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Effects of combined exercise and progesterone treatments on cocaine seeking in male and female rats

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Abstract

BACKGROUND—Individually, both treatment with progesterone and concurrent access to an exercise wheel reduce cocaine self-administration under long-access conditions and suppress cocaine-primed reinstatement in female rats. In the present study, wheel running and progesterone (alone and combined) were assessed for their effects on reinstatement of cocaine-seeking primed by yohimbine, cocaine, and cocaine-paired cues.

METHODS—Male and female rats were implanted with an intravenous catheter and allowed to self-administer cocaine (0.4 mg/kg/inf, iv) during 6-h sessions for 10 days. Subsequently, the groups of male and female rats were each divided into 2 groups that were given concurrent access to either a locked or unlocked running wheel under extinction conditions for 14 days. Next, all 4 groups were tested in a within-subjects design for reinstatement of cocaine-seeking precipitated by separate administration of cocaine-paired stimuli, yohimbine, or cocaine; or the combination of yohimbine + cocaine-paired stimuli or cocaine + cocaine-paired stimuli. These priming conditions were tested in the presence of concurrent wheel access (W), pretreatment with progesterone (P), or both (W+P).

RESULTS—In agreement with previous results, females responded more for cocaine than males during maintenance. Additionally, concurrent wheel running attenuated extinction responding and cocaine-primed reinstatement in females but not males. Across all priming conditions, W+P reduced reinstatement compared to control conditions, and for cocaine-primed reinstatement in male rats, the combined W+P treatment was more effective than W or P alone.

CONCLUSION—Under certain conditions, combined behavioral (exercise) and pharmacological (progesterone) interventions were more successful at reducing cocaine-seeking behavior than either intervention alone.

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Contributors

NEZ and MEC designed the study. NEZ wrote the protocol and collected data with the assistance of ATS. NEZ completed the data analysis and wrote the first draft of the manuscript. All authors contributed to and approved the final manuscript.

Conflicts of interest

None.

Keywords

exercise; progesterone; cocaine; reinstatement; relapse; addiction; sex differences

Introduction

Although progress has been made in identifying promising pharmacological or behavioral treatments for drug addiction, no one treatment has been completely effective on its own. Since clinical research suggests that the most successful treatment effects may be achieved using a combination of therapies (Potenza et al. 2011), the goal of the present study was to examine potential sex differences in the combination of pharmacological and behavioral treatment vs. each treatment alone. Several studies indicate that behavioral treatments like cognitive behavioral therapy and contingency management in combination with pharmacological treatments (e.g., methadone, disulfiram, nicotine replacement) helped patients maintain longer abstinence than pharmacological treatment alone (McLellan et al. 1993, Peirce et al. 2006, Ball and Ross et al. 1991, Carroll et al. 1998, Carroll et al. 2004, Bickel et al. 1997). Further, in animal experiments, combining the partial opioid agonist buprenorphine with concurrent access to the nondrug reinforcer saccharin led to an 80-90% reduction in drug use compared to less than 50% reduction for each of the treatments individually (Comer et al. 1996, Rodefer et al. 1997). These initial results suggest that, in both humans and animals, combined drug abuse treatment strategies are more effective than single treatments.

An increasing number of findings indicate that sex differences in drug abuse are influenced by the gonadal hormones estrogen and progesterone (P). While estrogen has apparent potentiating effects on positive subjective effects of drugs in humans (Evans et al. 2002, Sofuoglu et al. 1999, Justice and de Wit 2000) and drug seeking in animals (Larson et al. 2005, Becker 1990, Jackson et al. 2006), P attenuates drug-related responses in both humans and animals (Anker and Carroll 2010, Quinones-Jenab and Jenab 2010, Evans and Foltin 2010, Evans and Foltin 2006, Evans et al. 2002, Sofuoglu et al. 1999, Sofuoglu et al. 2002). In women, high endogenous levels of P were associated with lower cue- and stress-induced cocaine craving (Sinha et al. 2007). Animal models of the human drug abuse process also demonstrate P-mediated attenuation of cocaine-maintained behavior (Anker and Carroll 2011; Feltenstein and See 2007, Lynch 2008, Larson et al. 2007, Anker et al. 2012). Cocaine-primed reinstatement was reduced in female rats that were treated with P compared to vehicle-treated controls (Anker et al. 2007), and allopregnanolone, a major metabolite of P, attenuated stress-induced reinstatement of cocaine-seeking behavior in female but not male rats (Anker and Carroll 2010). Progesterone and allopregnanolone have been shown to alleviate anxiogenic-like behaviors (Bitran et al. 1995, Brot et al. 1997, Laconi et al. 2001) and blunt the release of CRF following exposure to stress (Drugan et al. 1993, Frye et al. 2006, Owens et al. 1992, Patchev et al. 1994, Purdy et al. 1991). During abstinence, endogenous P has been hypothesized to reduce cue- and stress-induced cravings and relapse by reducing withdrawal-induced HPA activation and mediating components of stress dysregulation (Fox and Sinha 2009, Anker and Carroll 2010).

