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Role of Dorsal Medial Prefrontal Cortex Dopamine D1-Family Receptors in Relapse to High-Fat Food Seeking Induced by the Anxiogenic Drug Yohimbine

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In humans, relapse to maladaptive eating habits during dieting is often provoked by stress. In rats, the anxiogenic drug yohimbine, which causes stress-like responses in both humans and nonhumans, reinstates food seeking in a relapse model. In this study, we examined the role of medial prefrontal cortex (mPFC) dopamine D1-family receptors, previously implicated in stress-induced reinstatement of drug seeking, in yohimbine-induced reinstatement of food seeking. We trained food-restricted rats to lever press for 35% high-fat pellets every other day (9–15 sessions, 3 h each); pellet delivery was accompanied by a discrete tone-light cue. We then extinguished operant responding for 10–16 days by removing the pellets. Subsequently, we examined the effect of yohimbine (2 mg/kg, i.p.) on reinstatement of food seeking and Fos (a neuronal activity marker) induction in mPFC. We then examined the effect of systemic injections of the D1-family receptor antagonist SCH23390 (10 μg/kg, s.c.) on yohimbine-induced reinstatement and Fos induction, and that of mPFC SCH23390 (0.5 and 1.0 μg/side) injections on this reinstatement. Yohimbine-induced reinstatement was associated with strong Fos induction in the dorsal mPFC and with weaker Fos induction in the ventral mPFC. Systemic SCH23390 injections blocked both yohimbine-induced reinstatement and mPFC Fos induction. Dorsal, but not ventral, mPFC injections of SCH23390 decreased yohimbine-induced reinstatement of food seeking. In addition, dorsal mPFC SCH23390 injections decreased pellet-priming-induced reinstatement, but had no effect on ongoing high-fat pellet self-administration or discrete-cue-induced reinstatement. Results indicate a critical role of dorsal mPFC dopamine D1-family receptors in stress-induced relapse to palatable food seeking, as well as relapse induced by acute re-exposure to food taste, texture, and smell.

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INTRODUCTION

Although many people attempt to control their excessive food intake, they typically relapse to old, unhealthy eating habits within a few months (Kramer *et al*, 1989; Peterson and Mitchell, 1999; Skender *et al*, 1996). This relapse is often induced by stress, anxiety, or negative mood states (Byrne *et al*, 2003; Grilo *et al*, 1989; Herman and Polivy, 1975; Polivy and Herman, 1999; Torres and Nowson, 2007). Despite evidence in humans of stress-induced relapse to unhealthy eating habits during dieting, until recently the mechanisms of this relapse have not been studied in animal models (Nair *et al*, 2009a). To address this issue, we adapted a rat reinstatement model, commonly used to study relapse to abused drugs (Shaham *et al*, 2003), to investigate mechanisms of stress-induced relapse to palatable food seeking during dieting (Ghitza *et al*, 2006). In these studies, we use the pharmacological stressor yohimbine to reinstate food seeking (Nair *et al*, 2009a).

We chose yohimbine (a prototypical α-2 adrenoceptor antagonist) as the stress manipulation, because this pharmacological stressor has been used in many studies to induce stress- and anxiety-like states in both humans (Bremner *et al*, 1996b; Holmberg and Gershon, 1961) and nonhumans (Bremner *et al*, 1996a; Lang and Gershon, 1963). In addition, yohimbine reliably reinstates food seeking in rats (Ghitza *et al*, 2006; Nair *et al*, 2009b; Richards *et al*, 2008) and drug seeking in rats (Cippitelli *et al*, 2010; Feltenstein and See, 2006; Kupferschmidt *et al*, 2009; Shepard *et al*, 2004) and monkeys (Lee *et al*, 2004).

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The brain mechanisms of yohimbine-induced reinstatement of food and drug seeking are unknown, and results from studies on the role of central noradrenergic systems in this reinstatement are mixed. The α-2 adrenoceptor agonist clonidine attenuates yohimbine-induced reinstatement of alcohol seeking in rats (Le *et al*, 2009) and cocaine seeking in monkeys (Lee *et al*, 2004). In contrast, clonidine exerts no effect on yohimbine-induced reinstatement of cocaine or food seeking in rats (Brown *et al*, 2009; Nair *et al*, 2009a), and yohimbine-induced reinstatement of cocaine conditioned place preference (CPP) in mice (Mantsch *et al*, 2010). In addition, in rats, 6-hydroxydopamine lesions of the ventral or dorsal noradrenergic bundles have no effect on yohimbine-induced reinstatement of alcohol seeking (Le *et al*, 2009). Furthermore, the effect of yohimbine on reinstatement of food or alcohol seeking is not mimicked by RS79948, a selective α-2 adrenoceptor antagonist (Le *et al*, 2009; Nair *et al*, 2009a). In contrast, in monkeys, the effect of yohimbine on reinstatement is mimicked by RS79948 (Lee *et al*, 2004), and in mice, this effect of yohimbine is mimicked by another selective α-2 adrenoceptor antagonist, BRL44408 (Mantsch *et al*, 2010). Mantsch *et al* (2010) also reported that yohimbine-induced cocaine CPP in mice is attenuated by the *β*-adrenoceptor antagonist, propranolol.

In this study, we studied the role of medial prefrontal cortex (mPFC) dopamine D1-family receptor in yohimbine-induced reinstatement of food seeking, because dorsal mPFC injections of the D1-family receptor antagonist SCH23390 or the mixed D1/D2 receptor antagonist fluphenazine decrease footshock-stress-induced reinstatement of cocaine seeking (Capriles *et al*, 2003; McFarland *et al*, 2004) and immobilization stress-induced reinstatement of cocaine CPP (Sanchez *et al*, 2003). In addition, stressors preferentially activate mesocortical dopaminergic projections from the ventral tegmental area to the mPFC (Deutch and Roth, 1990; Thierry *et al*, 1976). Finally, yohimbine increases mPFC extracellular dopamine levels (Tanda *et al*, 1996) and expression of Fos (a neuronal activity marker) (Bing *et al*, 1991, 1992; Funk *et al*, 2006; Singewald *et al*, 2003).

MATERIALS AND METHODS

Subjects and Apparatus

Male Long-Evans rats (total $n = 114$, Charles River, Raleigh, NC; 300–385 g) were maintained on a reverse 12-h:12-h light–dark cycle (lights off at 0900 or 0930 hours). In all, 16 rats were excluded from the study because of poor health, misplaced cannulae, intracranial injection procedure problems, or failure to meet the extinction criterion. All rats were weighed daily and food intake was restricted to 16 g/day of Purina rat chow (about 60–65% of their daily food intake) during the training phase and to 18–22 g/day (to maintain stable body weight) during the extinction and reinstatement test phases. All procedures followed the guidelines outlined in the 'Principles of Laboratory Animal Care' (NIH publication no. 85–23). Experiments were conducted in standard self-administration chambers (Med Associates, Georgia, VT). Each chamber had two levers 9 cm above the floor, but only one lever ('active,' retractable lever) activated the pellet dispenser, which delivered 45-mg food pellets containing 35% fat and 45.2% carbohydrate (catalog no. F05989, Bioserv, Frenchtown, NJ). Efforts were made to minimize the number of animals used and their suffering.

Drugs

SCH23390 hydrochloride and MK212 hydrochloride were purchased from Tocris (Ellisville, MO), yohimbine hydrochloride was purchased from Sigma (St Louis, MO), and M100907 was provided by Dr Kenner Rice (NIDA). SCH23390 (injection volume of 1 ml/kg or 0.5 μl/side for systemic or intracranial injections, respectively) and MK212 (0.5 μl/side) were dissolved in sterile saline; M100907 (0.5 μl/side) was dissolved in a minimal volume of 0.01 N HCl and sterile saline and the pH of the solution was adjusted to 6–7 with 0.1 N NaOH. Yohimbine (0.5 ml/kg) was dissolved in sterile water. Doses of yohimbine, SCH23390, MK212, and M100907 are based on our studies (Bossert *et al*, 2007, 2009; Ghitza *et al*, 2007; Nair *et al*, 2008, 2009b) and on studies of other investigators (Alleweireldt *et al*, 2006; Anderson *et al*, 2003; Bachtell *et al*, 2005; Bossert *et al*, 2009; Chaudhri *et al*, 2009; Filip and Cunningham, 2003; McMahon *et al*, 2001; Pentkowski *et al*, 2010; Ramos *et al*, 2005; See, 2009).

