Published in final edited form as:

Addict Biol. 2016 November ; 21(6): 1140–1150. doi:10.1111/adb.12287.

Effects of repeated yohimbine administration on reinstatement of palatable food seeking: involvement of dopamine D₁-like receptors and food-associated cues

Kevin T. Ball^{*}, Leah Miller, Christopher Sullivan, Ashleigh Wells, Olivia Best, Brittany Cavanaugh, Taylor Copus, Nathan Corrigan, Shaina Hawkins, Krista Kobbe, Ashley Schoener, Johnathan Steiger, and Lauren Vieweg

Department of Psychology, Bloomsburg University of Pennsylvania, 400 E. 2nd St., Bloomsburg, PA, 17815, USA

Abstract

Acute exposure to the pharmacological stressor yohimbine induces relapse to both food and drug seeking in a rat model. However, no systematic studies on the effects of *chronic* stress on relapse have been conducted. Because chronic stress causes changes in dopamine D₁-like receptor-mediated transmission in prefrontal cortex (a relapse node), we tested the hypothesis that chronic exposure to stress increases vulnerability to relapse via dopamine-mediated mechanisms. Additionally, to determine the role of food-conditioned cues in reinstatement of food seeking, we made discrete food-paired cues either available (CS Present) or not available (CS Absent) during extinction and reinstatement testing. Rats responded for palatable food reinforcers in daily 3-hr sessions, and the behavior was extinguished. To model chronic stress, rats were injected daily with yohimbine (0.0, 2.5, or 5.0 mg/kg; i.p.) during the first 7 days of extinction. Injections were combined with SCH-23390 (0.0, 5.0, or 10.0 µg/kg; i.p.), a D₁-like receptor antagonist. Rats were then tested for reinstatement of food seeking triggered by acute yohimbine (0.0, 1.0, or 2.0 mg/kg; i.p.) and pellet priming. Rats treated previously with chronic yohimbine displayed increased responding following acute yohimbine priming relative to non-chronically stressed rats, but in the CS Absent condition only. Conversely, the lower dose of chronic yohimbine caused an increase in pellet-primed reinstatement, but this effect was more pronounced in the CS Present condition. Importantly, SCH-23390 combined with repeated yohimbine injections attenuated these effects. Thus, chronic stress may increase vulnerability to relapse under specific circumstances via a dopamine D₁-like receptor mediated mechanism.

Keywords

CUE; DOPAMINE; REINSTATEMENT; RELAPSE; STRESS; YOHIMBINE

^{*}Please address correspondence to: Kevin T. Ball, Department of Psychology, Bloomsburg University of Pennsylvania, 400 E. 2nd St., Bloomsburg, PA 17815, TEL: 570-389-4513, FAX: 570-389-2019, kball@bloomu.edu.

Authors Contribution: KTB was responsible for the study concept and design and drafted the manuscript. KTB, LM, CS, AW, OB, BC, TC, NC, SH, KK, AS, JS, and LV performed experiments and analyzed and interpreted data. All authors approved the final version for publication.

Conflict of Interest: None declared.

INTRODUCTION

A growing literature suggests that excessive and compulsive eating has much in common with drug addiction, including behavioral phenotypes and underlying physiological and neuroanatomical mechanisms (Frascella et al., 2010; Gearhardt et al., 2011; Kenny, 2011; Volkow et al., 2012). Although it is clear that eating disorders are enormously heterogeneous, and that the addiction model has its limitations (Epstein and Shaham, 2010; Ziauddeen et al., 2012), it is nonetheless relevant that, similar to abstinence among drug addicts, dieting among individuals attempting to reduce their palatable food intake is associated with high rates of relapse (Kramer et al., 1989; Skender et al., 1996). Relapse may be triggered by a variety of stimuli, but it is often triggered by exposure to an acute stressor (Grilo et al., 1989; Torres and Nowson, 2007).

In recent years, stress-induced relapse to palatable food seeking has been investigated using a version of the reinstatement model originally designed to study drug relapse (Calu et al., 2014; Nair et al., 2009). In the modified model, the pharmacological stressor yohimbine is used to reinstate extinguished food-seeking behavior in animals with a history of operant responding for highly palatable food reinforcers. Yohimbine is an α -2 adrenoceptor antagonist that increases brain noradrenergic neuronal firing and release (Abercrombie et al., 1988; Aghajanian and VanderMaelen, 1982). Both humans (Bremner et al., 1996b; Charney et al., 1987; Southwick et al., 1999; Vasa et al., 2009) and laboratory animals (Banihashemi and Rinaman, 2006; Bijlsma et al., 2010; Bremner et al., 1996a; File, 1986; Johnston and File, 1989; Lee et al., 2004) display similar physiological and behavioral responses to yohimbine, including activation of the hypothalamic-pituitary-adrenal axis and anxiety-like behaviors. Moreover, yohimbine induces drug craving and enhances cue-induced craving in human drug addicts (Moran-Santa Maria et al., 2014; Stine et al., 2002; Umhau et al., 2011).

Although studies using the reinstatement model have provided valuable information regarding the neuropharmacological mechanisms of acute stress-induced relapse to both food and drug seeking, no systematic studies on the effects of repeated or chronic stress on relapse have been conducted. Because humans are exposed to multiple stressors, both acutely and chronically, it is important to understand the role of chronic stress in relapse vulnerability, as well as the interaction between chronic stress and acute stress during testing on reinstatement. Indeed, chronic stress induces lasting structural changes in brain regions implicated in relapse, namely medial prefrontal cortex (mPFC) (Cook and Wellman, 2004b; Izquierdo et al., 2006; Radley et al., 2006; Radley et al., 2004)). Given this overlapping circuitry, it is possible that chronic exposure to stress during dietary restriction increases one's vulnerability to relapse. Thus, a main goal of the present study was to test this possibility by adding a chronic (i.e., repeated, daily) stress component during the extinction phase of the reinstatement model.

