Published in final edited form as:

Psychopharmacology (Berl). 2015 January ; 232(1): 209–221. doi:10.1007/s00213-014-3659-2.

Effects of pramipexole on the acquisition of responding with opioid-conditioned reinforcement in the rat

Jeremiah W. Bertz¹, Jianyong Chen², and James H. Woods¹

¹University of Michigan Medical School, Departments of Pharmacology, Ann Arbor, MI, USA

²University of Michigan Medical School, Departments of Internal Medicine, Ann Arbor, MI, USA

Abstract

Rationale—Dopamine D3 receptor-preferring ligands may be able to modify the conditioned reinforcing effects of drug-associated stimuli. In evaluating the effects of these compounds, it is important to clarify the extent to which responding depends on (1) conditioned reinforcement vs. other behavioral mechanisms and (2) dopamine D3 vs. D2 receptor activity.

Objectives—Use behaviorally stringent new-response acquisition procedures to characterize the effects of the D3-preferring agonist, pramipexole, on the conditioned reinforcing effects of a stimulus paired with the opioid agonist, remifentanyl.

Methods—First, in Pavlovian conditioning (PAV) sessions, rats received response-independent IV injections of remifentanyl and presentations of a light–noise stimulus. In separate groups, injections and stimuli either always co-occurred (“paired PAV”) or occurred with no consistent relationship (“random PAV” control). Next, in instrumental acquisition (ACQ) sessions, all animals could respond in two nose-poke manipulanda: an active nose-poke, which produced the stimulus alone, or an inactive nose-poke. Pramipexole was injected SC prior to ACQ sessions with or without pretreatments of the D3-preferring antagonist, SB-277011A, or the dopamine D2-preferring antagonist, L-741,626.

Results—After paired PAV, but not random PAV, rats acquired nose-poke responding during ACQ (i.e., active > inactive). Pramipexole dose-dependently increased active responding without changing inactive responding. Pramipexole-induced increases in responding were blocked by pretreatment with L-741,626, but not SB-277011A.

Conclusions—Pramipexole specifically enhanced remifentanyl-conditioned reinforcement: active responding was selectively increased only after the stimulus was paired with remifentanyl.

Although pramipexole is D3-preferring, the antagonist effects obtained presently suggest an important role for the D2 receptor in opioid-conditioned reinforcement.

Keywords

conditioned reinforcement; cues; dopamine; D2; D3; drug abuse; opioid; Pavlovian conditioning; remifentanyl

Drug-associated environmental stimuli are thought to contribute not only to the compulsive drug-seeking and -taking behaviors that are hallmarks of drug abuse and dependence, but also to the chronic, relapsing nature of these disorders (e.g., Hogarth et al. 2013; Koob and Le Moal 2008, Table 1; Milton and Everitt 2010; Robinson and Berridge 2003; See 2005; Taylor et al. 2009). Even compared with other effects of Pavlovian drug-conditioned stimuli (e.g., Pavlovian conditioned approach, Pavlovian-to-instrumental transfer; Milton and Everitt 2010), conditioned reinforcement may exert especially robust control over behavior (Di Ciano and Everitt 2004; see also Taylor et al. 2009; Robinson et al. 2014). Drug-conditioned reinforcement can enhance both the persistence and diversity of drug-related behavior by maintaining responding over extended periods in the absence of the drug itself and by influencing other, novel types of behavior that had not previously been associated with drug (Bertz and Woods 2013; Di Ciano and Everitt 2004; Palmatier et al. 2007). More broadly, conditioned reinforcement can support responses made contrary to primary reinforcement contingencies (Pears et al. 2003), which may allow drug-related behaviors to continue despite the presence of alternative, non-drug reinforcers, and behavior maintained by a conditioned reinforcer can persist after the primary reinforcer that originally established it has been devalued (Burke et al. 2007; Robinson et al. 2014). Although the techniques advocated may vary (e.g., Peck and Ranaldi 2014; Troisi 2013), attenuating the effects of drug-associated stimuli is widely recognized as an important goal in treating drug abuse and dependence (Milton and Everitt 2010; Myers and Carlezon 2010; Taylor et al. 2009). Characterizing precisely the environmental and pharmacological factors that determine the conditioned reinforcing effects of drug-associated stimuli may, therefore, facilitate the development of refined treatment interventions.

Several researchers have suggested that dopamine D3-preferring ligands can modify the conditioned reinforcing effects of drug-associated 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). This idea has been supported by studies of drug self-administration under second order schedules of reinforcement (Di Ciano et al. 2003; Pilla et al. 1999) and of the extinction of drug self-administration responding (Cervo et al. 2003, 2007; Collins and Woods 2009; Collins et al. 2012; De Vries et al. 2002; Dias et al. 2004; Fuchs et al. 2002; Gál and Gyertyán 2006; Gilbert et al. 2005; Self et al. 1996). In both of these procedures, D3-preferring agonists increased, and D3-preferring antagonists decreased, responding that produced drug-associated stimuli but not the drug itself. These results are consistent with changes in the conditioned reinforcing effects of the stimuli. However, both responding under second-order schedules of reinforcement and the extinction of a previously trained response can be affected by a number of associative and non-associative processes other than conditioned reinforcement: primary reinforcing effects of the training drug; discriminative stimulus effects of the training drug, the test drug (i.e., the D3-preferring ligand), and the exteroceptive stimuli themselves; and generalization decrements between training and testing conditions, which can have a variety of causes (Collins et al. 2012; Mackintosh 1974; Schindler et al. 2002; Wike 1966, § 1.4; Williams 1994). In short, it is possible for stimulus presentations to change behavior in these procedures, yet for the stimuli to have no conditioned reinforcing effects at all (Kelleher and

Gollub 1962; Wike 1966; Williams 1994), and so it is difficult to make strong conclusions about the effects of D3-preferring ligands on drug-conditioned reinforcement.

To provide a behaviorally selective test for drug-conditioned reinforcement, the present study characterized the effects of the D3-preferring agonist, pramipexole, on rats' acquisition of a new response for a stimulus paired with the potent, short-acting opioid agonist, remifentanyl. To establish a new response with drug-conditioned reinforcement (e.g., Bertz and Woods 2013), animals are first given response-independent injections of drug and presentations of an exteroceptive stimulus, such that there either is or is not a consistent drug-stimulus pairing. Then, the animals are given access to novel instrumental responses that either do or do not produce the stimulus alone (i.e., without the drug), and the ability of animals to learn to make the response that produces the stimulus is assessed. These procedures provide a valid measure of conditioned reinforcement (Mackintosh 1974; Williams 1994): the response that produces the stimulus does not and did not also produce the primary reinforcer, and the behavior generated by stimulus presentation can be shown to depend on both (1) the Pavlovian contingency between the primary reinforcer and the environmental stimulus and (2) the instrumental contingency between a particular response and stimulus delivery. To our knowledge, the effects of D3-preferring ligands on new-response acquisition with drug-conditioned reinforcement have not been previously reported. However, new response-acquisition has been used to establish stringently that dopaminergic agonism can enhance water-conditioned reinforcement (Cador et al. 1991; Taylor and TW Robbins 1984). Therefore, the present study focused on pramipexole's ability to enhance remifentanyl-conditioned reinforcement. Experiments were conducted to characterize pramipexole's dose-effect function (Experiment 1) and timecourse (Experiment 2). To clarify the receptor subtypes involved in pramipexole's effects, pretreatments of the D3-preferring antagonist, SB-277011A, or the dopamine D2-preferring antagonist, L-741,626, were given prior to sessions of responding with conditioned reinforcement (Experiment 3) or sessions in which yawning and penile erection, two responses previously shown to depend specifically on D3 activity (Collins et al. 2005; 2007; 2009), were measured (Experiment 4).

