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Sex differences in the effects of a combined behavioral and pharmacological treatment strategy for cocaine relapse prevention in an animal model of cue exposure therapy

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Abstract

Brief interventions of environmental enrichment (EE) or the glycine transporter-1 inhibitor Org24598 administered with cocaine-cue extinction training were shown previously to inhibit reacquisition of cocaine self-administration in male rats trained to self-administer a moderate 0.3 mg/kg dose of cocaine. Determining how EE and Org24598 synergize in combination in an animal model of cue exposure therapy is novel. Important changes made in this investigation were increasing the cocaine training dose to 1.0 mg/kg and determining sex differences. Adult male and female rats self-administering 1.0 mg/kg cocaine for 35–40 daily sessions exhibited an addiction-like phenotype under a second-order schedule of cocaine delivery and cue presentation. Rats next underwent 6 weekly extinction training sessions for which treatments consisted of EE or NoEE and Vehicle or Org24598 (3.0 mg/kg in males; 3.0 or 7.5 mg/kg in females). Rats then were tested for reacquisition of cocaine self-administration for 15 daily sessions. In males, the combined EE + 3.0 mg/kg Org24598 treatment facilitated extinction learning and inhibited reacquisition of cocaine self-administration to a greater extent than no treatment and to individual EE or 3.0 mg/kg Org24598 treatments. In females, EE + 7.5 mg/kg Org24598 facilitated extinction learning, but did not inhibit reacquisition of cocaine self-administration. Thus, there were sex differences in the ability of EE + Org24598 administered in conjunction with extinction training to inhibit cocaine relapse in rats exhibiting an addiction-like phenotype. These findings suggest that this multimodal

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treatment approach might be a feasible option during cue exposure therapy in cocaine-dependent men, but not women.

Keywords

Cocaine; Cue Extinction; Environmental Enrichment; Org24598; Relapse; Sex Differences

1. Introduction

Drug addiction usurps brain mechanisms underlying learning and memory to promote persistent drug-seeking activity [1]. Following abstinence, reexposure to drug-paired cues alone (people, places, things, or an emotional state) serves as a powerful trigger for relapse [2–4], which is conceptualized as the reemergence of the original drug use patterns [5]. Consequently, modifying the impact of cues paired with drug use – e.g., through cue exposure therapy – is an important goal for effective treatment of drug addiction. As a standalone treatment, cue exposure therapy (*i.e.*, extinction training) can successfully address anxiety disorders [6]; however, it is minimally effective as a standalone treatment for substance use disorders [7]. Addiction scientists have attempted to overcome this limitation by combining cue exposure therapy with the memory-enhancing drug D-cycloserine, which is a partial agonist at the glycine site of the N-methyl-D-aspartate (NMDA) receptor that enhances glutamate neurotransmission [8,9] and augments cue exposure therapy for anxiety disorders [10]. However, early findings in drug-dependent individuals generally were disappointing, as cue exposure therapy with D-cycloserine did not facilitate a reduction in craving or inhibit relapse to drugs such as nicotine, cocaine, or alcohol [11–16]. It was revealed recently that successful trials with D-cycloserine in people rely on adherence to several essential procedural steps – steps missing in these earlier clinical reports. The optimized cue exposure therapy procedure includes pre-exposing subjects to cues before extinction training begins (*i.e.*, a memory retrieval period) and administering an appropriate dose of D-cycloserine prior to sufficiently long extinction sessions that are typically spaced at weekly intervals [10,17]. With an optimized procedure, D-cycloserine improved cue-exposure therapy for alcohol [18] and nicotine [19] to reduce craving and forestall relapse in alcohol-abusing individuals and smokers, respectively. Use of drug memory retrieval methods prior to extinction training also was shown to reduce cue-induced heroin craving in people and reinstatement of cocaine seeking in rats [20–22]. Research that builds upon these findings might afford a powerful therapeutic means for the clinical management of relapse to cocaine abuse. To address this issue, we developed an animal model of cue exposure therapy for cocaine addiction treatment through the use of a second-order schedule of cocaine delivery and cue presentation in rats and monkeys [23,24]. Advantages of using this type of schedule are that (1) each self-administration and extinction session begins with a cue-only (drug-free) fixed-interval (FI) component as a means to pre-expose subjects to drug-related cues prior to the first infusion and (2) it generates a high degree of exposure to drug-related cues throughout baseline sessions, thus providing a stringent test for how well drug-seeking responses are reduced during extinction training and self-administration reacquisition testing by the treatment strategies. In previous reports, this extinction procedure was combined with

memory enhancing strategies, either brief interventions of environmental enrichment [25,26] or administration of a glycine transporter-1 (GlyT-1) inhibitor [24,27].

Environmental enrichment (EE) is known to improve learning and memory across species through promotion of neuroplasticity [28–30]. Most studies examining the impact of EE for reducing different aspects of cocaine self-administration used male rats and relatively long-term enriched housing conditions spanning weeks to months [31–40]. EE needs to be brief to be of practical use in therapeutic settings. Beneficial effects of brief interventions of EE are supported by studies showing activation of limbic and cortical sites and memory improvement after as little as 1 hr of EE [41,42]. In adult male rats trained to self-administer 0.3 mg/kg cocaine under a second-order schedule, we demonstrated that use of two 4 hr periods of EE scheduled in conjunction with each extinction training session (the first 4 hr period was 24 hr before and the second 4 hr period was immediately after extinction training) was optimal for facilitating extinction learning and inhibiting cocaine self-administration to slow its reacquisition for a prolonged period [25,26].

Drugs that inhibit GlyT-1 increase synaptic glycine availability at NMDA receptors, resulting in enhanced memory, including extinction memory [43–46]. Low doses of GlyT-1 inhibitors having occupancies in the 30%–80% range at GlyT-1 sites produce optimal efficacy for memory enhancement [47,48]. In adult male rats trained to self-administer 0.3 mg/kg cocaine under a second-order schedule, 3.0 mg/kg of the GlyT-1 inhibitor Org24598 administered 30 min before each extinction training session was optimal in inhibiting subsequent cocaine self-administration to slow its reacquisition for a prolonged period relative to the effects of no-added treatment and 7.5 mg/kg Org24598 [24]. A similar dose of Org24598 (2.5 mg/kg) was shown to be optimal relative to higher and lower doses in producing memory enhancement in a variety of tasks in adult male rats [49].

As EE produced effects that overlapped with those of the GlyT-1 inhibitor Org24598 in cocaine self-administration experiments, an investigation into how EE and Org24598 synergize in combination with extinction training to inhibit cocaine relapse is novel and could have considerable scientific and clinical significance. The important changes made in the present investigation were increasing the cocaine training dose from 0.3 mg/kg [24–27] to 1.0 mg/kg, using a 2 min FI [25,26] rather than a 5 min FI [24,27], and determining sex differences in treatment efficacy. Use of a high, 1.0 mg/kg cocaine training dose for a prolonged period is thought to produce addictive behavior [50,51], and thus is likely to be more relevant than a more moderate 0.3 mg/kg cocaine training dose for evaluating the effects of the combined strategy for cocaine addiction treatment. Moreover, use of a 2 min FI allows for a greater hourly intake of cocaine to be achieved (a maximum of 30 infusions/hr) than a 5 min FI (a maximum of 12 infusions/hr), which also promotes an addiction phenotype [50,51]. We hypothesized that EE combined with Org24598 during extinction training will inhibit reacquisition of cocaine self-administration to a greater extent than no-added treatment and individual EE or Org24598 treatments in male rats trained to self-administer 1.0 mg/kg cocaine. Secondly, we hypothesized that the degree of reduction in self-administration of 1.0 mg/kg cocaine produced by the multimodal extinction treatment strategy will be less robust in female than male rats, given that EE housing as a standalone

treatment was shown to be ineffective in reducing self-administration of 0.3 and 1.0 mg/kg cocaine in female rats [52].

2. Materials and methods

2.1 Animals

Thirty-six male (251–275 g; 8 weeks old on arrival) and thirty female (176–200 g; 8 weeks old on arrival) Wistar rats (WIS/Crl) were obtained from Charles River Laboratories, USA and housed individually in standard cages in separate climate-controlled colony rooms under a 12 hr light/dark cycle (08:00 hr on, 22:00 hr off). All procedures took place during the light phase. Rats had free access to food and water in home cages, unless otherwise noted. All procedures complied with the 8th edition of the NIH Guide for Care and Use of Laboratory Animals and were approved by the Boston University Institutional Animal Care and Use Committee.

2.2 Procedures

2.2.1 Drugs—Cocaine hydrochloride (National Institute on Drug Abuse Drug Supply, Bethesda, MD, USA) was dissolved in a dilute heparinized 0.9% saline solution (3.0 IU/ml). The cocaine training dose was 1.0 mg/kg (5.4 mg/ml infused i.v. at 1.8 ml/min for 0.6 s/100 g body weight). Org24598 (Tocris Bio-Techne Corporation, Minneapolis, MN, USA) was dissolved in 45% 2-hydroxypropyl- β -cyclodextrin. Org24598 (3.0 or 7.5 mg/kg) and vehicle were injected i.p. (1.0 ml/kg) 30-min prior to each extinction session. We selected a dose of 3.0 mg/kg Org24598 for evaluation in male rats, as it was shown previously to be optimal for inhibiting reacquisition of cocaine self-administration following its administration during extinction training in male rats trained to self-administer 0.3 mg/kg cocaine [24]. Both doses of Org24598 were evaluated in female rats, as no prior studies have documented if this GlyT-1 inhibitor can produce memory-enhancing effects in female rats.