Intracranial Surgery

Rats were anesthetized with a mixture of sodium pentobarbital and chloral hydrate (60 and 25 mg/kg, i.p.). For intracranial injection studies, rats were implanted with bilateral guide cannulae (23 G, Plastics One, Roanoke, VA) 1 mm above the dorsal mPFC (cingulate area 1 and dorsal part of the prelimbic cortex) (AP: + 2.8 mm, ML: + 1.2 mm, and DV: −2.4 mm, 10° angle) or the ventral mPFC (ventral part of the prelimbic cortex and infralimbic cortex) (AP: + 2.8 mm, ML: + 1.5 mm, and DV: −4.2 mm, 10° angle) (Paxinos and Watson, 2005). These coordinates are based on a previous study from our laboratory (Koya *et al*, 2009). The analgesic buprenorphine (0.1 mg/kg, s.c.) was administered after surgery and rats were allowed to recover for at least 7 days.

Intracranial Injections

Intracranial injections of SCH23390, MK212, M100907, or their vehicles were prepared with Harvard infusion pumps, using 10-μl Hamilton syringes connected to 30-G injectors (Plastics One) by polyethylene-50 tubing. Injections lasted 1 min and injectors were left in place for an additional minute before being replaced with cannula blockers. After the last reinstatement test, rats in experiments 2–4 were deeply anesthetized, decapitated, and their brains were removed and stored in 10% formalin. The brains were sliced into coronal sections (of 50-μm thickness) and stained with cresyl violet. The sections were then examined for cannulae placement under a microscope.

Fos Immunohistochemistry

All experiments described below were carried out at room temperature unless specified. Ninety minutes after the start of the test sessions (~120 min after yohimbine injections),

rats were deeply anesthetized with isoflurane (~80 s) and perfused transcardially with 100 ml of 0.1 M phosphate-buffered saline (PBS), followed by 400 ml of 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4). The brains were removed, post fixed in 4% paraformaldehyde for 2 h, and transferred to 30% sucrose in 0.1 M sodium phosphate (pH 7.4) for 48 h at 4 °C. The brains were subsequently frozen in powdered dry ice and stored at −80 °C until sectioning. Coronal sections of 40-μm thickness, approximately +2.5 to 3.5 mm from the bregma (Paxinos and Watson, 2005), containing the dorsal and ventral mPFCs were cut on a cryostat (Leica Microsystems, Bannockburn, IL), collected in cryoprotectant (20% glycerol and 2% dimethylsulfoxide in 0.1 M sodium phosphate, pH 7.4), and stored at −80 °C until further processing.

Free-floating sections were washed (3 times for 10 min each) in PBS, incubated for 1 h in 3% normal goat serum (NGS) in PBS with 0.2% Triton X-100 (PBS-Tx), and incubated overnight at 4 °C with the anti-c-Fos primary antibody (c-Fos sc-52, Lot F2209, Santa Cruz Biotechnology) diluted 1 : 4000 in 1% NGS in PBS-Tx. The sc-52 antibody was raised against amino acids 3–16 of human c-Fos: SGFNADYEASSSRC. Sections were then washed in PBS and incubated for 2 h with the biotinylated anti-rabbit IgG secondary antibody (BA-1000, Vector Laboratories) diluted 1 : 600 in 1% NGS in PBS-Tx. Sections were washed in PBS and incubated in avidin–biotin–peroxidase complex (ABC Elite kit, PK-6100, Vector Laboratories) in PBS containing 0.5% Triton X-100 for 1 h, and washed in PBS. Sections were developed in 3,3′-diaminobenzidine for ~4 min, washed in PBS, mounted onto chrom-alum/gelatin-coated slides, and air dried. The slides were dehydrated through a graded series of alcohol (30, 60, 90, 95, 100, 100% ethanol), cleared with Citrasolv (Fisher Scientific, Pittsburgh, PA), and coverslipped with Permount (Sigma).

Bright field images of the dorsal and ventral mPFCs were digitally captured using a CCD Camera (Coolsnap Photometrics, Roper Scientific, Trenton, NJ) attached to a Zeiss Axioskop 2 microscope with a ×5 objective. Labeled Fos-immunoreactive nuclei from 1–2 sections from the left and right hemispheres of each rat under different experimental conditions were automatically counted using IPLab software (version 3.9.4 r5; Scanalytics, Fairfax, VA) for Macintosh. Image capture and quantification of Fos-positive nuclei were conducted in a blind manner by CC and independently verified in selected sections by JMB (inter-rater reliability $r = 0.81$, $p < 0.05$).

Double Labeling with Fos and NeuN Immunohistochemistry

Double-labeling experiments for Fos and the neuronal marker NeuN were used to estimate the proportion of neurons expressing Fos. Sections from 6 rats from experiment 1 ($n = 3$ for vehicle and yohimbine conditions) were thawed and washed (3 times for 10 min each) in Tris-buffered saline (TBS; 0.025 M Tris-HCl, 0.5 M NaCl, pH 7.5) and incubated for 20 min in TBS with 0.2% Triton X-100 (TBS-Tx). Sections were washed in TBS and incubated for 48 h with antibody to Fos (1 : 500 dilution, sc-52) and antibody to NeuN (1 : 2000 dilution of MAB377, Millipore) in TBS-Tx. Sections were washed in TBS and incubated for 1 h with secondary antibodies Alexa 488-labeled donkey anti-rabbit antibody and Alexa 568-labeled goat anti-mouse antibody (1 : 200 dilution in TBS-Tx for both antibodies, Invitrogen). Finally, sections were washed in TBS, mounted on to chrom-alum/gelatin-coated slides, air dried, and coverslipped using the Vectashield fluorescent mounting medium (H-1400, Vector Laboratories). Fluorescent images of the dorsal and ventral mPFCs were captured using the same CCD camera and microscope using a ×20 objective. The number of Fos-labeled, NeuN-labeled, and double-labeled immunoreactive nuclei in these images were manually counted in a blind manner by CC.

Behavioral Procedures

We used a reinstatement procedure that included 3 phases: training for food self-administration (9–15 sessions), extinction of food-reinforced behavior (10–16 sessions), and tests for reinstatement under extinction conditions (2–4 sessions). During all phases, the sessions started 30 min after the beginning of the dark cycle (0930 or 1000 hours). Below, we first describe the training and extinction procedures for all experiments, and then provide specific details for the testing phase of each experiment. During testing, the experimental conditions were counterbalanced.

Food Self-Administration Training

All rats underwent 3-h daily sessions of 'autoshaping' for 2–3 days during which pellets were administered noncontingently every 5 min into a receptacle located near the active lever. Pellet delivery was accompanied by a compound 5-s tone (2900 Hz)-light (7.5-W white light located above the active lever) cue. Subsequently, rats were trained to self-administer the pellets on a fixed-ratio 1, 20-s timeout reinforcement schedule. Training sessions were conducted for 9–15 days, every other day, 3 h/day over 18–30 days. Rats were chronically housed in operant self-administration chambers for the entire duration of training, with the exception of 19 rats (10 in experiment 2a and 9 in experiment 2b). Due to scheduling issues, for 10 training days, these rats were housed in the animal facility and transferred to the self-administration chambers before the training sessions, and returned to the facility at the end of the 3-h sessions. From training day 11 onwards, these rats were housed in operant chambers.

At the start of each 3-h session, the red houselight was turned on and the active lever was extended. After each pellet delivery, the tone-light cue was turned on for 5 s. During the training days, regular food (16 g Purina rat chow) was given immediately after the daily session (~3.5 h into the dark cycle). During the days off, 16 g regular food was given at the start of the dark cycle. We chose this training schedule and these diet conditions because previous home-cage food-consumption studies have shown that rats placed on a restricted diet and given intermittent access to palatable food develop binge-like eating behavior (Avena *et al*, 2008; Boggiano *et al*, 2007; Colantuoni *et al*, 2002; Corwin and Buda-Levin, 2004; Figlewicz *et al*, 2007) and become hypersensitive to the effect of stress on palatable food intake (Cifani *et al*, 2009; Hagan *et al*, 2002, 2003).

Extinction of Food-Reinforced Responding

After training, rats underwent 10–16 daily 3-h extinction sessions until active lever responding decreased to 30 presses/3 h or less for 3 consecutive sessions (the extinction criterion). In experiments 1, 3a, and 3b, during the extinction phase, lever presses led to tone-light cue presentations, but not to pellet delivery. In experiment 3c (cue-induced reinstatement), lever presses during the extinction phase had no programmed consequences (ie, neither the tone-light cue nor pellets were made available). During the extinction and reinstatement phases, regular food (18–22 g) was given approximately at the same time as during training phases ($\sim$3.5 h after the onset of the dark cycle). In all experiments, the amount of food given during the extinction phase was higher than that given during the training phase to maintain rats on a stable body weight during this phase and the subsequent reinstatement phase.