Methods

Subjects

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing at least 250 g served as the experimental subjects. Animals were housed individually in a climate controlled facility under a 12 h light-dark cycle (lights on at 7:00 am) and were allowed to acclimate to the facility for at least 7 days before experimental procedures began. All animals had unrestricted access to standard pellet chow and tap water in the home cage. Experimental sessions were conducted 5–7 days/week during the light phase of the cycle. All experimental groups contained 8 rats, except where noted in Experiment 2. All studies were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Research, 2011), 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

Remifentanyl 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). Remifentanyl 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. Remifentanyl injections were delivered IV in a volume of 100 μ l/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.

Surgery

After acclimating to the facility, animals in Experiments 1–3 were catheterized 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, USA). 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 scapulae, where it was secured to a Dacron mesh back-plate (DC95BS, Instech Laboratories; Plymouth Meeting, PA, USA) and attached to 22 ga stainless steel tubing for externalization. Rats were allowed to recover for at least 5 days following surgery. Catheters were flushed daily with 0.25 ml of heparinized saline (50 U/ml) to ensure patency.

Apparatus

In Experiments 1–3, all sessions were conducted in two experimental chambers (ENV-008, Med Associates; St. Albans, VT, USA) controlled by Med-PC IV software (Med Associates). Each experimental chamber was housed in a light- and sound-attenuating cubicle and was located in a separate room of the laboratory. The right wall of each 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) of the chamber. The houselight was centered horizontally between the nose-pokes 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 replaced the nose-pokes when the nose-pokes were removed from the chamber.

Motorized syringe drivers (PHM-107, Med Associates) were located outside of the light-and sound-attenuating cubicles to deliver IV drug injections. Syringes were attached to Tygon tubing (S-54-HL, Norton Performance Plastics), which was connected to a fluid swivel (375/22PS, Instech Laboratories or QCS-D, Strategic Applications Inc.; Lake Villa, IL, USA) and spring tether mounted to a counterbalanced arm.

In Experiment 4, animals were observed in transparent plastic chambers (48 x 23 x 20 cm), which 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 position in the chamber.

Pavlovian conditioning

After recovery from surgery, animals in Experiments 1–3 received either “paired” or “random” Pavlovian conditioning (PAV) for five consecutive sessions. During PAV sessions, the nose-pokes were removed from the experimental chambers, and all animals received response-independent IV injections of 3.2 µg/kg/injection remifentanyl and response-independent deliveries of a light-noise compound stimulus consisting of houselight illumination and 80±5 db white noise (measured at the chamber's center). Injections and stimuli lasted 2.0±0.5 s, depending on the weight of the animal. In paired PAV groups, a single variable time (VT) 3 min (range: 0.0–6.0 min) schedule controlled both remifentanyl injection and stimulus delivery, and injections and stimuli always co-occurred. In the random PAV control group, remifentanyl injection and stimulus delivery were each controlled by independently running VT 3 min schedules; injections and stimuli were not explicitly unpaired. PAV sessions lasted until 20 injections and 20 stimuli were delivered, approximately 60 min. These parameters were chosen based on a previous study of remifentanyl-conditioned reinforcement (Bertz and Woods 2013).

Instrumental acquisition

For all animals in Experiments 1–3 (i.e., regardless of PAV type), instrumental acquisition (ACQ) sessions began the day after the conclusion of PAV. For all ACQ sessions, the two nose-pokes were present in the chamber. Responses in the inactive nose-poke had no scheduled consequences. Responses in the active nose-poke produced the light-noise stimulus alone under a modified random ratio (RR) 2 schedule: the first response in each session produced the stimulus with a probability of 1.0, whereas each subsequent response produced the stimulus with a probability of 0.5. In each group, the side of the active nose-poke (left vs. right) was counterbalanced across animals. Illumination of the stimulus lights inside both nose-pokes signaled the start of each ACQ session, and both nose-pokes remained illuminated continuously throughout the session. The number and duration of ACQ sessions conducted differed among experiments, as described below. In each experiment, animals were returned to their home cages between pretreatment injection(s) and the start of the session.

In Experiment 1 (pramipexole dose-effect function), each group was tested in 14 ACQ sessions (ACQ1–14). ACQ1 lasted 60 min, and no pretreatment injection was given. From ACQ2–14, each session lasted 240 min, and a pretreatment injection was given 10 min before the start of each session. The increased session duration was based on the session durations used in previous studies of extinction responding (Collins et al. 2012; De Vries et al. 2002). After paired PAV, pramipexole (0.1 mg/kg, 0.32 mg/kg, or 1.0 mg/kg) or its vehicle was administered to separate groups of animals on ACQ2–8 and ACQ10–14. On ACQ9, all groups were pretreated with vehicle. A control group was given 0.32 mg/kg pramipexole according to the same schedule after random PAV.

In Experiment 2 (pramipexole time-course), each group was tested in 8 ACQ sessions (ACQ1-8). All ACQ sessions lasted 60 min. No pretreatment injection was given before ACQ1. From ACQ2-8, animals were injected with either vehicle or 0.32 mg/kg pramipexole either 10 min or 190 min before the start of each session, giving four groups: 10 min vehicle ($n = 4$), 190 min vehicle ($n = 4$), 10 min PRAM ($n = 6$), and 190 min PRAM ($n = 8$).

In Experiment 3 (antagonist administration), 10 ACQ sessions (ACQ1-10) were conducted. ACQ1 lasted for 60 min, and no pretreatment injection was given. Session duration increased to 240 min for ACQ2-10. An injection of 0.32 mg/kg pramipexole 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: (1) SB-277011A, L-741,626, or their respective vehicles were given 40 min before the start of the session, and (2) 0.32 mg/kg pramipexole or its vehicle was given 10 min before the start of the session. Two groups of animals were tested with L-741,626: a “high dose” group (High L) received vehicle, 0.32 mg/kg, or 3.2 mg/kg, whereas a “low dose” group (Low L) received vehicle, 0.1 mg/kg, or 1.0 mg/kg. One group of animals received SB-277011A (SB): vehicle, 5.6 mg/kg, or 56.0 mg/kg. Each antagonist dose was tested for one ACQ session. In each group, antagonist doses were given in either ascending or descending order before 0.32 mg/kg pramipexole. The vehicle + vehicle condition was tested first or last (i.e., on ACQ7 or ACQ10) in each group. This latter condition was included to verify that vehicle + 0.32 mg/kg pramipexole enhanced responding. Antagonists were classified as D3- vs. D2-preferring, and pretreatment doses and times were selected based on previous work in the laboratory (Collins et al. 2007, 2012).

