Pharmacology, Biochemistry and Behavior 91 (2009) 473–480

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effects of repeated yohimbine on the extinction and reinstatement of cocaine seeking

David A. Kupferschmidt, Erin Tribe, Suzanne Erb *

Centre for the Neurobiology of Stress, Department of Psychology, University of Toronto Scarborough, Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Received 11 July 2008
Received in revised form 25 August 2008
Accepted 28 August 2008
Available online 9 September 2008

Keywords:
Yohimbine
Extinction
Relapse
Cocaine
Stress
Footshock

ABSTRACT

Acute exposure to the pharmacological stressor yohimbine (YOH) reinstates drug seeking in rats. The present experiments investigated whether repeated exposure to YOH during extinction training affects the time-course of extinction and the magnitude of subsequent YOH- or footshock-induced reinstatement of cocaine seeking. Rats trained to self-administer cocaine were given five days of extinction training, during which they were injected with YOH (1.25 mg/kg, i.p.) either before or after daily extinction sessions. Following additional extinction training in the absence of YOH, animals were tested for reinstatement to a YOH (1.25 mg/kg, i.p.) or footshock (20 min, intermittent, 0.9 mA per 0.5 s shock) challenge. Animals injected with YOH before daily extinction sessions showed an attenuated rate of extinction, relative to control animals. Following additional extinction training in the absence of YOH treatment, these same animals showed a marked attenuation of YOH-induced reinstatement of cocaine seeking. YOH treatment during extinction did not, however, affect the magnitude of reinstatement induced by footshock. These findings demonstrate that repeated exposure to a stressor during extinction training can modulate the processes governing extinction learning and the subsequent reinstatement of drug seeking induced by that stressor.

1. Introduction

Stress has long been considered to play a key role in perpetuating the cycle of drug use, withdrawal, and relapse to drug use that characterizes addiction. In recent years, controlled experiments carried out in humans and laboratory animals have made significant advances towards characterizing the complex role of stress in relapse to drug use (Sinha, 2001; Shaham et al., 2000). Central to this effort are studies based on an animal model of relapse, known as the reinstatement procedure. In this procedure, the subject is trained to perform an operant response, such as a lever press, to obtain intravenous infusions of a drug, or oral access to alcohol. Following training, extinction of the drug-reinforced behavior is achieved by withholding response-contingent reinforcement (De Wit and Stewart, 1981). Subsequently, the reinstatement of drug seeking is triggered by an acute event, such as re-exposure to the previously self-administered drug or exposure to a stressor (De Wit and Stewart, 1981; Shaham and Stewart, 1995; Shaham et al., 2003).

Various stressors reliably induce the reinstatement of drug seeking in rats. These include intermittent, electric footshocks (Shaham and Stewart, 1995; Erb et al., 1996), food deprivation stress (Shalev et al., 2000, 2003), and some pharmacological stressors that mimic aspects of the mammalian stress response (Shaham et al., 1997; Erb et al., 2006). Among the pharmacological stressors that reliably induce reinstatement is the alpha-2 adrenoceptor antagonist yohimbine (YOH). YOH induces anxiety- and stress-like responses in both human (Gurguis et al., 1997; Holmberg and Gershon, 1961) and non-human (Davis et al., 1979; Johnston and File, 1989) subjects, by activating stress-responsive neurotransmitter systems, including noradrenaline, serotonin, and dopamine (Millan et al., 2000), as well as the hypothalamic–pituitary–adrenal axis (Charney et al., 1983, 1989). YOH also triggers drug craving in abstinent opioid-dependent subjects (Stine et al., 2002), enhances oral self-administration of alcohol in rats (Lê et al., 2005), and reinstates drug seeking in rats with a history of methamphetamine (Shepard et al., 2004), alcohol (Lê et al., 2005), or cocaine (Feltenstein and See, 2006) self-administration. Thus, YOH serves as a reliable stressor for studying neurobiological and phenomenological aspects of stress and relapse.

To date, the preponderance of experiments in which stress has been studied as a trigger of relapse to drug seeking has focused on the effects of exposure to an acute and novel stressor at the time of testing for reinstatement (Shaham et al., 2003). In the human experience, however, stress is typically recurrent. This is almost certainly the case for individuals with a history of drug dependence, who invariably struggle with the adverse social, medical, and financial consequences of addiction. In animal studies, repeated exposure to stress is associated with profound and long-lasting neuroadaptations that are manifested as changes in an extensive repertoire of behaviors, not the least being drug-related behaviors (Mangiavacchi et al., 2001; Haile et al., 2001; Lepsch et al., 2005). Thus, the effects of repeated exposure to stress on long-term reinstatement of drug seeking would

* Corresponding author. Centre for the Neurobiology of Stress, Department of Psychology, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON, Canada, M1C 1A4. Tel.: +1 416 287 7454; fax: +1 416 287 7642.
E-mail address: erb@utsc.utoronto.ca (S. Erb).

0091-3057/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.08.026

seem a highly warranted, and surprisingly undeveloped, focus of investigation.

Another area in which investigation of the effects of repeated stress is relatively unexplored to date is that of the extinction of previously drug-reinforced behavior. One report indicates that in animals previously trained to self-administer heroin, 10-min exposures to intermittent footshock before daily extinction sessions modestly attenuate the rate of extinction learning over a 10-day period (Highfield et al., 2000). Likewise, in animals with a history of heroin self-administration, pretreatment with the corticosterone synthesis inhibitor metyrapone (which acts acutely to activate the endocrine stress response and other stress-responsive neurotransmitter systems, e.g., noradrenaline and glutamate) produces a strong facilitatory effect on responding during the first day of extinction (Shaham et al., 1997). Although these findings suggest that exposure to stressors can, at least to some degree, interrupt the processes governing the extinction of a drug-maintained behavior, no studies to date have been carried out to specifically address how exposure to stress during extinction might affect subsequent reinstatement of drug seeking. Given that extinction-based therapies are routinely employed in the treatment of anxiety disorders and addictions (Morris and Bouton, 2007; Conklin and Tiffany, 2002), there would seem to be value in studying the effects of stress during extinction on extinction learning itself, and on subsequent measures of drug craving and relapse to drug seeking.

