi: 10.1038/sj.npp.1300148

Dias C, Lachize S, Boilet V, Huitelec, and Cador M (2004) Differential effects of dopaminergic agents on locomotor sensitization and on the reinstatement of cocaine-seeking and food-seeking behaviour. *Psychopharmacology (Berl)* **175:** 414–427. doi: 10.1007/s00213-004-1839-1

Dockstader CL, Rubinstein M, Grandy DK, Low MJ, and van der Kooy D (2001) The D2 receptor is critical in mediating opiate motivation only in opiate-dependent and withdrawn mice. *Eur J Neurosci* **13:** 995–1001. doi: 10.1046/j.1460-9568.2001.01455.x

Edwards S, Whisler KN, Fuller DC, Orsulak PJ, and Self DW (2007) Addiction-related alterations in D1 and D2 dopamine receptor behavioral responses following chronic cocaine self-administration. *Neuropsychopharmacology* **32:** 354–366. doi: 10.1038/sj.npp.1301062

Elmer GI, Pieper JO, Rubinstein M, Low MJ, Grandy DK, and Wise RA (2002) Failure of intravenous morphine to serve as an effective instrumental reinforcer in dopamine D2 receptor knock-out mice. *J Neurosci* **22:** RC224.

Elmer GI, Pieper JO, Levy J, Rubinstein M, Low MJ, Grandy DK, and Wise RA (2005) Brain stimulation and morphine reward deficits in dopamine D2 receptor-deficient mice. *Psychopharmacology (Berl)* **182:** 33–44. doi: 10.1007/s00213-005-0051-2

Filip M, Alenina N, Bader M, and Przegaliński E (2010) Behavioral evidence for the significance of serotoninergic (5-HT) receptors in cocaine addiction. *Addict Biol* **15:** 227–249. doi: 10.1111/j.1369-1600.2010.00214.x

Freedman SB, Patel S, Marwood R, Emms F, Seabrook GR, Knowles MR, and McAllister G (1994) Expression and pharmacological characterization of the human D3 dopamine receptor. *J Pharmacol Exp Ther* **286:** 417–426.

Fuchs RA, Tran-Nguyen LTL, Weber SM, Khroyan TV, and Neisewander JL (2002) Effects of 7-OH-DPAT on cocaine-seeking behavior and on re-establishment of cocaine self-administration. *Pharmacol Biochem Behav* **72:** 623–632. doi: 10.1016/S0091-3057(02)00731-1

Gál K and Gyertyán I **(**2006) Dopamine D3 as well as D2 receptor ligands attenuate the cue-induced cocaine-seeking in a relapse model in rats. *Drug Alcohol Depend* **81:** 63–70. doi: 10.1016/j.drugalcdep.2005.05.011

Garcia-Ladona FJ and Cox BF (2003) BP 897, a selective dopamine D3 receptor ligand with therapeutic potential for the treatment of cocaine-addiction. *CNS Drug Rev* **9:** 141–158. doi: 10.1111/j.1527-3458.2003.tb00246.x

Gerlach M, Double K, Arzberger T, Leblhuber F, Tatschner T, and Riederer P (2003) Dopamine receptor agonists in current clinical use: comparative dopamine receptor binding profiles defined in the human striatum. *J Neural Transm* **110:** 1119–1127. doi: 10.1007/s00702-003-0027-5

Gilbert JG, Newman AH, Gardner EL, Ashby Jr CR, Heidbreder CA, Pak AC, Peng X-Q, and Xi Z-X (2005). Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: role of dopamine D3 receptors. *Synapse* **57:** 17–28. doi: 10.1002/syn.20152

Heidbreder C (2013) Rationale in support of the use of selective dopamine D3 receptor antagonists for the pharmacotherapeutic management of substance use disorders. *Naunyn Schmiedebergs Arch Pharmacol* **386:** 167–176. doi: 10.1007/s00210-012-0803-6

Heidbreder C and Newman AH (2010) Current perspectives on selective dopamine D3 receptor antagonists as pharmacotherapeutics for addictions and related disorders. *Ann N Y Acad Sci* **1187:** 4–34. doi: 10.1111/j.1749-6632.2009.05149.x

Higley AE, Kiefer SW, Li X, Gaál J, Xi Z-X, and Gardner EL (2011) Dopamine D3 receptor antagonist SB-277011A inhibits methamphetamine self-administration and methamphetamine-induced reinstatement of drug-seeking in rats. *Eur J Pharmacol* **659:** 187–92. doi: 10.1016/j.ejphar.2011.02.046

Institute of Laboratory Animal Research CoLS, National Research Council (1996) *Guide for the Care and Use of Laboratory Animal*s, 7th ed. National Academies Press, Washington DC.

Kelleher RT and Gollub LR (1962) A review of positive conditioned reinforcement. *J Exp Anal Behav* **5:** 543–597. doi: 10.1901/jeab.1962.5-s543

Koeltzow TE and Vezina P (2005) Locomotor activity and cocaine-seeking behavior during acquisition and reinstatement of operant self-administration behavior in rats. *Behav Brain Res* **160:** 250–259. doi: 10.1016/j.bbr.2004.12.005

Lagos P, Scorza C, Monti JM, Jantos H, Reyes-Parada M, Silveira R, and Ponzoni A (1998) Effects of the D3 preferring dopamine agonist pramipexole on sleep and waking, locomotor activity and striatal dopamine release in rats. *Eur Neuropsychopharmacol* **8:** 113–120. doi: 10.1016/S0924-977X(97)00054-0

Langlois X, Megens A, Lavreysen H, Atack J, Cik M, te Riele P, Peeters L, Wouters R, Vermeire J, Hendrickx H, Macdonald G, and De Bruyn M (2012) Pharmacology of JNJ-37822681, a specific and fast-dissociating D2 antagonist for the treatment of schizophrenia. *J Pharmacol Exp Ther* **342:** 91–105. doi: 10.1124/jpet.111.190702.

Le Foll B, Goldberg SR, and Sokoloff P (2005) The dopamine D3 receptor and drug dependence: effects on reward or beyond? *Neuropharmacology* **49:** 525–541. doi: 10.1016/j.neuropharm.2005.04.022

Luedtke RR, Mishra Y, Wang Q, Griffin SA, Bell-Horner C, Taylor M, Vangveravong S, Dillon GH, Huang RQ, Reichert DE, and Mach RH (2012) Comparison of the binding and functional properties of two structurally different D2 dopamine receptor subtype selective compounds. *ACS Chem Neurosci* **3:** 1050–1062. doi: 10.1021/cn300142q

Mackintosh NJ (1974) *The Psychology of Animal Learning.* Academic Press, New York.

Maj J, Rogóż Z, Skuza G, and Kołodziejczyk K (1997) The behavioural effects of pramipexole, a novel dopamine receptor agonist. *Eur J Pharmacol* **324:** 31–37. doi: 10.1016/S0014-2999(97)00066-6

Maldonado R, Saiardi A, Valverde O, Samad TA, Roques BP, and Borrelli E (1997) Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. *Nature* **388:** 586–589.

Millan MJ, Dekeyne A, Rivet JM, Dubuffet T, Lavielle G, and Brocco M (2000) S33084, a novel, potent, selective, and competitive antagonist at dopamine D(3)-receptors: II. functional and behavioral profile compared with GR218,231 and L741,626. *J Pharmacol Exp Ther* **293:** 1063–1073.