Observation of yawning and penile erection

In Experiment 4, yawning and penile erection were measured in two groups of animals. Yawning was defined as the prolonged (~1 s), wide opening of the mouth followed by rapid closure, whereas penile erection was defined as an emerging, engorged penis, typically followed by upright body posture and genital grooming (Collins et al., 2009). Each animal was observed in one session. At the start of the session, animals were placed individually into the observation chambers and allowed to acclimate to the chamber for 30 min. Separate groups were then injected with vehicle or 56.0 mg/kg SB-277011A. Thirty min after antagonist injection, all animals were injected with 0.32 mg/kg pramipexole. Unique yawns and penile erections were counted by a trained observer at the following times after pramipexole injection: 55–70 min, 115–130 min, 175–190 min, and 235–250 min. These observation periods were chosen to correspond to the final 15 min of the first, second, third, and fourth hours, respectively, of the ACQ sessions used in Experiment 3. Animals remained in the observation chambers between injections and among observation periods.

Data analysis

In Experiment 1, the mean number of active and inactive nose-pokes made in each ACQ session were analyzed in four separate phases, corresponding to when animals did or did not receive pramipexole: ACQ1, ACQ2-8, ACQ9, and ACQ10-14. Analyses were performed using mixed model ANOVA with factors for manipulandum (active vs. inactive) and, when appropriate, group (i.e., pretreatment dose) and/or session.

If the stimulus served as a conditioned reinforcer, animals should make more active than inactive responses. If pramipexole enhanced the conditioned reinforcing effects of the stimulus, animals receiving pramipexole should make significantly more active responses than vehicle-treated animals. Therefore, if significant effects involving manipulandum and group were found, responding was averaged across sessions to perform two sets of pairwise comparisons: (1) paired *t*-tests were used to compare active vs. inactive responding within groups, and (2) unpaired *t*-tests were used to compare the active and inactive responses of each pramipexole-treated group to the active and inactive responses, respectively, of the vehicle-treated group.

To characterize the effects of pramipexole on the intrasession allocation of responding in Experiment 1, the active responses made by the animals treated with vehicle or 0.32 mg/kg pramipexole during ACQ2-8 were re-analyzed. Rates of responding (responses/min) in each hour (Hour 1–4) of each session were calculated and were analyzed using three-way ANOVA with factors for hour, session, and group. Following significant effects of hour and group, the data were averaged across sessions for pairwise comparison of the groups in each hour using unpaired *t*-tests.

In Experiment 2, the responses of the vehicle-treated animals were first analyzed using three-way ANOVA with factors for manipulandum, session, and pretreatment interval (10 min vs. 190 min). Because no effect of pretreatment interval was significant, the two vehicle groups were combined for subsequent analyses. Responding in ACQ1 and ACQ2-8 was then analyzed separately using mixed-model ANOVA followed by pairwise comparisons as in Experiment 1.

In Experiment 3, to check that pramipexole did not have a different effect among groups before the start of the antagonist administration, the mean active and inactive nose-pokes made in each session from ACQ2-6 were analyzed using three-way ANOVA with factors for manipulandum, session, and group. In the antagonist test sessions, the mean active and inactive nose-pokes of each group were analyzed separately using two-way ANOVA with factors for manipulandum and pretreatment condition. Based on a previous study in the laboratory of D3-vs. D2-preferring antagonists (Collins et al. 2012) and on the range of doses found to be effective in Experiment 1 (i.e., higher doses that produce D2-mediated effects in rats, Collins et al. 2007), it was hypothesized *a priori* that both SB-277011A and L-741,626 would attenuate the effects of pramipexole. Therefore, planned comparisons with paired *t*-tests were used to determine if active or inactive responses in the other pretreatment conditions differed from the responding on the same manipulandum in the vehicle + 0.32 mg/kg pramipexole condition. Finally, to determine if L-741,626 suppressed responding before the stimulus was presented, the latency to the first active response in each antagonist test session was calculated in min and then log-transformed to reduce the heterogeneity of variance. If an animal failed to respond, its latency was recorded as 240 min. Mean latencies were then analyzed separately for each group using one-way ANOVA with repeated measures.

In Experiment 4, the number of yawns and penile erections made were summed to produce a D3-mediated behavioral score for each animal in each observation period. The mean

behavioral scores from each observation period were analyzed using mixed-model ANOVA with factors for SB-277011A pretreatment dose and observation period.

Analyses were performed using Prism 5.0 or 6.0 (GraphPad Software; La Jolla, CA, USA) or SPSS Statistics 20.0 (IBM; Armonk, NY, USA). Differences were considered significant when $p < .05$, two-tailed. Except where noted for Experiment 3, the Holm-Bonferroni method was used to correct for multiple pairwise comparisons. Only significant effects are described numerically below. All data are presented graphically as the mean $\pm$ SEM.

Results

Experiment 1: Effects of pramipexole on new-response acquisition for a remifentanyl-paired stimulus

Figure 1 presents the active and inactive nose-poke responses of rats treated with pramipexole after paired PAV. Figures 1a and 1b present the active and inactive responses, respectively, made in each of the 14 ACQ sessions. Mean responding in each of the four phases of ACQ is presented in Figures 1c-1f.

In ACQ1 (Figure 1c) and ACQ9 (Figure 1e), when pramipexole was not given, responding did not differ significantly among groups. Collapsed across groups, animals made significantly more active responses than inactive responses in both ACQ1 [manipulandum: $F(1,28) = 5.73$, $p = .023$; $t(31) = 2.46$, $p = .019$] and ACQ9 [manipulandum: $F(1,28) = 23.49$, $p < .001$; $t(31) = 4.86$, $p < .001$].

In ACQ2-8, the first period of pramipexole treatment, animals responded differently in the active vs. inactive nose-pokes [manipulandum: $F(1,28) = 44.79$, $p < .001$]. The manipulandum X session interaction was not significant. Averaged across sessions (Figure 1d), animals pretreated with vehicle [$t(7) = 3.55$, $p = .027$], 0.1 mg/kg pramipexole [$t(7) = 3.17$, $p = .031$], and 0.32 mg/kg pramipexole [$t(7) = 5.77$, $p = .002$] made significantly more active responses than inactive responses. Pramipexole pretreatment significantly affected responding [group: $F(3,28) = 11.43$, $p < .001$; group X session: $F(18,168) = 2.25$, $p = .004$], and the effects of pramipexole differed for active vs. inactive responding [group X manipulandum: $F(3,28) = 14.75$, $p < .001$; group X manipulandum X session: $F(18,168) = 2.83$, $p < .001$]. By pairwise comparison, 0.32 mg/kg pramipexole significantly increased active responding compared to vehicle [$t(14) = 4.97$, $p = .0012$]. No dose of pramipexole significantly changed inactive responding compared to vehicle.