A second goal was to determine the role of dopamine D₁-like receptors (D₁Rs) in any chronic stress-related effects on food seeking because D₁Rs play a critical role in several types of food-seeking behaviors. For example, yohimbine-induced reinstatement of food seeking is blocked by both systemic and intra-dorsal mPFC injections of the D₁R antagonist SCH-23390 (Nair et al., 2011). Systemic administration of SCH-23390 also blocks discrete

cue-induced reinstatement of food seeking during extinction (Ball et al., 2011) and sucrose seeking after forced abstinence (Grimm et al., 2011). Given that chronic stress has been shown to cause changes in mesocortical dopamine transmission (Moore et al., 2001) and D₁R signaling (Mizoguchi et al., 2008; Mizoguchi et al., 2000), we tested the possibility that blockade of D₁Rs with SCH-23390 during repeated yohimbine administration would attenuate any effects of this treatment on later reinstatement.

A final goal was to investigate the role of extinguished food-conditioned cues in yohimbine- and food-primed reinstatement of food seeking. Thus, some studies have demonstrated that stress- and cocaine-primed reinstatement of cocaine seeking occurs only when responding during reinstatement testing results in presentation of extinguished cocaine-conditioned discrete cues (Shelton and Beardsley, 2005; Shelton et al., 2004). The role of food-conditioned cues in stress- and food-primed reinstatement of food seeking has not been systematically studied. Therefore, we conducted experiments in separate groups of rats in which discrete food-paired cues were either present or absent during extinction and reinstatement testing.

MATERIALS AND METHODS

Subjects and Apparatus

Adult male, Sprague-Dawley rats weighing 250-350 g at the commencement of experiments were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Rats were housed individually under standard laboratory conditions (12-hr light cycle from 7:00 AM to 7:00 PM) with *ad libitum* access to water. Rats were restricted to 16 g/day of standard rat chow (~65% of their daily food intake when freely feeding) during the self-administration phase and to 18-30 g/day during the extinction and reinstatement testing phases (to maintain stable body weight) according to the protocol of Shaham and coworkers (Nair et al., 2011; Pickens et al., 2012). Rats were fed immediately following each daily test session. All testing was conducted between 7:00 AM and 7:00 PM and occurred in standard modular operant conditioning chambers (Coulbourn Instruments, Whitehall, PA) that were housed in sound-attenuating, ventilated cubicles and connected to a PC with the Graphic State software interface system (Coulbourn Instruments). Each chamber is equipped with an active and an inactive response lever. Responses on the inactive lever are recorded, but have no programmed consequences. Chambers also include a house light, a row of multicolored LED cue lamps (above active lever), a tone generator, and a food tray (between the two response levers).

Food Self-Administration

Rats ($n = 77$ and 76 for Experiments 1 and 2, respectively) were trained to press the lever for food reinforcers contingent upon a fixed-ratio (FR)-1 schedule of reinforcement for 14 days in daily 3-hr sessions. Each lever press resulted in delivery of a 45-mg food pellet containing 12.7% fat, 66.7% carbohydrate, and 20.6% protein (Catalogue # 1811155, TestDiet). This pellet type was chosen based on pellet preference tests conducted by Pickens et al. (2012), in which it was determined to be the most preferred pellet. Delivery of the pellet was

accompanied by a tone + flashing cue light conditioned stimulus (CS) presented for 5 s, which was followed by a 20-s time-out period signaled by illumination of the house light.

Extinction and chronic yohimbine administration

Experiment 1: CS Absent—On the day following the last self-administration session, daily 3-hr extinction sessions began and continued for 10 days. During the extinction sessions, responses were recorded but had no programmed consequences (i.e., no CS or pellets). Beginning on the first day of extinction, rats were injected daily with yohimbine (0.0, 2.5, or 5.0 mg/kg $\times$ 7 days; i.p.) approximately 2 hr after extinction sessions. We chose 5.0 mg/kg yohimbine as the highest dose during the chronic stress phase because this dose induces significant anxiogenic effects (Cole et al., 1995), central Fos activation (Myers et al., 2005), and elevation of plasma corticosterone levels (Banihashemi and Rinaman, 2006). Yohimbine was dissolved in distilled water at a concentration of 2.5 or 5.0 mg/ml. To assess dopaminergic involvement in chronic stress effects, chronic yohimbine injections were combined with injections of SCH-23390 (0.0, 5.0, or 10.0 μ g/kg; i.p.), a D₁R antagonist. SCH-23390 was dissolved in physiological saline at a concentration of 5.0 or 10.0 μ g/ml, and the doses are based on previous research (Hamlin et al., 2006; Nair et al., 2011). Thus, rats were randomly assigned to one of nine chronic treatment conditions: saline + water, saline + yohimbine (2.5 mg/kg), saline + yohimbine (5.0 mg/kg), SCH-23390 (5.0 μ g/kg) + water, SCH-23390 (5.0 μ g/kg) + yohimbine (2.5 mg/kg), SCH-23390 (5.0 μ g/kg) + yohimbine (5.0 mg/kg), SCH-23390 (10.0 μ g/kg) + water, SCH-23390 (10.0 μ g/kg) + yohimbine (2.5 mg/kg), or SCH-23390 (10.0 μ g/kg) + yohimbine (5.0 mg/kg) (n = 6-12/ treatment condition). Rats were returned to their home cages immediately following injections.

Experiment 2: CS Present—A separate group of rats underwent the exact procedures described in Experiment 1, except that presses on the active lever resulted in presentations of the CS during extinction sessions.

Acute Yohimbine-Induced Reinstatement Tests

Rats in both Experiments 1 and 2 continued to be tested under their respective extinction conditions (CS Absent or CS Present) for 5 more days, during which yohimbine-induced reinstatement testing occurred using a repeated-measures design. Thus, each rat received injections of all three doses of yohimbine (0.0, 1.0, and 2.0 mg/kg; i.p.) 30 min prior to the commencement of sessions on 3 different days during reinstatement testing (Days 1, 3, and 5). Doses were given in a counterbalanced order based on active lever responding during the last three days of food self-administration. On Days 2 and 4, no injections were administered. The doses of yohimbine used for reinstatement testing are based on previous research (Ghitza et al., 2006; Nair et al., 2006; Shepard et al., 2004).

Pellet Priming-Induced Reinstatement Tests

Following acute yohimbine-induced reinstatement testing rats were tested under counterbalanced conditions for pellet priming-induced reinstatement. On separate days each rat was tested under extinction conditions (CS Absent and CS Present for Experiments 1 and 2, respectively), except that they received zero (no pellet) or four non-contingent pellets

within the first min of the session. The test sessions were separated by 2 days during which regular extinction sessions occurred.