2.2.2 Cocaine Self-Administration—Fig. 1 illustrates the experimental timeline for procedures 2.2.2 through 2.2.5. After acclimation to the animal facility for at least 72 hr, rats first were trained under a fixed-ratio 1 (FR 1) schedule to rapidly earn 100 food pellets (45 mg chocolate-flavored; Bio-Serv, Frenchtown, NJ, USA) in order to facilitate operant lever pressing for cocaine. To maintain motivation for lever pressing during these daily sessions, the 100 food pellets were supplemented with limited amounts of rodent chow in the home cages (8–10 g in male rats and 6–8 g in female rats). After 2–3 days of training, rats typically lever pressed for 100 food pellets within 20–30 min. Catheters then were implanted [53] into either the right femoral vein (male rats) or right jugular vein (female rats), and our customary post-surgical care and catheter maintenance procedures were followed [26]. Catheters were tested periodically for patency by i.v. infusion of 1.0 mg/0.1 ml of methohexital sodium (Brevital; JHP Pharmaceuticals, Rochester, MI, USA), which induces rapid transient loss of muscle tone. Daily 1 hr cocaine self-administration sessions (Monday-Friday) began one week after surgery, with home cage food restriction during the first few sessions. The self-administration chambers [53] were located in different testing rooms for male and female rats. All rats first self-administered cocaine under an FR 1 schedule and then under an FR 5 schedule, with a 2-sec cue light paired with each cocaine infusion. Once

FR 5 responding was reliably maintained, cocaine delivery and cue presentation transitioned to a second-order (FI 2 min [FR 5: S]) schedule. Under this schedule, every fifth press of the active lever (FR 5) during the 2 min FI produced a 2-sec cue light (S), and the first FR 5 completed after the 2 min FI elapsed produced the 2-sec cue light paired with an infusion of cocaine (a maximum of 30 during a 1 hr session). Responses on the inactive lever (right or left, counterbalanced across subjects) were counted separately, but had no scheduled consequences. In addition, white noise (70-db) was presented in the background for the duration of each session. Rats were acclimated in weekly 15 min periods to the EE chamber and companion animals on three or four occasions to ensure compatibility prior to incorporating the 4 hr EE periods during extinction training (see section 2.2.4). Cocaine self-administration was well-established (35–40 daily sessions) in order to provide a long cocaine history before extinction training began.

2.2.3 Extinction Training—Beginning 48 hr after the last cocaine self-administration session, lever pressing was extinguished. To pre-expose rats to drug cues prior to extinction training, each extinction session began with the 2 min FI component during which the drug-paired cue light was presented for 2 sec after completing each FR 5 on the active lever. However, the anticipated i.v. infusion of cocaine at the end of the initial cue-only component was withheld and rats received an i.v. infusion of saline instead. Extinction training with saline infusions continued for the remainder of the 1 hr session under the FI 2 min [FR 5:S] second-order contingencies. Six extinction training sessions (1hr/week for 6 weeks) were conducted, analogous to clinical protocols utilizing 1 hr sessions spaced at weekly intervals [10,17].

2.2.4 Environmental Enrichment—EE consisted of 4 hr periods in the enrichment chambers [25], which were located in different testing rooms for male and female rats. Each chamber was equipped with two running wheels, three levels of ramps and platforms, movable tunnel structures, and numerous manipulatable items and chew toys. Items were changed weekly to maintain novelty. Commercial pulp fiber bedding was used as nesting material on the cage bottom and pieces of sweetened cereal were hidden in various locations to encourage foraging. EE periods were provided both 24 hr before and immediately after each of the extinction training sessions. Three to four compatible rats were placed together during each of these 4 hr EE periods. We previously established this EE protocol as optimal for facilitating extinction and inhibiting reacquisition of cocaine self-administration in male rats [25]. Some rats remained in their home cages before and after extinction training sessions without receiving EE (NoEE).

2.2.5 Reacquisition of Cocaine Self-Administration—Reacquisition of cocaine self-administration was evaluated beginning 1 week after the last extinction training session. The FI 2 min [FR 5: S] second-order contingencies were identical to those established during the baseline cocaine self-administration phase, as described in section 2.2.1. The reacquisition phase lasted for 15 consecutive daily sessions (Monday-Friday).

2.3. Experiments

2.3.1 Experiment 1: Effects of EE + Org24598 in Male Rats—Of the 36 adult male rats, 2 lost catheter patency early in training. Following initial self-administration training, the remaining 34 rats were assigned randomly to 4 groups that received different treatments during extinction training. Group 1 received EE + 3.0 mg/kg Org (n=8); Group 2 received EE + Veh (n=9); Group 3 received NoEE + 3.0 mg/kg Org (n=8); and Group 4 received NoEE + Veh (n=9). Org or Veh was injected i.p. 30 min prior to each of the extinction training sessions and EE was provided in 4 hr periods, both 24 hr before and immediately after each extinction training session. This design allowed testing the effects of the EE + Org treatments combined (Group 1) and the effects of the EE or Org treatments individually (Groups 2 and 3) against a no-added treatment control (Group 4) on cocaine-cue extinction learning and reacquisition of cocaine self-administration in male rats.

2.3.2 Experiment 2: Effects of EE + Org24598 in Female Rats—This experiment determined if the combined EE + Org treatments had the same or different effects on cocaine-cue extinction learning and reacquisition of cocaine self-administration in female rats as in male rats. Of the 30 adult female rats, 6 lost catheter patency early in training. Following initial self-administration training, the remaining 24 rats were assigned randomly to 3 groups that received different treatments during extinction training. Group 1 received EE + 3.0 mg/kg Org (n=8); Group 2 received EE + 7.5 mg/kg Org (n=7); and Group 3 received NoEE + Veh (n=9). Org or Veh were injected i.p. 30 min prior to each of the extinction training sessions and EE was provided in 4 hr periods, both 24 hr before and immediately after each extinction training session. Given that EE housing as a standalone treatment was ineffective in reducing self-administration of 1.0 mg/kg cocaine in female rats in a previous study [52], only the EE + Org treatment strategy was evaluated. This design allowed testing the effects of the EE + Org treatments combined (Group 1 and Group 2) against a no-added treatment control (Group 3) and for determining whether 3.0 or 7.5 mg/kg Org produced the more optimal effect in combination with EE on extinction learning and reacquisition of cocaine self-administration in female rats. Two female rats assigned to Group 2 lost catheter patency before completing the reacquisition phase. Their data were included in analyses of the baseline and extinction phases, but were excluded from analyses of the reacquisition phase.

2.4 Statistical Analysis

The behavioral measures in Experiments 1 and 2 first were analyzed separately in male and female rats. To analyze sex differences in the effects of EE + Org for cocaine relapse prevention, we selected the NoEE + Veh control groups and the EE + Org treatment groups to compare in a balanced factorial design. The group for whom the combined EE + Org treatment was behaviorally active during extinction training (EE + 3.0 mg/kg Org for males and EE + 7.5 mg/kg Org for females) was used in these analyses. Active and inactive lever responses at baseline (last 5 training sessions under the second-order schedule) were analyzed by two-factor (Group x Lever) or three-factor (Sex x Group x Lever) repeated measures (RM) analysis of variance (ANOVA). The average daily mg/kg cocaine intakes at baseline were analyzed by one-factor (Group) or two-factor (Sex x Group) ANOVA. Due to 2- to 5-fold differences in baseline responses among individual rats within each of the

groups (see individual data points in Fig. 1A and Fig. 4A), active lever responses during the weekly extinction sessions and the daily reacquisition sessions were expressed as percent of baseline responses prior to analysis (see raw data in Supplementary Table S1). The percentages of baseline active lever responses were analyzed by two-factor (Group x Session) or three-factor (Sex x Group x Session) RM ANOVA for the extinction and reacquisition phases. The average daily mg/kg cocaine intakes across the 15 reacquisition sessions were analyzed by a one-factor (Group) or two-factor (Sex x Group) ANOVA. For the sex-specific analyses, Dunnett tests were applied for post-hoc comparisons, using the NoEE + Veh group as the control reference in the group analyses and session 1 as the control reference in the session number analyses. For analyses examining sex differences, Tukey tests were applied for post-hoc comparisons.

3. Results

3.1 Experiment 1: Effects of EE + Org24598 in Male Rats

3.1.1 Cocaine Baseline—Cocaine self-administration behavior was similar across the four groups of male rats at baseline, before extinction treatments were initiated (Fig. 2). As revealed by 2-factor RM ANOVA, there was a greater number of active than inactive lever responses across groups (Fig. 2A), as shown by the significant main effect for lever ($F[1,30] = 182.9, p=0.001$). Lever responses did not differ significantly as a main effect of group ($F[3,30] = 2.0, p=0.13$), nor was there an interaction between group and lever ($F[3,30] = 0.9, p=0.47$). Analysis of the average daily mg/kg cocaine intakes at baseline (Fig. 2B) showed no significant differences among the four groups of male rats as revealed by 1-factor ANOVA ($F[3,30] = 2.4, p=0.08$).

3.1.2. Cocaine-Cue Extinction—The combined EE + 3.0 mg/kg Org treatment facilitated cocaine-cue extinction learning in male rats (Fig. 3). As revealed by 2-factor RM ANOVA, active lever responding declined across the course of extinction training (main effect of session number; $F[5,150] = 9.87, p=0.001$), with significantly less responding during sessions 3–6 compared to session 1 ($ps=0.001$). However, the magnitude of active lever responding during extinction training was different among the four groups (main effect of group; $F[3,30] = 6.92, p=0.001$). The group that received the combined treatment (EE + 3.0 mg/kg Org) responded significantly less than the control group that did not receive treatment (NoEE + Veh) during extinction training ($p=0.001$). In contrast, responding in the groups that only received EE (EE + Veh) and only received the GlyT-1 inhibitor (NoEE + 3.0 mg/kg Org) during extinction training was not significantly different from the NoEE + Veh control ($p=0.15$ and 0.71 , respectively). There was no group x session number interaction during extinction training in the male rats ($F[15,150] = 0.7, p=0.78$).