During ACQ10-14, the second period of pramipexole treatment, animals continued to respond differently in the active vs. inactive nose-pokes [manipulandum: $F(1,28) = 32.45$, $p < .001$]. No effect of session was significant during this period. Collapsed across sessions (Figure 1f), animals in all groups made significantly more active responses than inactive responses [vehicle: $t(7) = 4.24$, $p = .011$; 0.1 mg/kg: $t(7) = 4.32$, $p = .014$; 0.32 mg/kg: $t(7) = 3.69$, $p = .007$; 1.0 mg/kg: $t(7) = 3.93$, $p = .011$]. Pramipexole pretreatment significantly affected responding [group: $F(3,28) = 6.91$, $p < .001$], and the effects of pramipexole differed for active vs. inactive responding [group X manipulandum: $F(3,28) = 7.87$, $p < .001$]. Animals treated with 0.32 mg/kg pramipexole [$t(14) = 3.33$, $p = .024$] or 1.0 mg/kg

pramipexole [$t(14) = 3.49, p = .021$] made significantly more active responses than vehicle-treated animals. No dose of pramipexole changed inactive responding compared to vehicle.

Figure 2 presents the active and inactive nose-poke responses of animals treated with 0.32 mg/kg pramipexole after random PAV. Responding in this group did not differ by manipulandum or session in any phase of ACQ.

Figure 3 presents the rate of active responding in each hour of ACQ1 and ACQ2-8 of the groups pretreated with vehicle (Figure 3a) or 0.32 mg/kg pramipexole (Figure 3b). In ACQ2-8, response rate differed among the hours of the session [hour: $F(3,42) = 8.94, p < .001$; hour X session: $F(18,252) = 1.72, p = .036$] and by pretreatment [group: $F(1,14) = 24.30, p < .001$; group X session: $F(6,84) = 3.52, p = .004$]. However, the effect of pretreatment depended on the hour of the session [group X hour: $F(3,42) = 13.08, p < .001$]. No other effects of session were significant. Averaged across sessions (Figure 3c), pramipexole significantly increased responding only in Hour 3 [$t(14) = 6.06, p < .001$] and Hour 4 [$t(14) = 4.06, p = .003$].

Experiment 2: Effects of pramipexole exposure without extended ACQ session duration

Because pramipexole had a slow onset of effect within the extended (i.e., 240 min long) ACQ sessions in Experiment 1, the animals in Experiment 2 were given pramipexole with more or less time before the start of shorter (i.e., 60 min long) ACQ sessions in order to determine the sufficiency of extended exposure to pramipexole *per se* to enhance responding. In the vehicle-treated animals, pretreatment time did not affect responding; therefore, the vehicle groups were combined for presentation and analysis. Figure 4 presents the active (Figure 4a) and inactive (Figure 4b) nose-poke responses of the vehicle-treated animals and animals treated with 0.32 mg/kg pramipexole either 10 min or 190 min before the start of the session.

In ACQ1 (Figure 4c), before the start of pretreatments, responding did not differ among the groups. The main effect of manipulandum only approached significance [$F(1,19) = 4.08, p = .057$]; however, collapsed across groups, animals made significantly more active than inactive responses [$t(21) = 2.09, p = .048$]. In ACQ2-8, when a pretreatment was given before each session, animals continued to respond differently in the active vs. inactive nose-pokes [manipulandum: $F(1,19) = 19.92, p < .001$]. Averaged across sessions (Figure 4d), 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 was affected by pretreatment condition [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 pramipexole-treated groups responded less than the vehicle-treated group; however, neither the mean active responses nor the mean inactive responses of either pramipexole-treated group differed from those of the vehicle-treated group.

Experiment 3: D3 vs. D2 receptor involvement in pramipexole-induced enhancement of remifentanil-conditioned reinforcement

Figure 5 presents the effects of pretreatment with SB-277011A or L-741,626 on the response-enhancing effects of 0.32 mg/kg pramipexole. Figure 5a presents the nose-poke responding of the three antagonist pretreatment groups (SB, Low L, High L) before the start of antagonist administration. Active responding increased over the course of pramipexole administration during ACQ2-6, whereas inactive responding remained low throughout [manipulandum: $F(1,21) = 34.10, p < .001$; session: $F(4,84) = 5.07, p < .001$; session X manipulandum: $F(4,84) = 6.34, p < .001$]. There were no significant effects of group during this period.

Figure 5b presents the responses of animals pretreated with SB-277011A. Across pretreatment conditions, animals made more active responses than inactive responses [manipulandum: $F(1,7) = 32.78, p < .001$]. Overall, the effects of antagonist pretreatment condition were not significant. By pairwise comparison to vehicle + 0.32 mg/kg pramipexole, animals made significantly fewer active responses only when pretreated with vehicle + vehicle [$t(7) = 3.32, p = .012$]. There were no significant differences in inactive responding.

Figures 5c and 5d present the responses of the animals pretreated with L-741,626. Animals in the Low L group (Figure 5c) made more active responses than inactive responses across pretreatment conditions [manipulandum: $F(1,7) = 16.74, p = .005$]. Antagonist pretreatment condition affected responding [pretreatment: $F(3,21) = 4.16, p = .018$] differently for the active vs. inactive responses [pretreatment X manipulandum: $F(3,21) = 4.62, p = .012$]. Compared to vehicle + 0.32 mg/kg pramipexole, animals made significantly fewer active responses when given 1.0 mg/kg L-741,626 + 0.32 mg/kg pramipexole [$t(7) = 2.49, p = .041$] or vehicle + vehicle [$t(7) = 2.57, p = .036$]. There were no significant differences in inactive responding. Animals in the High L group (Figure 5d) also made more active responses than inactive responses across pretreatment conditions [manipulandum: $F(1,7) = 30.70, p < .001$]. Antagonist pretreatment condition affected active and inactive responding differently [pretreatment: $F(3,21) = 6.19, p = .004$; pretreatment X manipulandum: $F(3,21) = 5.52, p = .006$]. Compared to vehicle + 0.32 mg/kg pramipexole, animals made significantly fewer active responses when given 3.2 mg/kg L-741,626 + 0.32 mg/kg pramipexole [$t(7) = 3.52, p = .009$] or vehicle + vehicle [$t(7) = 2.64, p = .033$]. There were no significant differences in inactive responding. In both the Low L group and High L group, pretreatment condition did not affect animals' latency to initiate active responding at the start of the session (data not shown).

Experiment 4: Effects of SB-277011A on D3-mediated elicited responses

Figure 6 presents the combined number of yawns and penile erections made by animals given 0.32 mg/kg pramipexole after pretreatment with 56.0 mg/kg SB-277011A or its vehicle. Rats made significantly fewer yawns and penile erections when given 56.0 mg/kg SB-277011A [$F(1,14) = 15.51, p = .001$]. No effects involving observation period were significant.