The major objective of the present experiments was to determine whether repeated exposure to a stressor during extinction training might serve to interfere in the subsequent effects of that stressor, or a different stressor, on the reinstatement of drug seeking. In these studies, YOH was used as a general pharmacological stressor, because of its known propensity to activate stress-related systems (Millan et al., 2000), as well as to reliably and robustly induce the reinstatement of drug seeking (e.g. Feltenstein and See, 2006). In Experiment 1, we investigated the effects of repeated exposure to YOH, either during or following daily extinction sessions, on the rate of extinction learning and subsequent magnitude of YOH-induced reinstatement of cocaine seeking. We predicted that YOH would initially slow the rate of extinction, but that this effect would become attenuated over successive trials, as extinction learning progressed. Although YOH, purportedly via its influences on noradrenergic transmission, has been shown to *facilitate* the extinction of conditioned fear responses (Cain et al., 2004; Morris and Bouton, 2007), our prediction that it would *slow* the rate of extinction of a drug-maintained behavior was based on two findings: 1) that YOH serves as a reliable and robust stimulus for the reinstatement of drug seeking (e.g. Feltenstein and See, 2006), and 2) that another stressor, footshock, slows the initial rate of extinction in previously drug-trained animals (Highfield et al., 2000). We further predicted that, following a period of extinction training in the absence of YOH, animals that had initially experienced extinction training in its presence would not exhibit reinstatement of responding to a YOH challenge at test. This prediction was based on the idea that YOH during extinction training should become part of a complex of contextual factors associated with that training such that, at test, the drug should elicit a conditioned response consistent with extinction learning (Cunningham, 1979; Bouton, 2002; Bouton et al., 1990; see also Discussion for further explanation of this prediction). Experiment 2 was designed to determine whether exposure to YOH during extinction training would alter reinstatement responding to footshock, another reliable trigger of reinstatement.

2. Materials and methods

2.1. Subjects

Fourty-six male Long–Evans rats (Charles River, Montreal, QC; 300–325 g initial weight) served as subjects. Rats were individually housed in plastic cages in a temperature- (21 ± 1 °C) and humidity-controlled vivarium, where they were maintained on a reverse light–dark schedule (lights on 1900–0700) with free access to water and standard laboratory rat chow. All procedures were performed in accordance with Canadian Council of Animal Care guidelines, and were approved by the University of Toronto animal care committee.

2.2. Surgery

Under isoflurane anesthesia (3–5%; Benson Medical, Markham, ON), rats were implanted with a silastic intravenous catheter (Dow Corning, Midland, MI; inner diameter: 0.51 mm; outer diameter: 0.94 mm) into the right jugular vein. The catheter was secured to the vein with silk sutures and passed subcutaneously to the skull surface where it was connected to a modified 22-gauge cannula (Plastics One, Roanoke, VA). The cannula was mounted on the skull using jeweler's screws and dental cement. The open end of the cannula was fitted with a plastic blocker to maintain patency. Animals were given seven days to recover from surgery before experimental procedures began.

2.3. Drugs

Cocaine HCl (Medisca Pharmaceuticals, St. Laurent, QC) was dissolved in sterile, physiological saline at a dose of 3.5 mg/mL. Yohimbine HCl (Sigma-Aldrich, Oakville, ON) was dissolved in distilled water at a dose of 1.25 mg/mL. The dose of YOH was chosen based on a dose–response pilot reinstatement study carried out in our laboratory (unpublished data), and the previously published findings of others (Shepard et al., 2004; Feltenstein and See, 2006).

2.4. Apparatus

The self-administration chambers (Med Associates, St. Albans, VT) were equipped with a white house light and two retractable levers, both elevated 6.5 cm above a stainless steel rod floor. Responses on one lever, the so-called “active” lever, resulted in the activation of an infusion pump (Razel Scientific Instruments, St. Albans, VT) and the illumination of a white stimulus light, located just above the lever. Responses on the other lever, the “inactive” lever, were without consequence. All lever responses were recorded using software and equipment from Med Associates. Each chamber was also equipped to deliver constant-current, intermittent, inescapable, electric footshock through a scrambler to the steel rod floor (Med Associates). Footshock was delivered according to a variable time schedule at a mean interval of 40 s (10–70 s range). Each shock (0.9 mA) was 0.5 ms in duration.

2.5. Behavioral procedures

Both Experiments 1 and 2 consisted of four phases: 1) self-administration training, 2) extinction with YOH, 3) extinction without YOH, and 4) testing for reinstatement. A timeline of procedures is presented in Fig. 1.

2.5.1. Self-administration training

Self-administration training procedures were identical in both experiments. Rats were first habituated to the self-administration chambers in a single 2-h session, during which the active lever was retracted. Twenty-four hours later, self-administration training sessions commenced. Rats were allowed to self-administer cocaine for 8–10 days, during daily 3-h sessions under a fixed-ratio-1 schedule of reinforcement. Self-administration sessions alternated between the morning (9:00–12:00) and afternoon (1:00–4:00). Because the procedures in subsequent phases of the experiment (i.e. extinction and testing) occurred at both times of day for all animals, it was important that self-administration training be given at both times in

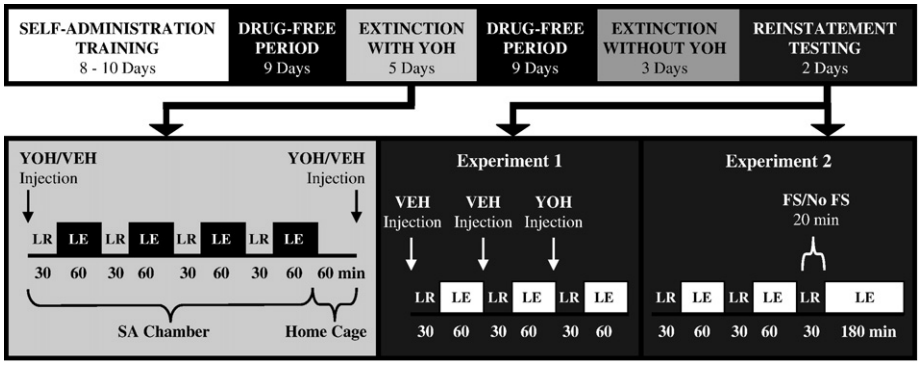

Fig. 1. Procedural timeline for Experiments 1 and 2: The top bar provides an overview of all phases of the experiment. The bottom bar provides a more detailed description of the daily treatment procedures during the first extinction phase and testing for reinstatement. FS: Footshock. LR: Lever Retracted. LE: Lever Extended.

order to prevent any confounding effects of the time of day on drug-seeking behaviour.

Each training session was preceded by a 5-min acclimatization period, during which animals were placed in the self-administration chambers with the active lever retracted. Subsequently, the active lever was extended and the stimulus light illuminated for 30 s. In addition, the houselight was activated and remained illuminated throughout the session. Collectively, these events signaled the availability of cocaine. Active lever responses during the self-administration session resulted in a 3-s infusion of cocaine (0.23 mg/65 μL infusion, i.v.) and a 20-s activation of the stimulus light, which corresponded to a "time-out" period during which additional active lever responses were recorded but not reinforced. Animals were allowed a maximum of 50 infusions per 3-h session.