3.1.3 Cocaine Reacquisition—The combined EE + 3.0 mg/kg Org treatment administered during extinction training inhibited subsequent reacquisition of cocaine self-administration in male rats (Fig. 4). As revealed by 2-factor RM ANOVA, the magnitude of active lever responding during reacquisition sessions was different among the four groups (main effect of group; $F[3,30] = 12.29, p=0.001$). Responding across the daily reacquisition sessions (Fig. 4A) was significantly lower in the EE + 3.0 mg/kg Org group compared to the

NoEE + Veh control group ($p=0.001$), which did not differ from the EE + Veh and NoEE + 3.0 mg/kg Org treatment groups ($p=1.0$ and 0.93 , respectively). These latter groups returned to baseline levels of cocaine self-administration responding within 1–2 reacquisition sessions, whereas the EE + 3.0 mg/kg Org group required over 13 reacquisition sessions to return to baseline levels of responding. Session number also was a significant factor ($F[14,420] = 2.38$, $p=0.003$), with responding trending upward during sessions 2–15 compared to session 1 ($ps<0.05$). This session pattern was less apparent in the EE + 3.0 mg/kg Org group, but the session number $\times$ group interaction for responding was not significant ($F[42,420] = 1.37$, $p=0.067$). The average daily mg/kg cocaine intake across the 15 reacquisition sessions (Fig. 4B) was significantly lower ($p<0.003$) in the group receiving EE + 3.0 mg/kg Org (representing $63 \pm 8\%$ of baseline intake) compared to the NoEE + Veh control group (representing $103 \pm 3\%$ of baseline intake), as revealed by the significant effect of group ($F[3,30] = 6.55$, $p<0.002$) in the 1-factor ANOVA. Cocaine intakes in the groups receiving EE + Veh (representing $95 \pm 6\%$ of baseline intake) and the NoEE + 3.0 mg/kg Org (representing $110 \pm 3\%$ of baseline intake) treatments were not significantly different from the NoEE + Veh control group ($p=0.97$ and 0.96 , respectively).

3.2 Experiment 2: Effects of EE + Org24598 in Female Rats

3.2.1. Cocaine Baseline—Cocaine self-administration behavior was similar across the three groups of female rats at baseline, before extinction treatments were initiated (Fig. 5). As revealed by 2-factor RM ANOVA, there was a greater number of active than inactive lever responses across groups (Fig. 5A), as shown by the significant main effect for lever ($F[1,21] = 144.4$, $p=0.001$). Lever responses did not differ significantly as a main effect of group ($F[2,21] = 0.3$, $p=0.76$), nor was there an interaction between group and lever ($F[2,21] = 0.3$, $p=0.75$). Analysis of the average daily mg/kg cocaine intakes at baseline (Fig. 5B) showed no significant differences among the three groups of female rats as well ($F[2,21] = 0.6$, $p=0.55$) as revealed by 1-factor ANOVA.

3.2.2 Cocaine-Cue Extinction—The combined EE + 7.5 mg/kg Org treatment facilitated cocaine-cue extinction learning in female rats (Fig. 6). As revealed by 2-factor RM ANOVA, active lever responding declined across the course of extinction training (main effect of session number; $F[5,105] = 2.33$, $p=0.047$), with significantly less responding during sessions 3 and 4 compared to session 1 ($ps<0.001$). However, the magnitude of active lever responding during extinction training was different among the three groups (main effect of group; $F[2,21] = 4.63$, $p=0.022$). The group that received the combined treatment with the higher dose of Org (EE + 7.5 mg/kg Org) responded significantly less than the control group that did not receive treatment (NoEE + Veh) during extinction training ($p=0.05$). In contrast, responding in the group that received the combined treatment with the lower dose of Org (EE + 3.0 mg/kg Org) during extinction training was not significantly different from the NoEE + Veh control ($p=0.68$). There was no interaction between session number and group during extinction training in the female rats ($F[10,105] = 1.2$, $p=0.29$).

3.2.3 Cocaine Reacquisition—Neither dose of Org administered in combination with EE during extinction training altered subsequent reacquisition of cocaine self-administration in female rats (Fig. 7). As revealed by 2-factor RM ANOVA, the magnitude of active lever

responding during reacquisition sessions was not significantly different ($F[2,19] = 0.3$, $p=0.73$) among the groups receiving EE + 3.0 mg/kg Org, EE + 7.5 mg/kg Org, and NoEE + Veh, with the three groups returning to baseline levels of cocaine self-administration responding within 1–3 reacquisition sessions (Fig. 7A). In addition, session number and the session number x group interaction were not significant factors ($F[14,266] = 1.2$, $p<0.27$ and $F[28,266] = 1.1$, $p<0.40$, respectively). The average daily mg/kg cocaine intakes across the 15 reacquisition sessions (Fig. 7B) were not significantly different among the groups receiving NoEE + Vehicle (representing 84 ± 8 % of baseline intake), EE + 3.0 mg/kg Org (representing 98 ± 8 % of baseline intake), and EE + 7.5 mg/kg Org (representing 99 ± 13 % of baseline intake) as well ($F[2,19] = 0.8$, $p=0.37$) as revealed by 1-factor ANOVA.

It should be noted that one female rat in the EE + 7.5 mg/kg Org group who had emitted near baseline levels of responding during reacquisition sessions 1–3 suddenly responded between 200% - 1200% above baseline during reacquisition sessions 4 through 15, causing the large variability in reacquisition responding in this group of female rats (Fig 7A). The Grubbs test showed that this rat had significant outlying values for sessions 6–11 and 13–14. For this rat, most of the active lever responses were made during the 2 min drug-free FI components of the second-order schedule, during which time FR 5 responding was maintained solely by the 2-sec brief stimulus light. As a result, the excess responding made by this rat did not impact its average daily mg/kg cocaine intake, which was within normal limits. Also, because this rat's catheter was determined to be functional via complete loss of muscle tone immediately after i.v. infusion of 1.0 mg Brevital, data from this rat were retained in the above 2-factor RM ANOVA analysis. The remaining rats in the EE + 7.5 mg/kg Org group maintained responding at or near baseline levels throughout the reacquisition phase (see Supplementary Table S1). In an analysis of reacquisition responding after removal of this rat's data, the main effects for group ($F[2,18] = 2.0$, $p=0.16$) and session number ($F[14, 252] = 1.1$, $p=0.34$) as well as the group x session number interaction ($F[28, 252] = 0.7$, $p=0.90$) remained nonsignificant.

3.2.4 Sex Differences—At baseline (Supplementary Fig. S1), there were no statistical differences between male and female rats. As revealed by 3-factor RM ANOVA, there was a similar number of active and a similar number of inactive lever responses across groups ($F[1, 29] = 1.9$, $p=0.17$ for the sex x group x lever interaction). As expected, there were more active than inactive lever responses (main effect of lever, $F[1, 29] = 174.2$, $p=0.001$). As revealed by 2-factor ANOVA, there was a similar average daily mg/kg cocaine intake across sexes and groups ($F[1, 29] = 1.8$, $p=0.19$ for the sex x group interaction). Thus, male and female rats had comparable baseline cocaine self-administration behavior before initiating treatments during extinction training.

During extinction training (Supplementary Fig. S2), the 3-factor RM ANOVA revealed a significant sex x group x session number interaction for responding ($F[5,145] = 2.67$, $p=0.025$). Within the NoEE + Veh control groups, female rats responded significantly less than male rats ($F[1,16] = 8.3$, $p=0.01$), with no interaction of sex x session number ($F[5, 80] = 1.8$, $p=0.12$). Within the EE + Org groups, there was a sex x session number interaction ($F[5,65] = 5.1$, $p=0.001$), with female rats responding significantly less than male rats during session 1 ($p=0.021$), session 2 ($p=0.008$), and session 4 ($p=0.047$) of the weekly sessions.

This profile of results suggests a faster decline in extinction responding in non-treated female than male rats and a greater efficacy of EE + Org for facilitating extinction learning in females relative to males. Despite the EE + Org treatment favoring the female rats during extinction training, this combined treatment strategy was ineffective for inhibiting cocaine relapse in the female rats.

During reacquisition of cocaine self-administration (Supplementary Fig. S3), the 3-factor RM ANOVA revealed a significant sex x group x session number interaction for responding ($F[14,378] = 1.94, p=0.022$). Within the NoEE + Veh control groups, female rats responded significantly less than male rats over the daily sessions (main effect of sex; $F[1,16] = 12.4, p=0.003$), with no interaction of sex x session number ($F[14, 182] = 1.5, p=0.13$). Within the EE + Org groups, female rats responded significantly more than male rats over the daily sessions (main effect of sex; $F[1,13] = 5.8, p=0.032$), with no interaction of sex x session number ($F[14, 252] = 1.7, p=0.057$). As revealed by 2-factor ANOVA, the average daily mg/kg cocaine intake during reacquisition sessions showed a significant sex x group interaction ($F[1,29] = 9.9, p=0.004$). Within the NoEE + Veh control groups, female rats consumed less cocaine than male rats ($p=0.042$) and within the EE + Org groups, female rats consumed more cocaine than male rats ($p=0.027$). The average daily mg/kg cocaine intakes during reacquisition did not differ between the two groups of female rats ($p=0.12$), but did differ between the two groups of male rats whereby the EE + Org group consumed less cocaine than the NoEE + Veh control group ($p=0.007$). This profile of results suggests that EE + Org treatment during extinction training had greater efficacy for inhibiting cocaine relapse in male relative to female rats.