Discussion

The present study characterized the ability of the dopamine D3-preferring agonist, pramipexole, to enhance the conditioned reinforcing effects of a remifentanyl-paired stimulus. Whereas previous studies have shown that selective dopamine D2-like receptor agonists can enhance rats' new-response acquisition with food- or water-paired stimuli (Beninger and Ranaldi 1992; Sutton et al. 2001; Wolterink et al. 1993), the present experiments provide, to our knowledge, the first demonstration that a selective, direct dopaminergic agonist can enhance the acquisition of responding with drug-conditioned reinforcement. After paired PAV, all groups of animals significantly preferred the active response, which produced the stimulus but never remifentanyl, to the inactive response. Compared to vehicle, higher doses of pramipexole significantly increased active responding without changing inactive responding. In contrast, animals treated with pramipexole after random PAV, which provided the same amount of exposure to remifentanyl and the stimulus as paired PAV, did not respond differently between the nose-pokes, which still either produced (active response) or did not produce (inactive response) the stimulus alone. Thus, the effects of pramipexole on nose-poking during ACQ depended on not only the instrumental contingency between a particular response and stimulus delivery, but also the Pavlovian contingency between the stimulus and remifentanyl, as required to conclude that pramipexole changed conditioned reinforcement (Mackintosh 1974).

Pramipexole enhanced active responding with an inverted *U*-shaped dose-response function, consistent with previous studies of the effects of the D3-preferring agonist, quinpirole, on new-response acquisition with food- or water-paired stimuli (Beninger and Ranaldi 1992; Wolterink et al. 1993). Rather than indicating a loss of the stimulus' conditioned reinforcing effects or of pramipexole's response-enhancing effects, the descending limb of the dose-response function likely resulted from an interaction of pramipexole's response-enhancing mechanisms (discussed below) with a response-disrupting mechanism, e.g., pramipexole's locomotor effects. The response-enhancing effects of pramipexole in the present study cannot be attributed only to nonspecific hyperlocomotion, given the importance of animals' Pavlovian histories and the selectivity of changes for the active, but not inactive, response. However, pramipexole's locomotor effects may have contributed to the overall shape of the dose-effect function. In the range of doses tested in Experiment 1, pramipexole has complex locomotor effects that depend on both dose and time after administration (Chang et al. 2011; Lagos et al. 1998; Maj et al. 1997): broadly, in open-field tests, lower doses produce persistent hypolocomotion, whereas higher doses produce an initial period of hypolocomotion followed by hyperlocomotion. Higher doses of pramipexole can also increase focused behaviors (e.g., sniffing; Chang et al. 2011). Either locomotor sedation (as observed by Wolterink et al. 1993, p 361), or the eliciting of competing locomotor responses could have interfered with nose-poke responding. The concurrent collection of locomotor measures in future studies of conditioned reinforcement may help resolve this issue.

In characterizing a drug's behavioral effects, it is important to distinguish the contributions of behavioral experience with the drug from exposure to the drug *per se* (e.g., Jonkman et al. 2012; Zimmer et al. 2014). Presently, extended exposure to pramipexole without extended exposure to the ACQ session was insufficient to enhance responding after paired PAV. In

Experiment 1, animals treated with pramipexole showed increases in active responding in the final 60 min of ACQ sessions lasting 240 min (i.e., 190–250 min after pramipexole injection); however, in Experiment 2, animals did not show enhanced active responding when given pramipexole 190 min before ACQ sessions lasting only 60 min (i.e., in the same 190–250 min post-injection interval). Therefore, the development of pramipexole's effects over time, both within and across sessions, in Experiment 1 was not determined exclusively by pharmacokinetic factors (e.g., slow absorption and/or distribution) or to receptor-level changes that can be induced by drug treatment without behavioral testing (e.g., receptor trafficking; Min et al. 2013). In complement, active responding was not enhanced when animals were exposed to an extended ACQ session without pramipexole pretreatment: in ACQ9 in Experiment 1, when animals previously administered pramipexole were given vehicle, and in the vehicle + vehicle condition in Experiment 3. Enhanced responding depended on both pramipexole administration and extended ACQ session access.

This pattern focuses attention on the learning mechanisms operating during behavioral testing. Analyzing responding with conditioned reinforcement is complicated by the fact that, by definition, conditioned reinforcement depends on both Pavlovian and instrumental learning (Mackintosh 1974; O'Brien and Gardner 2005; Williams 1994). For example, Pavlovian extinction has been thought to significantly limit new-response acquisition: each response-contingent presentation of the stimulus necessarily occurs in the absence of the primary reinforcer with which it was originally paired, potentially reducing or eliminating the conditioned reinforcing effects of the stimulus (Mackintosh 1974; Williams 1994). D3-preferring agonists can attenuate the effects of extinction in other behavioral preparations (Dubrovina and Zinova 2010; Kurylo and Tanguay 2003; Nader and LeDoux 1999). Therefore, it is possible that pramipexole interfered with the extinction of the remifentanyl-stimulus association during ACQ (or the expression of that extinction learning), allowing the “full strength” of the conditioned reinforcer to determine responding. If so, active responding would have increased across ACQ sessions as animals acquired the novel instrumental contingency between the active response and the stimulus, and the reductions in responding when pramipexole was withheld could be attributed to a pramipexole-state dependency of the instrumental acquisition.

Pramipexole may also have impacted instrumental incentive learning processes during ACQ, leading to an increase in the reinforcing efficacy or value of the stimuli (e.g., Collins et al. 2012). Studies of instrumental reinforcer revaluation using food reinforcement have found that dopaminergic manipulations do not cause or affect reinforcer value changes (Dickinson et al. 2000; Lex and Hauber 2010; Trifilieff et al. 2013; Wassum et al. 2011; but see Hitchcott et al. 2007). However, more work is needed to understand how drugs, like or unlike foods, act as instrumental incentives (e.g., Hutcheson et al. 2001), and to determine whether or not dopamine is involved in the revaluation of drug reinforcers, both primary and conditioned.

Considering the particular dopamine receptor subtypes involved in pramipexole's effects, the present study indicates a significant role for the D2 receptor in remifentanyl-conditioned reinforcement. At a dose, 1.0 mg/kg, previously shown to have D2-selective effects *in vivo* (Collins et al. 2007), L-741,626, attenuated the response-enhancing effects of pramipexole,

mimicking the effects of substituting saline for pramipexole. It is unlikely that this effect was due to general locomotor suppression, as animals' latency to initiate responding at the start of the session was not changed by L-741,626 pretreatment. SB-277011A, in contrast, did not alter the response-enhancing effects of pramipexole. The highest dose of SB-277011A tested presently, 56.0 mg/kg, has been shown to produce D3-selective effects *in vivo* (Collins et al. 2007) and is equal to or larger than doses of SB-277011A that have been effective in other, different behavioral tasks involving drug self-administration and/or drug-paired stimuli (e.g., Cervo et al. 2007; Di Ciano et al. 2003; Higley et al. 2011; Ross et al. 2007). Nevertheless, Experiment 4 was performed to demonstrate that 56.0 mg/kg of SB-277011A can block responses associated specifically with D3 activity (Collins et al. 2005; 2007; 2009) following pramipexole administration for a length of time corresponding to the antagonist test sessions of Experiment 3. The yawning and penile erection measured in Experiment 4 are not necessarily directly related to responding with drug-conditioned reinforcement, but they do provide an important independent measure of SB-277011A's activity at D3 receptors.