Experiment 1: Effect of Systemic SCH23390 Injections on Yohimbine-Induced Reinstatement and mPFC Fos Induction

Experiment 1a: In this initial experiment, we studied the effect of yohimbine or vehicle injections ($n = 7$ per group) on reinstatement of lever responding and Fos induction in the dorsal and ventral mPFCs. Rats were injected with yohimbine (2 mg/kg, i.p.) or distilled water 25–30 min before the start of 90-min test sessions. The test session duration was shorter than that of the subsequent experiments (3 h), because of the time course of Fos protein expression, which is maximal 90–120 min after neuronal activation (Curran and Morgan, 1995). At the end of testing, rats were anesthetized, perfused, and their brains were removed and assayed for Fos-IR (see above).

Experiment 1b. Fos induction is dependent on activation of D1-family receptor signaling (Graybiel *et al*, 1990; Robertson *et al*, 1989; Young *et al*, 1991), and we previously found that systemic SCH23390 injections (5 and 10 μg/kg, s.c.) decrease yohimbine-induced reinstatement of food seeking (Figure 2 in Nair *et al*, 2009a). Therefore, we assessed whether yohimbine-induced reinstatement and mPFC Fos induction are reversed by systemic SCH23390 injections. We used 4 groups of rats ($n = 6$–7 per group) in a 2 (SCH23390 dose: 0, 10 μg/kg) $\times$ 2 (yohimbine dose: 0, 2 mg/kg) experimental design. Rats were injected with SCH23390 or saline immediately before yohimbine or distilled water injections, administered 25–30 min before the start of test sessions. At the end of testing, rats were anesthetized, perfused, and their brains were removed and assayed for Fos-IR.

Experiment 2: Effect of Dorsal and Ventral mPFC Injections of SCH23390 on Yohimbine-Induced Reinstatement

Experiment 2a: dorsal mPFC. In experiment 1, we found that systemic yohimbine injections preferentially increased Fos expression in the dorsal mPFC and that this effect was blocked by systemic SCH23390 injections. In this experiment, we tested whether local SCH23390 would mimic the systemic effect of the drug on yohimbine-induced reinstatement. We tested the effect of dorsal mPFC SCH23390 injections on yohimbine-induced reinstatement in four test sessions with two sessions run consecutively and one extinction day between sets of tests. We used two groups ($n = 8$–12 per group) in a mixed experimental design that included the between-subjects factor of SCH23390 dose (0.5, 1.0 μg/site), and the within-subjects factors of pretreatment condition (vehicle, SCH23390 (0.5 or 1.0 μg/site)), and yohimbine dose (0, 2 mg/kg). Rats were injected intracranially with SCH23390 or saline, followed immediately by i.p. injections of yohimbine or distilled water 25–30 min before the start of test sessions. The experimental conditions were counterbalanced. The rationale for using the mixed experimental design described above, which was also used in our previous work (Ghitza *et al*, 2007; Nair *et al*, 2008, 2009b), is to minimize the number of rats used while limiting the number of repeated yohimbine reinstatement tests. At the end of the experiment, rats were anesthetized, decapitated, and their brains were removed for verification of cannulae placements.

Experiment 2b: ventral mPFC. In experiment 2a, we found that dorsal mPFC SCH23390 injections decreased yohimbine-induced reinstatement. In experiment 2b, we tested the anatomical selectivity of this effect by injecting SCH23390 into the ventral mPFC. Although both SCH23390 doses were effective in the dorsal mPFC (Figure 4), we used the higher dose (1 μg) to assess anatomical specificity (experiment 2b) and behavioral specificity (experiment 3), because negative results from intracranial injections of a given receptor antagonist (or agonist) can be interpreted with more confidence than negative findings with half the drug dose. This is because of the fact that for a constant injection volume, the higher drug dose can achieve higher receptor occupancy and can diffuse further away from the injection site (Wise and Hoffman, 1992). We tested the effect of ventral mPFC SCH23390 injections on yohimbine-induced reinstatement in four test sessions with two sessions run consecutively and one extinction day between sets of tests. We used a within-subjects experimental design that included the factors of pretreatment condition (vehicle, SCH23390 (1.0 μg/site)) and yohimbine dose (0, 2 mg/kg) ($n = 9$). Rats were injected intracranially with SCH23390 or saline, followed immediately by i.p. injections of yohimbine or distilled water 25–30 min before the start of test sessions. The experimental conditions were counterbalanced. At the end of the experiment, rats were anesthetized, decapitated, and their brains were removed for verification of cannulae placements.

Experiment 3: Effect of Dorsal mPFC Injections of SCH23390 on Food Self-Administration, and Pellet-Priming- and Cue-Induced Reinstatement

The purpose of experiment 3 was to determine the behavioral specificity of the effect of dorsal mPFC SCH23390 on yohimbine-induced reinstatement by assessing its effect on ongoing pellet self-administration, as well as reinstatement of food seeking induced by pellet priming and discrete cues. These stimuli reliably reinstate lever

presses under our experimental conditions (Nair *et al*, 2008, 2009b).

Experiment 3a: pellet self-administration. We tested the effect of dorsal mPFC injections of SCH23390 (1.0 μg/side) or saline (0.5 μl/side) on ongoing food self-administration. A single group of rats ($n = 10$) was used in a within-subjects design in which rats were tested, under counterbalanced conditions, on training days 11 and 14. The mean pellet intake and active lever responding for the two training sessions before the test days were 249 ± 25 and 1085 ± 258 per 3 h, respectively. Rats were injected intracranially with SCH23390 or saline 25–30 min before the start of test sessions.

Experiment 3b: pellet-priming-induced reinstatement. We tested the effect of dorsal mPFC injections of SCH23390 (1.0 μg/side) or saline on pellet-priming-induced reinstatement of lever responding. It is well established that noncontingent exposure to a food pellet after extinction of operant responding reinstates food seeking (Baker *et al*, 1991; Horvitz and Ettenberg, 1988; Skinner, 1938). We used the same rats ($n = 10$) that were used in experiment 3a. After the second test session in experiment 3a, these rats underwent 15 extinction sessions during which lever responding led to tone-light cue presentations, but not pellets. Rats were then tested under counterbalanced conditions for pellet-priming-induced reinstatement. The test sessions were separated by 3 days and rats underwent regular extinction sessions on the days between testing. During testing, saline or SCH23390 was injected into the dorsal mPFC 25–30 min before exposure to the pellet priming manipulation: noncontingent delivery of 3 pellets, every 20 s, at the onset of the sessions. After the second test session, rats were anesthetized, decapitated, and their brains were removed for verification of cannulae placements. In experiment 2, we found that dorsal mPFC saline or SCH23390 injections had no effect on baseline extinction responding. Thus, to limit the number of intracranial injections, we did not assess this in experiment 3b (and in experiments 3c, 4a, and 4b).

Experiment 3c: cue-induced reinstatement. We tested the effect of dorsal mPFC injections of SCH23390 (1.0 μg/side) or saline on cue-induced reinstatement of lever responding. For this purpose, we used a well-established cue-induced reinstatement procedure that has been used in studies involving food (De Vries *et al*, 2005; Floresco *et al*, 2008; McLaughlin and Floresco, 2007) and drug (Davis and Smith, 1976; Meil and See, 1996) rewards. In this procedure, rats are first trained to self-administer a drug or nondrug reward; each reward delivery is temporally paired with a discrete cue (eg, tone, light). Lever pressing is then extinguished in the absence of the reinforcer and the cue. During reinstatement testing, exposure to the cue, which is earned contingently during testing, serves as a conditioned reinforcer and reliably reinstates operant responding (See, 2005). A single group of rats ($n = 10$) was trained to lever press for food pellets for nine sessions as described above. Next, rats underwent 10 extinction sessions during which neither pellets nor tone-light cues were delivered after the lever press. Rats were then tested under counterbalanced conditions for cue-induced reinstatement. Rats were injected intracranially with SCH23390 or saline 25–30 min before the start of test sessions; during the sessions, lever responding led to contingent presentations of the cue under a fixed-ratio 1, 20-s timeout reinforcement schedule. The test sessions were separated by 4 days and rats underwent extinction sessions (without the discrete cue) on the days between testing. Rats that did not initiate lever responding within 10 min of the start of the session were given a single noncontingent exposure to the tone-light cue.