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin J-A, and Newman-Tancredi A (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. a multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. *J Pharmacol Exp Ther* **303:** 791–804. doi: 10.1124/jpet.102.039867

Newman-Tancredi A, Cussac D, Quentric Y, Touzard M, Verrièle L, Carpentier N, and Millan MJ (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes. *J Pharmacol Exp Ther* **303:** 815–822. doi: 10.1124/jpet.102.039883

Peng X-Q, Ashby Jr CR, Spiller K, Li X, Li J, Thomasson N, Millan MJ, Mocaër E, Muńoz C, Gardner EL, and Xi Z-X (2009) The preferential dopamine D3 receptor antagonist S33138 inhibits cocaine reward and cocaine-triggered relapse to drug-seeking behavior in rats. *Neuropharmacology* **56:** 752–760. doi: 10.1016/j.neuropharm.20 08.12.007

Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz J-C, Everitt BJ, and Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* **400:** 371–375. doi: 10.1038/22560

Ross JT, Corrigall WA, Heidbreder CA, and LeSage MG (2007) Effects of the selective dopamine D3 receptor antagonist SB-277011A on the reinforcing effects of nicotine as measured by a progressive-ratio schedule in rats. *Eur J Pharmacol* **22:** 173–179. doi: 10.1016/j.ejphar.2007.01.004

Sautel F, Griffon N, Lévesque D, Pilon C, Schwartz J-C, and Sokoloff P (1995) A functional test identifies dopamine agonists selective for D3 versus D2 receptors. *Neuroreport* **6:** 329–332.

Seeman P, Ko F, Willeit M, McCormick P, and Ginovart N (2005) Antiparkinson concentrations of pramipexole and PHNO occupy dopamine D2(high) and D3(high) receptors. *Synapse* **58:** 122–128. doi: 10.1002/syn.20193

Schindler CW, Panlilio LV, and Goldberg SR (2002) Second-order schedules of drug self-administration in animals. *Psychopharmacology (Berl)* **163:** 327–344. doi: 10.1007/s00213-002-1157-4

Self DW, Barnhart WJ, Lehman DA, and Nestler EJ (1996) Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. *Science* **271:** 1586–1589. doi: 10.1126/science.271.5255.1586

Shafer RA and Levant B (1998) The D3 dopamine receptor in cellular and organismal function. *Psychopharmacology (Berl)* **135:** 1–16. doi: 10.1007/s002130050479

Shaham Y and Stewart J (1996) Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats. *Psychopharmacology (Berl)* **125:** 385–391. doi: 10.1007/BF02246022

Smith JW, Fetsko LA, Xu R, and Wang Y (2002) Dopamine D2L receptor knockout mice display deficits in positive and negative reinforcing properties of morphine and in avoidance learning. *Neuroscience* **113:** 755–765. doi: 10.1016/S0306-4522(02)00257-9,

Sokoloff P, Diaz J, Le Foll B, Guillin O, Leriche L, Bezard E, and Gross C (2006) The dopamine D3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders. *CNS Neurol Disord Drug Targets* **5:** 25–43. doi: 10.2174/187152706784111551

Song R, Yang R-F, Wu N, Su R-B, Li J, Peng X-Q, Li X, Gaál J, Xi Z-X, and Gardner EL (2011). YQA14: a novel dopamine D3 receptor antagonist that inhibits cocaine self-administration in rats and mice, but not in D3 receptor-knockout mice. *Addict Biol* **17:** 259–73. doi: 10.1111/j.1369-1600.2011.00317.x

Sutton MA, Rolfe NG, and Beninger RJ (2001) Biphasic effects of 7-OH-DPAT on the acquisition of responding for conditioned reward in rats. *Pharmacol Biochem Behav* **69:** 195–200. doi: 10.1016/S0091-3057(01)00540-8

Tzschentke TM (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade *Addict Biol* **12:** 227–462. doi: 10.1111/j.1369-1600.2007.00070.x

Vorel SR, Ashby Jr CR, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, and Gardner EL (2002) Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci* **22:** 9595–9603.

Wike EL (1966) *Secondary Reinforcement: Selected Experiments*. Harper & Row, New York.

Williams BA (1994) Conditioned reinforcement: experimental and theoretical issues. *Behav Anal* **17:** 261–285.

Wise RA, Murray A, and Bozarth MA (1990) Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing in rats. *Psychopharmacology (Berl)* **100:** 355–360. doi: 10.1007/BF02244606

Xi Z-X, Newman AH, Gilbert JG, Pak AC, Peng X-Q, Ashby Jr CR, Gitajn L, and Gardner EL (2006) The novel dopamine D3 receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. *Neuropsychopharmacology* **31:** 1393–1405. doi: 10.1038/sj.npp.1300912

Figure 4.1. Effects of the D3-preferring antagonist, SB-277011A, or the D2-preferring antagonist, L-741,626, on PRAM-induced increases in responding with the remifentanil-paired stimulus