The lack of effect of SB-277011A in the present study may be related to the particular training drug (remifentanyl) and/or to the particular stimulus function (conditioned reinforcement) under investigation. Whereas SB-277011A can attenuate opioid-conditioned place preference (Ashby et al. 2003), the effects of D3 antagonism on opioid self-administration and responding with opioid associated stimuli in operant procedures have not been well established. Far more work has been done in cocaine- or nicotine-trained animals (reviewed by Heidbreder 2013). To resolve this issue, it will be necessary (1) to test D3 antagonists using procedures that can rigorously establish the conditioned reinforcing effects of stimuli paired with these other drugs and (2) to test opioids in the other behavioral preparations (e.g., second order schedules of reinforcement) in which D3 antagonists have been effective.

Whereas the present results do not implicate the D3 receptor in the enhancement of remifentanyl-conditioned reinforcement, they are consistent with several pieces of evidence indicating that D2 receptor activity is important for opioid self-administration and responding with opioid-associated stimuli. First, quinpirole can reinstate rats' heroin-trained responding (De Vries et al. 2002), but does so only at larger doses that have been shown to produce significant D2-mediated effects (i.e., 0.1 mg/kg; Collins et al. 2005, 2007). Second, D2 receptor knockout mice fail to acquire both morphine self-administration (Elmer et al. 2002) and morphine-conditioned place preference (Maldonado et al. 1997; Smith et al. 2002; but see Dockstader et al. 2001). These latter results also suggest the importance of the training drug, as D2 knockout mice can acquire cocaine self-administration (Caine et al. 2002) and cocaine-conditioned place preference (Bello et al. 2011; Smith et al. 2002). Future studies of the effects of drug-associated stimuli may benefit particularly from more direct comparisons of the effects of D3 vs. D2 receptor manipulations (e.g., Collins et al. 2012). Ultimately, resolving both the associative learning mechanisms and receptor mechanisms by which dopaminergic manipulations affect drug-conditioned reinforcement may be important to both treating drug abuse disorders and understanding basic learning processes.

Acknowledgments

We thank Davina Barron, Alyssa Cunningham, and Tomas Davalos for their excellent technical assistance. We thank Gail Winger for her comments on an earlier draft of the manuscript. This research was supported by the National Institute on Drug Abuse under grants T32DA07268, R01DA020669, R01DA024897, R01DA032943 and by the National Science Foundation Graduate Research Fellowship Program under grant DGE 0718128. The authors declare no conflicts of interest. All experiments comply with the current laws of the United States, the country in which they were performed.

Works cited

- Ashby CR Jr, Paul M, Gardner EL, Heidbreder CA, Hagan JJ. 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*. 2003; 48:154–156. [PubMed: 12645041]
- Bello EP, Mateo Y, Gelman DM, Noain D, Shin JH, Low MJ, Alvarez VA, Lovinger DM, Rubinstein M. Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nat Neurosci*. 2011; 14:1033–1038. [PubMed: 21743470]
- Beninger RJ, Rinaldi R. The effects of amphetamine, apomorphine, SKF 38393, quinpirole and bromocriptine on responding for conditioned reward in rats. *Behav Pharmacol*. 1992; 3:155–163. [PubMed: 11224114]
- Bertz JW, Woods JH. Acquisition of responding with a remifentanyl-associated conditioned reinforcer in the rat. *Psychopharmacology (Berl)*. 2013; 229:235–243. [PubMed: 23609770]
- Burke KA, Franz TM, Miller DN, Schoenbaum G. Conditioned reinforcement can be mediated by either outcome-specific or general affective representations. *Front Integr Neurosci*. 2007; 1:2. [PubMed: 18958230]
- Cador M, Taylor JR, Robbins TW. Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. *Psychopharmacology (Berl)*. 1991; 104:377–385. [PubMed: 1924645]
- Caine SB, Negus SS, Mello NK, Patel S, Bristow L, Kulagowski J, Vallone D, Saiardi A, Borrelli E. Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. *J Neurosci*. 2002; 22:2977–2988. [PubMed: 11923462]
- Cervo L, Carnovali F, Stark JA, Mennini T. Cocaine-seeking behavior in response to drug-associated stimuli in rats: involvement of D3 and D2 dopamine receptors. *Neuropsychopharmacology*. 2003; 28:1150–1159. [PubMed: 12700684]
- Cervo L, Cocco A, Petrella C, Heidbreder CA. Selective antagonism at dopamine D3 receptors attenuates cocaine-seeking behaviour in the rat. *Int J Neuropsychopharmacol*. 2007; 10:167–181. [PubMed: 16426478]
- Chang W-L, Breier MR, Yang A, Swerdlow NR. Disparate effects of pramipexole on locomotor activity and sensorimotor gating in Sprague-Dawley rats. *Pharmacol Biochem Behav*. 2011; 99:634–638. [PubMed: 21683731]
- Collins GT, Witkin JM, Newman AH, Svensson KA, Grundt P, Cao J, Woods JH. Dopamine agonist-induced yawning in rats: a dopamine D3 receptor-mediated behavior. *J Pharmacol Exp Ther*. 2005; 314:310–319. [PubMed: 15833897]
- Collins GT, Newman AH, Grundt P, Rice KC, Husbands SM, Chauvignac C, Cheng J, Wang S, Woods JH. Yawning and hypothermia in rats: effects of dopamine D3 and D2 agonists and antagonists. *Psychopharmacology (Berl)*. 2007; 193:159–170. [PubMed: 17393143]
- 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, Woods JH. Proerectile effects of dopamine D2-like agonists are mediated by the D3 receptor in rats and mice. *J Pharmacol Exp Ther*. 2009; 329:210–217. [PubMed: 19136638]
- Collins GT, Woods JH. Influence of conditioned reinforcement on the response-maintaining effects of quinpirole in rats. *Behav Pharmacol*. 2009; 20:492–504. [PubMed: 19696656]
- Collins GT, Cunningham AR, Chen J, Wang S, Newman AH, Woods JH. Effects of pramipexole on the reinforcing effectiveness of stimuli that were previously paired with cocaine reinforcement in rats. *Psychopharmacology (Berl)*. 2012; 219:123–135. [PubMed: 21701814]