Another growing body of research has evaluated the physical and psychological benefits of exercise and physical activity and has begun exploring potential treatment applications for exercise as a behavioral intervention (USDHHS 1996, Ussher et al. 2012, Smith and Lynch 2011). Controlled laboratory studies in humans demonstrated that moderate-intensity aerobic exercise decreased cravings for alcohol (Ussher et al. 2004), cigarettes (Daniel et al. 2004), and cannabis (Buchowski et al. 2011), while brief episodes of isometric (Ussher et al. 2006, Ussher et al. 2009) and aerobic (Ussher et al. 2001, Daniel et al. 2004, Williams et al. 2011) exercise also alleviated symptoms of tobacco withdrawal. Studies with animals have also revealed promising treatment effects of exercise on drug-motivated behaviors (Smith and Pitts 2011, Kanarek et al. 1995, Miller et al. 2011, Cosgrove et al. 2002, McMillan et al. 1995, Ehringer et al. 2009, Smith et al. 2011, Zlebnik et al. 2012). Reinstatement of cocaine seeking precipitated by exposure to cocaine (Zlebnik et al. 2010, Smith et al. 2012) or cocaine-paired cues (Lynch et al. 2010, Smith et al. 2012) was attenuated in exercising rats compared to sedentary rats, and exercise over a withdrawal period also decreased subsequent cocaine seeking (Lynch et al. 2010). Several studies have investigated sex differences in the efficacy of exercise as a treatment for drug abuse, and as with pharmacological agents (Campbell et al. 2002, Carroll et al. 2001, Cosgrove and Carroll 2004), results suggest that exercise attenuates drug seeking more in female than male rats. For example, concurrent access to W decreased intake of cocaine (Cosgrove et al. 2002) and ethanol (Ehringer et al. 2009) more effectively in females than males, and female rats exhibited faster extinction of responding for cocaine self-administration than male rats following chronic W (Smith et al. 2012).

While these results indicate that exercise is an effective sex-specific treatment intervention for drug use and relapse-related behaviors, the mechanism of the effects of exercise on addiction have not been well-studied. However, like P, evidence suggests that exercise reduces stress and anxiety (Asmundson et al. 2013, Sciolino and Holmes 2012). While P has not been used in combination with any other drug abuse treatment, exercise been recommended as an adjunctive therapy for standard treatment of anxiety disorders (Sciolino and Holmes 2012) and may also be effective as a supplement to standard behavioral therapies for addiction. Exercise enhanced the effectiveness of behavioral counseling in traditional tobacco cessation programs (Martin et al. 1997) and also had an additive effect with contingency management for the treatment of substance use disorders in an outpatient setting (Weinstock et al. 2008). It is not known whether exercise would improve drug abuse treatment with pharmacological agents such as P and whether these effects would be sex-specific. However, given the promising individual treatment effects of P and exercise in humans and animals, an investigation of their combined therapeutic efficacy is warranted. In the present study, we investigated the treatment effects of W, P, or their combination (W+P) on cocaine-primed, cue-primed, and stress-primed reinstatement of cocaine seeking-behavior in male and female rats. We hypothesized that both W and P would have significant individual treatment effects, but that their combination would be more effective than either individual treatment alone. Further, while both W and P have shown greater treatment efficacy in females compared to males, we hypothesized that the combined treatment would be effective in both sexes, although with a greater effect size in females.

Materials and methods

Animals

Twenty-nine female and 22 male adult Wistar rats were obtained from Harlan Sprague-Dawley, Inc. (Madison, WI, USA) and began behavioral testing around postnatal day 90. Sex differences were investigated in this study because previous work had shown sex differences in avidity for W (Boakes et al. 1999, Cosgrove et al. 2002, Eikelboom and Mills 1988, Lambert and Kinsley 1993) and in the ability of concurrent W to decrease cocaine self-administration (Cosgrove et al. 2002). These studies suggested possible differential exercise treatment effects between males and females. Estrous cycle was not monitored in the females, as animals were housed in their operant conditioning chambers with the attached wheel apparatus during the experiment, and vaginal lavage could have disrupted the cycle as well as cocaine- and wheel-reinforced behavior. Additionally, allowing cycles to vary randomly would permit results to be generalized across all phases of the estrous cycle.