Statistical Analyses

Total number of lever responses/3-h session were analyzed separately for Experiments 1 and 2 using mixed factorial ANOVAs. The between-subjects factors were chronic yohimbine dose and chronic SCH-23390 dose. The within-subjects factors were day (for extinction training), acute yohimbine dose (for yohimbine-induced reinstatement testing), and number of pellets (for pellet priming-induced reinstatement testing). In addition, for yohimbine- and pellet priming-induced reinstatement data, lever (active and inactive) was included as a within-subjects factor. To verify the chronic stress manipulation, amount of daily food ration required to maintain weight across extinction days was determined for each chronic treatment group and was calculated as g/100 g body weight. Food ration data were analyzed with a mixed factorial ANOVA (chronic yohimbine dose $\times$ chronic SCH-23390 dose $\times$ day). All ANOVAs were followed by Bonferroni post-tests.

RESULTS

Experiment 1: CS Absent

Self-Administration and Extinction—All rats demonstrated reliable pellet self-administration (see Fig. 1a). During subsequent extinction training, lever pressing decreased over days [main effect of day; $F(9, 603) = 206.89, p < .001$], with a similar time course for all chronic treatment groups (see Fig. 1b). As shown in Fig. 1c, body weight increased across the 2 weeks of self-administration and remained relatively stable during the extinction and reinstatement phases. Relatively stable body weight during the extinction and reinstatement phases was maintained in all animals by individually adjusting their daily food ration during this time. As shown in Fig. 1d, more food/100 g body weight was necessary during extinction in rats receiving repeated yohimbine injections [main effect of chronic yohimbine dose; $F(2, 67) = 24.37, p < .001$]. There also was a significant chronic yohimbine dose $\times$ chronic SCH dose $\times$ Day interaction [$F(32, 536) = 2.19, p < .001$], indicating that the effect of yohimbine stress on body weight was attenuated when SCH-23390 was co-administered. Finally, there was a significant main effect of extinction day and a significant extinction day $\times$ chronic yohimbine dose interaction [$F(8, 536) = 57.47, p < .001$ and $F(16, 536) = 6.90, p < .001$, respectively] with regard to daily food ration.

Acute Yohimbine-Induced Reinstatement Tests—Acute yohimbine injections increased responding [main effect of acute yohimbine dose; $F(2, 134) = 28.16, p < .001$]. Relative to active-lever responding, inactive-lever responding was low [main effect of lever; $F(1, 67) = 71.61, p < .001$]. There also was an acute yohimbine dose $\times$ lever interaction [$F(2, 134) = 13.00, p < .001$], in that increases in responding as a function of acute yohimbine dose were greater on the active lever relative to the inactive lever. Although baseline responding (0.0 mg/kg yohimbine prime) was similar across all chronic treatment groups, Bonferroni post-tests indicated that responding increased significantly with acute yohimbine injections only in rats treated previously with chronic yohimbine (see Fig. 2). Moreover, this

effect was attenuated in rats receiving chronic co-administration of SCH-23390 and yohimbine.

Pellet Priming-Induced Reinstatement Tests—Active- and inactive-lever responses during pellet-primed reinstatement tests are shown in Figs. 3a and 3b, respectively. Relative to yohimbine-induced reinstatement, pellet priming-induced reinstatement was less robust; nonetheless, there was a significant main effect of pellet priming, $F(1, 67) = 31.34, p < .001$. There also was a main effect of lever [$F(1, 67) = 138.26, p < .001$] and a pellet priming $\times$ lever interaction [$F(1, 67) = 36.25, p < .001$]. There was a main effect of chronic yohimbine dose [$F(2, 67) = 5.41, p < .01$] and a chronic yohimbine dose $\times$ lever interaction [$F(2, 67) = 6.50, p < .01$], indicating that pellet priming-induced active lever pressing increased to a greater extent in rats chronically treated with 2.5 mg/kg yohimbine relative to the other chronic treatment groups; this was particularly evident in the SCH-23390 (5 μ g/kg) + yohimbine (2.5 mg/kg) chronic treatment group.

Experiment 2: CS Present

Self-Administration and Extinction—As in Experiment 1, all rats demonstrated reliable pellet self-administration (see Fig. 4a), and lever pressing decreased over days during subsequent extinction training [main effect of day; $F(9, 612) = 173.76, p < .001$]. In contrast to Experiment 1, however, overall extinction responding differed depending on chronic treatment condition. ANOVA revealed a significant main effect of chronic yohimbine dose [$F(2, 68) = 5.03, p < .01$] and a significant main effect of chronic SCH-23390 dose [$F(2, 68) = 5.65, p < .01$]. Thus, rats in the saline + yohimbine (5.0 mg/kg) chronic treatment group displayed the lowest extinction responding overall, and this effect was attenuated in rats given daily co-administration of SCH-23390 and yohimbine (see Fig. 4b). The SCH-23390 (10 μ g/kg) + water chronic treatment group displayed the highest levels of extinction responding overall. As in Experiment 1, body weight increased across the 2 weeks of self-administration and remained relatively stable during the extinction and reinstatement phases (see Fig. 4c). Also as in Experiment 1, more food/100 g body weight was necessary during extinction in rats receiving repeated yohimbine injections [main effect of chronic yohimbine dose; $F(2, 68) = 19.53, p < .001$; see Fig. 4d]. There also was a significant main effect of chronic SCH-23390 dose [$F(2, 68) = 3.21, p < .05$] and a significant SCH-23390 dose $\times$ chronic yohimbine dose interaction [$F(4, 68) = 3.18, p < .05$], which reflects an attenuated effect of yohimbine on body weight when SCH-23390 was co-administered. Finally, there was a significant main effect of extinction day [$F(8, 544) = 32.40, p < .001$] and significant extinction day $\times$ chronic yohimbine dose and extinction day $\times$ SCH-23390 dose interactions [$F(16, 544) = 7.04, p < .001$ and $F(16, 544) = 1.97, p < .05$, respectively] with regard to daily food ration.