2.6. Experiment 1: effects of YOH during extinction on YOH-induced reinstatement

2.6.1. Extinction Phase 1: extinction with YOH

Extinction sessions began nine days after the final self-administration training session. This intervening period was imposed for two reasons. One, it ensured that testing for reinstatement occurred following a period of time that exceeded the initial cocaine withdrawal period. Indeed, our interest in performing these studies was to examine the effects of stress on the long-term reinstatement of drug seeking. The second reason related to the fact that the experiment was carried out in two squads of animals at a time. Because some phases of the experiment (i.e., extinction and testing) required animals to be housed in the self-administration chambers for an extended time each day, it was not possible to run both squads simultaneously in all phases. Thus, the two squads of animals began the experiment in a staggered fashion, and a 9-day period conveniently accommodated their individual schedules.

During this phase of the experiment, animals were given four 60-min extinction sessions each day for five consecutive days. Each session was separated by 30-min intervals, during which the active lever was retracted. During these sessions, all conditions that were present during self-administration training were maintained, except that active lever responses were not reinforced with cocaine infusions. On each day, animals were given two injections (1.25 mg/kg YOH or VEH, i.p.). One injection was given 30 min before the first extinction session, in the self-administration chamber, and the other injection was given 60 min after the final session, in the home cage. Based on the order of YOH and VEH injections, three treatment conditions were formed: YOH/VEH (n=9), VEH/YOH (n=14), and VEH/VEH (n=12). Animals were assigned to one of the three conditions using matched assignment based on total active lever responding during self-administration training.

2.6.2. Extinction Phase 2: extinction without YOH

Following another nine-day period in the home cage, animals underwent three additional days of extinction training. In this case, an intervening period between the two experimental phases was included to allow for the opportunity to observe spontaneous recovery of extinguished responding following a time delay. A period of 9 days was selected because it conveniently accommodated the staggered training and extinction schedules of two squads of animals (see explanation for Extinction Phase 1 above). The extinction conditions were identical to those described in Extinction Phase 1, except that no injections were given. This second extinction phase was included to give YOH/VEH animals extinction training in the absence of YOH, such that the effects of the drug on reinstatement of cocaine seeking could be subsequently tested.

2.6.3. Reinstatement testing

In the two days following extinction, animals were given YOH and VEH tests for reinstatement of cocaine seeking. On each day, animals were given two 60-min extinction sessions. Thirty minutes before each session, animals were given an injection of VEH (i.p.) to familiarize them with the testing procedures. Animals that exhibited 20 or fewer total active lever responses during these extinction sessions were subsequently tested for reinstatement. Animals that did not reach the extinction criterion were given up to two additional extinction sessions the following day, and were tested that day (all animals reached the extinction criterion with these additional sessions). In the tests for reinstatement, animals were given an injection of YOH (1.25 mg/kg, i.p.) or VEH, followed 30 min later by 60-min access to the previously active lever. During this test session, lever presses were recorded but not reinforced. The order in which animals were given YOH and VEH tests for reinstatement was counterbalanced.

2.7. Experiment 2: effects of YOH during extinction on footshock-induced reinstatement

2.7.1. Extinction Phase 1: extinction with YOH

Nine days after the final self-administration training session, animals were given five days of extinction training, under conditions identical to those described in Experiment 1 (see rationale in Experiment 1 for including a 9-day intervening period). Since there were no differences in extinction or reinstatement responding between VEH/VEH and VEH/YOH control groups in Experiment 1, only YOH/VEH (n=6) and VEH/YOH (n=5) groups were included in this experiment.

2.7.2. Extinction Phase 2: extinction without YOH

Following a nine-day period in their home cage, animals underwent three additional days of extinction training, as described in

Experiment 1 (see rationale in Experiment 1 for including a 9-day intervening period between extinction phases).

2.7.3. Reinstatement testing

In the two days following Extinction Phase 2, animals were given footshock (FS) and No FS tests for reinstatement. As in Experiment 1, on each test day, animals were first given two 60-min extinction sessions, and animals that exhibited 20 or fewer active lever responses during these sessions were subsequently given a test for reinstatement. Animals that did not reach the extinction criterion were given up to two additional extinction sessions the following day, and were tested that day (all animals reached the extinction criterion with these additional sessions). During FS tests for reinstatement, animals were exposed to 20 min of intermittent footshock stress (0.9 mA; 0.5 ms/shock, range of 10–70 s between shocks) immediately prior to a 3-h test for reinstatement; during No FS tests, animals were left undisturbed in their self-administration chambers for the 20 min prior to testing. The order in which animals were given the FS and No FS tests was counterbalanced.

2.8. Statistical analysis

In both experiments, the main dependent variable was the number of active lever responses during extinction and testing for reinstatement. Data from Extinction Phases 1 and 2 were analyzed using repeated measures ANOVAs for the within-subjects factors of Session (1–4) and Day (1–5 [Phase 1]; 1–3 [Phase 2]), and the between-subjects factor of Extinction Group (YOH/VEH, VEH/YOH, VEH/VEH [Experiment 1]; YOH/VEH, VEH/YOH [Experiment 2]). In addition, as a measure of the spontaneous recovery of responding between Phases 1 and 2, responses during the last session of Extinction Phase 1 were compared with responses during the first session of Phase 2, using a repeated measures ANOVA for the factors of Session (last vs. first session of Phase 1 and 2, respectively) and Extinction Group. Significant interactions from these analyses were followed by additional ANOVAs, Fisher's LSD post hoc analyses and paired-sample *t*-tests, as appropriate. Data from the tests for reinstatement were analyzed using repeated measures ANOVAs for the within-subjects factor of Test Challenge (YOH, VEH [Experiment 1]; FS, No FS [Experiment 2]) and the between-subjects factor of Extinction Group, followed by planned comparisons for responding in the YOH test condition.

3. Results

3.1. Experiment 1: effects of YOH during extinction on YOH-induced reinstatement

3.1.1. Self-administration training

Animals in the three extinction groups showed comparable rates of cocaine self-administration. Average daily infusions (±SEM) over the 10-day training period were 32.4 (±1.0), 34.0 (±1.1), and 29.4 (±1.2), corresponding to average daily cocaine intakes of 7.4 (±0.2), 7.7 (±0.3), and 6.7 (±0.3) mg, for groups VEH/VEH, YOH/VEH, and VEH/YOH, respectively.