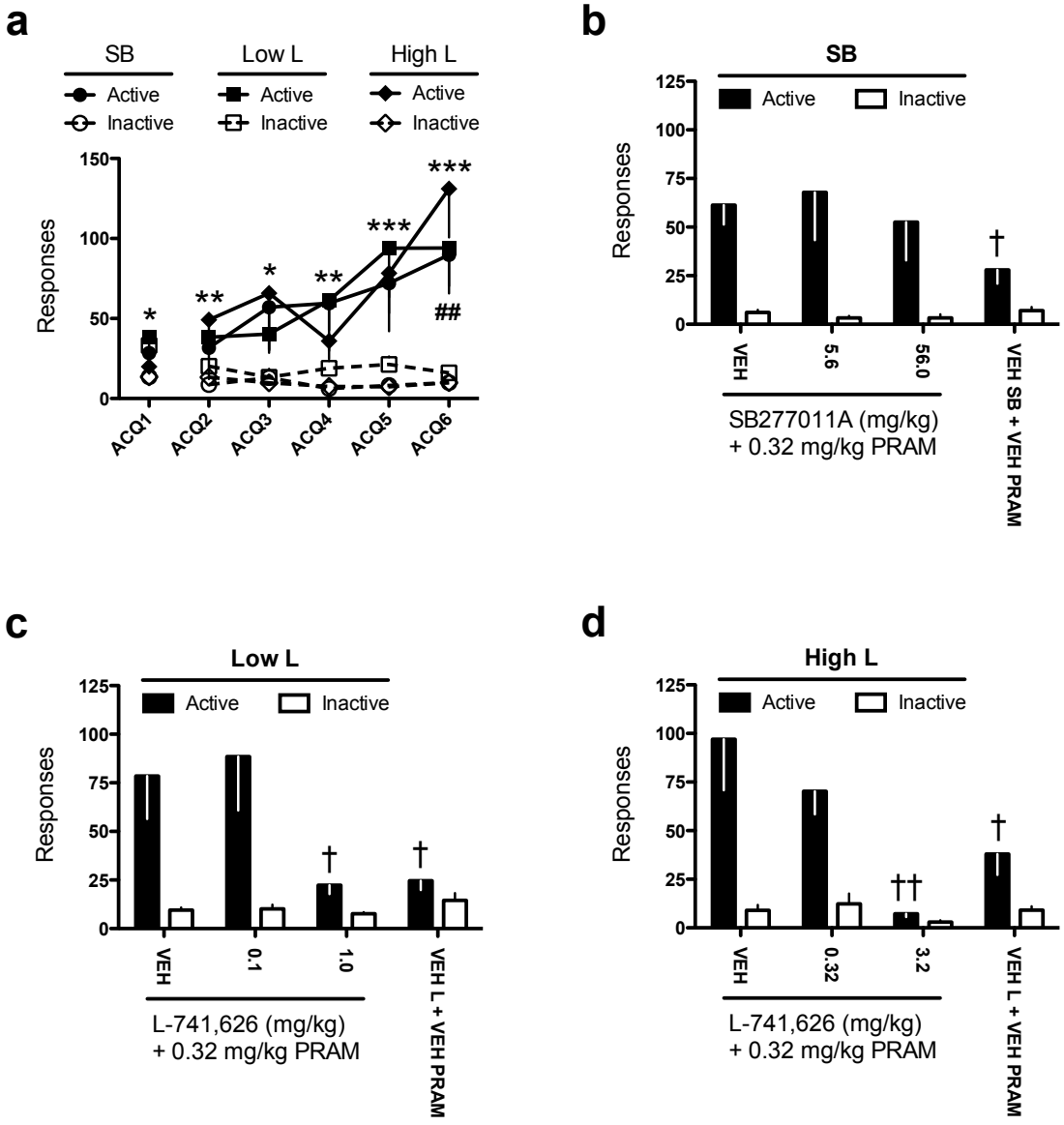

Figure 4.1. Effects of the D3-preferring antagonist, SB-277011A, or the D2-preferring antagonist, L-741,626, on PRAM-induced increases in responding with the remifentanil-paired stimulus. *a:* Acquisition of nose-poke responding with the remifentanil-paired stimulus before the start of antagonist administration. No pretreatment was given on ACQ1, whereas 0.32 mg/kg PRAM alone was administered before each session from ACQ2-6. *b*: The D3-preferring antagonist, SB-277011A, did not alter the response-enhancing effects of PRAM. *c-d:* A lower or higher dose, respectively, of the D2-preferring antagonist, L-741,626, attenuated the ability of PRAM to increase responding with the remifentanil-paired stimulus. All data are presented as the mean±SEM. *, $p < .05$; **, $p < .01$; ***, $p < .001$. Significant difference after collapsing across groups between active and inactive responses, as assessed by paired *t*-test with the Holm-Bonferroni correction. ##, $p < .01$. Significant difference from ACQ2 in responding on the same manipuladum (active vs. active or inactive vs. inactive), as assessed by paired *t*-test with the Holm-Bonferroni correction. †, $p < .05$; ††, $p < .01$. Significant difference from the vehicle antagonist + 0.32 mg/kg PRAM condition in responding on the same manipulandum (active vs. active or inactive vs. inactive), as assessed by planned comparisons using paired *t*-tests.

Figure 4.2. Effects of SB-277011A or L-741,626 on the latency the first active response in the session

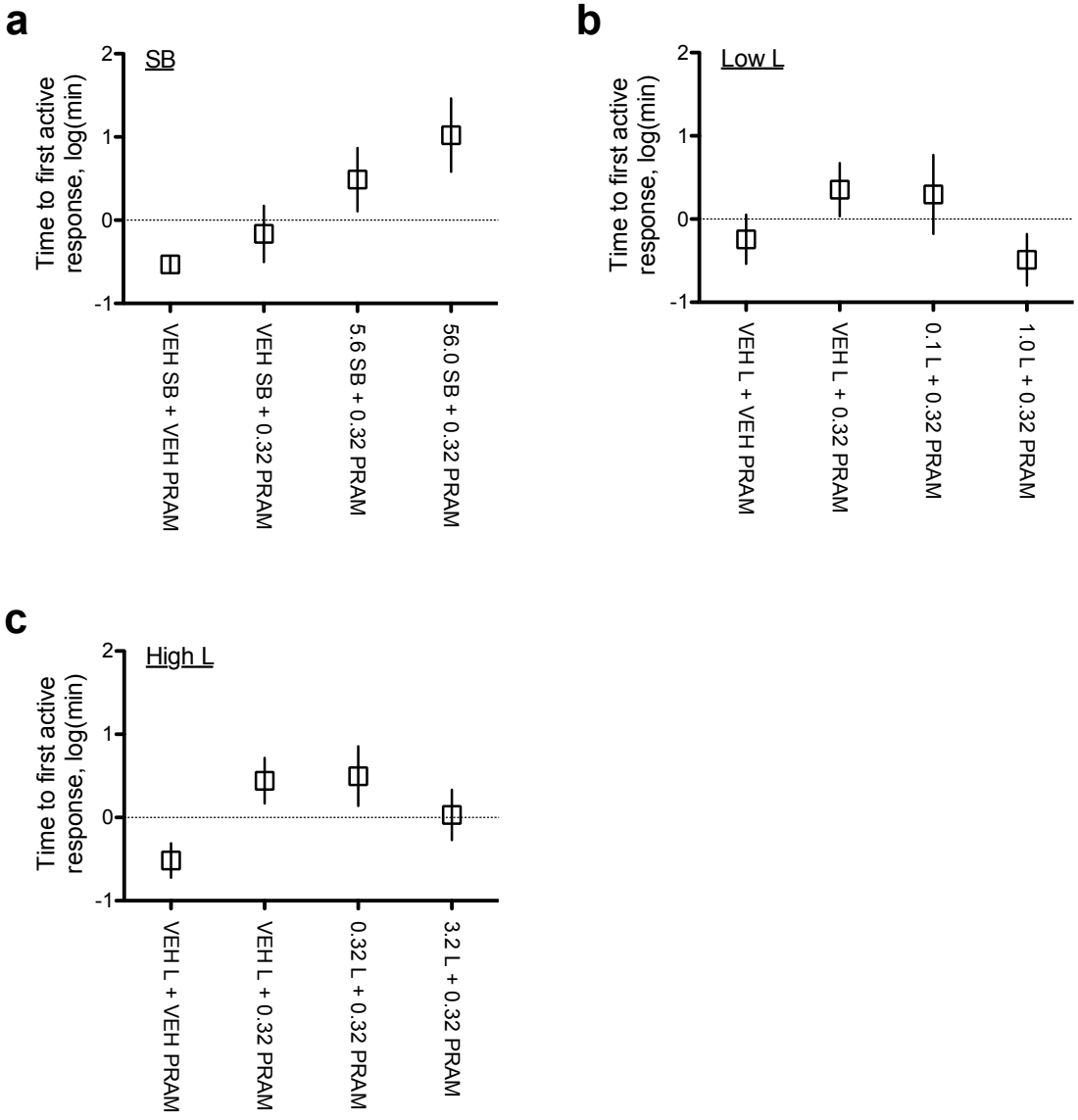

Figure 4.2. Neither SB-277011A nor L-741,626 significantly increased the latency for animals to begin responding at the start of the session. *a:* Animals treated with SB-277011A. *b:* Animals treated with lower doses of L-741,626. *c:* Animals treated with higher doses of L-741,626. All data were log-transformed before analysis to correct for extreme heterogeneity of variance and are presented as the mean±SEM of the transformed values. In each group, no antagonist dose differed from vehicle + 0.32 mg/kg PRAM when compared pairwise using post hoc Dunnett's tests following one-way ANOVA.