4. Discussion

4.1 Male and Female Rats Exhibited an Addiction-Like Phenotype at Baseline

Baseline performance under the FI 2 min [FR 5:S] second order schedule of 1.0 mg/kg cocaine delivery and cue presentation was robust and similar between male and female rats. A finding of no sex differences in baseline cocaine self-administration behavior is consistent with a previous report utilizing a 1.0 mg/kg cocaine training dose under FR schedules of cocaine delivery [54]. In the present study, male and female rats on average made over 100 active lever responses during the 1 -hr long baseline sessions and maintained hourly cocaine intake at ~12 mg/kg. This level of active lever responding during baseline sessions indicates that a large portion of the responses (at least 40%) were maintained by the 2-sec brief stimulus light presented during the 2 min drug-free intervals, given that a total of only 60 active lever responses are necessary to earn 12 infusions of 1.0 mg/kg cocaine during the hour-long sessions under the second-order schedule. This reflects a high degree of drug seeking and cue reactivity, which are hallmarks of addictive behavior [55,56]. Moreover, hourly cocaine intakes fell within the range of hourly cocaine intakes (12–20 mg/kg) reported after escalation in extended access models of addiction [57–60]. These findings support the use of a prolonged training history with 1.0 mg/kg cocaine to model addictive behavior in cocaine self-administration experiments [50,51]. It was against this background that rats were evaluated for the capacity of EE + Org to facilitate cocaine-cue extinction

learning and inhibit reacquisition of self-administration after training with a high dose of i.v. cocaine.

4.2 EE + Org24598 Inhibited Reacquisition of Cocaine Self-Administration in Male Rats

The hypothesis that EE combined with Org during extinction training will inhibit reacquisition of cocaine self-administration to a greater extent than no treatment and individual EE or Org treatments in male rats trained to self-administer 1.0 mg/kg cocaine was supported. The combined EE + Org 3 treatment in male rats facilitated the rate of cocaine-cue extinction learning and inhibited reacquisition of cocaine self-administration, whereas the EE treatment alone and the Org 3 treatment alone were ineffective in this regard. These findings contrast with our earlier observations in male rats trained to self-administer 0.3 mg/kg cocaine, showing that both the EE treatment alone and the Org 3 treatment alone inhibited subsequent reacquisition of cocaine self-administration and that the EE treatment alone facilitated the rate of cocaine-cue extinction learning [24–26]. Differences in hourly cocaine consumption at baseline might help explain these different effects on extinction and relapse behavior under the 0.3 mg/kg and 1.0 mg/kg cocaine training dose conditions. The 0.3 mg/kg cocaine training dose generated average hourly cocaine intakes of ~6 mg/kg at baseline, likely resulting in a more moderate degree of addiction vulnerability and relapse potential than the 1.0 mg/kg cocaine training dose, which generated average hourly cocaine intakes of ~12 mg/kg at baseline. Thus, individual treatments might be successful if addiction vulnerability and relapse potential is moderate, but a combined treatment strategy might be required if addiction vulnerability and relapse potential is high.

Work by other investigators using individual memory enhancing treatments targeting the glycine site or involving EE during extinction training supports this perspective on relapse potential for male rats with high cocaine intake at baseline. In male rats with average daily hourly cocaine intakes of ~15 mg/kg at baseline, treatment with 100 mg/kg of the glycine site full agonist D-serine vs. saline during the first four sessions of operant extinction training facilitated the rate of extinction learning, but was ineffective in reducing reinstatement of cocaine seeking following a priming injection of cocaine [61]. In a different study in which male rats had average daily hourly cocaine intakes of ~12.5 mg/kg, treatment with 100 mg/kg of D-serine vs. saline during 5 sessions of operant extinction training was ineffective in altering the rate of extinction learning and for reducing cue-induced reinstatement of cocaine seeking, although D-serine produced a significant reduction in weakly generated (10 vs. 3 responses) reinstatement of cocaine seeking following a priming injection of cocaine [62]. In male rats with average daily hourly cocaine intakes of ~10.5 mg/kg, an EE intervention facilitated the rate of extinction learning, but did not significantly reduce cue-induced reinstatement of cocaine-seeking 1-day or 7-days later compared to extinction training alone [34]. In contrast, an EE intervention in male rats with average daily hourly cocaine intakes of ~5.5 mg/kg not only facilitated the rate of extinction learning, but also reduced cue-induced reinstatement of cocaine seeking compared to extinction training alone [31]. A clinical implication of these findings is that for treatment of cocaine addiction in highly dependent men undergoing cue-exposure therapy, a synergistic behavioral and pharmacological treatment strategy might be required to successfully augment the retrieval

of extinction memory to forestall relapse if individuals are re-exposed to cocaine and cocaine-paired cues following a period of abstinence.

There remains a possibility that treatment with EE and Org devalued the cocaine environment in which extinction training took place rather than augmenting extinction memory to inhibit subsequent reacquisition of cocaine self-administration. For example, it has been shown that EE can reduce the incentive salience of cocaine-associated environmental cues [31,34]. If these memory enhancing strategies that were applied during extinction training inhibited reacquisition of cocaine self-administration by reducing the value of the cocaine environment, then application of these strategies in the cocaine environment without extinction training should have inhibited reacquisition of cocaine self-administration as well. In [23], [24], and [27], when a glycine site partial agonist (D-cycloserine) or a GlyT-1 inhibitor (RO4543338 or Org24598) was combined with weekly cocaine self-administration sessions or with weekly abstinence sessions in the cocaine environment instead of weekly extinction sessions, reacquisition of cocaine self-administration was not inhibited, arguing against these drugs devaluing the cocaine environment. The same outcome occurred in [25] when EE was combined with weekly abstinence sessions in the cocaine environment instead of weekly extinction sessions. In all cases, these behavioral and pharmacological strategies had to be combined with cocaine-cue extinction training to inhibit subsequent reacquisition of cocaine self-administration.

4.3 EE + Org24598 Failed to Inhibit Reacquisition of Cocaine Self-Administration in Female Rats: Sex Differences

The hypothesis that the degree of reduction in self-administration of 1.0 mg/kg cocaine produced by EE combined with Org during extinction training will be less robust in female than male rats was supported. The combined EE + Org 7.5 treatment in female rats facilitated the rate of cocaine-cue extinction learning, but did not inhibit subsequent reacquisition of cocaine self-administration. The combined EE + Org 3 treatment was ineffective for altering responding during the extinction and reacquisition phases in female rats, unlike in male rats. One interesting sex difference observed in the NoEE + Veh control groups was that during cocaine-cue extinction training and reacquisition testing, female rats had significantly lower rates of responding (and lower cocaine consumption) than male rats despite similar baseline self-administration behavior (Figures S1–S3). Others have reported greater or no difference in extinction responding, lower or no difference in cue-induced reinstatement of responding, and greater or no difference in cocaine prime-induced reinstatement of responding in female than male rats trained to self-administer cocaine [63–67]. Although these findings make it difficult to predict how female rats will react to relative to male rats in general during extinction and relapse testing, the lower rates of responding in female than male rats under the control treatment condition in the present study makes the lack of an effect of the EE + Org 7.5 treatment on reacquisition of cocaine self-administration in female rats more notable.

In a consideration of each treatment modality separately, EE can generally assist with memory improvement equally in both male and female rats, as shown in past studies of spatial memory in a Morris Water Maze task [68] and preference for a novel object in a

novel object recognition task [69]. No prior studies have documented if Org24598 can produce memory-enhancing effects in female rats, however, reports utilizing the glycine site partial agonist D-cycloserine have revealed some interesting sex differences. Treatment with 15 mg/kg D-cycloserine improved memory retention in an eyeblink conditioning task in male but not female rats [70]. In studies of conditioned fear, 30 mg/kg D-cycloserine, but not 15 mg/kg D-cycloserine, facilitated fear extinction in female rats [71], whereas 15 mg/kg D-cycloserine was sufficient to facilitate fear extinction in male rats [72]. In cocaine self-administration studies, 30 mg/kg D-cycloserine, but not 15 mg/kg D-cycloserine, facilitated cocaine-cue extinction learning in male rats [23,73]. These findings collectively suggest that memory improvement via activation of the glycine site on the NMDA receptor is dose-dependent, with female rats generally requiring a higher treatment dose than male rats. Such sex differences help to explain why the higher dose of Org (7.5 mg/kg) was required in combination with EE to facilitate the rate of cocaine-cue extinction learning in female rats, whereas the lower dose of Org (3.0 mg/kg) in combination with EE was effective in facilitating cocaine-cue extinction learning in the males but not females. However, unlike in male rats, the combination of EE with a suitable dose of Org was ineffective for inhibiting cocaine relapse in the female rats after extinction training. It could be considered a limitation that the effects of Org alone and EE alone with extinction training were not evaluated in female rats, as they were in male rats. However, it is unlikely that Org alone and EE alone would inhibit reacquisition of cocaine self-administration in female rats, based on the null effects of the combined treatment on this measure in female rats (present findings) and on the inability of an EE intervention to modify the reinforcing effect of 1.0 mg/kg cocaine [52] in female rats.

Other investigators have evaluated a combined behavioral and pharmacological treatment approach for cocaine relapse prevention. In [74], the behavioral strategy (wheel running, an aspect of EE in rats) was implemented during operant extinction training (14-daily sessions) and during reinstatement tests (5 four-session tests with extinguished responding reinstated by stress, stress + cues, cues, a cocaine priming injection, or a cocaine priming injection + cues), while the pharmacological strategy (progesterone) was implemented only during the reinstatement tests. Rats were trained to self-administer 0.4 mg/kg cocaine over ten 6 hr extended access sessions, generating average hourly cocaine intakes of ~10 mg/kg in female rats and ~8.6 mg/kg in male rats. During operant extinction training, female rats with running wheel access extinguished lever responding faster than female rats without running wheel access, but running wheel access did not benefit the rate of extinction in the male rats. Running wheel access + progesterone was more successful overall than either individual treatment alone in reducing reinstatement of cocaine-seeking behavior in both female and male rats. In a later study [75], only female rats were evaluated and the behavioral strategy (wheel running) and the pharmacological strategy (atomoxetine) were implemented only during the reinstatement tests (2 four-session tests with extinguished responding reinstated by a cocaine priming injection or cues). As above, rats were trained to self-administer 0.4 mg/kg cocaine over ten 6 hr extended access sessions, generating average hourly cocaine intakes of ~10 mg/kg. Running wheel access + atomoxetine was more successful overall than either individual treatment alone in reducing reinstatement of cocaine-seeking behavior in female rats.