Acute Yohimbine-Induced Reinstatement Tests—Acute yohimbine injections dose-dependently increased active-lever responding in all chronic treatment groups [main effect of acute yohimbine dose; $F(2, 136) = 62.05, p < .001$]. Inactive-lever responding was low [main effect of lever; $F(1, 68) = 168.36, p < .001$], and there was an acute yohimbine dose $\times$ lever interaction [$F(2, 136) = 37.38, p < .001$]. Bonferroni post-tests indicated that acute yohimbine-induced responding was reduced in rats treated previously with the highest dose

of chronic yohimbine and that this effect was reversed in rats receiving chronic co-administration of the highest dose of SCH-23390 (see Fig. 5).

Pellet-Primed Reinstatement Tests—There were significant main effects of pellet priming and lever [$F(1, 68) = 60.59, p < .001$ and $F(1, 68) = 301.39, p < .001$, respectively] and a pellet priming $\times$ lever interaction, $F(1, 68) = 77.94, p < .001$. There was a main effect of chronic yohimbine dose [$F(2, 68) = 3.21, p < .05$] and several interactions with chronic yohimbine dose: chronic yohimbine dose $\times$ priming [$F(2, 68) = 5.83, p < .01$], chronic yohimbine dose $\times$ SCH-23390 dose $\times$ lever [$F(4, 68) = 2.77, p < .05$], and chronic yohimbine dose $\times$ priming $\times$ lever [$F(2, 68) = 11.29, p < .001$]. Thus, as shown in Fig. 6, pellet priming caused an increase in active lever responding in rats treated previously with 2.5 mg/kg of chronic yohimbine, and this effect was attenuated in rats receiving chronic co-administration of the highest dose of SCH-23390.

DISCUSSION

We investigated the effects of repeated yohimbine stress (2.5 or 5.0 mg/kg/day for 7 days) on subsequent reinstatement of extinguished palatable food seeking primed by acute yohimbine (1.0 and 2.0 mg/kg) or food priming. We found that rats treated previously with chronic yohimbine displayed increased responding following acute yohimbine priming relative to non-chronically stressed rats, but in the CS Absent condition only. Conversely, the lower dose of chronic yohimbine caused an increase in pellet-primed reinstatement, but this effect was more pronounced in the CS Present condition. Importantly, SCH-23390 combined with repeated yohimbine injections attenuated these effects.

Chronic Yohimbine Administration: Comparison to Other Forms of Chronic Stress

In the present study, daily treatment with yohimbine resulted in decreased ability to gain body weight as indicated by a significant increase in daily food ration necessary to maintain body weight. This is consistent with the effects of repeated exposure to non-pharmacological stressors, such as restraint and immobilization stress, (e.g., (Baran et al., 2009; Cook and Wellman, 2004a)). Interestingly, we found that when the D₁R antagonist SCH-23390 was combined with repeated yohimbine administration the effect on body weight was attenuated. This parallels findings from a recent study by Lin et al. (2014) showing that intra-prelimbic infusions of SCH-23390 during repeated restraint stress (3 h/day $\times$ 10 days) reduced several indices of chronic stress, including attenuation of weight gain, increases in adrenal weight, and dendritic retraction in prelimbic cortex. Thus, yohimbine appears to have induced a form of stress that is similar in some ways to that of other, non-pharmacological, stressors.

Role of D₁Rs in Chronic Stress Effects on Reinstatement

Rats that received prior chronic saline + yohimbine injections in Experiment 1 displayed significant increases in active lever pressing following acute yohimbine priming. Rats that did not receive prior yohimbine injections (saline + water and SCH + water groups) showed only a very modest increase in lever pressing at both doses of acute yohimbine. Importantly, the potentiating effect of prior yohimbine administration on reinstatement was reversed (1.0 mg/kg prime) or attenuated (2.0 mg/kg prime) in rats that had SCH-23390 combined with

the chronic yohimbine injections. This supports the hypothesis that excessive dopamine D₁R stimulation due to repeated yohimbine administration contributed to the observed potentiation of acute stress-induced reinstatement that we observed in Experiment 1.

Chronic stress reduces the number of active dopamine cells in ventral tegmental area (Moore et al., 2001), resulting in reduced baseline dopamine levels in mPFC (Gresch et al., 1994; Mizoguchi et al., 2008; Mizoguchi et al., 2000; Ramkumar et al., 2012) and a compensatory increase in D₁Rs in mPFC (Mizoguchi et al., 2000). Given that yohimbine-induced reinstatement of food seeking requires activation of D₁Rs in mPFC (Nair et al., 2011) and that acute stressors, including yohimbine (Chen et al., 2014; Tanda et al., 1996), selectively increase dopamine in the mPFC—an effect that is sensitized in chronically stressed animals (Di Chiara et al., 1999; Gresch et al., 1994)—we speculate that the enhanced yohimbine-induced responding observed in saline + yohimbine rats in Experiment 1 was due to augmented D₁R activation in mPFC relative to controls. Blockade of D₁Rs during yohimbine stress may have attenuated the stress-induced changes in dopaminergic transmission in mPFC.

We hypothesize that repeated antagonism of D₁Rs by SCH-23390 caused a compensatory increase in D₁Rs. Given that acute SCH-23390 administration attenuates responding for discrete food-associated cues (Ball et al., 2011; Grimm et al., 2011), such plasticity would be predicted to enhance responding for food cues. This prediction was supported in the present study in that SCH-23390-treated rats displayed increased responding during extinction training and yohimbine-induced reinstatement tests, but only in Experiment 2, when discrete food cues were available. Although chronic stress also has been shown to cause an increase in D₁Rs in mPFC (Mizoguchi et al., 2000), this is superimposed on a background of reduced basal dopamine levels (Gresch et al., 1994; Mizoguchi et al., 2008; Mizoguchi et al., 2000; Ramkumar et al., 2012), and thus should only invigorate responding under conditions of stimulated dopamine release. This prediction was supported in the present study in that chronically stressed rats showed low baseline levels of responding and increased yohimbine-primed responding in both Experiments 1 and 2.

Interestingly, on all measures for which chronic yohimbine had an effect (i.e., maintenance of weight and reinstatement responding in both experiments and extinction responding in Experiment 2), SCH-23390 when combined with chronic yohimbine reversed or attenuated chronic stress effects, resulting in behavior that looked more like non-stressed rats. Thus, the effect of SCH-23390 might best be described as a normalization of chronic yohimbine-induced changes.