Experiment 4: Effect of Dorsal mPFC Injections of the 5-HT2c Agonist MK212 and the 5-HT2a Antagonist M100907 on Yohimbine-Induced Reinstatement

Experiment 4a: MK212. SCH23390 is also a 5-HT2c (formerly 5-HT1c) receptor agonist (Briggs *et al*, 1991; Millan *et al*, 2001), and Fletcher *et al* (2008) found that systemic injections of the 5-HT2c receptor agonist Ro60–0175 decrease yohimbine-induced reinstatement of cocaine seeking. Thus, we determined whether dorsal mPFC SCH23390 effects on yohimbine-induced reinstatement are mimicked by the 5-HT2c receptor agonist MK212 (Ramos *et al*, 2005). At the end of experiment 3c (cue-induced reinstatement), rats underwent seven extinction sessions in the presence of the tone-light cue and then tested for yohimbine-induced reinstatement in two counterbalanced sessions (separated by 4 days) after pretreatment with MK212 (0.1 μg/0.5 μl per side) or vehicle (sterile saline) into the dorsal mPFC. MK212 was injected just before yohimbine (2 mg/kg, i.p.), which was injected 25–30 min before the test sessions. After the second test session, rats were anesthetized, decapitated, and their brains dissected for cannulae placement verification.

Experiment 4b: M100907. SCH23390 also binds to 5-HT2a receptors with high affinity (Neumeyer *et al*, 2003; Porter *et al*, 1999). Results from pharmacological studies indicate that SCH23390 is an antagonist at 5-HT2a receptors, because it blocks behavioral and physiological effects of 5-HT2a receptor stimulation (Monti *et al*, 1990; Schreiber *et al*, 1995). Thus, we determined whether the effects of dorsal mPFC SCH23390 on yohimbine-induced reinstatement are mimicked by the 5-HT2a receptor antagonist M100907 (McMahon *et al*, 2001). Rats ($n = 9$) were trained to self-administer high-fat food pellets as described above. Next, lever responding was extinguished in the presence of the tone-light cue for 10 days. Rats were then tested for yohimbine-induced reinstatement in two counterbalanced sessions (separated by 2 days) after pretreatment with M100907 (0.3 μg/0.5 μl per side) or vehicle (sterile saline) into the dorsal mPFC. M100907 was injected just before yohimbine (2 mg/kg, i.p.), which was injected 25–30 min before the test sessions. After the second test session, rats were anesthetized, decapitated, and their brains were removed for verification of cannulae placements.

Statistical Analyses

Data were analyzed using mixed ANOVAs in SPSS version 15.0 statistical software Proc GLM procedure. In experiment 1,

the number of Fos-IR and NeuN nuclei for the dorsal and ventral mPFCs was determined by calculating the mean value of two hemispheres per rat. The factors used in statistical analyses are described in the 'Results' section. Significant overall effects ($p<0.05$) in the different ANOVAs were followed by *post hoc* Fisher PLSD tests.

RESULTS

Rats (total $n=98$) were trained to self-administer food pellets for 9–15 days (3 h/day) and demonstrated reliable pellet self-administration and, as in our previous studies (Ghitza *et al*, 2006; Nair *et al*, 2008, 2009b), a progressive escalation of timeout active lever presses across sessions (Figure 1; p-values <0.01 for a training session effect for both pellet intake and timeout responding). After self-administration training, rats underwent 10–16, 3-h extinction sessions either in the presence (experiment 1, 2, 3b, 4a, and 4b, total $n=88$) or absence (experiment 3c, $n=10$) of the discrete tone-light cue, during which lever pressing decreased over days under both extinction conditions (Figure 1; p-values <0.01 for an extinction session effect).

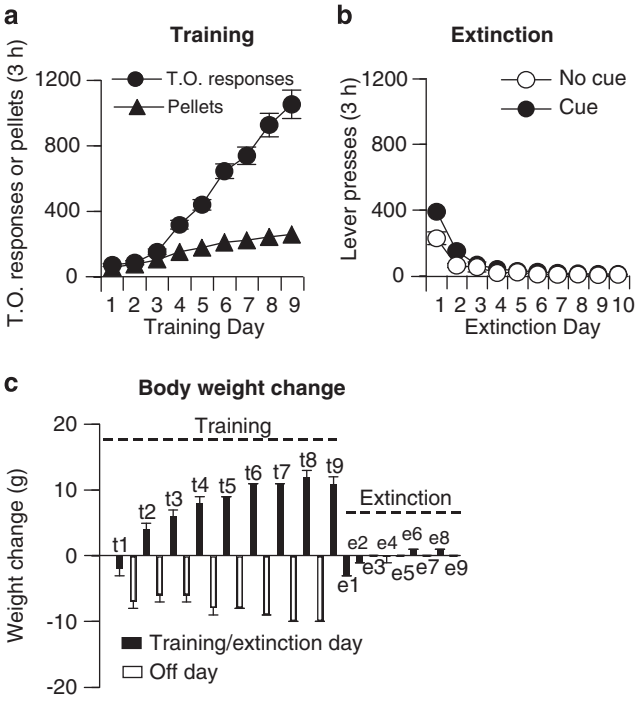


Figure 1 Food pellet self-administration training, extinction of food-reinforced lever responding, and body weight change. (a) Training (first 9 days): mean ± SEM number of 35% fat pellets earned and timeout (T.O.) responses on the active lever (total lever presses minus pellets earned) during the training sessions, which occurred on alternate days (one 3-h session per day, every other day). Rats were trained on a fixed-ratio 1 (FR-1) 20-s timeout reinforcement schedule ($n=98$). (b) Extinction (first 9 days): mean ± SEM number of responses on the previously active lever in the presence ($n=88$) or absence ($n=10$, experiment 3c) of a discrete tone-light cue. (c) Body weight fluctuations during training and extinction phases ($n=98$). During the training phase, rats were maintained on 16 g/day of regular chow food and were given 3-h access to food pellets every other day. During the extinction (and reinstatement) phases, food pellets were not available in the self-administration chambers, and rats were maintained on 18–22 g/day of regular chow to maintain stable body weight.

Experiment 1: Effect of systemic SCH23390 Injections on Yohimbine-Induced Reinstatement and mPFC Fos Induction

Experiment 1a. Yohimbine reinstated active lever responding after extinction; this effect was associated with strong and moderate Fos induction in the dorsal and ventral mPFCs, respectively (Figure 2). Behavioral data were analyzed using the between-subjects factor of yohimbine dose (0, 2 mg/kg) and the within-subjects factor of lever (active, inactive). ANOVA revealed significant effects of yohimbine dose ($F_{1,12}=47.6$, $p<0.01$), lever ($F_{1,12}=66.1$, $p<0.01$), and yohimbine dose × lever ($F_{1,12}=46.3$, $p<0.01$). Yohimbine had no effect on inactive lever presses, a potential measure of nondirected activity and/or response generalization (Shalev *et al*, 2002) (Figure 2). *Post hoc* differences and the time course (30-min intervals) of yohimbine-induced reinstatement are provided in Figure 2. Analysis of Fos quantification (Figure 2) included the between-subjects factor of yohimbine dose and the within-subjects factor of the mPFC region (ventral, dorsal). ANOVA revealed significant effects of yohimbine dose ($F_{1,12}=17.4$, $p<0.01$), mPFC region ($F_{1,12}=32.5$, $p<0.01$), and yohimbine dose × mPFC region ($F_{1,12}=35.7$, $p<0.01$). The significant interaction reflects a stronger effect of yohimbine on Fos-IR induction in the dorsal vs the ventral mPFC. This conclusion is qualitatively confirmed in a small sample ($n=3$ per group) in which we assessed the percentage of double-labeled Fos-NeuN neurons in the ventral and dorsal mPFCs after water or yohimbine injections (dorsal mPFC: water: 3.8 ± 1.1%, yohimbine: 24.6 ± 2.1%; ventral mPFC: water: 4.0 ± 1.0%, yohimbine: 11.5 ± 1.3%) (Figure 2).