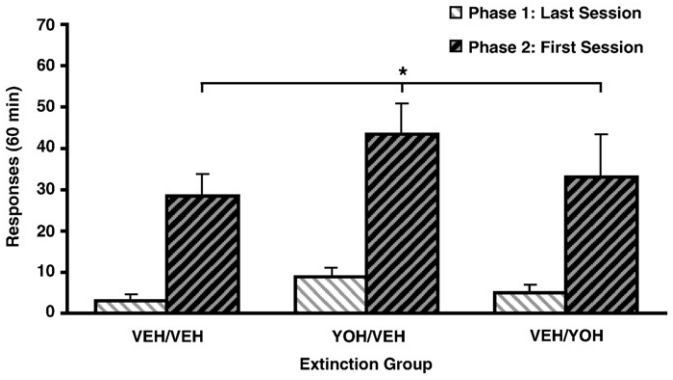

Fig. 3. Spontaneous recovery, Experiment 1: Mean (±SEM) number of responses on the active lever during the final session of Extinction Phase 1 and the first session of Extinction Phase 2. *Phase 2 different from Phase 1, $p<0.001$.

3.1.2. Extinction Phase 1: extinction with YOH

Fig. 2 shows the mean (±SEM) number of responses on the previously active lever during each of the 4 sessions on each of the 5 days of extinction. To better reflect the outcome of the statistical analyses (see below), an inset has been included in the figure that shows the mean number of responses (±SEM) on the active lever during each day of extinction, averaged across sessions. A repeated measures ANOVA revealed significant interactions of Session×Day [$F(12,284)=7.24$, $p<0.001$] and Day×Extinction Group [$F(8,128)=9.66$, $p<0.001$]. Inspection of Fig. 2 reveals that the Session×Day interaction can be attributed to progressively reduced levels of responding both within and between extinction days, regardless of extinction group. The Day×Extinction Group interaction, on the other hand, can be attributed to a comparatively greater difference in responding between YOH/VEH and the other extinction groups on Day 1 of extinction, relative to the other days of extinction (see inset Fig. 2).

3.1.3. Extinction Phase 2: extinction without YOH

Fig. 3 shows the mean (±SEM) number of responses on the previously active lever during the last session of Extinction Phase 1 and the first session of Extinction Phase 2. It can be seen that, relative to the number of responses in the last session of Phase 1, all groups showed comparable spontaneous recovery of responding during the first session of Phase 2. This observation was confirmed by a repeated measures ANOVA that revealed only a significant effect of Session [$F(1,31)=40.743$, $p<0.001$]. Thus, regardless of Extinction Group, animals responded more during the first session of Phase 2 than the last session of Phase 1. Likewise, all groups demonstrated comparable rates of extinction over the 3-day period of Extinction

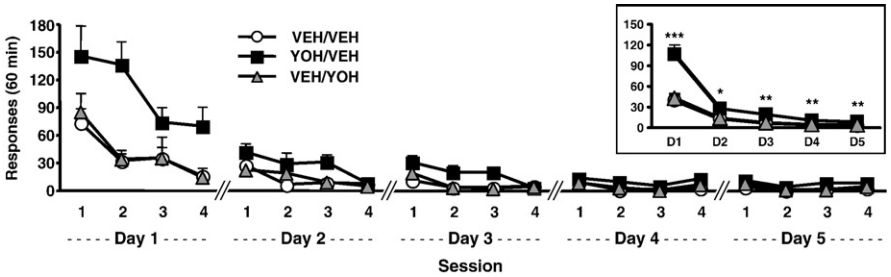

Fig. 2. Extinction Phase 1, Experiment 1: Mean (±SEM) number of responses on the active lever during the four daily extinction sessions on each of the five days of extinction. *Inset*: Mean (±SEM) active lever responses during the five extinction days (averaged across sessions). YOH/VEH different from VEH/VEH and VEH/YOH, *$p<0.05$, **0.01, and ***0.001.

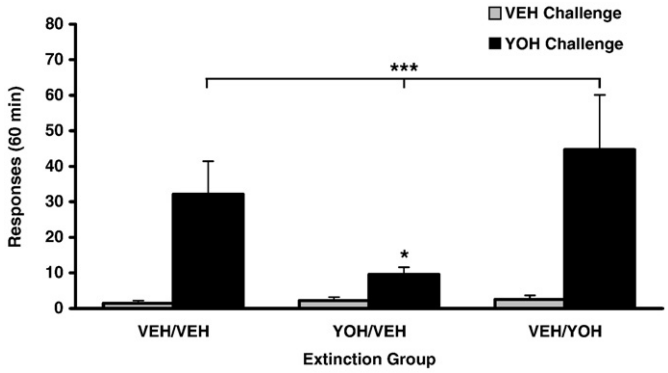

Fig. 4. Testing for reinstatement, Experiment 1: Mean (±SEM) number of responses on the active lever in 60-min tests for reinstatement after injections of YOH (1.25 mg/kg, i.p.) or VEH. ***YOH different from VEH test challenge, $p<0.001$. *YOH/VEH different from VEH/VEH and VEH/YOH, $p<0.05$.

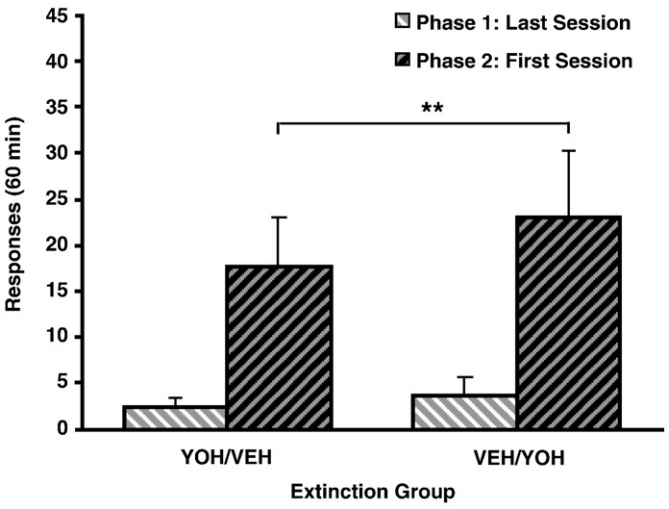

Fig. 6. Spontaneous recovery, Experiment 2: Mean (±SEM) number of responses on the active lever during the final session of Extinction Phase 1 and the first session of Extinction Phase 2. **Phase 2 different from Phase 1, $p<0.01$.