Modulation of Chronic Yohimbine Effects by Discrete Food-Associated Cues

Although numerous studies have shown that yohimbine reliably induces reinstatement of food seeking (for review see (Calu et al., 2014; Nair et al., 2009)), it appears that contingent presentation of the discrete food-paired cues may be necessary for this effect. For example, Lin and Pratt (2014) reported recently that yohimbine (2.0 mg/kg) was ineffective in inducing reinstatement of sugar seeking, but sugar-paired cues that were present during self-administration were omitted during extinction and reinstatement testing as in Experiment 1 in the present study. In drug studies with alcohol, cocaine, and heroin it has been shown that

reinstatement induced by both yohimbine and footshock stress is weaker or completely absent when drug-paired discrete cues are omitted (Banna et al., 2010; Feltenstein and See, 2006; Liu and Weiss, 2002; Shelton and Beardsley, 2005). From a psychological perspective, these results suggest that stress triggers reinstatement by enhancing the saliency or incentive motivational properties of reward-associated cues. Assuming this interpretation, our findings suggest that, specifically in rats with a history of repeated yohimbine exposure, acute yohimbine was able to enhance the incentive motivational properties of either contextual cues or discrete, but less salient, cues that could not be omitted (e.g., clicking of the response lever).

An alternative interpretation is that in the chronically stressed animals, yohimbine, per se, was able to increase the motivation to seek food. This interpretation is supported by the fact that the presence of discrete food-associated cues had little effect on yohimbine-induced reinstatement in the saline + yohimbine (5.0 mg/kg) group, that is, responding in this group increased negligibly when cues were available [mean ($\pm$ SEM) responses/3 hr for 1.0 and 2.0 mg/kg yohimbine prime = 65.91 (12.54) and 81.45 (27.97) and = 70 (9.69) and 85.92 (12.98) for Experiments 1 and 2, respectively]. This stands in stark contrast to the effect of CS availability in the other chronic treatment groups, in which yohimbine-induced responding increased greatly (sometimes doubling or tripling) when cues were available. Although these data appear to be consistent with the hypothesis that distinct neural mechanisms underlie the relapse-triggering vs. -promoting effects of stress (Mantsch et al., 2014), it is noteworthy that caution should be taken when drawing conclusions from between-experiment comparisons. In the present study, for example, rats displayed large differences in baseline responding between experiments.

It is assumed in reinstatement studies that responding is critically dependent on primary reinforcement history. However, a recent study challenged this assumption by showing that rats with a history of responding for discrete tone + light cues in the absence of any food reinforcement displayed increased responding when primed with yohimbine or methamphetamine injections, and that for both drugs, the magnitude of responding was similar to that of rats with a history of responding for food reinforcers + cues (Chen et al., 2014). These data suggest that reinstatement induced by sympathomimetic drugs may not necessarily reflect reinstatement of responding for the previously delivered food or drug reward per se. Although we cannot rule out this possibility in Experiment 2 in the present study, it is noteworthy that acute yohimbine administration increased lever pressing significantly in Experiment 1 in chronically stressed rats when discrete cues were absent. In addition, whereas higher doses of yohimbine (5.0 mg/kg) cause strong activation of stress response systems (Banihashemi and Rinaman, 2006), and significant anxiogenic effects (Cole et al., 1995), Chen et al. (2014) reported that a dose of yohimbine commonly used in reinstatement studies (2.0 mg/kg) did not induce conditioned place aversion. Thus, whether yohimbine-induced responding in reinstatement studies reflects a stress-induced response is unclear. Nonetheless, based on the present results, it appears that a history of repeated activation of stress response systems (i.e., with 5.0 mg/kg yohimbine) impacts reinstatement induced by acute yohimbine, and that activation of D₁Rs is critical for this effect.

Conclusions

The present results suggest that the effects of chronic yohimbine on subsequent reinstatement are complex, depending on many factors, including dose of yohimbine, presence or absence of food cues, and the stimulus used to induce reinstatement. Importantly, however, in all cases, we found that the effects of chronic yohimbine were reversed or attenuated in rats receiving combined SCH-23390 and yohimbine injections. Thus, our results support and extend previous findings (Lin et al., 2014; Mizoguchi et al., 2000; Nikiforuk, 2012; Sorg et al., 2004) suggesting dopamine D₁R involvement in a wide range of behavioral, physiological, and anatomical changes induced by chronic stress. From a clinical perspective, our findings suggest that individuals on a dietary treatment plan who also are exposed to chronic stress may be vulnerable to relapse under conditions that would not otherwise promote robust food seeking. In future studies it will be important to determine whether other types of stressors produce a similar pattern of results and whether chronic stress similarly affects reinstatement of drug seeking.

Acknowledgements

This work was supported by the National Institutes of Health (NIDA R15 DA035432 to KTB).

The authors thank Dr. Yavin Shaham for valuable discussions regarding methodological and conceptual issues related to this study.

REFERENCES

- Abercrombie ED, Keller RWJ, Zigmond MJ. Characterization of hippocampal norepinephrine release as measured by microdialysis perfusion: pharmacological and behavioral studies. *Neuroscience*. 1988; 27:897–904. [PubMed: 3252176]
- Aghajanian GK, VanderMaelen CP. alpha 2-adrenoceptor-mediated hyperpolarization of locus coeruleus neurons: intracellular studies in vivo. *Science*. 1982; 215:1394–1396. [PubMed: 6278591]
- Ball KT, Combs T, Beyer D. Opposing roles for dopamine D1- and D2-like receptors in discrete cue-induced reinstatement of food seeking. *Behav Brain Res*. 2011; 222:390–393. [PubMed: 21497172]
- Banihashemi L, Rinaman L. Noradrenergic inputs to the bed nucleus of the stria terminalis and paraventricular nucleus of the hypothalamus underlie hypothalamic-pituitary-adrenal axis but not hypophagic or conditioned avoidance responses to systemic yohimbine. *J Neurosci*. 2006; 26:11442–11453. [PubMed: 17079674]
- Banna KM, Back SE, Do P, See RE. Yohimbine stress potentiates conditioned cue-induced reinstatement of heroin-seeking in rats. *Behav Brain Res*. 2010; 208:144–148. doi: 110.1016/j.bbr.2009.1011.1030. Epub 2009 Nov 1029. [PubMed: 19931573]
- Baran SE, Armstrong CE, Niren DC, Hanna JJ, Conrad CD. Chronic stress and sex differences on the recall of fear conditioning and extinction. *Neurobiol Learn Mem*. 2009; 91:323–332. doi: 310.1016/j.nlm.2008.1011.1005. Epub 2009 Jan 1016. [PubMed: 19073269]
- Bijlsma EY, de Jongh R, Olivier B, Groenink L. Fear-potentiated startle, but not light-enhanced startle, is enhanced by anxiogenic drugs. *Pharmacol Biochem Behav*. 2010; 96:24–31. Epub 2010 Apr 2013. [PubMed: 20394767]
- Bremner JD, Krystal JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. *Synapse*. 1996a; 23:28–38. [PubMed: 8723133]
- Bremner JD, Krystal JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety: II. Clinical studies. *Synapse*. 1996b; 23:39–51. [PubMed: 8723134]
- Calu DJ, Chen Y-W, Kawa AB, Nair SG, Shaham Y. The use of the reinstatement model to study relapse to palatable food seeking during dieting. *Neuropharmacology*. 2014; 76:395–406. doi: 310.1016/j.neuropharm.2013.1004.1030. Epub 2013 May 1016. [PubMed: 23660229]