Experiment 1b. Systemic SCH23390 injections blocked yohimbine-induced reinstatement of lever responding and yohimbine-induced Fos induction in the dorsal and ventral mPFCs. As in experiment 1a, yohimbine-induced Fos induction was more pronounced in the dorsal than in the ventral mPFC (Figure 3). Behavioral data (active lever presses) were analyzed using the between-subjects factors of yohimbine dose (0, 2 mg/kg) and SCH23390 dose (0, 10 μg/kg). ANOVA revealed significant effects of yohimbine dose ($F_{1,22}=17.9$, $p<0.01$), SCH23390 dose ($F_{1,22}=12.9$, $p<0.01$), and yohimbine dose × SCH23390 dose ($F_{1,22}=10.3$, $p<0.01$). *Post hoc* differences and the time course (30-min intervals) of yohimbine-induced reinstatement are provided in Figure 3. Analysis of Fos quantification included the between-subjects factors of yohimbine dose and SCH23390 dose, and the within-subjects factor of the mPFC region (ventral, dorsal). ANOVA revealed significant effects of yohimbine dose ($F_{1,22}=23.8$, $p<0.01$), SCH23390 dose ($F_{1,22}=16.4$, $p<0.01$), mPFC region ($F_{1,22}=27.9$, $p<0.01$), SCH23390 dose × mPFC region ($F_{1,22}=8.3$, $p<0.01$), yohimbine dose × SCH23390 dose ($F_{1,22}=10.8$, $p<0.01$), yohimbine dose × mPFC region ($F_{1,22}=5.4$, $p<0.05$), and yohimbine dose × SCH23390 dose × mPFC region ($F_{1,22}=35.7$, $p<0.01$). These significant interactions reflect (1) a stronger effect of yohimbine on Fos induction in the dorsal vs the ventral mPFC, (2) a stronger attenuation of yohimbine-induced Fos expression by SCH23390 in the dorsal vs the ventral mPFC, and

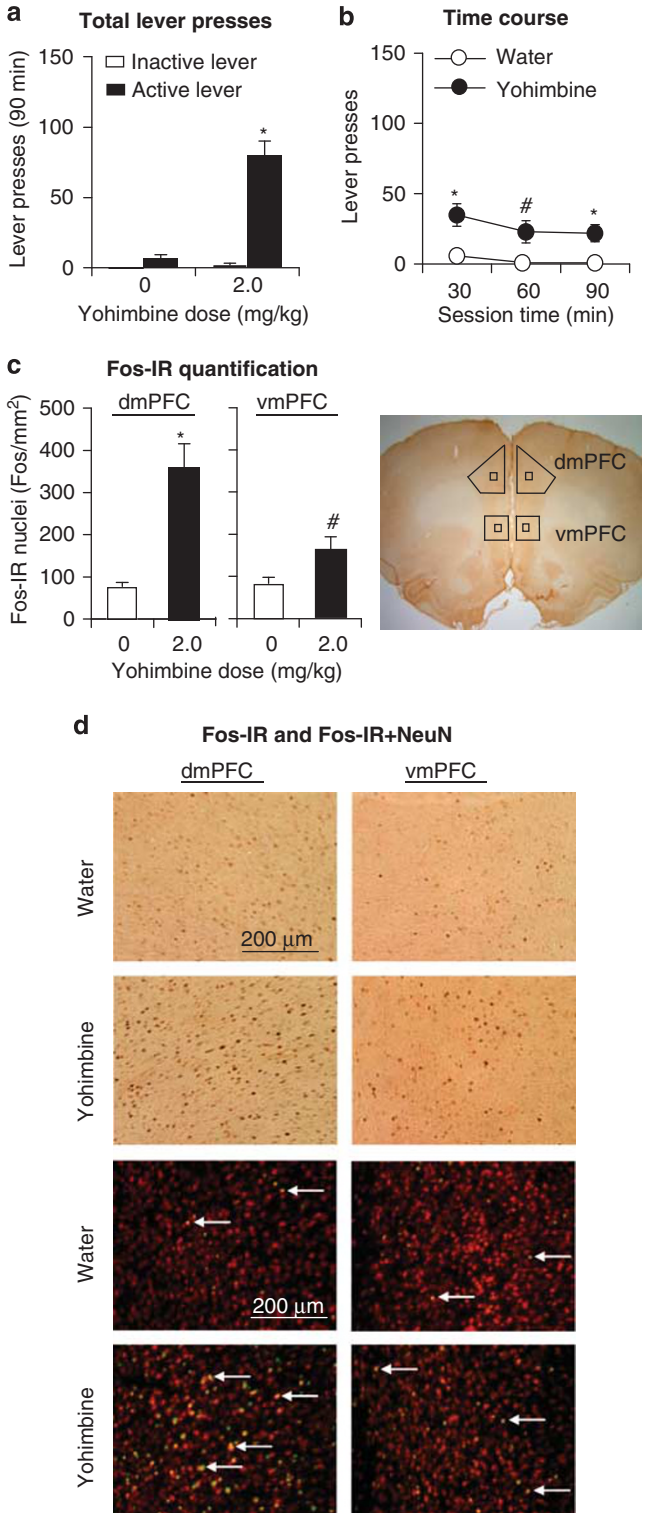


Figure 2 Effect of systemic injections of yohimbine (2 mg/kg) or water (vehicle) on reinstatement of lever responding and Fos induction in the dorsal and ventral mPFCs. (a, b) Mean ± SEM number of active and inactive lever presses after systemic injections of water or yohimbine (total per 90-min and 30-min time course ($n = 7$ per group). (c) Mean ± SEM number of Fos-IR nuclei (per mm^2) in the dorsal (left) and ventral (right) mPFCs 2 h after systemic injections of water or yohimbine. (d) Representative pictures of Fos-IR nuclei or Fos-NeuN double labeling in the dorsal (left column) and ventral (right column) mPFCs from rats pretreated with water (top row) or yohimbine (bottom row). Different from the water condition, $^{*}p < 0.01$, $^{\#}p < 0.05$.