Figure 4.3. SB-277011A, 56.0 mg/kg, inhibits the yawning and PE elicited by 0.1 mg/kg PRAM

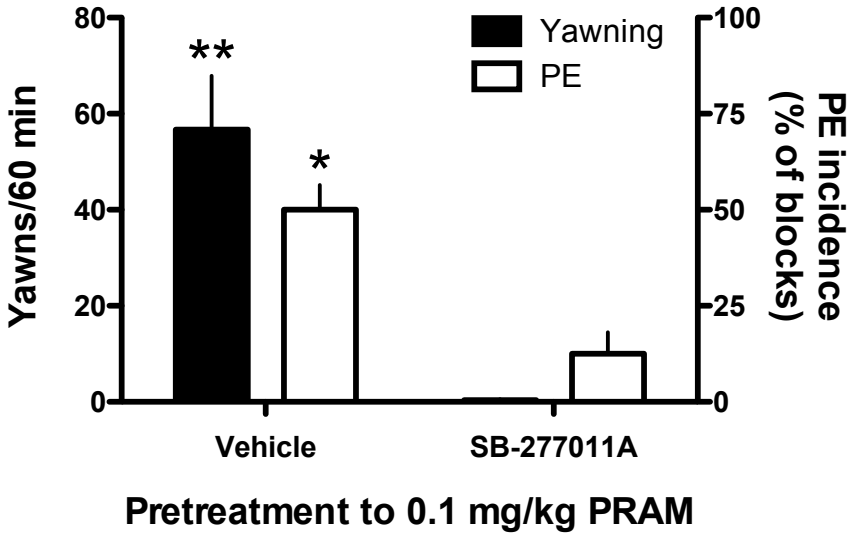

Figure 4.3. SB-277011A, 56.0 mg/kg, inhibits the yawning and PE elicited by 0.1 mg/kg PRAM. Animals were pretreated with either SB-277011A or its vehicle prior to being administered 0.1 mg/kg PRAM. All animals were exposed to both antagonist conditions in counterbalanced order. Both yawning and PE were recorded from all animals in the same observation periods. All data are presented as the mean±SEM. * $p < .05$; ** $p < .01$. Significant difference between vehicle and SB-277011A pretreatment as assessed by paired *t*-test.

CHAPTER V

GENERAL DISCUSSION

The potential importance of conditioned reinforcement to human and non-human animal behavior generally, and to drug self-administration behaviors more specifically, has been long recognized (e.g., Everitt et al. 2008; Hull 1943; Koob and Le Moal 2008; Skinner 1953; Wikler 1971; Williams 1994). Many of the laboratory methods that have been used to study conditioned reinforcement, however, present significant interpretational difficulties: the behaviors they generate may actually be influenced by a combination of conditioned reinforcement and other associative and nonassociative behavioral processes, and in some cases, the target stimuli may have no conditioned reinforcing effects at all (Cunningham 1993; Kelleher and Gollub 1962; Mackintosh 1974; Schindler et al. 2002; Shahan 2010; Wike 1966; Williams 1994). Therefore, it has been difficult to characterize the influence over behavior that drug-paired stimuli can exert specifically because of their conditioned reinforcing effects, with a particular lack of work on the conditioned reinforcing effects of opioid-paired stimuli (Davis and Smith 1987; Di Ciano and Everitt 2004).

Outside of the laboratory, many behaviors do, at some point, involve contact with a primary reinforcer, even if this contact follows only after a complicated chain of responses, a considerable temporal delay from a given response, and/or various types of experiences with environmental stimuli. To model these complex interactions, it is often most fitting in laboratory

procedures to study behavior as it is influenced by multiple mechanisms simultaneously or in sequence (e.g., by the combined effects of primary and conditioned reinforcement contingencies acting together). Nonetheless, it is also important to characterize the separate contributions made by individual learning mechanisms or stimulus functions. There are, in particular, many different associations that could possibly be made when a laboratory animal or human learns to self-administer a drug and engage in various kinds of drug-seeking and drug-taking behaviors, and it is important to determine which associations actually are made in different circumstances (cf., Hogarth and Duka 2006; Milton and Everitt 2010). If precisely characterized, these mechanisms can be more effectively targeted by behavioral and/or pharmacological interventions designed to reduce self-administration and related maladaptive behaviors while minimizing changes in other, more adaptive forms of learning or memories (reviewed by Milton and Everitt 2010; Myers and Carlezon 2010; Taylor et al. 2009). Even considering only Pavlovian drug-conditioned stimuli, it may be possible to differentiate, both behaviorally and neurobiologically, several different ways that responding can be changed by stimulus presentation: Pavlovian conditioned approach, Pavlovian-to-instrumental transfer, and conditioned reinforcement (Milton and Everitt 2010). It is possible, therefore, that different kinds of environmental or pharmacological interventions may be more or less effective at reducing a target behavior based on the mechanism(s) generating the behavior in the first place. The present study focused on several different environmental and pharmacological determinants of performance with drug-associated conditioned reinforcement.

Rats acquired a novel nose-poke response that produced a light-noise compound stimulus that had been paired with the potent, short-acting μ-opioid agonist, remifentanil. Control procedures were designed to verify that performance met the criteria of Mackintosh (1974) for a

sufficient demonstration of conditioned reinforcement. First, to prevent the association of responding with remifentanil as a primary reinforcer, Pavlovian conditioning procedures alone (i.e., only response-independent events) were used to establish the drug-stimulus pairing, and animals had no operant training history before the start of nose-poke acquisition. Second, to establish that acquisition depended on, or was sensitive to, the Pavlovian contingency between the stimulus and remifentanil, animals exposed to stimulus-remifentanil pairings were compared to animals given remifentanil injections and stimulus presentations without consistent pairing. Third, to establish that acquisition depended on the instrumental contingency between a particular response and the stimulus, animals were allowed to choose between an active nose-poke manipulandum, which produced the stimulus, and an inactive nose-poke manipulandum, which had no scheduled consequences. Significantly greater active responding was found with the remifentanil-paired stimulus, but not the randomly presented control stimulus. Therefore, the remifentanil-paired stimulus was, indeed, acting as a conditioned reinforcer. Whereas previous studies have produced responding with nicotine-paired stimuli (Palmatier et al. 2007, 2008) and cocaine-paired stimuli (Di Ciano and Everitt 2004; Panlilio et al. 2007) that meets the criteria of Mackintosh (1974), the present studies are, to my knowledge, the first demonstration of the conditioned reinforcing effects of opioid-paired stimuli that does so.

Unresolved issues

Most broadly, the same questions can be raised about responding with conditioned reinforcement as with primary reinforcement: any operation known to alter the acquisition of responding with primary reinforcement may potentially also alter the acquisition of responding

with conditioned reinforcement (e.g., see Campbell and Carroll 2000 for a number of variables that can influence the acquisition of drug self-administration). The present discussion will focus on issues related to (1) potentially unique features of responding with conditioned reinforcement, (2) the effects of the dopaminergic manipulations pursued, and (3) evidence that the behavior of human drug users is acutally reinforced by drug-paired stimuli.