- Charney DS, Woods SW, Goodman WK, Heninger GR. Neurobiological mechanisms of panic anxiety: biochemical and behavioral correlates of yohimbine-induced panic attacks. *Am J Psychiat*. 1987; 144:1030–1036. [PubMed: 3037926]
- Chen Y-W, Fiscella KA, Bacharach SZ, Tanda G, Shaham Y, Calu DJ. Effect of yohimbine on reinstatement of operant responding in rats is dependent on cue contingency but not food reward history. *Addict Biol*. 2014; 27:12164.
- Cole BJ, Hillmann M, Seidelmann D, Klewer M, Jones GH. Effects of benzodiazepine receptor partial inverse agonists in the elevated plus maze test of anxiety in the rat. *Psychopharmacology (Berl)*. 1995; 121:118–126. [PubMed: 8539336]
- Cook SC, Wellman CL. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol*. 2004a; 60:236–248. [PubMed: 15266654]
- Cook SC, Wellman CL. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol*. 2004b; 60:236–248. [PubMed: 15266654]
- Di Chiara G, Loddo P, Tanda G. Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. *Biol Psychiatry*. 1999; 46:1624–1633. [PubMed: 10624543]
- Epstein DH, Shaham Y. Cheesecake-eating rats and the question of food addiction. *Nat Neurosci*. 2010; 13:529–531. doi: 510.1038/nn0510-1529. [PubMed: 20421898]
- Feltenstein MW, See RE. Potentiation of cue-induced reinstatement of cocaine-seeking in rats by the anxiogenic drug yohimbine. *Behav Brain Res*. 2006; 174:1–8. Epub 2006 Aug 2021. [PubMed: 16920204]
- File SE. Aversive and appetitive properties of anxiogenic and anxiolytic agents. *Behav Brain Res*. 1986; 21:189–194. [PubMed: 2876714]
- Frascella J, Potenza MN, Brown LL, Childress AR. Shared brain vulnerabilities open the way for nonsubstance addictions: carving addiction at a new joint? *Ann N Y Acad Sci*. 2010; 1187:294–315. [PubMed: 20201859]
- Gearhardt AN, Yokum S, Orr PT, Stice E, Corbin WR, Brownell KD. Neural correlates of food addiction. *Archives of General Psychiatry*. 2011; 68:808–816. Epub 2011 Apr 2014. [PubMed: 21464344]
- Ghitza UE, Gray SM, Epstein DH, Rice KC, Shaham Y. The anxiogenic drug yohimbine reinstates palatable food seeking in a rat relapse model: a role of CRF1 receptors. *Neuropsychopharmacology*. 2006; 31:2188–2196. Epub 2005 Dec 2187. [PubMed: 16341025]
- Gresch PJ, Sved AF, Zigmond MJ, Finlay JM. Stress-induced sensitization of dopamine and norepinephrine efflux in medial prefrontal cortex of the rat. *J Neurochem*. 1994; 63:575–583. [PubMed: 8035182]
- Grilo CM, Shiffman S, Wing RR. Relapse crises and coping among dieters. *J Consult Clin Psychol*. 1989; 57:488–495. [PubMed: 2768608]
- Grimm JW, Harkness JH, Ratliff C, Barnes J, North K, Collins S. Effects of systemic or nucleus accumbens-directed dopamine D1 receptor antagonism on sucrose seeking in rats. *Psychopharmacology (Berl)*. 2011; 216:219–233. Epub 2011 Feb 2012. [PubMed: 21318562]
- Hamlin AS, Blatchford KE, McNally GP. Renewal of an extinguished instrumental response: neural correlates and the role of D1 dopamine receptors. *Neuroscience*. 2006; 143:25–38. [PubMed: 16949214]
- Izquierdo A, Wellman CL, Holmes A. Rapid dendritic retraction in medial prefrontal neurons and impaired fear extinction following exposure to uncontrollable stress. *J Neurosci*. 2006; 26:5733–5738. [PubMed: 16723530]
- Johnston AL, File SE. Yohimbine's anxiogenic action: evidence for noradrenergic and dopaminergic sites. *Pharmacol Biochem Behav*. 1989; 32:151–156. [PubMed: 2567522]
- Kenny PJ. Common cellular and molecular mechanisms in obesity and drug addiction. *Nat Rev Neurosci*. 2011; 12:638–651. doi: 610.1038/nrn3105. [PubMed: 22011680]
- Kramer FM, Jeffery RW, Forster JL, Snell MK. Long-term follow-up of behavioral treatment for obesity: patterns of weight regain among men and women. *International Journal of Obesity*. 1989; 13:123–136. [PubMed: 2663745]