Phase 2. In this case, a repeated measures ANOVA revealed only a significant interaction of Day×Session [$F(6,186)=3.18$, $p<0.01$]; regardless of extinction group, responding gradually decreased over sessions and days (data not shown). In the last extinction session, prior to testing for reinstatement, the average numbers of responses on the previously active lever were 3.6 (±1.6), 2.8 (±0.9), and 3.8 (±2.3) for groups VEH/VEH, YOH/VEH, and VEH/YOH, respectively.

3.1.4. Reinstatement testing

Fig. 4 shows the mean (±SEM) number of responses on the previously active lever for each extinction group during the 60-min YOH and VEH tests for reinstatement. All extinction groups exhibited enhanced responding on the previously active lever in the YOH, relative to VEH, test for reinstatement [$F(1,32)=13.44$, $p<0.001$]. However, YOH-induced reinstatement of responding was attenuated in the YOH/VEH, relative to other extinction groups, as revealed by planned comparisons between YOH/VEH and VEH/VEH groups, and between YOH/VEH and VEH/YOH groups ($ps<0.05$). Notably, responding on the inactive lever during the YOH tests for reinstatement was very low in all groups, corresponding to 6.1 (±2.4), 3.7 (±1.2) and 8.1 (±2.6) lever presses in VEH/VEH, YOH/VEH and VEH/YOH groups, respectively.

3.2. Experiment 2: effects of YOH during extinction on footshock-induced reinstatement

3.2.1. Self-administration training

Animals in the two extinction groups showed comparable rates of cocaine self-administration. The average daily numbers of infusions (±SEM) over the 10-day training period were 25.8 (±2.3) and 25.1 (±2.8), corresponding to average daily cocaine intakes of 5.9 (±0.5) and 5.7 (±0.6) mg, for groups YOH/VEH and VEH/YOH, respectively.

3.2.2. Extinction Phase 1: extinction with YOH

Fig. 5 shows the mean (±SEM) number of responses on the previously active lever during each of 4 sessions on each of 5 days of extinction. Consistent with Experiment 1, a repeated measures ANOVA revealed significant interactions of Day×Session [$F(12,108)=4.95$, $p<0.001$] and Day×Extinction Group [$F(4,108)=3.06$, $p<0.05$]. In addition, the three-way interaction of Session×Day×Extinction Group was significant [$F(12,108)=3.13$, $p<0.001$]. To further analyze this interaction, separate two-way ANOVAs were carried out for each day of extinction. These analyses revealed a significant interaction of Session×Extinction Group only on Day 1 of extinction [$F(3,27)=4.34$, $p<0.05$]. As depicted in Fig. 5, the interaction on Day 1 can be attributed to a difference in responding between extinction groups during Sessions 1–3.

3.2.3. Extinction Phase 2: extinction without YOH

Fig. 6 shows the mean (±SEM) number of responses on the previously active lever during the last session of Extinction Phase 1 and the first session of Extinction Phase 2. Consistent with Experiment 1, all groups showed comparable spontaneous recovery of responding during the first session of Phase 2, relative to responding in the last session of Phase 1. Indeed, a repeated measures ANOVA revealed a significant main effect of Session [$F(1,9)=16.66$, $p<0.01$], but no effect of Extinction Group. Thus, regardless of extinction group, animals responded more in the first session of Extinction Phase 2 than the last session of Phase 1. Also consistent with Experiment 1, all groups demonstrated comparable rates of extinction over the 3-day period of the second extinction phase. A repeated measures ANOVA revealed only a significant interaction of Day×Session [$F(3,54)=6.54$, $p<0.01$].

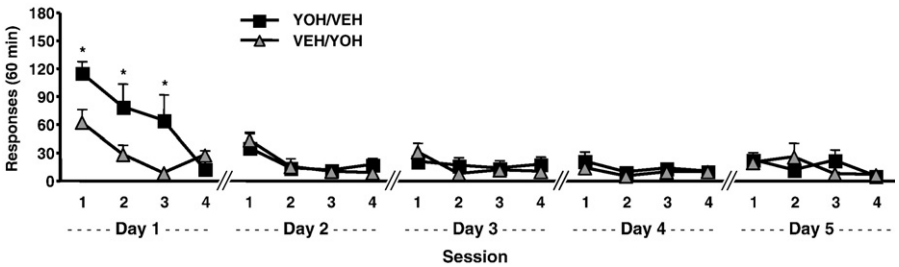

Fig. 5. Extinction Phase 1, Experiment 2: Mean (±SEM) number of responses on the active lever during the four daily extinction sessions on each of the five days of extinction. *YOH/VEH different from VEH/VEH and VEH/YOH, $p<0.05$.

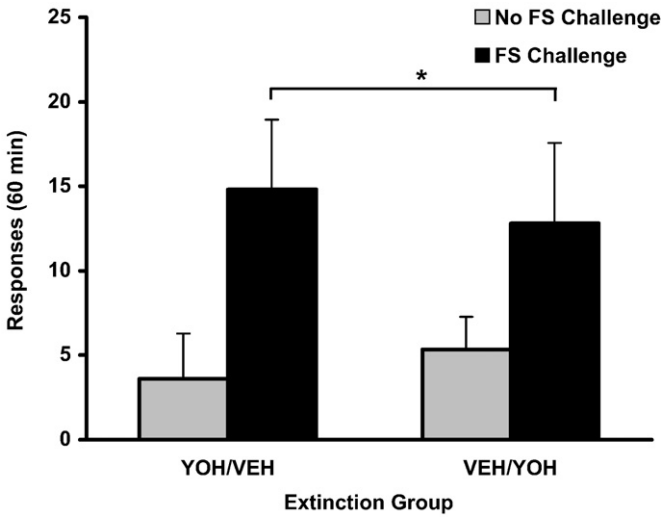

Fig. 7. Testing for reinstatement, Experiment 2: Mean (±SEM) number of responses on the active lever in the first 60 min of tests for reinstatement after 20-min exposure to intermittent footshock (FS) or No FS. *FS different from No FS test challenge, $p<0.05$.

Regardless of extinction group, responding gradually decreased over sessions and days (data not shown). In the last extinction session, prior to testing for reinstatement, the average numbers of responses on the previously active lever were 2.8 (±1.8) and 1.4 (±1.0) for groups YOH/VEH and VEH/YOH, respectively.