- De Vries TJ, Schoffelmeer ANM, Binnekade R, Raasø H, Vanderschuren LJMJ. Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. *Neuropsychopharmacology*. 2002; 26:18–26. [PubMed: 11751029]
- Di Ciano P, Underwood RJ, Hagan JJ, Everitt BJ. Attenuation of cue-controlled cocaine-seeking by a selective D3 dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology*. 2003; 28:329–338. [PubMed: 12589386]
- Di Ciano P, Everitt BJ. Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin, or sucrose: implications for the persistence of addictive behaviour. *Neuropharmacology*. 2004; 47:202–213. [PubMed: 15464138]
- Dias C, Lachize S, Boilet V, Huitelec , Cadore M. Differential effects of dopaminergic agents on locomotor sensitization and on the reinstatement of cocaine-seeking and food-seeking behaviour. *Psychopharmacology (Berl)*. 2004; 175:414–427. [PubMed: 15064914]
- Dickinson A, Smith J, Mirenowicz J. Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. *Behav Neurosci*. 2000; 114:468–483. [PubMed: 10883798]
- Dockstader CL, Rubinstein M, Grandy DK, Low MJ, van der Kooy D. The D2 receptor is critical in mediating opiate motivation only in opiate-dependent and withdrawn mice. *Eur J Neurosci*. 2001; 13:995–1001. [PubMed: 11264672]
- Dubrovina NI, Zinov'eva DV. Effects of activation and blockade of dopamine receptors on the extinction of a passive avoidance reaction in mice with a depressive-like state. *Neurosci Behav Physiol*. 2010; 40:55–59. [PubMed: 20012492]
- Fuchs RA, Tran-Nguyen LTL, Weber SM, Khroyan TV, Neisewander JL. Effects of 7-OH-DPAT on cocaine-seeking behavior and on re-establishment of cocaine self-administration. *Pharmacol Biochem Behav*. 2002; 72:623–632. [PubMed: 12175459]
- Gál K, Gyertyán I. Dopamine D3 as well as D2 receptor ligands attenuate the cue-induced cocaine-seeking in a relapse model in rats. *Drug Alcohol Depend*. 2006; 81:63–70. [PubMed: 16005579]
- Gilbert JG, Newman AH, Gardner EL, Ashby CR Jr, Heidbreder CA, Pak AC, Peng X-Q, Xi Z-X. 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*. 2005; 57:17–28. [PubMed: 15858839]
- Heidbreder C. Rationale in support of the use of selective dopamine D3 receptor antagonists for the pharmacotherapeutic management of substance use disorders. *Naunyn Schmiedeberg's Arch Pharmacol*. 2013; 386:167–176. [PubMed: 23104235]
- Higley AE, Kiefer SW, Li X, Gaal J, Xi Z-X, Gardner EL. Dopamine D3 receptor antagonist SB-277011A inhibits methamphetamine self-administration and methamphetamine-induced reinstatement of drug-seeking in rats. *Eur J Pharmacol*. 2011; 659:187–192. [PubMed: 21466803]
- Hitchcott PK, Quinn JJ, Taylor JR. Bidirectional modulation of goal-directed actions by prefrontal cortical dopamine. *Cereb Cortex*. 2007; 17:2820–2827. [PubMed: 17322558]
- Hogarth L, Balleine BW, Corbit LH, Killcross S. Associative learning mechanisms underpinning the transition from recreational drug use to addiction. *Ann N Y Acad Sci*. 2013; 1282:12–24. [PubMed: 23126270]
- Hutcheson DM, Everitt BJ, Robbins TW, Dickinson A. The role of withdrawal in heroin addiction: enhances reward or promotes avoidance? *Nat Neurosci*. 2001; 4:943–947. [PubMed: 11528427]
- Institute of Laboratory Animal Research CoLS, National Research Council. Guide for the Care and Use of Laboratory Animals. 8th ed.. Washington DC: National Academies Press; 2011.
- Jonkman S, Pelloux Y, Everitt BJ. Drug intake is sufficient, but conditioning is not necessary for the emergence of compulsive cocaine seeking after extended self-administration. *Neuropsychopharmacology*. 2012; 37:1612–1619. [PubMed: 22334124]
- Kelleher RT, Gollub LR. A review of positive conditioned reinforcement. *J Exp Anal Behav*. 1962; 5:543–597. [PubMed: 14031747]
- Koob GF, Le Moal M. Neurobiological mechanisms for opponent motivational processes in addiction. *Philos Trans R Soc Lond B Biol Sci*. 2008; 363:3113–3123. [PubMed: 18653439]
- Kurylo DD, Tanguay S. Effects of quinpirole on behavioral extinction. *Physiol Behav*. 2003; 80:1–7. [PubMed: 14568302]

- Lagos P, Scorza C, Monti JM, Jantos H, Reyes-Parada M, Silveira R, Ponzoni A. Effects of the D3 preferring dopamine agonist pramipexole on sleep and waking, locomotor activity and striatal dopamine release in rats. *Eur Neuropsychopharmacol.* 1998; 8:113–120. [PubMed: 9619689]
- Le Foll B, Goldberg SR, Sokoloff P. The dopamine D3 receptor and drug dependence: effects on reward or beyond? *Neuropharmacology.* 2005; 49:525–541. [PubMed: 15963538]
- Lex B, Hauber W. The role of nucleus accumbens dopamine in outcome encoding in instrumental and Pavlovian conditioning. *Neurobiol Learn Mem.* 2010; 93:283–290. [PubMed: 19931626]
- Mackintosh, NJ. *The Psychology of Animal Learning.* New York: Academic Press; 1974.
- Maj J, Rogó Z, Skuza G, Kołodziejczyk K. The behavioural effects of pramipexole, a novel dopamine receptor agonist. *Eur J Pharmacol.* 1997; 324:31–37. [PubMed: 9137910]
- Maldonado R, Saiardi A, Valverde O, Samad TA, Roques BP, Borrelli E. Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. *Nature.* 1997; 388:586–589. [PubMed: 9252189]
- Milton AL, Everitt BJ. The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *Eur J Neurosci.* 2010; 31:2308–2319. [PubMed: 20497475]
- Min C, Zheng M, Zhang X, Caron MG, Kim KM. Novel roles for β -arrestins in the regulation of pharmacological sequestration to predict agonist-induced desensitization of dopamine D3 receptors. *Br J Pharmacol.* 2013; 170:1112–1129. [PubMed: 23992580]
- Myers KM, Carlezon WA. Extinction of drug- and withdrawal-paired cues in animal models: relevance to the treatment of addiction. *Neurosci Biobehav Rev.* 2010; 35:285–302. [PubMed: 20109490]
- Nader K, LeDoux J. The dopaminergic modulation of fear: quinpirole impairs the recall of emotional memories in rats. *Behav Neurosci.* 1999; 113:152–165. [PubMed: 10197915]
- O'Brien CP, Gardner EL. Critical assessment of how to study addiction and its treatment: human and non-human animal models. *Pharmacol Ther.* 2005; 108:18–58. [PubMed: 16183393]
- Palmatier MI, Liu X, Matteson GL, Donny EC, Caggiula AR, Sved AF. Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. *Psychopharmacology (Berl).* 2007; 195:235–243. [PubMed: 17676401]
- Pears A, Parkinson JA, Hopewell L, Everitt BJ, Roberts AC. Lesions of the orbitofrontal but not medial prefrontal cortex disrupt conditioned reinforcement in primates. *J Neurosci.* 2003; 23:11189–11201. [PubMed: 14657178]
- Peck JA, Ranaldi R. Drug abstinence: exploring animal models and behavioral treatment strategies. *Psychopharmacology (Berl).* 2014; 231:2045–2058. [PubMed: 24633446]
- Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz J-C, Everitt BJ, Sokoloff P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature.* 1999; 400:371–375. [PubMed: 10432116]
- Robinson TE, Berridge KC. Addiction. *Ann Rev Psychol.* 2003; 54:25–53. [PubMed: 12185211]
- Robinson TE, Yager LM, Cogan ES, Saunders BT. On the motivational properties of reward cues: Individual differences. *Neuropharmacology.* 2014; 76:450–459. [PubMed: 23748094]
- Ross JT, Corrigan WA, Heidbreder CA, LeSage MG. 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.* 2007; 22:173–179. [PubMed: 17303116]
- Schindler CW, Panlilio LV, Goldberg SR. Second-order schedules of drug self-administration in animals. *Psychopharmacology (Berl).* 2002; 163:327–344. [PubMed: 12373434]
- See RE. Neural substrates of cocaine-cue associations that trigger relapse. *Eur J Pharmacol.* 2005; 526:140–146. [PubMed: 16253228]
- Self DW, Barnhart WJ, Lehman DA, Nestler EJ. Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. *Science.* 1996; 271:1586–1589. [PubMed: 8599115]
- Smith JW, Fetsko LA, Xu R, Wang Y. Dopamine D2L receptor knockout mice display deficits in positive and negative reinforcing properties of morphine and in avoidance learning. *Neuroscience.* 2002; 113:755–765. [PubMed: 12182883]