After arrival at the laboratory, rats were pair-housed in plastic cages with free access to laboratory chow (Teklad 2018, Harlan Laboratories, Madison, WI, USA) and water for at least 3-days of acclimation. Upon commencement of behavioral testing, each rat was removed from the plastic cages and placed in individual operant conditioning chambers where it remained for the duration of the study. Once transferred to the operant conditioning chambers, rats continued to have free access to water, and they were fed about 15 min after their daily sessions at 3:15 pm. Females and males were fed 16 g or 20 g, respectively, of rodent meal (Teklad 2018 ground meal, Harlan Laboratories) to maintain them at 85% of their free-feeding body weight. Mean body weights throughout the experiment did not differ among the female groups (FLW = 263 ± 4.62 , FW = 254 ± 5.56) or male groups (MLW = 401 ± 6.64 , MW = 399 ± 6.01). All rodent holding rooms were maintained at 24°C and at 40-50% humidity under a light/dark cycle (12/12-h) with room lights on at 6:00 am. The experimental protocol (1008A87755) was approved by the University of Minnesota Institutional Animal Care and Use Committee. The experiment was conducted in compliance with the Principles of Laboratory Animal Care (National Academies Press 2011), and all laboratory facilities were accredited by the American Association for the Accreditation of Laboratory Animal Care.

Apparatus

Animals were housed and tested in custom-built, octagonally-shaped operant conditioning chambers enclosed in wooden sound-attenuating boxes equipped with ventilation fans as previously described (Zlebnik et al. 2010, Zlebnik et al. 2012). During cocaine self-administration sessions, responding on the active/drug-paired lever activated the syringe pump (PHM-100, MedAssociates Inc.) to deliver cocaine infusions through a swivel-tether (375/22PS, Instech, Plymouth Meeting, PA, USA, C313CS-MN, PlasticsOne, Roanoke, VA, USA) infusion system that was attached to an infusion harness (CIH95AB, Instech) worn by the rat. The swivel and tether allowed free movement within the operant conditioning chamber and easy access to the adjoining running wheel to accommodate concurrent drug self-administration and W. Data collection and programming were conducted using PC computers with a Med-PC interface (MedAssociates, Inc.).

Drugs

Cocaine HCl (National Institute of Drug Abuse, Research Triangle Institute, Research Triangle Park, NC, USA) was dissolved in 0.9 % NaCl at a concentration of 1.6 mg cocaine HCl/1 ml saline, and heparin (5 USP/ml) was added to the cocaine solution to prevent catheter occlusion from thrombin accumulation. The flow rate of each cocaine infusion was 0.025 ml/sec, and the duration of pump activation (1 sec/100 g of body weight) was adjusted weekly to provide a 0.4 mg/kg cocaine dose throughout self-administration testing. Progesterone (Sigma Aldrich, St. Louis, MO, USA) was dissolved in peanut oil (0.625 mg/ml) and administered at the 0.5 mg/kg (sc) dose that previously had been shown to decrease reinstatement of cocaine seeking (Anker et al. 2007). Yohimbine (Lloyd Laboratories, Shenandoah, IA, USA) was administered at a dose of 2.5 mg/kg (ip), as prior work demonstrated that this dose reliably reinstated cocaine seeking alone (Feltenstein and See 2006; Anker and Carroll 2010) and in combination with cocaine-paired cues (Feltenstein and See 2006).

Catheterization surgery

One to three days after achieving wheel-running behavior, rats were implanted with an indwelling catheter in the right jugular vein following previously published methods (Carroll and Boe 1982, Zlebnik et al. 2010, Zlebnik et al. 2012). Following the surgical procedure, doors to the wheels remained closed, and each rat was fitted with an infusion harness and tether that remained in place throughout the remainder of the study.

Procedure

Maintenance of cocaine self-administration

The experimental procedure (Table 1) consisted of 5 phases: 1) wheel training, 2) self-administration training, 3) maintenance, 4) extinction, and 5) reinstatement of cocaine seeking. Wheel running acquisition and training of cocaine self-administration followed methods previously published (Zlebnik et al. 2010). During self-administration training and maintenance, sessions began with illumination of the house light, and responses on the active/drug-paired lever started the infusion pump and illuminated the stimulus lights located directly above the lever for the duration of the infusion. Responses on the active lever during the length of the infusion (2-4 seconds for females and 3-5 seconds for males) were recorded but had no programmed consequences. Responses on the inactive lever illuminated the stimulus lights above that lever for the same duration as an infusion but did not activate the infusion pump. Rats were allowed to self-administer iv cocaine (0.4 mg/kg/infusion) for ten 6-h sessions. The door leading to the wheel remained closed throughout the self-administration training and maintenance (no wheel access) phases.