The above reports [74,75] bear some similarities, but also some differences, to findings in the present study. In terms of similarities, EE + Org administered during extinction training was more effective in reducing extinction responding in female than male rats. This finding suggests a better facilitation of extinction learning in the females after EE + Org treatment. An alternative interpretation is that EE + Org reduced motivation to lever press in the absence of cocaine reinforcement to a greater extent in female than male rats. However, in a study examining male and female rats selectively bred for low or high responding, motivation to continue responding was greater in low-responding females compared to low-responding males when cocaine reinforcement was withheld for short 15 min periods under a progressive ratio schedule [76]. There was no difference in motivation to continue responding when cocaine reinforcement was withheld in high-responding females and males. These findings suggest it unlikely that reduced motivation to lever press in the female rats was the basis for sex differences in the effects of EE + Org during extinction.

It also is unlikely that sex differences in the effects of EE + Org during extinction training was related to female rats having a greater predisposition for goal tracking (responding maintained primarily by reward) than sign-tracking (responding maintained primarily by reward-paired cues). As shown previously, there were no sex differences in the distributions of goal-tracking vs. sign-tracking traits within cohorts of male and female rats [77]. Moreover, rats with goal-tracking and sign-tracking traits were shown to extinguish cocaine-maintained responding at the same rate [78]. Other similarities with [74] and [75] were that EE alone was ineffective in facilitating extinction in male rats and that the combined EE + Org treatment strategy was more successful than either individual treatment alone in male rats for inhibiting relapse. As discussed above (section 4.2), a need for a combined behavioral and pharmacological treatment for successful relapse prevention might be related to a high degree of addiction vulnerability and relapse potential in male rats when baseline cocaine intake is high. The main difference from [74] and [75] was that the combined EE + Org treatment strategy was not successful for relapse prevention in female rats. Differences in reacquisition testing (15 sessions with i.v. cocaine availability throughout each session) vs. reinstatement testing (4 session per test with a single i.p. cocaine injection prior to the 1 or 2 priming tests) might be a critical factor for this difference. Although baseline hourly cocaine intakes were similarly high in these three studies, it might be more difficult for a combined behavioral and pharmacological treatment strategy to suppress cocaine self-administration behavior (present findings) than cocaine reinstatement behavior [74,75] in female rats.

Some insight into why the combined EE + Org treatment strategy administered with cocaine-cue extinction training inhibited cocaine relapse in male rats but not female rats stems from a consideration of sex differences within mesolimbic reward circuitry of 1) the molecular changes produced by EE, particularly as it applies to brain-derived neurotrophic factor (BDNF) and its high-affinity receptor tropomyosin-related kinase B (TrkB); 2) the behavioral changes produced by genetic deletion of GlyT-1, a functional proxy for GlyT-1 inhibition; and 3) the basal expression levels of NMDA receptors. In mice, EE exposure for 7–8 weeks was shown to enhance BDNF protein and/or mRNA expression in the hippocampus and frontal cortex of male wild-type mice, but not in female wild-type mice [79]. Sex differences in the effects of EE on BDNF might be crucial because recently, we

demonstrated that inhibition of reacquisition of cocaine self-administration in male rats receiving brief periods of EE with weekly extinction training was associated with increased TrkB signaling (receptor for BDNF) in dorsal hippocampus and ventromedial prefrontal cortex, but not nucleus accumbens and basolateral amygdala [26]. Others demonstrated that when TrkB signaling within the ventromedial prefrontal cortex was blocked in female rats, extinction of cocaine seeking was impaired [80]. Sex differences also occurred for the behavioral effects of genetic deletion of forebrain GlyT-1. During extinction of appetitive Pavlovian conditioning (nose pokes during conditioned stimulus presentations in absence of reward), male mice with genetic deletion of GlyT-1 exhibited facilitated extinction compared to male control mice, whereas female mice with genetic deletion of GlyT-1 exhibited resistance to extinction compared to female control mice [81]. In terms of the NMDA receptor, female rats were shown to have higher levels of the NR2B subunit (glutamate binding site) in the hippocampus and higher levels of the NR1 subunit (glycine binding site) in the hippocampus, prefrontal cortex, and amygdala compared to male rats [82]. Such sex differences in basal NMDA receptor expression could influence the mnemonic properties of EE and Org24598 differentially in males and females.

Hormonal differences also might play a role. For example, levels of 17 β -estradiol (E2) were shown to be substantially higher in the hippocampus of male than female rats, even during proestrus [83]. Neuron-derived E2 regulates hippocampal synaptic plasticity and improves memory, but there are sex differences in the cell-signaling pathways through which E2 facilitates memory consolidation [84,85]. Collectively, these findings suggest that female rats might have been insensitive to EE + Org treatment for cocaine relapse prevention because, in part, EE exposure did not produce a long-lasting increase in TrkB signaling in dorsal hippocampus and ventromedial prefrontal cortex and Org administration did not sufficiently augment extinction memory retrieval in female rats. A future direction includes determining if TrkB, NR1, NR2B, and other signaling molecules associated with EE + Org treatment during cocaine-cue extinction are the same or different in male vs. female rats. Such studies might help elucidate why cocaine-dependent men are more successful than cocaine-dependent women at establishing control over cue-induced arousal during cue exposure therapy [86] and why women exhibit greater vulnerability for cocaine dependence and relapse than men [87]. Other potentially fruitful areas of investigation include studies that determine if the combined effects of EE + Org with cue-extinction training generalize to other drugs of abuse or to consumption of food or sucrose, as shown for the anti-relapse effects of EE alone in reinstatement studies [88]. Imbedded within these investigations should be an analysis of sex differences to determine if the failure of this treatment approach to inhibit relapse in female rats applies just to cocaine or if it extends to other drug and non-drug reinforcers.

5. Conclusions

Use of a behavioral and pharmacological intervention, EE + a GlyT-1 inhibitor, strategically administered in conjunction with cocaine-cue extinction training was effective in inhibiting reacquisition of cocaine self-administration in male rats. Responding was reduced to approximately 50% of baseline levels and cocaine intake was reduced to approximately 63% of baseline levels for a prolonged period. This multimodal treatment approach was

ineffective for relapse prevention in female rats, suggesting EE + a GlyT-1 inhibitor might be a feasible option in cocaine-dependent men, but not women, undergoing cue exposure therapy. Although cocaine self-administration was not completely suppressed in male rats, non-abstinence endpoints for cocaine use disorder treatment are gaining traction. In a recent evaluation of outpatient-based behavioral treatment and/or pharmacotherapy in a pooled dataset [89], participants with a high level of cocaine use at baseline and who reduced cocaine use to a lower level by end-of-treatment had improvements in functional outcomes at 12-month follow up (assessed by the Addiction Severity Index) that were of similar magnitude to the improvements observed in participants who were completely abstinent by end-of-treatment. In relation to cue exposure therapy, it is likely that cocaine-dependent patients also will undergo Coping Skills Training and other Cognitive Behavioral Therapies [90,91]. These adjunct therapies teach patients to cognitively control reactions during craving episodes and help to prevent drug lapses from spiraling into a full-fledged relapse to addiction. Cognitive Behavioral Therapy cannot be modeled in animals, which is a limitation. It is possible that Cognitive Behavioral Therapy coupled with pharmacological and behavioral strategies that augment retrieval of extinction memory will afford longer-term relapse prevention in cocaine-dependent men than what our experiments in male rats predict, as the rats received just the extinction treatment strategy followed by daily cocaine availability and reexposure. Clearly, a treatment strategy other than EE + a GlyT-1 inhibitor would be needed to improve cue exposure therapy in cocaine-dependent women to deter cocaine relapse for a prolonged period. The discovery of novel ways to optimize TrkB and NMDA receptor signaling in mesolimbic brain regions during cue-exposure therapy could lead to the development of treatment tools that are beneficial for relapse prevention in cocaine-dependent women (as well as men).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Hyman SE, Addiction: a disease of learning and memory, *Am J Psychiatry* 162(8) (2005) 1414–22. [PubMed: 16055762]
- [2]. O'Brien CP, Childress AR, Ehrman R, Robbins SJ, Conditioning factors in drug abuse: can they explain compulsion?, *J Psychopharmacol* 12(1) (1998) 15–22. [PubMed: 9584964]
- [3]. Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP, Limbic activation during cue-induced cocaine craving, *Am J Psychiatry* 156(1) (1999) 11–8. [PubMed: 9892292]
- [4]. Walton MA, Castro FG, Barrington EH, The role of attributions in abstinence, lapse, and relapse following substance abuse treatment, *Addict Behav* 19(3) (1994) 319–31. [PubMed: 7942249]
- [5]. Brandon TH, Vidrine JJ, Litvin EB, Relapse and relapse prevention, *Annu Rev Clin Psychol* 3 (2007) 257–84. [PubMed: 17716056]