Responding with conditioned reinforcement necessarily has a complex associative basis. The present experiments were designed to generate responding that depended on the conditioned reinforcing effects of the remifentanil-paired stimulus as distinguished from other stimulus functions. Even if, under ideal circumstances, the conditioned reinforcing effects of a stimulus can be studied in genuine isolation, this single stimulus function still results from multiple associative processes (Berridge et al. 2009; Mackintosh 1974; O'Brien and Gardner 2005; cf., Schuster 1969). Responding with conditioned reinforcement necessarily depends on not only the Pavlovian contingency between the stimulus and a primary reinforcer, but also the instrumental contingency between the response and the stimulus (Mackintosh 1974). This dual dependency constrains attempts to interpret the effects of environmental or pharmacological manipulations on responding with conditioned reinforcement. When responding with conditioned reinforcement is increased or decreased, these behavioral changes may depend on changes in Pavlovian and/or instrumental learning (Gerdjikov et al. 2011; Palmatier et al. 2008). In the present experiments, for example, pramipexole pretreatment may have increased the reinforcing effectiveness (or value) of the stimulus as an outcome in the instrumental contingency or reduced the extinction of the Pavlovian drug-stimulus association. Functionally, this distinction may be irrelevant, as relatively greater reinforcer effectiveness results from an increase or the lack of a

decrease. However, changes in acquisition responding alone cannot be used to identify the source of the change (cf., Downs and Woods 1975, p 426).

Separate behavioral procedures designed to assess specifically the effects of Pavlovian learning (e.g., Pavlovian-to-instrumental transfer) or instrumental learning (e.g., reinforcer devaluation) may be adaptable to test the effects of pramipexole (and other manipulations) on responding with opioid-paired stimuli in a way that would allow for inferences about specific associative structures. For example, Pavlovian-to-instrumental transfer effects have recently been demonstrated with cocaine-paired stimuli (LeBlanc et al. 2012). Because the stimulus is presented response-independently during both the training and test phases of the experiment, Pavlovian-to-instrumental transfer may provide an index of the motivational effects of the stimulus that does not depend on instrumental learning about the stimulus. Dopamine receptor antagonists can attenuate the ability of food-paired stimuli to produce Pavlovian-to-instrumental transfer (Smith et al. 2000), but to my knowledge, it remains to be determined if selective dopamine receptor agonists or antagonists can modify Pavlovian-to-instrumental transfer with drug-paired stimuli.

New-response acquisition may be influenced by other behavioral processes, including discrimination between the active and inactive response manipulanda and attention to the stimuli. In human opioid users, opioid-paired stimuli have been shown to have significant effects on attention-related neuropsychological tasks, such as the Stroop task and dot-probe task (e.g., Garland et al. 2012; Lubman et al. 2000; Marissen et al. 2006; Waters et al. 2012). Generally, these studies show that human opioid users attend more intensely to opioid-associated stimuli than other stimuli. Nonetheless, manipulations or interventions that change the extent to which the stimuli are attended during the Pavlovian conditioning phase or instrumental acquisition

phase could change responding with conditioned reinforcement. Forgetting, a modification of the drug-stimulus contingency or response-stimulus contingency that occurs without programmed training, may also alter responding with conditioned reinforcement, although experiments designed to assess this possibility in rats have not shown strong forgetting effects (Di Ciano and Everitt 2004; but see Samaha et al. 2011 for results compatible with either extinction or forgetting).

Finally, it is important to consider the use of Pavlovian conditioning procedures to pair remifentanil injection and the light-noise stimulus. In designing the present new-response acquisition experiments, it was a priority that the animals have as restricted an operant training history as possible, i.e., to prevent the animals from having experimental experience with any particular response manipulandum that was or was not programmed to have a particular consequence. Restricting the animals' history in this way provides an assessment of their ability to make a new response in the most comprehensive or stringent sense of *new*. One potential disadvantage of this approach, however, is that human drug users often experience their drug-stimulus pairings in the context of drug self-administration. Most human drug users do not get all of their experience with either drugs or stimuli through response-independent events, as the rats in the present new-response acquisition experiments did, and so these Pavlovian procedures potentially lack (at least) face validity with human drug use. There may be important behavioral and neurobiological differences in animals exposed to response-dependent vs. response-independent opioid injections. In rats, for example, response-dependent vs. response-independent heroin administration can produce different behavioral effects (Lecca et al. 2007) and different effects on dopamine release in the nucleus accumbens (Hemby et al. 1995; Lecca et al. 2007).

However, other-administered drug may play an important role in at least some cases of human drug abuse and dependence, with drug administered by a physician to a patient (Musto 1985; Walker 1978, Case #2) or, among "street" users, by a more experienced/skilled drug user to a less experienced/skilled drug user (Crofts et al. 1996; Day et al. 2005; Faupel 1991; Kermode et al. 2007; Levine 1974; McBride et al. 2001). For example, in a recent study of opioid users, 88% of participants were injected by another person in their first experience with opioid injection (Barry et al. 2012), and similar, high rates of initial injection by another person (73-94% of participants) were found in several earlier studies of individuals whose first injection experience involved opioids or psychomotor stimulants (Crofts et al. 1996; Doherty et al. 2000; Roy et al. 2002). In certain cases or certain subpopulations of drug users, injection by another may persist well beyond the first experience with injection (Doherty et al. 2000; Faupel 1991; Levine 1974, Case #1; Walker 1978, Case #2). This is not to discount the importance of self-administration, but it is noteworthy that many human drug users may actually have histories that include both self-administered and other-administered drug. Therefore, thorough characterization of the motivational effects of a drug in laboratory animals should ultimately encompass the consequences of both response-dependent and response-independent drug delivery.

Are human drug self-administration behaviors influenced by conditioned reinforcement?

The roles proposed for drug-associtated conditioned reinforcers in human drug abuse and dependence (e.g., as reviewed by Milton and Everitt 2010) are quite similar to the roles long proposed for conditioned reinforcers in human and non-human animal behavior generally:

maintaining responding in the absence of the primary reinforcer until the primary reinforcer is ultimately delivered (e.g., Hull 1943; Skinner 1953). It is also thought that conditioned reinforcement is responsible for "flexible drug-seeking" (Milton and Everitt 2010, Figure 4). The drug-seeking behavior maintained by conditioned reinforcement is considered flexible because it is sensitive to its consequences, and this flexible drug-seeking can be contrasted with behaviors that are elicited by antecedent stimuli without regard to the events that follow, as with stimulus-response or "habit" learning mechanisms. Broadly, patterns of behavior consistent with these two functions have been observed in human drug users, but the evidence for specific instances of conditioned reinforcement (i.e., changes in the rate of particular behaviors that lead to the delivery of particular stimuli) in human opioid use is presently limited.

Ethnographic accounts or case histories of human opioid users emphasize the long, complex sequences of behavior in which these individuals will engage to obtain (1) money to buy drugs, (2) drugs themselves, and (3) a safe location in which the drugs can be consumed (e.g., Biernacki 1979; Dickson-Gómez et al. 2004; Faupel 1991; Fernandez 1998, Chapter 4; Fields and Walters 1985; Lalander 2003). These behaviors can last for hours or (sometimes) days before the drugs are obtained and consumed (Faupel 1991; Lex 1990). Upon securing the necessary elements, drug users will often prepare their drugs for consumption using complex "rituals" or "ceremonies." Particular sensory stimuli are often given special emphasis or importance during drug preparation, including the sound of aluminum foil being handled for users who smoke heroin (Lalander 2003) and the sight of blood in the syringe prior to injection for IV drug users (McBride et al. 2001). These long sequences of pre-consumption behavior may be supported significantly by the conditioned reinforcing effects of drug-associated stimuli.