- Sutton MA, Rolfe NG, Beninger RJ. Biphasic effects of 7-OH-DPAT on the acquisition of responding for conditioned reward in rats. *Pharmacol Biochem Behav.* 2001; 69:195–200. [PubMed: 11420086]
- Taylor JR, Robbins TW. Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. *Psychopharmacology (Berl).* 1984; 84:405–412. [PubMed: 6440188]
- Taylor JR, Olausson P, Quinn JJ, Torregrossa MM. Targeting extinction and reconsolidation mechanisms to combat the impact of drug cues on addiction. *Neuropharmacology.* 2009; 56:186–195. [PubMed: 18708077]
- Trifilieff P, Feng B, Urizar E, Winiger V, Ward RD, Taylor KM, Martinez D, Moore H, Balsam PD, Simpson EH, Javitch JA. Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. *Mol Psychiatry.* 2013; 18:1025–1033. [PubMed: 23711983]
- Troisi JR 2nd. Perhaps more consideration of Pavlovian–operant interaction may improve the clinical efficacy of behaviorally based drug treatment programs. *Psychol Rec.* 2013; 63:863–894. [PubMed: 25346551]
- Wassum KM, Ostlund SB, Balleine BW, Maidment NT. Differential dependence of Pavlovian incentive motivation and instrumental incentive learning processes on dopamine signaling. *Learn Mem.* 2011; 18:475–483. [PubMed: 21693635]
- Wike, EL. *Secondary Reinforcement: Selected Experiments.* New York: Harper & Row; 1966.
- Williams BA. Conditioned reinforcement: experimental and theoretical issues. *Behav Anal.* 1994; 17:261–285. [PubMed: 22478192]
- Wolterink G, Phillips G, Cador M, Donselaar-Wolterink I, Robbins TW, Everitt BJ. Relative roles of ventral striatal D1 and D2 dopamine receptors in responding with conditioned reinforcement. *Psychopharmacology (Berl).* 1993; 110:355–364. [PubMed: 7831431]
- Zimmer BA, Chiodo KA, Roberts DCS. Reduction of the reinforcing effectiveness of cocaine by continuous D-amphetamine treatment in rats: importance of active self-administration during treatment period. *Psychopharmacology (Berl).* 2014; 231:949–954. [PubMed: 24146137]

Fig. 1.

Effects of pramipexole on the acquisition of a novel nose-poke response which produced a stimulus previously paired with remifentanyl injection. **a-b** Session-by-session record of active and inactive responding, respectively, by separate groups of rats ($n = 8/\text{group}$) pretreated with pramipexole or vehicle (0.0 mg/kg). **c** Responding in ACQ1, when no pretreatment was given. **d** Mean responding in ACQ2-8, when a pretreatment of pramipexole or vehicle was given before each session. **e** Responding in ACQ9, when all groups received a vehicle pretreatment. **f** Mean responding in ACQ10-14, when

pretreatments of pramipexole or vehicle were resumed. *, $p < .05$; **, $p < .01$; ***, $p < .001$. Significant difference between active and inactive responding either collapsed across groups (for ACQ1 and ACQ9) or within each group (for ACQ2-8 and ACQ10-14). #, $p < .05$; ##, $p < .01$; ###, $p < .001$. Significant difference in responding on the same manipulandum compared to vehicle treatment.

Fig. 2.

Nose-poke responses made by rats ($n = 8$) treated with pramipexole after random PAV, when remifentanyl injections and stimulus presentations were not consistently paired. Responding did not differ significantly between manipulanda or across sessions.

Fig. 3.

Intrasection allocation of active nose-poke responding during ACQ1 and ACQ2-8 by the animals in Experiment 1 treated with 0.32 mg/kg pramipexole or vehicle (0.0 mg/kg) after paired PAV. **a** Rate in each hour of each ACQ session by vehicle-treated animals. **b** Rate in each hour of each ACQ session by pramipexole-treated animals. **c** Rate in each hour averaged among ACQ2-8. **, $p < .01$; ***, $p < .001$. Significant difference between groups within the hour.

Fig. 4.

Acquisition of nose-poke responding for a remifentanyl paired stimulus by rats treated with pramipexole either 10 min or 190 min before ACQ sessions lasting 60 min. **a–b** Session-by-session record of active and inactive responding, respectively, by animals treated 0.32 mg/kg pramipexole or vehicle ($n = 6$ or 8 /group). **c** Responding in ACQ1, when no pretreatment was given. **d** Mean responding in ACQ2-8, when a pretreatment was given before each session. *, $p < .05$. Significant difference between active and inactive responding either collapsed across groups (ACQ 1) or within each group (for ACQ2-8).

Fig. 5.

Effects of SB-277011A or L-741,626 on the ability of pramipexole (PRAM) to enhance nose-poke responding for the remifentanyl-paired stimulus. **a** Acquisition of responding for the remifentanyl-paired stimulus before the start of antagonist administration. **b** Responding of animals ($n = 8$) when treated with SB-277011A prior to pramipexole or treated with vehicle (0.0 mg/kg) only. **c** Responding of animals ($n = 8$) when treated with lower doses of L-741,626 prior to pramipexole or treated with vehicle only. **c** Responding of animals ($n = 8$) when treated with higher doses of L-741,626 prior to pramipexole or treated with vehicle

only. *, $p < .05$; **, $p < .01$. Significant difference in responding on the same manipulandum compared to vehicle + 0.32 mg/kg PRAM.

Fig. 6.

Effects of SB-277011A or its vehicle (0.0 mg/kg) on yawning and penile erections (PE) by pramipexole-treated animals ($n = 8/\text{group}$). **, $p < .01$. Significant main effect of SB-277011A dose.