- [6]. Hofmann SG, Smits JA, Cognitive-behavioral therapy for adult anxiety disorders: a meta-analysis of randomized placebo-controlled trials, *J Clin Psychiatry* 69(4) (2008) 621–32. [PubMed: 18363421]
- [7]. Conklin CA, Tiffany ST, Applying extinction research and theory to cue-exposure addiction treatments, *Addiction* 97 (2002) 155–167. [PubMed: 11860387]
- [8]. Bowery NG, Glycine-binding sites and NMDA receptors in brain, *Nature* 326(6111) (1987) 338. [PubMed: 3031504]
- [9]. Hood WF, Compton RP, Monahan JB, D-cycloserine: a ligand for the N-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics, *Neurosci Lett* 98(1) (1989) 91–5. [PubMed: 2540460]
- [10]. Hofmann SG, Carpenter JK, Otto MW, Rosenfield D, Smits JAJ, Pollack MH, Dose timing of d-cycloserine to augment cognitive behavioral therapy for social anxiety: Study design and rationale, *Contemporary Clinical Trials* 43 (2015) 223–230. [PubMed: 26111923]
- [11]. Price KL, McRae-Clark AL, Saladin ME, Maria MM, DeSantis SM, Back SE, Brady KT, D-cycloserine and cocaine cue reactivity: preliminary findings, *Am J Drug Alcohol Abuse* 35(6) (2009) 434–8. [PubMed: 20014913]
- [12]. Santa Ana EJ, Rounsaville BJ, Frankforter TL, Nich C, Babuscio T, Poling J, Gonsai K, Hill KP, Carroll KM, D-Cycloserine attenuates reactivity to smoking cues in nicotine dependent smokers: a pilot investigation, *Drug Alcohol Depend* 104(3) (2009) 220–7. [PubMed: 19592176]
- [13]. Watson BJ, Wilson S, Griffin L, Kalk NJ, Taylor LG, Munafo MR, Lingford-Hughes AR, Nutt DJ, A pilot study of the effectiveness of D-cycloserine during cue-exposure therapy in abstinent alcohol-dependent subjects, *Psychopharmacology (Berl)* 216(1) (2011) 121–9. [PubMed: 21318564]
- [14]. Hofmann SG, Huweler R, MacKillop J, Kantak KM, Effects of D-cycloserine on craving to alcohol cues in problem drinkers: preliminary findings, *Am J Drug Alcohol Abuse* 38(1) (2012) 101–7. [PubMed: 21851195]
- [15]. Price KL, Baker NL, McRae-Clark AL, Saladin ME, Desantis SM, Santa Ana EJ, Brady KT, A randomized, placebo-controlled laboratory study of the effects of D-cycloserine on craving in cocaine-dependent individuals, *Psychopharmacology (Berl)* 226(4) (2013) 739–46. [PubMed: 22234379]
- [16]. Santa Ana EJ, Prisciandaro JJ, Saladin ME, Mcrae-Clark AL, Shaftman SR, Nietert PJ, Brady KT, D-cycloserine combined with cue exposure therapy fails to attenuate subjective and physiological craving in cocaine dependence, *American Journal on Addictions* 24 (2015) 217–224. [PubMed: 25808169]
- [17]. Otto MW, Kredlow MA, Smits JAJ, Hofmann SG, Tolin DF, de Kleine RA, van Minnen A, Evins AE, Pollack MH, Enhancement of Psychosocial Treatment With D-Cycloserine: Models, Moderators, and Future Directions, *Biological Psychiatry* 80 (2016) 274–283. [PubMed: 26520240]
- [18]. MacKillop J, Few LR, Stojek MK, Murphy CM, Malutinok SF, Johnson FT, Hofmann SG, McGeary JE, Swift RM, Monti PM, D-cycloserine to enhance extinction of cue-elicited craving for alcohol: A translational approach, *Translational Psychiatry* 5 (2015) e544. [PubMed: 25849983]
- [19]. Otto MW, Pachas GN, Cather C, Hoepfner SS, Moshier SJ, Hearon BA, Ward HB, Laffer AB, Smits JAJ, Evins AE, A placebo-controlled randomized trial of D-cycloserine augmentation of cue exposure therapy for smoking cessation, *Cogn Behav Ther* 48(1) (2019) 65–76. [PubMed: 30111253]
- [20]. Xue YX, Luo YX, Wu P, Shi HS, Xue LF, Chen C, Zhu WL, Ding ZB, Bao YP, Shi J, Epstein DH, Shaham Y, Lu L, A memory retrieval-extinction procedure to prevent drug craving and relapse, *Science* 336(6078) (2012) 241–5. [PubMed: 22499948]
- [21]. Luo YX, Xue YX, Liu JF, Shi HS, Jian M, Han Y, Zhu WL, Bao YP, Wu P, Ding ZB, Shen HW, Shi J, Shaham Y, Lu L, A novel UCS memory retrieval-extinction procedure to inhibit relapse to drug seeking, *Nat Commun* 6 (2015) 7675. [PubMed: 26169171]
- [22]. Sartor GC, Aston-Jones G, Post-retrieval extinction attenuates cocaine memories, *Neuropsychopharmacology* 39(5) (2014) 1059–65. [PubMed: 24257156]

- [23]. Nic Dhonnchadha BÁ, Szalay JJ, Achat-Mendes C, Platt DM, Otto MW, Spealman RD, Kantak KM, D-cycloserine deters reacquisition of cocaine self-administration by augmenting extinction learning, *Neuropsychopharmacology* 35 (2010) 357–67. [PubMed: 19741593]
- [24]. Achat-Mendes C, Nic Dhonnchadha BÁ, Platt DM, Kantak KM, Spealman RD, Glycine transporter-1 inhibition preceding extinction training inhibits reacquisition of cocaine seeking, *Neuropsychopharmacology* 37 (2012) 2837–45. [PubMed: 22948980]
- [25]. Gauthier JM, Lin A, Nic Dhonnchadha BÁ, Spealman RD, Man H-Y, Kantak KM, Environmental enrichment facilitates cocaine-cue extinction, deters reacquisition of cocaine self-administration and alters AMPAR GluA1 expression and phosphorylation, *Addiction Biology* 22 (2017).
- [26]. Hastings MH, Gauthier JM, Mabry K, Tran A, Man HY, Kantak KM, Facilitative effects of environmental enrichment for cocaine relapse prevention are dependent on extinction training context and involve increased TrkB signaling in dorsal hippocampus and ventromedial prefrontal cortex, *Behav Brain Res* 386 (2020) 112596. [PubMed: 32194188]
- [27]. Nic Dhonnchadha BÁ, Pinard E, Alberati D, Wettstein JG, Spealman RD, Kantak KM, Inhibiting glycine transporter-1 facilitates cocaine-cue extinction and attenuates reacquisition of cocaine-seeking behavior, *Drug and Alcohol Dependence* 122 (2012) 119–26. [PubMed: 21992874]
- [28]. van Praag H, Kempermann G, Gage FH, Neural consequences of environmental enrichment, *Nature Reviews Neuroscience* 1 (2000) 191–198. [PubMed: 11257907]
- [29]. Will B, Galani R, Kelche C, Rosenzweig MR, Recovery from brain injury in animals: relative efficacy of environmental enrichment, physical exercise or formal training (1990–2002), *Prog Neurobiol* 72(3) (2004) 167–82. [PubMed: 15130708]
- [30]. Nithianantharajah J, Hannan AJ, The neurobiology of brain and cognitive reserve: mental and physical activity as modulators of brain disorders, *Prog Neurobiol* 89(4) (2009) 369–82. [PubMed: 19819293]
- [31]. Chauvet C, Lardeux V, Goldberg SR, Jaber M, Solinas M, Environmental enrichment reduces cocaine seeking and reinstatement induced by cues and stress but not by cocaine, *Neuropsychopharmacology* 34(13) (2009) 2767–78. [PubMed: 19741591]
- [32]. Green TA, Alibhai IN, Roybal CN, Winstanley CA, Theobald DE, Birnbaum SG, Graham AR, Unterberg S, Graham DL, Vialou V, Bass CE, Terwilliger EF, Bardo MT, Nestler EJ, Environmental enrichment produces a behavioral phenotype mediated by low cyclic adenosine monophosphate response element binding (CREB) activity in the nucleus accumbens, *Biol Psychiatry* 67(1) (2010) 28–35. [PubMed: 19709647]
- [33]. Ranaldi R, Kest K, Zellner M, Hachimine-Semprebom P, Environmental enrichment, administered after establishment of cocaine self-administration, reduces lever pressing in extinction and during a cocaine context renewal test, *Behav Pharmacol* 22(4) (2011) 347–53. [PubMed: 21673566]
- [34]. Thiel KJ, Engelhardt B, Hood LE, Peartree NA, Neisewander JL, The interactive effects of environmental enrichment and extinction interventions in attenuating cue-elicited cocaine-seeking behavior in rats, *Pharmacol Biochem Behav* 97(3) (2011) 595–602. [PubMed: 20869391]
- [35]. Chauvet C, Goldberg SR, Jaber M, Solinas M, Effects of environmental enrichment on the incubation of cocaine craving, *Neuropharmacology* 63(4) (2012) 635–41. [PubMed: 22634364]
- [36]. Puhl MD, Blum JS, Acosta-Torres S, Grigson PS, Environmental enrichment protects against the acquisition of cocaine self-administration in adult male rats, but does not eliminate avoidance of a drug-associated saccharin cue, *Behav Pharmacol* 23(1) (2012) 43–53. [PubMed: 22157144]
- [37]. Li C, Frantz KJ, Abstinence environment contributes to age differences in reinstatement of cocaine seeking between adolescent and adult male rats, *Pharmacol Biochem Behav* 158 (2017) 49–56. [PubMed: 28601602]
- [38]. Ewing S, Ranaldi R, Environmental enrichment facilitates cocaine abstinence in an animal conflict model, *Pharmacol Biochem Behav* 166 (2018) 35–41. [PubMed: 29407873]
- [39]. Frankowska M, Miszkil J, Pomierny-Chamiolo L, Pomierny B, Giannotti G, Suder A, Filip M, Alternation in dopamine D2-like and metabotropic glutamate type 5 receptor density caused by differing housing conditions during abstinence from cocaine self-administration in rats, *J Psychopharmacol* 33(3) (2019) 372–382. [PubMed: 30644313]