In the laboratory, several studies have examined opioid self-administration in humans under second-order schedules of reinforcement, which can also require long periods of responding before drug is obtained (Lamb et al. 1991; Mello et al. 1981, 1982). These studies indicate that human participants will engage in extended episodes of behavior, including responding for long time intervals (90 min of sustained performance; Mello et al. 1981, 1982) or with high work requirements (3000 responses, Lamb et al. 1991) for a single opioid injection. However, these experiments did not address the specific contributions of drug-associated stimuli to responding, as performance with drug-associated stimuli was not compared to performance without stimuli or with stimuli that had not been associated with drug. Such manipulations could not establish unambiguously the conditioned reinforcing effects of the stimuli (e.g., Williams 1994), but they would be an important step in systematically establishing and studying the influence exerted (by whatever mechanism or mechanisms) over human behavior by drug-associated stimuli.

The ability of conditioned reinforcers to train new responses (including responses that have never led to the drug itself) may help to account for the diversity and flexibility of human drug-seeking behaviors. Again, ethnographic accounts emphasize the range of different behaviors in which human opioid users will engage to gain drugs and the money to buy drugs. To the extent they are able, human opioid users attempt to acquire the most drug with the least effort expended, and so they will change behavior as conditions in the drug marketplace change (Lex 1990). Human drug users may engage in a variety of different activities to maximize gains and minimize losses of drugs and money (Lalander 2003; Lex 1990). Sources of income for substance users can include formal employment, informal employment (i.e., "under the table" or "off the books" employment), criminal activity, government entitlements or benefits, loans, and

gifts (Biernacki 1979; Fields and Walters 1985; Zlotnick and Robertson 1996). Different kinds of behavior are necessary to access and maintain these various sources. There may be some degree of behavioral specialization, such that many opioid users have a "main hustle" by which they typically raise money for drugs or procure the drugs themselves; however, these invididuals will also engage in alternative behaviors based on negative or positive life events (Faupel 1991; Fields and Walters 1985; Lex 1990). Changes in behavior may be prompted by law enforcement intervention or other challenges that limit drug availability (Fields and Walters 1985; Lalander 2003) or because particular opportunities are present (Faupel 1991; Fields and Walters 1985; Lex 1990). These opportunities may recur cyclically (e.g., outdoor work that is possible during the summer but not winter), or they may be more unpredictable, as an idiosyncratic opportunity is identified and exploited (e.g., property that is momentarily unattended, an accidental injury that could lead to monetary compensation or access to prescription opioids). Individuals may also create opportunities, for example, by causing a diversion or distraction so that otherwise attended property becomes unattended, such that "almost any social setting can have potential for hustling" (Lex 1990, p 399). Even within a particular type of "hustle," a drug user may occupy different roles or participate in different activities at different times (Faupel 1991), and so a simple division of behavior into "hustles" may not fully capture its true diversity. The flexibility of behavior described in these qualitative data may or may not map onto a specific difference between response-outcome learning and stimulus-response learning as studied in animals, but it is clear that human drug users can engage in a number of different behaviors and, at least in some ways, alter their responses based on environmental outcomes.

Altogether, therefore, human opioid users have been observed to, or have reported themselves to, seek and take drugs in ways that are consistent with the proposed effects of

conditioned reinforcers. Nonetheless, direct evidence is lacking for specific behaviors that change in frequency because of stimulus delivery. Considering particular behaviors that may lead to the delivery of particular drug-associated stimuli, the "needle fixation" reported among some injection drug users may be caused, at least in part, by conditioned reinforcement (Levine 1974; McBride et al. 2001; Pates and Gray 2009; Pates et al. 2001). Needle fixation describes a phenomenon in which injection drug users will repeatedly puncture the skin whether or not drug is actually consumed and whether or not the user expects to experience drug effects (Pates et al. 2001, p 15). This independence of injection behavior from drug intake suggests that needle use has itself become a goal, or that "the means of administration has in itself become rewarding" (McBride et al. 2001, p 1050). Laboratory studies involving persistent self-injection without drug administration are rare (see O'Brien et al. 1979 for a study involving repeated saline self-injection), and as a result, most of the evidence for the existence and features of needle fixation comes from case reports and other forms of qualitative research with drug users. For example, some injection drug users report that they feel addicted to the needle itself, as well as to the drug (Pates et al. 2001). Such users report that the prospect of giving up injecting makes it more difficult to consider discontinuing drug use, and/or they would not use drug if injecting were impossible (McBride et al. 2001). However, it is difficult to assess the relative importance of the injection itself from the drug that typically follows in these cases. In the course of actually taking drug, some injection drug users report feeling strong drug-like effects when preparing the drug for consumption and, more specifically, during the injection experience when the skin is broken (McBride et al. 2001). Moreover, some users report greater subjective pleasure during the injection than after (i.e., when the drug is actually in the body), and needle fixation has been ascribed particularly to individuals who inject drugs and report no experience of drug effects

(Levine 1974, Case #1; McBride et al. 2001). As reported by one individual, drug-like subjective effects can begin "as soon as the needle hits your skin even though it can not have possibly entered your blood stream or hit your brain, you do feel it and they call that needle buzzing" (McBride et al. 2001, p 1052-1053).

This reported lack of subjective effects does not rule out primary reinforcement by the drug, as positive emotional experiences or subjective pleasure is not the same as behavioral reinforcement. Stronger evidence for the reinforcing effects of the injection itself comes from reports of individuals who inject themselves with water or break the skin without delivering an injection (Levine 1974, Case #2, McBride et al. 2001; Pates and Gray 2009; Pates et al. 2001). For example, Levine (1974, Case #2) reports a patient who would inject tap water IM "as often as every five minutes" (p 298), and a number of the users surveyed by McBride and colleagues (2001) reported either injecting water themselves or (more commonly) knowing other drug users who inject water. In a more recent study that classified drug users as either needle-fixated or not needle-fixated, the needle-fixated participants self-reported greater willingness to inject water when drug is unavailable and/or a history of having injected water (Pates and Gray 2009). These results are consistent with the injection having become a consequence that that can sustain behavior on its own, but additional work is needed to determine whether the injection (or some of its components) can be shown to be a reinforcer that is amenable to experimental manipulation and analysis, and if so, whether the conditioned reinforcing effects of the drug-associated injection stimuli can be separated from other behavioral mechanisms (e.g., primary reinforcement from the pain of the injection and/or its resolution) that could maintain injection behavior in the absence of the drug.