3.2.4. Reinstatement testing

Fig. 7 shows the mean (±SEM) number of responses on the previously active lever for each extinction group during the first hour of the 3-h FS and No FS tests for reinstatement. Only the data from the first hour of testing are shown, given that virtually all reinstatement of responding occurred in this hour. Whether analyses were carried out for active lever responses in the full 3-h testing period or only in the first hour of testing, the outcomes were the same. Specifically, FS induced reinstatement of responding (relative to No FS) to a comparable degree in both the YOH/VEH and VEH/YOH extinction groups ($ps>0.05$). As in Experiment 1, responding on the inactive lever during FS tests for reinstatement was very low in both groups, corresponding to 6.6 (±1.9) and 5.0 (±1.9) lever presses in the YOH/VEH and VEH/YOH groups, respectively.

4. Discussion

The major findings to emerge from the present experiments are that: 1) pretreatment with the pharmacological stressor YOH during extinction training slows the initial rate at which the extinction of cocaine-seeking behavior occurs; 2) YOH given during extinction attenuates the effectiveness with which a YOH challenge induces the reinstatement of cocaine seeking, following a subsequent period of extinction training in the absence of YOH; and 3) the effects of YOH during extinction on reinstatement do not generalize to another stressor, footshock. The effects of YOH on extinction and reinstatement responding were dependent on it being administered prior to daily extinction sessions. That is, the present findings cannot be attributed to a non-specific pharmacological effect of YOH, because the drug was ineffective in altering extinction and reinstatement responding when given after daily extinction sessions in the home cage.

Our finding that YOH administration during extinction training slowed the rate at which extinction occurred is consistent with a previous finding that presentations of footshock before daily extinction sessions slow the rate at which the extinction of heroin seeking occurs (Highfield et al., 2000). This finding is also consistent with the idea that stress acts during extinction, as it does during reinstatement testing, to disinhibit responding that is under inhibitory control (Highfield et al., 2000). With repeated exposures, however, the initial excitatory effect of the stressor on extinction responding is reduced (Highfield et al., 2000; see also Figs. 2 and 5, present study). This reduced effect of the stressor on extinction responding may be due to the disinhibitory effects of the stressor being overcome by the progressive inhibition of the response. It is also conceivable that the reduced effect reflects a progressive habituation to the neuronal and endocrine effects of the stressor (Stamp and Herbert, 1999; De Boer et al., 1990), a habituation that extends to the effects of the stressor on extinction responding. Another possibility is that as extinction progresses, the stressor becomes part of a complex of inhibitory stimuli that comprise the extinction context. For example, there is recent evidence that contexts paired with YOH can display inhibitory properties and, thereby, facilitate the extinction of conditioned fear responses (Morris and Bouton, 2007). Although in the present experiments YOH initially slowed rather than accelerated the rate at which extinction occurred, it is conceivable that over time it contributed to the contextual inhibition characterizing the extinction environment. From this perspective, not only would the presence of YOH during extinction training have served to gradually facilitate extinction learning, and counter the excitatory effects of the stressor on responding, it would also have contributed to the reduced reinstatement of responding to a YOH challenge at test.

It may be argued that the increased responding that we observed in YOH/VEH animals during Extinction Phase 1 reflects an unconditioned stimulatory effect of YOH on locomotor activity, an effect that habituates with repeated exposures to the drug. Although plausible, we do not believe that this argument provides a likely explanation for our findings. First, although YOH at the dose that we administered is known to increase locomotor activity (Mason et al., 1998; Schroeder et al., 2003), this effect appears to remain constant (Jiménez-Rivera et al., 2006) or, if anything, sensitize (Schroeder et al., 2003) with repeated exposures. In contrast, in our study, the excitatory effect of YOH on extinction responding was markedly reduced over the 5-day extinction period. In addition, animals in the YOH/VEH group tended to show very low levels of responding on the inactive lever during the daily extinction sessions. In fact, their response levels on the inactive lever were comparable to those exhibited by animals in the other extinction groups and were, for all groups, less than 15% of responding on the active lever during the same session.

A consideration of the important role of contextual factors in extinction learning may be central to explaining why YOH experienced during extinction training interfered in the reinstatement of cocaine seeking by a YOH challenge, but not footshock. Extinction learning is known to be highly context dependent, such that its expression can be suppressed if extinction of a conditioned stimulus occurs in one context and that stimulus is presented in an alternate context at test. This so-called "renewal" effect has been well established in studies of conditioned fear responses, whereby an animal that has undergone extinction of fear conditioning in one context shows renewed responding to the conditioned stimulus upon its presentation in an alternate context at test (see Bouton, 2004). Likewise, rats trained to self-administer cocaine or a cocaine-heroin mixture, and subsequently subjected to extinction of drug seeking in either the drug-associated context or a new context, show renewed responding when returned to the original drug-associated context at test (Crombag and Shaham, 2002; Crombag et al., 2002; see also Brooks and Bouton, 1993, 1994).

In addition to the physical features of the extinction context, the internal state, or "interoceptive cues", of an organism can contribute to the context dependency of extinction learning, such that re-experiencing the internal state associated with extinction learning can cue the retrieval of that learning (Bouton et al., 1990, 2006; Self and

Choi, 2004). This effect can be considered as an example of a more general phenomenon known as state-dependent learning, whereby a response that was learned in the presence of a distinct internal state is only expressed if testing occurs in that state. For example, in one study, animals that had undergone extinction of a conditioned fear response in the presence of a benzodiazepine showed renewed responding when tested in the absence, but not presence, of the benzodiazepine (Bouton et al., 1990). In another study, conditioned suppression of operant responding was established via repeated presentations of a tone/footshock stimulus in association with food-reinforced lever presses. Subsequently, extinction of the conditioned response to the shock-associated stimulus occurred in either the presence or absence of alcohol. When animals were tested in the absence of alcohol, only animals that had undergone extinction in the presence of the drug showed renewed responding to the conditioned stimulus (Cunningham, 1979). Based on these and other similar findings, it could be argued that in Experiment 1 animals that had originally undergone extinction in the presence of YOH showed reduced responding to the YOH challenge at test because the challenge served to re-create the internal state associated with the original extinction learning. On the other hand, when animals were challenged with footshock at test (Experiment 2), the stressor elicited an internal state that was distinct enough from that elicited by YOH such that responding was not suppressed. Indeed, YOH and footshock are distinct forms of stress (pharmacological versus physical) that are associated with distinct discriminative properties (Leidenheimer and Schechter, 1992) and the induction of distinct patterns of neuronal activation (Funk et al., 2006; Millan et al., 2000).