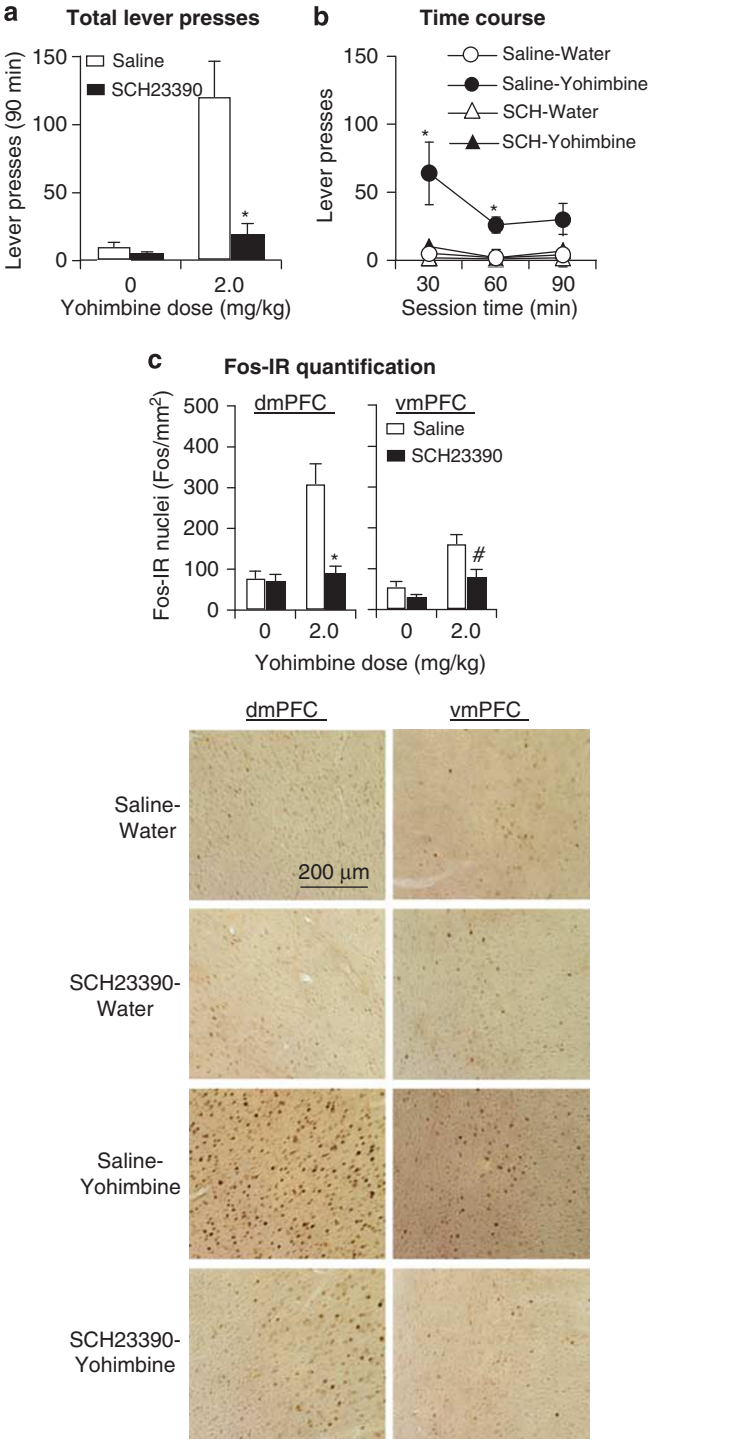


Figure 3 Effect of systemic injections of SCH23390 (10 µg/kg) on yohimbine-induced reinstatement of lever responding and Fos induction in the dorsal and ventral mPFCs. (a, b) Mean ± SEM number of active lever responses after systemic injections of saline or SCH23390, followed by water or yohimbine (total per 90-min and 30-min time course ($n = 6$–7 per group). (c) Mean ± SEM number of Fos-IR nuclei (per mm^2) in the dorsal (left) and ventral (right) mPFCs 2 h after systemic injections of saline or SCH23390, followed by water or yohimbine. (d) Representative pictures of Fos-IR nuclei in the dorsal (left column) and ventral (right column) mPFCs from rats pretreated with saline-water (row 1), SCH23390-water (row 2), saline-yohimbine (row 3), or SCH23390-yohimbine (row 4). Different from the water condition, $^{*}p < 0.01$, $^{\#}p < 0.05$. dmPFC and vmPFC, dorsal and ventral mPFC, respectively.

(3) a modest reduction by SCH23390 of basal Fos expression in the ventral but not in the dorsal mPFC (the 0 dose of the yohimbine condition). Neither yohimbine nor SCH23390 had an effect on inactive lever responding (data not shown). *Post hoc* differences are indicated in Figure 3.

Experiment 2: Effect of Dorsal and Ventral mPFC Injections of SCH23390 on Yohimbine-Induced Reinstatement

Dorsal mPFC. Dorsal mPFC SCH23390 injections decreased yohimbine-induced reinstatement of lever responding (Figure 4). Statistical analysis included the between-subjects factor of SCH23390 dose (0.5 or 1 μg/side) and the within-subjects factors of pretreatment condition (vehicle, SCH23390 (0.5 or 1 μg/side)), and yohimbine dose (0, 2 mg). ANOVA revealed significant effects of yohimbine dose ($F_{1,18} = 58.9$, $p < 0.01$), pretreatment condition ($F_{1,18} = 20.8$, $p < 0.01$), and pretreatment condition × yohimbine dose ($F_{1,18} = 25.7$, $p < 0.01$). This mixed AVOVA also revealed that the effect of SCH23390 dose was not significant ($p > 0.1$), because both the low (0.5 μg/side) and the high (1.0 μg/side) dose decreased yohimbine-induced reinstatement to a similar degree. *Post hoc* differences and the time course of yohimbine-induced reinstatement are indicated in Figure 4. Neither yohimbine nor SCH23390 had an effect on inactive lever responding (data not shown).

Ventral mPFC. Ventral mPFC SCH23390 injections (1 μg/site) had no effect on yohimbine-induced reinstatement of lever responding (Figure 4). ANOVA revealed significant effects of *y*ohimbine dose ($F_{1,8} = 62.7$, $p < 0.01$) but not of SCH23390 dose or SCH23390 dose × yohimbine dose ($p > 0.1$). Neither yohimbine nor SCH23390 had an effect on inactive lever responding (data not shown).

Experiment 3: Effect of Dorsal mPFC Injections of SCH23390 on Food Self-Administration, and Pellet-Priming- and Cue-Induced Reinstatement

Experiment 3a: pellet self-administration. Injections of SCH23390 in the dorsal mPFC had no effect on pellet intake or timeout responses on the active lever during training (p-values > 0.1; Figure 5).

Experiment 3b: pellet-priming-induced reinstatement. Injections of SCH23390 in the dorsal mPFC decreased pellet-priming-induced reinstatement of active lever responding (Figure 5). Statistical analysis included the within-subjects factors of SCH23390 dose (0 or 1 μg/side) and lever (active, inactive). ANOVA revealed significant effects of SCH23390 dose ($F_{1,9} = 11.2$, $p < 0.01$), lever ($F_{1,9} = 66.1$, $p < 0.01$), and SCH23390 dose × lever ($F_{1,9} = 8.9$, $p < 0.01$). *Post hoc* differences and the time course (60-min intervals) of the effect of SCH23390 on pellet-priming-induced reinstatement are indicated in Figure 5.

Experiment 3c: cue-induced reinstatement. Injections of SCH23390 in the dorsal mPFC had no effect on cue-induced

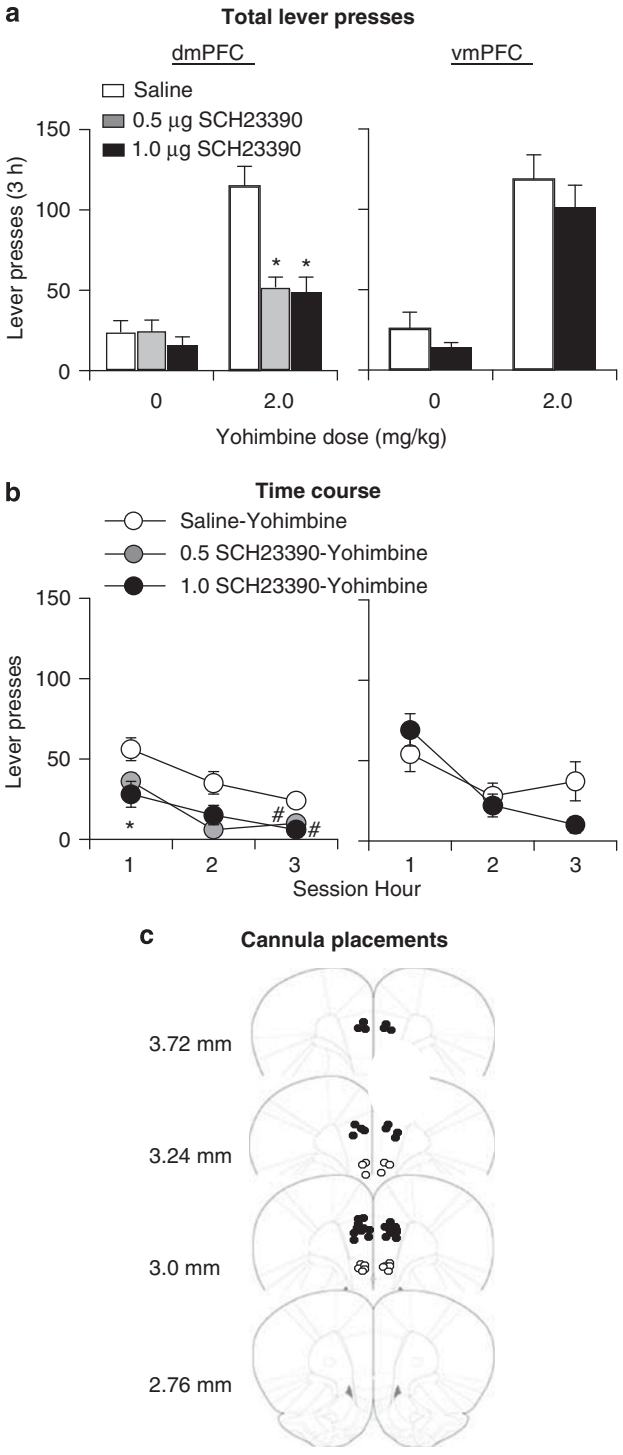
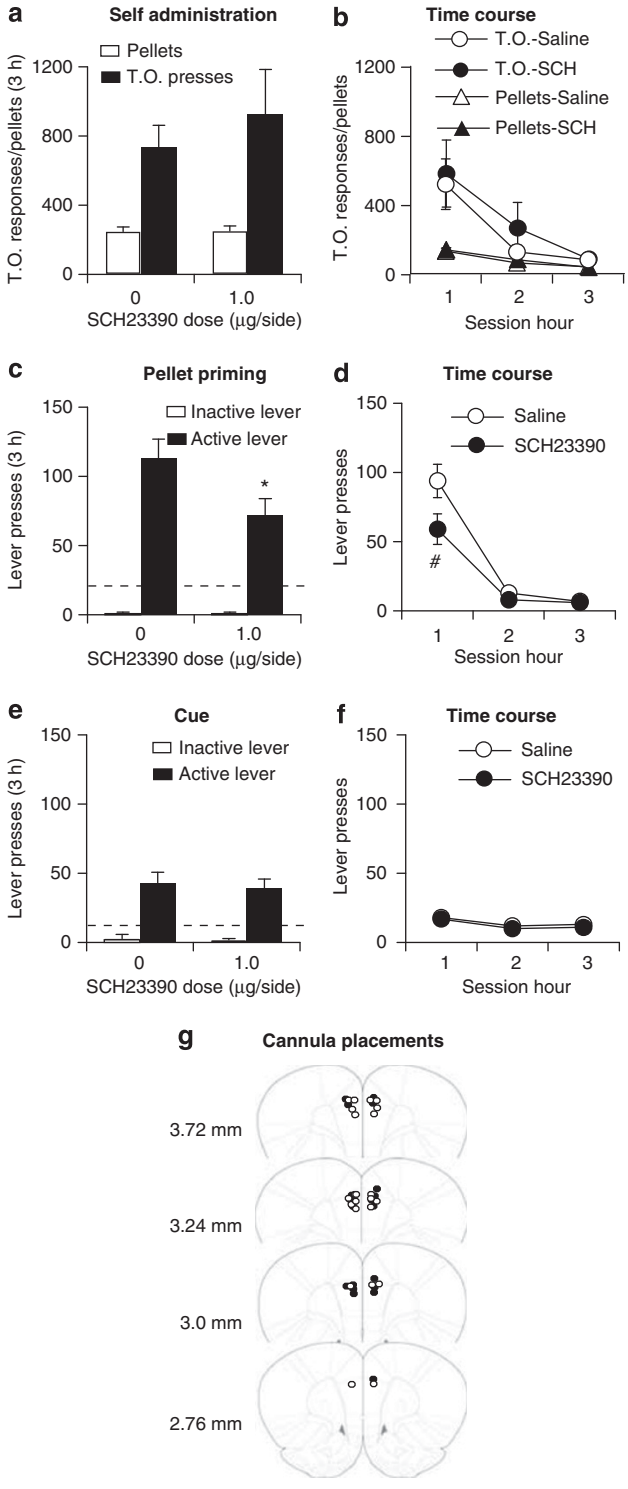