Rather than focusing on stimuli that have been paired with drug during human drug users' individual natural histories, a potentially fruitful alternative experimental approach could establish the conditioned reinforcing effects of stimuli paired with drug taken in the laboratory. For example, Foltin and Haney (2000) paired a novel combination of visual, auditory, and olfactory stimuli with smoked cocaine self-administration in a study of human cocaine users. After this history, the participants chose to expose themselves to the cocaine-paired stimuli over a control set of placebo-paired stimuli at a rate significantly greater than chance. Thus, participants would make a choice behavior that delivered the cocaine-paired stimuli, consistent with a conditioned reinforcing effect. Similar results were found by Mucha and colleagues (1998) in a study of tobacco smokers. Auditory stimuli were first paired with periods of smoking or no smoking, and then the participants could activate a switch to hear one of two sounds instead of white noise. Smokers spent more time listening to the sound that was paired with cigarette smoking, compared to the sound that was paired with no smoking periods, and this effect seemed to depend on the number of smoking-stimulus pairings. These results are promising, but it remains to be determined whether human opioid users would make similar choices for an opioid-paired stimulus over other stimuli or otherwise work to produce an opioid-paired stimulus. Generally, this approach is noteworthy because the experimenter can ensure that all participants have the same history of conditioning with the same stimuli and drug exposures and could potentially program unique combinations of stimuli with unique combinations of responses to create a human analogue of a new-response acquisition task. Of course, there may also be important ethical limitations to the work that could be done if it involves creating new drug-stimulus associations that could later promote drug-seeking or drug-taking behaviors outside of the laboratory.

Conclusion

The present experiments indicate that new-response acquisition procedures can provide valid measures of opioid-based conditioned reinforcement in the rat. These procedures are comparatively simple to implement and rapid in generating the behavior of interest, without the need for complex schedules of reinforcement or two separate operant acquisition phases, and they can produce responding that is persistent enough across acquisition test sessions to be practically useful. Furthermore, the basic behavioral procedures are flexible enough to accommodate several different environmental or pharmacological interventions. In particular, the present use of pramipexole provides, to my knowledge, the most direct evidence to date that D2-like dopaminergic agonism enhances the conditioned reinforcing effects of a drug-paired stimulus. Furthermore, among the D2-like subtypes, the present antagonist experiments highlight the potential importance of the D2 receptor specifically in responding with (drug-associated) conditioned reinforcement. These effects may be particularly important given the current widespread use of pramipexole and other D2-like agonists in the clinic for treating movement disorders and fibromyalgia. Future studies could easily implement other environmental or pharmacological manipulations during the Pavlovian conditioning phase, during the instrumental acquisition phase, or in a gap between the two phases of the procedure. Considerable work remains to be done to elucidate the environmental and neurobiological determinants of drug-based conditioned reinforcement, but new-response acquisition procedures should be particularly useful in pursuing this work.

Works cited

Barry D, Syed H, and Smyth BP (2012) The journey into injecting heroin use. *Heroin Addict Relat Clin Probl* **14:** 89–100.

Berridge KC, Robinson TE, and Aldridge JW (2009) Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol* **9:** 65–73. doi: 10.1016/j.coph.2008.12.014

Biernacki P (1979) Junkie work, "hustles" and social status among heroin addicts. *J Drug Issues* **9:** 535–551.

Campbell UC and Carroll ME (2000) Acquisition of drug self-administration: environmental and pharmacological interventions. *Exp Clin Psychopharmacol* **8:** 312–325. doi: 10.I037//1064-1297.8.3.312

Crofts N, Louie R, Rosenthal D, and Jolley D (1996) The first hit: circumstances surrounding initiation into injecting. *Addiction* **91:** 1187–1196. doi: 10.1046/j.1360-0443.1996.918118710.x

Cunningham CL (1993) Pavlovian drug conditioning, in *Methods in Behavioral Pharmacology* (van Haaren F ed) pp 349–381, Elsevier, New York.

Davis WM and Smith SG (1987) Conditioned reinforcement as a measure of the rewarding properties of drugs, in *Methods of Assessing the Reinforcing Properties of Abused Drugs* (Bozarth MA ed) pp 199–210, Springer-Verlag, New York.

Day CA, Ross J, Dietze P, and Dolan K (2005) Initiation to heroin injecting among heroin users in Sydney, Australia: cross sectional survey. *Harm Reduct J* **2**: 2. doi: 10.1186/1477-7517-2-2

Di Ciano P and 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. doi: 10.1016/j.neuropharm.2004.06.005

Dickinson A, Smith J, and Mirenowicz J (2000) Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. *Behav Neurosci* **114:** 468–483. doi: I0.1037M735-7044.114.3.46S

Dickson-Gómez J, Weeks MR, Martinez M, and Radda K (2004) Reciprocity and exploitation: social dynamics in private drug use sites. *J Drug Issues* **34:** 913–932. doi: 10.1177/002204260403400410

Doherty MC, Garfein RS, Monterroso E, Latkin C, Vlahov D (2000) Gender differences in the initiation of injection drug use among young adults. *J Urban Health* **77:** 396–414. doi: 10.1007/BF02386749

Downs DA and Woods JH (1975) Fixed-ratio escape and avoidance-escape from naloxone in morphine-dependent monkeys: effects of naloxone dose and morphine pretreatment. *J Exp Anal Behav* **23:** 415–427. doi: 10.1901/jeab.1975.23-415

Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, and Robbins TW (2008) Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos Trans R Soc Lond B Biol Sci* **363:** 3125–3135. doi: 10.1098/rstb.2008.0089

Faupel CE (1991) *Shooting Dope: Career Patterns of Hard-Core Heroin Users*. University of Florida Press, Gainesville, Florida.

Fields A and Walters JM (1985) Hustling: supporting a heroin habit, in *Life With Heroin: Voices from the Inner City* (Hanson B, Beschner G, Walters JM, and Bovelle E eds) pp 49–73, Lexington Books, Lexington, Massachusetts.

Fernandez H (1998) *Heroin*. Hazelden, Center City, Minnesota.

Foltin RW and Haney M (2000) Conditioned effects of environmental stimuli paired with smoked cocaine in humans. *Psychopharmacology (Berl)* **149:** 24–33. doi: 10.1007/s002139900340

Garland EL, Froeliger BE, Passik SD, and Howard MO (2012) Attentional bias for prescription opioid cues among opioid dependent chronic pain patients. *J Behav Med*. doi: 10.1007/s10865-012-9455-8

Gerdjikov TV, Baker TW, and Beninger RJ (2011) Amphetamine-induced enhancement of responding for conditioned reward in rats: interactions with repeated testing. *Psychopharmacology (Berl)* **214:** 891–899. doi: 10.1007/s00213-010-2099-x

Hemby SE, Martin TJ, Co C, Dworkin SI, and Smith JE (1995) The effects of intravenous heroin administration on extracellular nucleus accumbens dopamine concentrations as determined by *in vivo* microdialysis. *J Pharmacol Exp Ther* **273:** 591–598.

Hogarth L and Duka T (2006) Human nicotine conditioning requires explicit contingency knowledge: is addictive behavior cognitively mediated? *Psychopharmacology (Berl)* **184:** 553–566. doi: 10.1007/s00213-005-0150-0

Hull CL (1943) *Principles of Behavior: An Introduction to Behavior Theory*. Appleton-Century-Crofts, New York.

Kelleher RT and Gollub LR (1962) A review of positive conditioned reinforcement. *J Exp Anal Behav* **5:** 543–597. doi: 10.1901/jeab.1962.5-s543

Kermode M, Longleng V, Singh BC, Hocking J, Langkham B, and Crofts N (2007) My frist time: initiation into injecting drug use in Manipur and Nagaland, north-east India. *Harm Reduct J* **4**: 19. doi: 10.1186/1477-7517-4-19

Koob GF and Le Moal M (2001) Drug addiction, dysregulation of reward and allostasis. *Neuropsychopharmacology* **24:** 97–129. doi: 10.1016/S0893-133X(00)00195-0

Lalander P (2003) *Hooked on Heroin: Drugs and Drifters in a Globalized World*. Berg, New York.