While state-dependent learning may well have contributed to our findings, such an explanation cannot fully account for all aspects of them. In particular, levels of spontaneous recovery of responding at the start of the second extinction phase, in which no YOH injections were given, were comparable for all extinction groups. If operant responding was fully state-dependent, one might have expected a relatively more robust level of responding in YOH/VEH animals at the start of Extinction Phase 2. This prediction is based on the idea that at the time of testing for spontaneous recovery (i.e., first session of Extinction Phase 2) the internal state of YOH/VEH animals, unlike that of animals in the other extinction groups, would have been distinct from that experienced during the original extinction training. Notably, levels of responding for all groups during the first session of Extinction Phase 2 (i.e., test for spontaneous recovery) were less than half of that observed during the first session of Extinction Phase 1 (see Figs. 3 and 6), ruling out the possibility that a ceiling effect masked potential group differences in spontaneous recovery of responding.

There are several alternative explanations of our reinstatement findings that warrant consideration. First, YOH-induced reinstatement may have been attenuated in YOH/VEH animals due to the development of context-dependent sensitization to the anxiogenic effects of YOH during extinction training. A sensitized anxiogenic response to YOH in these animals may have resulted in a heightened expression of behaviors, such as freezing, that are incompatible with drug seeking. In fact, it may be argued that a dose–response assessment of the effects of YOH on reinstatement in the present experiment could have served to reveal such an effect, with higher challenge doses producing greater attenuations of responding in the tests for reinstatement. Alternatively, YOH/VEH animals, through repeated exposure to YOH in the self-administration chamber, may have developed context-dependent tolerance to the excitatory effects of YOH, such that re-exposure to YOH at the time of testing failed to elicit a robust drug-seeking response. Although both explanations are reasonable, we do not believe that either provides a satisfactory explanation of our findings. First, repeated exposure to YOH in a distinct context has been associated with a progressive augmentation in YOH-induced locomotor activity (Schroeder et al., 2003). Such a behavioral effect is, itself, incompatible with freezing, arguing against the plausibility of an explanation based on a sensitization of YOH-induced anxiety. A sensitization of locomotor activity with repeated exposures also argues against the idea that tolerance develops to the excitatory effects of YOH. Furthermore, in animals that had been previously trained to self-administer cocaine, we carried out a general observational assessment of a variety of behaviors following pretreatment with YOH in the self-administration chamber. Across a range of doses of YOH (0.625 to 5.0 mg/kg, i.p.) we failed to observe systematic or dose-dependent effects of the drug on freezing, rearing, or general mobility, making it unlikely that a higher challenge dose of YOH than that used in the present experiment (i.e., 1.25 mg/kg, i.p.) would have produced a greater attenuation in the reinstatement of responding. This argument is further strengthened by observations that YOH doses in the 0.625–2.5 mg/kg (i.p.) range are associated with comparable magnitudes of reinstatement of drug seeking (Kupferschmidt & Erb, unpublished data; Shepard et al., 2004; Feltenstein and See, 2006).

Another explanation for our findings is that the attenuated effect of YOH on reinstatement in YOH/VEH animals reflects a strengthened inhibitory learning in these animals due to their relatively elevated levels of non-reinforced responding during the early extinction sessions (Bouton, 2004). This explanation, however, is largely unsupported by the outcomes of both fear and appetitive conditioning studies which have failed to observe correlations between levels of responding during extinction learning and the subsequent expression of that learning (Moody et al., 2006; Drew et al., 2004). Similarly, in the present study, a correlational analysis performed between total number of responses during extinction training and during YOH tests for reinstatement was non-significant [$r(69) = -0.077$, $p = 0.541$]. Furthermore, YOH/VEH animals in Experiment 2 displayed no attenuation of footshock-induced reinstatement, despite showing relatively higher levels of responding during extinction, when compared to the other groups.

Finally, although the role of extinction cues in drug-cue reactivity in humans is complex, and not all findings are in agreement (see Stasiewicz et al., 2007), our findings may have relevance in the design of extinction-based programs aimed at treating drug addicts (Marlatt, 1990). To date, such programs are associated with limited therapeutic efficacy, possibly due to their shortcomings in creating an extinction context that sufficiently resembles the context in which relapse ultimately occurs (Conklin and Tiffany, 2002). Given that relapse is frequently triggered under conditions of stress, some have recommended that cue exposure programs incorporate these conditions into their design (Marlatt, 1990). The present study offers some degree of validation for this idea by showing that repeated exposure to a stressor during extinction training can suppress subsequent reinstatement of responding by that stressor. From this perspective, YOH during extinction training may have served to convert the stressor from a trigger of reinstatement into a retrieval cue for extinction learning. It must be borne in mind, however, that the effects of YOH during extinction on subsequent reinstatement responding did not generalize to another trigger of reinstatement (i.e. footshock). This lack of generality speaks to the highly context-dependent nature of extinction learning, and underscores the challenges associated with the long-term treatment of addiction.

Acknowledgements

This work was supported by grants to S. Erb from the Canadian Psychiatric Research Foundation and Natural Sciences and Engineering Research Council of Canada. We thank Dr. Douglas Funk for his insightful comments on the manuscript. We also thank Zenya Brown, Sarah Johnson, and Michael Misch for their careful reading of the manuscript.

References

Bouton ME. Context, ambiguity, and unlearning: sources of relapse after behavioural extinction. Soc Biol Psychiatry 2002;52:976–86.
Bouton ME. Context and behavioural processes in extinction. Learn Memory 2004;11:485–94.