- Lee B, Tiefenbacher S, Platt DM, Spealman RD. Pharmacological blockade of alpha2-adrenoceptors induces reinstatement of cocaine-seeking behavior in squirrel monkeys. *Neuropsychopharmacology*. 2004; 29:686–693. [PubMed: 14872205]
- Lin GL, Borders CB, Lundewall LJ, Wellman CL. D1 receptors regulate dendritic morphology in normal and stressed prefrontal cortex. *Psychoneuroendocrinology*. 2014; 51C:101–111. doi: 10.1016/j.psyneuen.2014.1009.1020. [PubMed: 25305546]
- Lin P, Pratt WE. Inactivation of the nucleus accumbens core or medial shell attenuates reinstatement of sugar-seeking behavior following sugar priming or exposure to food-associated cues. *PLoS One*. 2014; 9:e99301. doi: 10.1371/journal.pone.0099301. eCollection 2014. [PubMed: 24910996]
- Liu X, Weiss F. Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. *J Neurosci*. 2002; 22:7856–7861. [PubMed: 12223538]
- Mantsch JR, Vranjkovic O, Twining RC, Gasser PJ, McReynolds JR, Blacktop JM. Neurobiological mechanisms that contribute to stress-related cocaine use. *Neuropharmacology*. 2014; 76:383–394. doi: 10.1016/j.neuropharm.2013.1007.1021. Epub 2013 Aug 10. [PubMed: 23916481]
- Mizoguchi K, Shoji H, Ikeda R, Tanaka Y, Tabira T. Persistent depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission. *Pharmacol Biochem Behav*. 2008; 91:170–175. Epub 2008 Jul 13. [PubMed: 18675292]
- Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui D-H, Tabira T. Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *J Neurosci*. 2000; 20:1568–1574. [PubMed: 10662846]
- Moore H, Rose HJ, Grace AA. Chronic cold stress reduces the spontaneous activity of ventral tegmental dopamine neurons. *Neuropsychopharmacology*. 2001; 24:410–419. [PubMed: 11182536]
- Moran-Santa Maria MM, McRae-Clark A, Baker NL, Ramakrishnan V, Brady KT. Yohimbine administration and cue-reactivity in cocaine-dependent individuals. *Psychopharmacology (Berl)*. 2014; 231:4157–4165. doi: 10.1007/s00213-00014-03555-00219. Epub 2014 Apr 02. [PubMed: 24710621]
- Myers EA, Banihashemi L, Rinaman L. The anxiogenic drug yohimbine activates central viscerosensory circuits in rats. *The Journal of Comparative Neurology*. 2005; 492:426–441. [PubMed: 16228990]
- Nair SG, Adams-Deutsch T, Epstein DH, Shaham Y. The neuropharmacology of relapse to food seeking: methodology, main findings, and comparison with relapse to drug seeking. *Prog Neurobiol*. 2009; 89:18–45. [PubMed: 19497349]
- Nair SG, Gray SM, Ghitza UE. Role of food type in yohimbine- and pellet-priming-induced reinstatement of food seeking. *Physiology and Behavior*. 2006; 88:559–566. Epub 2006 Jun 27. [PubMed: 16806322]
- Nair SG, Navarre BM, Cifani C, Pickens CL, Bossert JM, Shaham Y. Role of dorsal medial prefrontal cortex dopamine D1-family receptors in relapse to high-fat food seeking induced by the anxiogenic drug yohimbine. *Neuropsychopharmacology*. 2011; 36:497–510. [PubMed: 20962767]
- Nikiforuk A. Dopamine D1 receptor modulation of set shifting: the role of stress exposure. *Behav Pharmacol*. 2012; 23:434–438. doi: 10.1097/FBP.1090b1013e328356522f. [PubMed: 22743605]
- Pickens CL, Cifani C, Navarre BM, Eichenbaum H, Theberge FR, Baumann MH, Calu DJ, Shaham Y. Effect of fenfluramine on reinstatement of food seeking in female and male rats: implications for the predictive validity of the reinstatement model. *Psychopharmacology*. 2012; 3:3.
- Radley JJ, Rocher AB, Miller M, Janssen WG, Liston C, Hof PR, McEwen BS, Morrison JH. Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cerebral Cortex*. 2006; 16:313–320. Epub 2005 May 18. [PubMed: 15901656]
- Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, McEwen BS, Morrison JH. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience*. 2004; 125:1–6. [PubMed: 15051139]

- Ramkumar K, Srikumar BN, Venkatasubramanian D, Siva R, Shankaranarayana Rao BS, Raju TR. Reversal of stress-induced dendritic atrophy in the prefrontal cortex by intracranial self-stimulation. *Journal of Neural Transmission*. 2012; 119:533–543. doi: 510.1007/s00702-00011-00740-00704. Epub 02011 Dec 00714. [PubMed: 22167578]
- Shelton KL, Beardsley PM. Interaction of extinguished cocaine-conditioned stimuli and footshock on reinstatement in rats. *Int J Comp Psych*. 2005; 18:154–166.
- Shelton KL, Hendrick E, Beardsley PM. Interaction of noncontingent cocaine and contingent drug-paired stimuli on cocaine reinstatement. *Eur J Pharmacol*. 2004; 497:35–40. [PubMed: 15302593]
- Shepard JD, Bossert JM, Liu SY, Shaham Y. The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. *Biol Psychiatry*. 2004; 55:1082–1089. [PubMed: 15158427]
- Skender ML, Goodrick GK, Del Junco DJ, Reeves RS, Darnell L, Gotto AM, Foreyt JP. Comparison of 2-year weight loss trends in behavioral treatments of obesity: diet, exercise, and combination interventions. *J Am Diet Assoc*. 1996; 96:342–346. [PubMed: 8598434]
- Sorg BA, Li N, Wu W, Bailie TM. Activation of dopamine D1 receptors in the medial prefrontal cortex produces bidirectional effects on cocaine-induced locomotor activity in rats: effects of repeated stress. *Neuroscience*. 2004; 127:187–196. [PubMed: 15219681]
- Southwick SM, Bremner JD, Rasmusson A, Morgan CA, Arnsten A, Charney DS. Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiatry*. 1999; 46:1192–1204. [PubMed: 10560025]
- Stine SM, Southwick SM, Petrakis IL, Kosten TR, Charney DS, Krystal JH. Yohimbine-induced withdrawal and anxiety symptoms in opioid-dependent patients. *Biol Psychiatry*. 2002; 51:642–651. [PubMed: 11955464]
- Tanda G, Bassareo V, Di Chiara G. Mianserin markedly and selectively increases extracellular dopamine in the prefrontal cortex as compared to the nucleus accumbens of the rat. *Psychopharmacology (Berl)*. 1996; 123:127–130. [PubMed: 8741935]
- Torres SJ, Nowson CA. Relationship between stress, eating behavior, and obesity. *Nutrition*. 2007; 23:887–894. Epub 2007 Sep 2017. [PubMed: 17869482]
- Umhau JC, Schwandt ML, Usala J, Geyer C, Singley E, George DT, Heilig M. Pharmacologically induced alcohol craving in treatment seeking alcoholics correlates with alcoholism severity, but is insensitive to acamprosate. *Neuropsychopharmacology*. 2011; 36:1178–1186. doi: 1110.1038/npp.2010.1253. Epub 2011 Feb 1172. [PubMed: 21289601]
- Vasa RA, Pine DS, Masten CL, Vythilingam M, Collin C, Charney DS, Neumeister A, Mogg K, Bradley BP, Bruck M, Monk CS. Effects of yohimbine and hydrocortisone on panic symptoms, autonomic responses, and attention to threat in healthy adults. *Psychopharmacology (Berl)*. 2009; 204:445–455. Epub 2009 Mar 2006. [PubMed: 19266185]
- Volkow ND, Wang GJ, Fowler JS, Tomasi D, Baler R. Food and drug reward: overlapping circuits in human obesity and addiction. *Current Topics in Behavioral Neurosciences*. 2012; 11:1–24. [PubMed: 22016109]
- Ziauddeen H, Farooqi IS, Fletcher PC. Obesity and the brain: how convincing is the addiction model? *Nat Rev Neurosci*. 2012; 13:279–286. doi: 210.1038/nrn3212. [PubMed: 22414944]