Figure 4 Effect of dorsal or ventral mPFC injections of SCH23390 (0.5 or 1 μg/side) on yohimbine-induced reinstatement. (a, b) Mean ± SEM number of active lever responses after dorsal or ventral mPFC injections of saline or SCH23390, followed by systemic water or yohimbine injections (total per 3-h and 1-h time course; $n = 8$–12 per SCH23390 dose). (c) Approximate placements of the tip of the injectors in the dorsal (closed circles) and ventral (open circles) mPFCs of rats in panels a, b; numbers refer to distance from the bregma (Paxinos and Watson, 2005). Different from the water condition, $^{*}p < 0.01$, $^{\#}p < 0.05$. dmPFC and vmPFC, dorsal and ventral mPFC, respectively.

reinstatement of lever responding (Figure 5). Statistical analysis included the within-subjects factors of SCH23390 dose (0 or 1 μg/side) and lever (active, inactive). ANOVA revealed significant effects of lever ($F_{1,9} = 48.0$, $p < 0.01$), but not of SCH23390 dose or SCH23390 dose × lever ($p > 0.1$).



Experiment 4: Effect of dorsal mPFC Injections of the 5-HT2c Agonist MK212 and the 5-HT2a Antagonist M100907 on Yohimbine-Induced Reinstatement

Experiment 4a: MK212. Dorsal mPFC injections of the 5-HT2c agonist MK212 had no effect on yohimbine-induced reinstatement of active lever responding. The mean ± SEM number of active and inactive lever presses for the last 3 extinction sessions before the first test was 15.0 ± 1.6 and 1.8 ± 1.4, respectively. During tests for yohimbine-induced reinstatement, the number of active and inactive lever presses under vehicle and MK212 pretreatment conditions was 125.1 ± 21.4 and 117.0 ± 23.0 vs 27.2 ± 9.5 and 32.1 ± 15.0, respectively. Statistical analysis included the within-subject factors of MK212 dose (0 or 0.1 μg/side) and lever (active, inactive). ANOVA revealed significant effects of lever ($F_{1,9} = 22.9$, $p < 0.01$), but not of MK212 dose or MK212 dose × lever ($p > 0.1$). The reasons for increased inactive lever responding after yohimbine injections, which was not observed in experiments 1–2 and in our previous work, are unknown.

Experiment 4b: M100907. Dorsal mPFC injections of the 5-HT2a antagonist M100907 had no effect on yohimbine-induced reinstatement of active lever responding. The mean ± SEM number of active and inactive lever presses for the last 3 extinction sessions before the first test was 16 ± 2 and 1 ± 1, respectively. During tests for yohimbine-induced reinstatement, the number of active and inactive lever presses under vehicle and M100907 pretreatment conditions was 86 ± 12 and 90 ± 8 vs 18 ± 10 and 8 ± 3, respectively. Statistical analysis included the within-subject factors of M100907 dose (0 or 0.3 μg/side) and lever (active, inactive). ANOVA revealed significant effects of lever ($F_{1,8} = 76.7$, $p < 0.01$), but not of M100907 dose or M100907 dose × lever ($p > 0.1$).

DISCUSSION

We studied the role of D1-family receptors in the reinstatement of high-fat food seeking induced by the anxiogenic drug yohimbine, which induces stress-like

Figure 5 Effect of dorsal mPFC injections of SCH23390 (1.0 μg/side) on food pellet self-administration, and pellet-priming- and cue-induced reinstatement. (a, b) Pellet self-administration: Mean ± SEM number of 35% fat pellets earned and timeout responses after dorsal mPFC injections of saline or SCH23390 (total per 3-h and 1-h time course, $n = 10$). (c, d) Pellet-priming-induced reinstatement: Mean ± SEM number of active and inactive lever responses after dorsal mPFC injections of saline or SCH23390 during testing ($n = 10$). The priming manipulation was noncontingent delivery of three pellets, every 20 s, at the onset of the test session. (e, f) Cue-induced reinstatement: Mean ± SEM number of active and inactive lever responses after dorsal mPFC injections of saline or SCH23390 during testing ($n = 10$). During testing, lever presses led to contingent presentations of the tone-light cue that was paired with pellet delivery during the training phase and was omitted during the extinction phase. (g) Approximate placements of the tip of the injectors in the dorsal mPFC for the data in panels a–d (closed circles) and in panels e and f (open circles); numbers refer to distance from the bregma (Paxinos and Watson, 2005). Different from the saline condition, $^{*}p < 0.01$, $^{\#}p < 0.05$. Dashed horizontal line: mean number of active lever presses during the last three extinction sessions before testing.

responses in both humans and nonhumans (Redmond and Huang, 1979). Yohimbine-induced reinstatement was associated with strong Fos (a neuronal activity marker) induction in the dorsal mPFC and weaker Fos induction in the ventral mPFC. The effect of yohimbine on reinstatement of food seeking and mPFC Fos induction was blocked by systemic SCH23390 injections, suggesting a role of mPFC D1-family receptors in yohimbine-induced reinstatement. This possibility was confirmed by demonstrating that dorsal but not ventral mPFC SCH23390 injections attenuated this reinstatement. These data established a causal role of dorsal mPFC D1-family receptors in yohimbine-induced reinstatement of food seeking.

In a follow-up experiment on the behavioral specificity of mPFC SCH23390 injections on reinstatement of food seeking, we found that D1-family receptor blockade decreased pellet-priming-induced reinstatement but had no effect on cue-induced reinstatement or on ongoing pellet self-administration. These data suggest that mechanisms of pellet-priming-induced reinstatement and cue-induced reinstatement are not identical, and support the notion that mechanisms of ongoing food intake and relapse to food seeking are dissociable (Nair *et al*, 2009a). In another follow-up experiment on the pharmacological specificity of SCH23390, we found that MK212 or M100907 dorsal mPFC injections had no effect on yohimbine-induced reinstatement. These data suggest that SCH23390 effects on yohimbine-induced reinstatement are not due to its actions on 5-HT2c or 5-HT2a receptors (Millan *et al*, 2001; Neumeyer *et al*, 2003).