- [40]. Yates JR, Bardo MT, Beckmann JS, Environmental enrichment and drug value: a behavioral economic analysis in male rats, *Addict Biol* 24(1) (2019) 65–75. [PubMed: 29178664]
- [41]. Ali AE, Wilson YM, Murphy M, A single exposure to an enriched environment stimulates the activation of discrete neuronal populations in the brain of the fos-tau-lacZ mouse, *Neurobiol Learn Mem* 92(3) (2009) 381–90. [PubMed: 19450699]
- [42]. Degroot A, Wolff MC, Nomikos GG, Acute exposure to a novel object during consolidation enhances cognition, *Neuroreport* 16(1) (2005) 63–7. [PubMed: 15618892]
- [43]. Bergeron R, Meyer TM, Coyle JT, Greene RW, Modulation of N-methyl-D-aspartate receptor function by glycine transport, *Proc Natl Acad Sci U S A* 95(26) (1998) 15730–4. [PubMed: 9861038]
- [44]. Mao SC, Lin HC, Gean PW, Augmentation of fear extinction by infusion of glycine transporter blockers into the amygdala, *Mol Pharmacol* 76(2) (2009) 369–78. [PubMed: 19411609]
- [45]. Shimazaki T, Kaku A, Chaki S, D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats, *Psychopharmacology (Berl)* 209(3) (2010) 263–70. [PubMed: 20198471]
- [46]. Harada K, Nakato K, Yarimizu J, Yamazaki M, Morita M, Takahashi S, Aota M, Saita K, Doihara H, Sato Y, Yamaji T, Ni K, Matsuoka N, A novel glycine transporter-1 (GlyT1) inhibitor, ASP2535 (4-[3-isopropyl-5-(6-phenyl-3-pyridyl)-4H-1,2,4-triazol-4-yl]-2,1,3-benzoxadiazol e), improves cognition in animal models of cognitive impairment in schizophrenia and Alzheimer's disease, *Eur J Pharmacol* 685(1–3) (2012) 59–69. [PubMed: 22542656]
- [47]. Castner SA, Murthy NV, Ridler K, Herdon H, Roberts BM, Weinzimmer DP, Huang Y, Zheng MQ, Rabiner EA, Gunn RN, Carson RE, Williams GV, Laruelle M, Relationship between glycine transporter 1 inhibition as measured with positron emission tomography and changes in cognitive performances in nonhuman primates, *Neuropsychopharmacology* 39(12) (2014) 2742–9. [PubMed: 24487737]
- [48]. Eddins D, Hamill TG, Puri V, Cannon CE, Vivian JA, Sanabria-Bohorquez SM, Cook JJ, Morrow JA, Thomson F, Uslander JM, The relationship between glycine transporter 1 occupancy and the effects of the glycine transporter 1 inhibitor RG1678 or ORG25935 on object retrieval performance in scopolamine impaired rhesus monkey, *Psychopharmacology (Berl)* 231(3) (2014) 511–9. [PubMed: 24051602]
- [49]. Fone KCF, Watson DJG, Billiras RI, Sicard DI, Dekeyne A, Rivet JM, Gobert A, Millan MJ, Comparative Pro-cognitive and Neurochemical Profiles of Glycine Modulatory Site Agonists and Glycine Reuptake Inhibitors in the Rat: Potential Relevance to Cognitive Dysfunction and Its Management, *Mol Neurobiol* 57(5) (2020) 2144–2166. [PubMed: 31960362]
- [50]. Deroche-Gamonet V, Belin D, Piazza PV, Evidence for addiction-like behavior in the rat, *Science* 305(5686) (2004) 1014–7. [PubMed: 15310906]
- [51]. Economidou D, Pelloux Y, Robbins TW, Dalley JW, Everitt BJ, High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence, *Biol Psychiatry* 65(10) (2009) 851–6. [PubMed: 19181308]
- [52]. Smith MA, Iordanou JC, Cohen MB, Cole KT, Gergans SR, Lyle MA, Schmidt KT, Effects of environmental enrichment on sensitivity to cocaine in female rats: importance of control rates of behavior, *Behav Pharmacol* 20(4) (2009) 312–21. [PubMed: 19584714]
- [53]. Szalay JJ, Jordan CJ, Kantak KM, Neural regulation of the time course for cocaine-cue extinction consolidation in rats, *European Journal of Neuroscience* 37 (2013) 267–77.
- [54]. Kantak KM, Goodrich CM, Uribe V, Influence of sex, estrous cycle, and drug-onset age on cocaine self-administration in rats (*Rattus norvegicus*), *Exp Clin Psychopharmacol* 15(1) (2007) 37–47. [PubMed: 17295583]
- [55]. Vanderschuren LJ, Ahmed SH, Animal studies of addictive behavior, *Cold Spring Harb Perspect Med* 3(4) (2013) a011932. [PubMed: 23249442]
- [56]. Dimet AL, Cisneros IE, Fox RG, Stutz SJ, Anastasio NC, Cunningham KA, Dineley KT, A Protocol for Measuring Cue Reactivity in a Rat Model of Cocaine Use Disorder, *J Vis Exp* (136) (2018).
- [57]. Liu Y, Roberts DC, Morgan D, Effects of extended-access self-administration and deprivation on breakpoints maintained by cocaine in rats, *Psychopharmacology (Berl)* 179(3) (2005) 644–51. [PubMed: 15650844]

- [58]. Allen RM, Dykstra LA, Carelli RM, Continuous exposure to the competitive N-methyl-D: - aspartate receptor antagonist, LY235959, facilitates escalation of cocaine consumption in Sprague-Dawley rats, *Psychopharmacology (Berl)* 191(2) (2007) 341–51. [PubMed: 17225167]
- [59]. Knackstedt LA, Kalivas PW, Extended access to cocaine self-administration enhances drug-primed reinstatement but not behavioral sensitization, *J Pharmacol Exp Ther* 322(3) (2007) 1103–9. [PubMed: 17601982]
- [60]. Carroll ME, Gao Y, Brimijoin S, Anker JJ, Effects of cocaine hydrolase on cocaine self-administration under a PR schedule and during extended access (escalation) in rats, *Psychopharmacology (Berl)* 213(4) (2010) 817–29. [PubMed: 20972552]
- [61]. Hafenbreidel M, Rafa Todd C, Twining RC, Tuscher JJ, Mueller D, Bidirectional effects of inhibiting or potentiating NMDA receptors on extinction after cocaine self-administration in rats, *Psychopharmacology (Berl)* 231(24) (2014) 4585–94. [PubMed: 24847958]
- [62]. Kelamangalath L, Wagner JJ, D-serine treatment reduces cocaine-primed reinstatement in rats following extended access to cocaine self-administration, *Neuroscience* 169(3) (2010) 1127–35. [PubMed: 20541592]
- [63]. Lynch WJ, Carroll ME, Reinstatement of cocaine self-administration in rats: sex differences, *Psychopharmacology (Berl)* 148(2) (2000) 196–200. [PubMed: 10663435]
- [64]. Fuchs RA, Evans KA, Mehta RH, Case JM, See RE, Influence of sex and estrous cyclicity on conditioned cue-induced reinstatement of cocaine-seeking behavior in rats, *Psychopharmacology (Berl)* 179(3) (2005) 662–72. [PubMed: 15682307]
- [65]. Feltenstein MW, Henderson AR, See RE, Enhancement of cue-induced reinstatement of cocaine-seeking in rats by yohimbine: sex differences and the role of the estrous cycle, *Psychopharmacology (Berl)* 216(1) (2011) 53–62. [PubMed: 21308466]
- [66]. Swalve N, Smethells JR, Zlebnik NE, Carroll ME, Sex differences in reinstatement of cocaine-seeking with combination treatments of progesterone and atomoxetine, *Pharmacol Biochem Behav* 145 (2016) 17–23. [PubMed: 27003832]
- [67]. Bechard AR, Hamor PU, Schwendt M, Knackstedt LA, The effects of ceftriaxone on cue-primed reinstatement of cocaine-seeking in male and female rats: estrous cycle effects on behavior and protein expression in the nucleus accumbens, *Psychopharmacology (Berl)* 235(3) (2018) 837–848. [PubMed: 29197981]
- [68]. Chamizo VD, Rodriguez CA, Sanchez J, Marmol F, Sex differences after environmental enrichment and physical exercise in rats when solving a navigation task, *Learn Behav* 44(3) (2016) 227–38. [PubMed: 26511132]
- [69]. Kokras N, Sotiropoulos I, Besinis D, Tzouveka EL, Almeida OFX, Sousa N, Dalla C, Neuroplasticity-related correlates of environmental enrichment combined with physical activity differ between the sexes, *Eur Neuropsychopharmacol* 29(1) (2019) 1–15. [PubMed: 30497839]
- [70]. Waddell J, Mallimo E, Shors T, d-cycloserine reverses the detrimental effects of stress on learning in females and enhances retention in males, *Neurobiol Learn Mem* 93(1) (2010) 31–6. [PubMed: 19666130]
- [71]. Woods AM, Bouton ME, D-cycloserine facilitates extinction but does not eliminate renewal of the conditioned emotional response, *Behav Neurosci* 120(5) (2006) 1159–62. [PubMed: 17014266]
- [72]. Ledgerwood L, Richardson R, Cranney J, Effects of D-cycloserine on extinction of conditioned freezing, *Behav Neurosci* 117(2) (2003) 341–9. [PubMed: 12708530]
- [73]. Thanos PK, Bermeo C, Wang GJ, Volkow ND, D-cycloserine facilitates extinction of cocaine self-administration in rats, *Synapse* 65(9) (2011) 938–44. [PubMed: 21360592]
- [74]. Zlebnik NE, Saykao AT, Carroll ME, Effects of combined exercise and progesterone treatments on cocaine seeking in male and female rats, *Psychopharmacology (Berl)* 231(18) (2014) 3787–98. [PubMed: 24595506]
- [75]. Zlebnik NE, Carroll ME, Effects of the combination of wheel running and atomoxetine on cue- and cocaine-primed reinstatement in rats selected for high or low impulsivity, *Psychopharmacology (Berl)* 232(6) (2015) 1049–59. [PubMed: 25258161]