Fig. 1.

Experiment 1: CS Absent. (a) Responding in all animals across 14 days of self-administration. Rats were trained on an FR-1 schedule of reinforcement in daily 3-h sessions. Delivery of the pellet was accompanied by a tone + flashing cue light conditioned stimulus (CS) presented for 5 s, which was followed by a 20-s time-out period signaled by illumination of the house light. (b) Active lever responses across 10 days of extinction. Responses had no programmed consequences (i.e., no CS or pellets). Rats received one of nine treatments (saline + water, saline + yohimbine (2.5 mg/kg), saline + yohimbine (5.0 mg/kg), SCH-23390 (5.0 µg/kg) + water, SCH-23390 (5.0 µg/kg) + yohimbine (2.5 mg/kg), SCH-23390 (5.0 µg/kg) + yohimbine (5.0 mg/kg), SCH-23390 (10.0 µg/kg) + water, SCH-23390 (10.0 µg/kg) + yohimbine (2.5 mg/kg), or SCH-23390 (10.0 µg/kg) + yohimbine (5.0 mg/kg) once each day (~2 hr after extinction session) for the first 7 days of extinction training ($n = 6-12/\text{treatment condition}$). (c) Body weight during the self-administration, extinction, and reinstatement testing phases in all animals. During the self-administration phase, rats were given 16 g/day of regular chow in their home cages. During the extinction and testing phases, rats were given 18-30 g/day chow to maintain stable body weight. (d) Amount of food (g/100 g body weight) provided in home cage to maintain body weight during extinction in the nine chronic treatment groups. All data in figure are represented as mean $\pm$ SEM.

Fig. 2.

Experiment 1: CS Absent. Effect of repeated yohimbine (2.5 or 5.0 mg/kg $\times$ 7 days) and repeated SCH-23390 (5.0 or 10.0 µg/kg $\times$ 7 days) on subsequent active (a) and inactive (b) lever pressing during acute yohimbine-induced reinstatement testing. Each rat received injections of all three doses of acute yohimbine (0.0, 1.0, and 2.0 mg/kg; i.p.; counterbalanced), on separate days, 30 min prior to the commencement of sessions that were identical to extinction sessions. * $p < .05$ and # $p < .001$ compared to 0.0 mg/kg prime in same chronic treatment group, Bonferroni post-test. All data in figure are represented as mean + SEM.

Fig. 3.

Experiment 1: CS Absent. Effect of repeated yohimbine (2.5 or 5.0 mg/kg $\times$ 7 days) and repeated SCH-23390 (5.0 or 100 µg/kg $\times$ 7 days) on subsequent active (a) and inactive (b) lever pressing during pellet priming-induced reinstatement testing. Following acute yohimbine-induced reinstatement testing rats were tested under counterbalanced conditions for pellet priming-induced reinstatement. On separate days each rat was tested under extinction conditions, except that they received zero (no pellet) or four non-contingent pellets within the first min of the session. $^+p < .01$ compared to control condition in same chronic treatment group and $^{\dagger}p < .001$ compared to saline + water group in priming condition, Bonferroni post-test. All data in figure are represented as mean + SEM.

Fig. 4.

Experiment 2: CS Present. (a) Responding in all animals across 14 days of self-administration. See Fig. 1a for other details. (b) Active lever responses across 10 days of extinction. Responses resulted in delivery of the CS, but not pellets. Rats received one of nine chronic treatments once each day (~2 hr after extinction session) for the first 7 days of extinction training ($n = 6-12$ /treatment condition). See Fig. 1b for other details. (c) Body weight during the self-administration, extinction, and reinstatement testing phases in all animals. See Fig. 1c for other details. (d) Amount of food (g/100 g body weight) provided in home cage to maintain body weight during extinction in the nine chronic treatment groups. All data in figure are represented as mean $\pm$ SEM.

Fig. 5.

Experiment 2: CS Present. Effect of repeated yohimbine (2.5 or 5.0 mg/kg $\times$ 7 days) and repeated SCH-23390 (5.0 or 10.0 µg/kg $\times$ 7 days) on subsequent active (a) and inactive (b) lever pressing during acute yohimbine-induced reinstatement testing. See Fig. 2 for other details. * p < .05, + p < .01, and # p < .001 compared to 0.0 mg/kg prime in same chronic treatment group, Bonferroni post-test. All data in figure are represented as mean + SEM.

Fig. 6.

Experiment 2: CS Present. Effect of repeated yohimbine (2.5 or 5.0 mg/kg $\times$ 7 days) and repeated SCH-23390 (5.0 or 10.0 µg/kg $\times$ 7 days) on subsequent active (a) and inactive (b) lever pressing during pellet priming-induced reinstatement testing. See Fig. 3 for other details. * $p < .05$ and # $p < .001$ compared to control condition in same chronic treatment group and † $p < .01$ compared to saline + water group in priming condition, Bonferroni post-test. All data in figure are represented as mean + SEM.