Role of mPFC Dopamine D1-Family Receptors in Reinstatement of Food Seeking

A main finding in our study is that dorsal mPFC SCH23390 injections decreased reinstatement of food seeking induced by both yohimbine and pellet priming. It is unlikely that these effects are due to nonspecific disruption of lever responding, because SCH23390 injections had no effect on a much higher response rate for the food pellets during training (Figure 5a). Thus, our data suggest a general role of dorsal mPFC dopamine D1-family receptors in relapse to food seeking, as assessed in the reinstatement procedure. One caveat of this conclusion is that dorsal mPFC SCH23390 had no effect on discrete-cue-induced reinstatement. However, these negative data should be interpreted with caution, because the magnitude of responding in the discrete-cue-induced reinstatement tests was much lower than in the yohimbine- or pellet-priming-induced reinstatement tests. Thus, our cue-induced reinstatement manipulation may not be sufficiently sensitive to reliably detect effects of pharmacological manipulations on this reinstatement.

It is also unknown whether dorsal mPFC D1-family receptors have a role in other forms of relapse to food seeking, including the time-dependent increases in cue-induced reinstatement (incubation of food seeking) (Grimm *et al*, 2002, 2005), or reinstatement induced by contextual (Bossert *et al*, 2006; Hamlin *et al*, 2006) or discriminative (Baptista *et al*, 2004) food cues. In this regard, studies using drug rewards indicate that the mechanisms of reinstatement induced by discrete vs contextual drug cues only partially overlap (Crombag *et al*, 2008; Feltenstein and See, 2008). In addition, we found that reversibly inactivating the ventral but not the dorsal mPFC with a muscimol + baclofen mixture decreases the time-dependent increases in cue-induced cocaine seeking, as assessed in a single extinction test (Koya *et al*, 2009).

Our finding on the effect of dorsal mPFC SCH23390 injections on pellet-priming-induced reinstatement of food seeking is consistent with that of Sun and Rebec (2005). However, these findings are different from those of McFarland and Kalivas (2001) who reported that dorsal mPFC inactivation with muscimol + baclofen had no effect on pellet-priming-induced reinstatement. In interpreting these different findings it should be noted that there are several other examples in drug reinstatement studies in which the effect of pharmacological antagonism of D1-family receptors does not mimic the behavioral effects of muscimol + baclofen inactivation (Bossert *et al*, 2005). For example, McFarland and Kalivas (2001) reported that muscimol + baclofen inactivation of the nucleus accumbens core, but not shell, attenuates cocaine-priming-induced reinstatement. In contrast, Anderson *et al* (2003) reported that SCH23390 injections into the nucleus accumbens shell, but not core, attenuate this reinstatement.

Mechanisms Underlying the Role of Dorsal mPFC Dopamine in Yohimbine-Induced Reinstatement: A Neuropsychological Perspective

A question for future research is the identification of the neuropsychological processes or mechanisms underlying the role of dorsal mPFC dopamine in yohimbine-induced reinstatement in particular and more generally in stress-induced reinstatement. Below, we speculate on the putative role of four potential mechanisms related to mPFC's role in response to satiety signals, behavioral inhibition, stress responses, and reward seeking.

Food Satiety. In food-restricted rats, exposure to palatable food increases mPFC dopamine release. This effect is reduced by selective satiety (a brief previous exposure to the same but not different palatable food before a second exposure to the food), suggesting a role of dorsal mPFC dopamine in food satiety mechanisms (Ahn and Phillips, 1999, 2002). However, it is unlikely that the modulation of SCH23390 of mPFC-dependent satiety mechanisms mediates its effect on yohimbine-induced reinstatement, because dorsal mPFC SCH23390 injections had no effect on ongoing pellet self-administration.

Behavioral Inhibition. We previously suggested that stress may provoke relapse by interfering with a putative behavioral inhibition system the function of which is to stop ongoing activity when reinforcers are not available (Highfield *et al*, 2000b), as, eg, during extinction training (Bouton and Swartzentruber, 1991; Gray, 1987; Pavlov, 1927). Some support for this hypothesis is the finding that repeated exposure to footshock or yohimbine during the extinction phase increases resistance to extinction of drug seeking (Highfield *et al*, 2000a; Kupferschmidt *et al*, 2009). The 'behavioral inhibition' hypothesis might be relevant to the present findings, because there is evidence that mPFC

lesions interfere with behavioral responses in learning tasks assessing behavioral inhibition (Kolb, 1984). However, this hypothesis is not supported by the present data or previous data mentioned above (Kalivas and McFarland, 2003), because a main prediction of the behavioral inhibition hypothesis would be that interference with mPFC function in the absence of stress exposure would reinstate reward seeking, mimicking the effect of stressors.

Stress Responses. Dorsal mPFC SCH23390 injections may decrease yohimbine-induced reinstatement because they decrease the manifestation of physiological and psychological stress states induced by yohimbine. In this regard, tail-pinch-stress-induced secretion of the stress hormone corticosterone (Selye, 1936) is correlated with tail-pinch-induced increases in mPFC dopamine levels (Sullivan and Gratton, 1998). In addition, dorsal mPFC lesions decrease stress-induced corticosterone secretion and gastric ulcer development (Sullivan and Gratton, 1999). Yohimbine increases corticosterone secretion in both rats (Marinelli *et al*, 2007) and monkeys (Lee *et al*, 2004). However, it is unlikely that this effect and its potential modulation by dorsal mPFC dopamine are involved in yohimbine-induced reinstatement because the effects of yohimbine on corticosterone secretion can be dissociated pharmacologically: the CRF1 receptor antagonist antalarmin blocks yohimbine-induced reinstatement of alcohol seeking but has no effect on yohimbine-induced corticosterone secretion (Marinelli *et al*, 2007). In addition, stress-induced corticosterone secretion does not mediate stress-induced reinstatement of drug seeking (Erb *et al*, 1998; Shaham *et al*, 1997), a phenomenon critically dependent on dorsal mPFC dopamine (Capriles *et al*, 2003; McFarland *et al*, 2004). However, it is possible that blockade of D1-family receptors ameliorates the impact of stress-related psychological processes that are dependent on intact mPFC (eg, increasing the perception of control over stress experience; Maier and Watkins, 2005), which in turn leads to decreased stress-induced reinstatement.

Reward Seeking. The effect of dorsal mPFC SCH23390 injections on yohimbine-induced reinstatement might be due to interference with the normal functioning of the putative 'final common pathway' glutamatergic projection from the dorsal mPFC to the accumbens core that mediates reinstatement of cocaine seeking induced by drug priming, discrete cues, and footshock stress; activation of this pathway is critically dependent on mPFC dopamine transmission (Feltenstein and See, 2008; Kalivas and McFarland, 2003). The 'final common pathway' hypothesis can account for similar effects of SCH23390 injections on both yohimbine- and pellet-priming-induced reinstatement. However, a prediction of this hypothesis is that SCH23390 injections should also attenuate discrete-cue-induced reinstatement, which we did not observe.

Concluding Remarks

Since 2004 (Lee *et al*, 2004; Shepard *et al*, 2004), yohimbine has been used as a pharmacological stressor in many reinstatement studies with food and drug rewards (Feltenstein and See, 2008; Nair *et al*, 2009a), but the brain sites involved in this effect have not been identified. In this study, we demonstrate a critical role of dorsal but not ventral mPFC dopamine D1-family receptors in yohimbine-induced reinstatement, and also provide evidence for the role of these receptors in pellet-priming-induced reinstatement. The results of this study and our previous work on relapse to food seeking during dieting may have implications for medication development (Nair *et al*, 2009a).

Historically, dietary treatments have primarily been developed based on their effects on physiological mechanisms that regulate ongoing food intake or food metabolism (Bray and Greenway, 2007). Yet, it has been known for many years that the physiological states of hunger and satiety are often dissociable from human feeding behaviors. Instead, these behaviors are to a significant extent under the control of external stimuli, such as stress and food cues (Schachter, 1968, 1974). A common theme in our studies on reinstatement of food seeking is that our pharmacological manipulations have dissociable effects on reinstatement of food seeking vs food intake during self-administration training (Nair *et al*, 2009b). Thus, the use of the reinstatement procedure allows for the identification of pharmacological agents that can prevent relapse to food by targeting the impact of stress or food cues on food-seeking behaviors in humans. These putative potential medications may not reach clinical development if the targeted preclinical outcome is reduction of ongoing food intake in rodents.

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DISCLOSURE

The authors state no conflict of interest.

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