- [76]. Cummings JA, Gowl BA, Westenbroek C, Clinton SM, Akil H, Becker JB, Effects of a selectively bred novelty-seeking phenotype on the motivation to take cocaine in male and female rats, *Biol Sex Differ* 2 (2011) 3. [PubMed: 21396095]
- [77]. Pitchers KK, Flagel SB, O'Donnell EG, Woods LC, Sarter M, Robinson TE, Individual variation in the propensity to attribute incentive salience to a food cue: influence of sex, *Behav Brain Res* 278 (2015) 462–9. [PubMed: 25446811]
- [78]. Pitchers KK, Phillips KB, Jones JL, Robinson TE, Sarter M, Diverse Roads to Relapse: A Discriminative Cue Signaling Cocaine Availability Is More Effective in Renewing Cocaine Seeking in Goal Trackers Than Sign Trackers and Depends on Basal Forebrain Cholinergic Activity, *J Neurosci* 37(30) (2017) 7198–7208. [PubMed: 28659281]
- [79]. Chourbaji S, Hortnagl H, Molteni R, Riva MA, Gass P, Hellweg R, The impact of environmental enrichment on sex-specific neurochemical circuitries - effects on brain-derived neurotrophic factor and the serotonergic system, *Neuroscience* 220 (2012) 267–76. [PubMed: 22710068]
- [80]. Yousuf H, Smies CW, Hafenbreidel M, Tuscher JJ, Fortress AM, Frick KM, Mueller D, Infralimbic Estradiol Enhances Neuronal Excitability and Facilitates Extinction of Cocaine Seeking in Female Rats via a BDNF/TrkB Mechanism, *Front Behav Neurosci* 13 (2019) 168. [PubMed: 31417375]
- [81]. Dubroqua S, Boison D, Feldon J, Mohler H, Yee BK, Examining the sex- and circadian dependency of a learning phenotype in mice with glycine transporter 1 deletion in two Pavlovian conditioning paradigms, *Neurobiol Learn Mem* 96(2) (2011) 218–29. [PubMed: 21596148]
- [82]. Wang Y, Ma Y, Hu J, Cheng W, Jiang H, Zhang X, Li M, Ren J, Li X, Prenatal chronic mild stress induces depression-like behavior and sex-specific changes in regional glutamate receptor expression patterns in adult rats, *Neuroscience* 301 (2015) 363–74. [PubMed: 26071959]
- [83]. Kato A, Hojo Y, Higo S, Komatsuzaki Y, Murakami G, Yoshino H, Uebayashi M, Kawato S, Female hippocampal estrogens have a significant correlation with cyclic fluctuation of hippocampal spines, *Front Neural Circuits* 7 (2013) 149. [PubMed: 24151456]
- [84]. Koss WA, Haertel JM, Philippi SM, Frick KM, Sex Differences in the Rapid Cell Signaling Mechanisms Underlying the Memory-Enhancing Effects of 17beta-Estradiol, *eNeuro* 5(5) (2018).
- [85]. Lu Y, Sareddy GR, Wang J, Wang R, Li Y, Dong Y, Zhang Q, Liu J, O'Connor JC, Xu J, Vadlamudi RK, Brann DW, Neuron-Derived Estrogen Regulates Synaptic Plasticity and Memory, *J Neurosci* 39(15) (2019) 2792–2809. [PubMed: 30728170]
- [86]. Sterling RC, Dean J, Weinstein SP, Murphy J, Gottheil E, Gender differences in cue exposure reactivity and 9-month outcome, *J Subst Abuse Treat* 27(1) (2004) 39–44. [PubMed: 15223092]
- [87]. Volkow ND, Tomasi D, Wang GJ, Fowler JS, Telang F, Goldstein RZ, Alia-Klein N, Wong C, Reduced metabolism in brain “control networks” following cocaine-cues exposure in female cocaine abusers, *PLoS One* 6(2) (2011) e16573. [PubMed: 21373180]
- [88]. Sikora M, Nicolas C, Istin M, Jaafari N, Thiriet N, Solinas M, Generalization of effects of environmental enrichment on seeking for different classes of drugs of abuse, *Behav Brain Res* 341 (2018) 109–113. [PubMed: 29288750]
- [89]. Roos CR, Nich C, Mun CJ, Babuscio TA, Mendonca J, Miguel AQC, DeVito EE, Yip SW, Witkiewitz K, Carroll KM, Kiluk BD, Clinical validation of reduction in cocaine frequency level as an endpoint in clinical trials for cocaine use disorder, *Drug Alcohol Depend* 205 (2019) 107648. [PubMed: 31677490]
- [90]. Rohsenow DJ, Martin RA, Monti PM, Urge-specific and lifestyle coping strategies of cocaine abusers: relationships to treatment outcomes, *Drug Alcohol Depend* 78(2) (2005) 211–9. [PubMed: 15845325]
- [91]. McKay JR, Van Horn D, Rennert L, Drapkin M, Ivey M, Koppenhaver J, Factors in sustained recovery from cocaine dependence, *J Subst Abuse Treat* 45(2) (2013) 163–72. [PubMed: 23561331]

Highlights

- Male and female rats with an addiction-like phenotype for cocaine were evaluated
- A model of cue exposure therapy with a multimodal treatment strategy was tested
- The behavioral strategy was brief interventions of environmental enrichment
- The pharmacological strategy was Org24589, a glycine transporter-1 inhibitor
- Sex differences in the efficacy of this multimodal treatment strategy were revealed
- Reacquisition of cocaine self-administration was reduced in males but not females

Experimental Timeline

Figure 1.

Experimental timeline of procedures used in Experiments 1 and 2. Procedures are delineated relative to the post-natal day (PND) age of the male and female rats. Graphics illustrate the contingencies under the FR 1, FR 5 and FI 2 min [FR 5:S] schedules of drug delivery and cue presentation.

Figure 2.

Cocaine self-administration at baseline in groups of male rats prior to extinction training and treatment initiation, which consisted of no-added treatment (NoEE + Veh), EE treatment alone (EE + Veh), 3.0 mg/kg Org24589 treatment alone (NoEE + Org 3) and EE and 3.0 mg/kg Org24589 treatments combined (EE + Org 3). N = 8–9 rats per group. (A) Values are Individual data points and the mean $\pm$ s.e.m. for active and inactive lever responses averaged over the last 5 sessions of baseline self-administration training. * $p=0.001$ comparing active to inactive lever responses. (B) Values are individual data points and the mean $\pm$ s.e.m. for

daily mg/kg cocaine intake averaged over the last 5 sessions of baseline self-administration training.

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Figure 3.

Responding during the weekly extinction training sessions in the 4 groups of male rats described in the legend for Figure 2. $N = 8-9$ rats per group. Values are the mean $\pm$ s.e.m. for active lever responses expressed as percent of baseline. * $p=0.001$ comparing the EE + Org 3 group to the NoEE + Veh control group.

Figure 4.

Reacquisition of cocaine self-administration during the daily sessions in the 4 groups of male rats described in the legend for Figure 2. $N = 8-9$ rats per group. (A) Values are the mean $\pm$ s.e.m. for active lever responses expressed as percent of baseline. * $p=0.001$ comparing the EE + Org 3 group to the NoEE + Veh control group. (B) Values are individual data points and the mean $\pm$ s.e.m. for daily mg/kg cocaine intake averaged over the 15 self-administration reacquisition sessions. * $p=0.003$ comparing the EE + Org 3 group to the NoEE + Veh control group.

Figure 5.

Cocaine self-administration at baseline in groups of female rats prior to extinction training and treatment initiation, which consisted of no-added treatment (NoEE + Veh), EE and 3.0 mg/kg Org24598 treatments combined (EE + Org 3), and EE and 7.5 mg/kg Org24598 treatments combined (EE + Org 7.5). N = 7–9 rats per group. (A) Individual data points and the mean $\pm$ s.e.m. values for active and inactive lever responses averaged over the last 5 sessions of baseline self-administration training. * $p=0.001$ comparing active to inactive

lever responses. (B) Individual data points and the mean $\pm$ s.e.m. values for daily mg/kg cocaine intake averaged over the last 5 sessions of baseline self-administration training.

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Cocaine Extinction Female Rats

Active Lever Responses

Figure 6.

Responding during the weekly extinction training sessions in the 3 groups of female rats described in the legend for Figure 5. N = 7–9 rats per group. Values are the mean $\pm$ S.E.M. for active lever responses expressed as percent of baseline. * $p=0.05$ comparing the EE + Org 7.5 group to the NoEE + Veh control group.

Figure 7.

Reacquisition of cocaine self-administration during the daily sessions in the 3 groups of female rats described in the legend for Figure 5. $N = 5-9$ rats per group. (A) Values are the mean $\pm$ s.e.m. for active lever responses expressed as percent of baseline. (B) Values are individual data points and the mean $\pm$ s.e.m. for daily mg/kg cocaine intake averaged over the 15 self-administration reacquisition sessions.