Bouton ME, Kenney FA, Rosengard C. State-dependent fear extinction with two benzodiazepine tranquilizers. Behav Neurosci 1990;104:44–55.
Bouton ME, Westbrook RF, Corcoran KA, Maren S. Contextual and temporal modulation of extinction: behavioural and biological mechanisms. Biol Psychiatry 2006;60:352–60.
Brooks DC, Bouton ME. A retrieval cue for extinction attenuates spontaneous recovery. J Exp Psychol Anim Behav Process 1993;19:77–89.
Brooks DC, Bouton ME. A retrieval cue for extinction attenuates response recovery (renewal) caused by a return to the conditioning context. J Exper Psychol Anim Behav Processes 1994;20:366–79.
Cain CK, Blouin AM, Barad M. Adrenergic transmission facilitates extinction of conditional fear in mice. Learn Mem 2004;11:179–87.
Charney DS, Heninger GR, Redmond Jr DE. Yohimbine induced anxiety and increased noradrenergic function in humans: effects of diazepam and clonidine. Life Sci 1983;33:19–29.
Charney DS, Woods SW, Heninger GR. Noradrenergic function in generalized anxiety disorder: effects of yohimbine in healthy subjects and patients with generalized anxiety disorder. Psychiatry 1989;27:173–82.
Conklin CA, Tiffany ST. Applying extinction research and theory to cue-exposure addiction treatments. Addiction 2002;97:155–67.
Crombag HS, Shaham Y. Renewal of drug seeking by contextual cues after prolonged extinction in rats. Behav Neurosci 2002;116:169–73.
Crombag HS, Grimm JW, Shaham Y. Effect of dopamine receptor antagonists on renewal of cocaine seeking by re-exposure to drug-associated contextual cues. Neuropsychopharmacology 2002;27:1006–15.
Cunningham CL. Alcohol as a cue for extinction: state dependency produced by conditioned inhibition. Anim Learn Behav 1979;7:45–52.
Davis M, Redmond Jr DE, Baraban JM. Noradrenergic agonists and antagonists: effects on conditioned fear as measured by the potentiated startle paradigm. Psychopharmacology 1979;65:111–8.
De Boer SF, Koopmans SJ, Slangen JL, Van der Gugten J. Plasma catecholamine, corticosterone and glucose responses to repeated stress in rats: effect of interstressor interval length. Physiol Behav 1990;47:1117–24.
De Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 1981;75:134–43.
Drew MR, Yang C, Ohyama T, Balsam PD. Temporal specificity of extinction in autoshaping. J Exp Psychol Anim Behav Process 2004;30:163–76.
Erb S, Petrovic A, Yi D, Kayyali H. Central injections of CRF reinstate cocaine seeking in rats after post-injection delays of up to 3 hours: an influence of time and environmental context. Psychopharmacology 2006;187:112–20.
Erb S, Shaham Y, Stewart J. Stress reinstates cocaine-seeking behaviour after prolonged extinction and a drug-free period. Psychopharmacology 1996;158:360–5.
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.
Funk D, Li Z, Lê AD. Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: relationship to the reinstatement of alcohol seeking. Neuroscience 2006;138:235–43.
Gurguis GN, Vitton BJ, Uhde TW. Behavioural, sympathetic and adrenocortical responses to yohimbine in panic disorder and normal controls. Psychiatry Res 1997;71:27–39.
Haile CN, GrandPre T, Kosten TA. Chronic unpredictable stress, but not chronic predictable stress, enhances the sensitivity to the behavioral effects of cocaine in rats. Psychopharmacology 2001;154:213–20.
Highfield D, Clements A, Shalev U, McDonald R, Featherstone R, Stewart J, Shaham Y. Involvement of the medial septum in stress-induced relapse to heroin seeking in rats. Eur J Neurosci 2000;12:1705–13.
Holmberg G, Gershon S. Autonomic and psychic effects of yohimbine hydrochloride. Psychopharmacologia 1961;2:93–106.
Jiménez-Rivera CA, Feliu-Mojer M, Vázquez-Torres R. Alpha-noradrenergic receptors modulate the development and expression of cocaine sensitization. Ann N Y Acad Sci 2006;1074:390–402.
Johnston AL, File SE. Yohimbine's anxiogenic action: evidence for noradrenergic and dopaminergic sites. Pharmacol Biochem Behav 1989;32:151–6.
Lê AD, Harding S, Juzytsch W, Funk D, Shaham Y. Role of alpha-2 adrenoceptors in stress-induced reinstatement of alcohol seeking and alcohol self-administration in rats. Psychopharmacology 2005;179:366–73.
Leidenheimer NJ, Schechter MD. Evidence for noradrenergic involvement in mediating the FG 7142 discriminative stimulus. Pharmacol Biochem Behav 1992;43:77–83.
Lepsch LB, Gonzalo LA, Magro FJ, Delucia R, Scavone C, Planeta CS. Exposure to chronic stress increases the locomotor response to cocaine and the basal levels of corticosterone in adolescent rats. Addict Biol 2005;10:251–6.
Mangiavacchi S, Masi F, Scheggi S, Leggio B, De Montis MG, Gambarana C. Long-term behavioral and neurochemical effects of chronic stress exposure in rats. J Neurochem 2001;79:1113–21.
Marlatt GA. Cue exposure and relapse prevention in the treatment of addictive behaviours. Addict Behav 1990;15:395–9.
Mason K, Heal DJ, Stanford SC. The anxiogenic agents, yohimbine and FG 7142, disrupt the noradrenergic response to novelty. Pharmacol Biochem Behav 1998;60:321–7.
Millan MJ, Newman-Tancredi A, Audinot V, Cussac D, Lejeune F, Nicolas JP, et al. Agonist and antagonist actions of yohimbine as compared to fluparoxan at α2 adrenergic receptors, serotonin 5-HT1A, 5-HT1C, 5-HT1D and dopamine D2 and D3 receptors: significance for the modulation of frontocortical monoaminergic transmission and depressive states. Synapse 2000;35:79–95.
Moody EW, Sunsay C, Bouton ME. Priming and trial spacing in extinction: effects on performance, spontaneous recovery, and reinstatement in appetitive conditioning. Q J Exp Psychol 2006;59:809–29.
Morris RW, Bouton ME. The effect of yohimbine on the extinction of conditioned fear: a role for context. Behav Neurosci 2007;121:501–14.
Schroeder BE, Schiltz CA, Kelley AE. Neural activation profile elicited by cues associated with the anxiogenic drug yohimbine differs from that observed for reward-paired cues. Neuropsychopharmacology 2003;28:14–21.
Self DW, Choi KW. Extinction-induced neuroplasticity attenuates stress-induced cocaine seeking: a state-dependent learning hypothesis. Stress 2004;7:145–55.
Shaham Y, Stewart J. Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology 1995;119:334–41.
Shaham Y, Erb S, Stewart J. Stress induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Rev 2000;33:13–33.
Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J. Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. J Neurosci 1997;17:2605–14.
Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology 2003;168:3–20.
Shalev U, Highfield D, Yap J, Shaham Y. Stress and relapse to drug seeking in rats: studies on the generality of the effect. Psychopharmacology 2000;150:337–46.
Shalev U, Marinelli M, Baumann MH, Piazza PV, Shaham Y. The role of corticosterone in food deprivation-induced reinstatement of cocaine seeking in the rat. Psychopharmacology 2003;168:170–6.
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;55:1082–9.
Sinha R. How does stress increase risk of drug abuse and relapse? Psychopharmacology 2001;158:343–9.
Stamp JA, Herbert J. Multiple immediate-early gene expression during physiological and endocrine adaptation to repeated stress. Neuroscience 1999;94:1313–22.
Stasiewicz PR, Brandon TH, Bradizza CM. Effects of extinction context and retrieval cues on renewal of alcohol-cue reactivity among alcohol-dependent outpatients. Psychol Addict Behav 2007;21:244–8.
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;51:642–51.