Lamb RJ, Preston KL, Schindler CW, Meisch RA, Davis F, Katz JL, Henningfield JE, and Goldberg SR (1991) The reinforcing and subjective effects of morphine in post-addicts: a dose-response study. *J Pharmacol Exp Ther* **259:** 1165–1173.

LeBlanc KH, Ostlund SB, and Maidment NT (2012) Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behav Neurosci* **126:** 681–689. doi: 10.1037/a0029534

Lecca D, Valentini V, Cacciapaglia F, Acquas E, and Di Chiara G (2007) Reciprocal effects of response contingent and noncontingent intravenous heroin on in vivo nucleus accumbens shell versus core dopamine in the rat: a repeated sampling microdialysis study. *Psychopharmacology (Berl)* **194:** 103–116. doi: 10.1007/s00213-007-0815-y

Levine DG (1974) “Needle freaks”: compulsive self-injection by drug users. *Am J Psychiatry*. **131:** 297–300.

Lex BW (1990) Narcotic addicts’ hustling strategies: creation and manipulation of ambiguity. *J Contemp Ethnogr* **18:** 388–415. doi: 10.1177/089124190018004002

Lubman DI, Peters LA, Mogg K, Bradley BP, and Deakin JFW (2000) Attentional bias for drug cues in opiate dependence. *Psychol Med* **30:** 169–175. 10.1017/S0033291799001269

Mackintosh NJ (1974) *The Psychology of Animal Learning*. Academic Press, New York.

Marissen MA, Franken IH, Waters AJ, Blanken P, van den Brink W, and Hendriks VM (2006) Attentional bias predicts heroin relapse following treatment. *Addiction* **101:** 1306–1312. doi: 10.1111/j.1360-0443.2006.01498.x

McBride AJ, Pates RM, Arnold K, and Ball N (2001) Needle fixation, the drug user’s perspective: a qualitative study. *Addiction* **96:** 1049–1058. doi: 10.1046/j.1360-0443.2001.967104914.x

Mello NK, Mendelson JH, Kuehnle JC, and Sellers MS (1981) Operant analysis of human heroin self-administration and the effects of naltrexone. *J Pharmacol Exp Ther* **216:** 45–54.

Mello NK, Mendelson JH, and Kuehnle JC (1982) Buprenorphine effects on human heroin self-administration: an operant analysis. *J Pharmacol Exp Ther* **223:** 30–39.

Milton AL and Everitt BJ (2010) The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *Eur J Neurosci* **31:** 2308–2319. doi: 10.1111/j.1460-9568.2010.07249.x

Mucha RF, Pauli P, and Angrilli A (1998) Conditioned responses elicited by experimentally produced cues for smoking. *Can J Physiol Pharmacol* **76:** 259–268. doi: 10.1139/y98-022

Musto DF (1985) Iatrogenic addiction: the problem, its definition and history. *Bull N Y Acad Med* **61:** 694–705.

Myers KM and Carlezon Jr WA (2010) Extinction of drug- and withdrawal-paired cues in animal models: relevance to the treatment of addiction. *Neurosci Biobehav Rev* **35:** 285–302. doi: 10.1016/j.neubiorev.2010.01.011

O'Brien CP, Greenstein R, Ternes J, McLellan AT, and Grabowski J (1979) Unreinforced self-injections: effects on rituals and outcome in heroin addicts. *NIDA Res Monogr* **27:** 275–281.

O'Brien CP and Gardner EL (2005) Critical assessment of how to study addiction and its treatment: human and non-human animal models. *Pharmacol Ther* **108:** 18–58. doi: 10.1016/j.pharmthera.2005.06.018

Palmatier MI, Liu X, Matteson GL, Donny EC, Caggiula AR, and Sved AF (2007) Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. *Psychopharmacology (Berl)* **195:** 235–243. doi: 10.1007/s00213-007-0897-6

Palmatier MI, Coddington SB, Liu X, Donny EC, Caggiula AR, and Sved AF (2008) The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose. *Neuropharmacology* **55:** 1425–1430. doi: 10.1016/j.neuropharm.2008.09.002

Panlilio LV, Thorndike EB, and Schindler CW (2007) Blocking of conditioning to a cocaine-paired stimulus: testing the hypothesis that cocaine perpetually produces a signal of larger-than-expected reward. *Pharmacol Biochem Behav* **86:** 774–777. doi: 10.1016/j.pbb.2007.03.005

Pates RM, McBride AJ, Ball N, and Arnold K (2001) Towards an holistic understanding of injecting drug use: an overview of needle fixation. *Addict Res Theory* **9:** 3–17.

Pates RM and Gray N (2009) The development of a psychological theory of needle fixation. *J Subst Use* **14:** 312–324. doi: 10.3109/14659890903235876

Roy E, Haley N, Leclerc P, C**é**dras L, and Boivin J-F (2002) Drug injection among street youth: the first time. *Addiction* **97:** 1003–1009. doi: 10.1046/j.1360-0443.2002.00161.x

Samaha A-N, Minogianis E-A, and Nachar W (2011) Cues paired with either rapid or slower self-administered cocaine injections acquire similar conditioned rewarding properties. *PLoS One* **6:** e26481. doi: 10.1371/journal.pone.0026481

Schindler CW, Panlilio LV, and Goldberg SR (2002) Second-order schedules of drug self-administration in animals. *Psychopharmacology (Berl)* **163:** 327–344. doi: 10.1007/s00213-002-1157-4

Schuster RH (1969) A functional analysis of conditioned reinforcement, in *Conditioned Reinforcement* (Hendry DP ed) pp 192–234, Dorsey Press, Homewood, IL.

Shahan TA (2010) Conditioned reinforcement and response strength. *J Exp Anal Behav* **93:** 269–289. doi: 10.1901/jeab.2010.93-269

Skinner BF (1953) *Science and Human Behavior*. Macmillan, New York.

Taylor JR, Olausson P, Quinn JJ, Torregrossa MM (2009) Targeting extinction and reconsolidation mechanisms to combat the impact of drug cues on addiction. *Neuropharmacology* **56:** 186–195. doi: 10.1016/j.neuropharm.20 08.07.027

Walker LW (1978) Iatrogenic addiction and its treatment. *Int J Addict* **13:** 461–473. doi: 10.3109/10826087809045261

Waters AJ, Marhe R, and Franken IHA (2012) Attentional bias to drug cues is elevated before and during temptations to use heroin and cocaine. *Psychopharmacology (Berl)* **219:** 909–921. doi: 10.1007/s00213-011-2424-z

Wike EL (1966) *Secondary Reinforcement: Selected Experiments*. Harper & Row, New York.

Wikler A, Pescor FT, Miller D, and Norrell H (1971) Persistent potency of a secondary (conditioned) reinforcer following withdrawal of morphine from physically dependent rats. *Psychopharmacologia* **20:** 103–117. doi: 10.1007/BF00404365

Williams BA (1994) Conditioned reinforcement: experimental and theoretical issues. *Behav Anal* **17:** 261–285.

Zlotnick C and Robertson MJ (1996) Sources of income among homeless adults with major mental disorders or substance use disorders. *Psychiatr Serv* **47:** 147–151.