Extinction

Following maintenance, cocaine solutions were removed for the remainder of the study. For 14 sessions, auditory (infusion pump) and visual (house light and stimulus lights) stimuli associated with cocaine self-administration were discontinued to allow rats to extinguish lever pressing. During this period, females (F) and males (M) were each divided into 2

groups, one with access to a locked (LW) running wheel (FLW and MLW) and one with access to an unlocked (W) running wheel (FW and MW), to assess the effect of concurrent W on extinction and any potential carry-forward effect of W on subsequent reinstatement of cocaine-seeking behavior.

Reinstatement

Next, all groups were tested in a within-subjects procedure for reinstatement of cocaine seeking precipitated by yohimbine (2.5 mg/kg, ip) alone, yohimbine + cocaine-paired stimuli, cocaine (10 mg/kg, ip), cocaine + cocaine-paired stimuli, or cocaine-paired stimuli alone in the presence of W, P (P; 0.5 mg/kg, sc), or W+P. All rats were tested under all 5 priming conditions in nonsystematic order, with the exception that the yohimbine alone priming condition always came first. It was hypothesized that there may be a possible carry-forward effect of chronic W during extinction on yohimbine-primed cocaine seeking during later reinstatement tests, since prior research supported a role for exercise in reducing physiological responses to stress (Sasse et al. 2008, Masini et al. 2011). Nonsystematic treatment sequences within the 5 different priming condition blocks ("X"; e.g., yohimbine alone, yohimbine + cocaine-paired stimuli, cocaine alone, cocaine + cocaine-paired stimuli, and cocaine-paired stimuli alone) were as follows: LW+X, W+X, LW+P+X, W+P+X. On intervening days, saline priming injections were administered as a control condition and to allow responding to extinguish before the next priming injection. Subcutaneous vehicle (peanut oil) injections were administered to control for P injections, and on all days when W was not available, rats had access to a locked running wheel (LW).

Data Analysis

The primary dependent measures were responses during maintenance and responses and wheel revolutions during extinction and reinstatement. For maintenance and extinction, data were grouped into 2-day blocks to reduce daily variability and the number of post-hoc contrasts. These measures were analyzed with 3-factor mixed analyses of variance (ANOVA) with sex and extinction wheel condition (LW vs. W) as the between-subjects factors and blocks of days or treatment during reinstatement (e.g., LW, W, LW+P, W+P) as the repeated measure. Separate 3-factor ANOVA were performed for each priming condition (cocaine, yohimbine, cues, cocaine+cues, yohimbine+cues). Following significant interactions, post hoc tests were performed with Fisher's least significant difference (LSD) protected t-tests, and results were considered significant if $p < 0.05$. Statistical analyses were performed using GB Stat (Dynamic Microsystems, Inc., Silver Spring, MD, USA).

Results

Maintenance

Figure 1 depicts the mean number of responses for cocaine (0.4 mg/kg/inf) during daily 6-h sessions over the 10-day maintenance period. A significant main effect of sex ($F_{1, 254} = 7.86$, $p = 0.0072$) indicated that females responded more for cocaine than males, and a significant main effect of 2-day blocks ($F_{4, 254} = 2.61$, $p = 0.0367$) indicated that responding for cocaine escalated or increased in males and females over the course of the maintenance period. There was no sex X block interaction. To specifically assess escalation of

responding, block 1 (days 1-2) was compared to block 5 (days 9-10) in a separate 2-factor repeated-measures ANOVA. Results demonstrated a significant increase in responding at the end of the maintenance period compared to the beginning of the maintenance period (main effect: $F_{1, 101} = 6.77$, $p = 0.0122$), and this effect occurred for both males and females, as there was no significant main effect of sex or sex X block interaction. Therefore, females responded significantly more for cocaine than males, and both groups escalated their responding over the maintenance period.

Extinction

Overall, Figure 2 shows that females were more resistant to extinction than males, and concurrent access to W reduced extinction responding more in females than males. Mean responses during the 14-day extinction period are shown separately for males and females. A 3-factor (sex X W X block of days) ANOVA revealed significant main effects of W ($F_{1,356} = 9.35$, $p = 0.0037$) and block of days ($F_{6,356} = 30.10$, $p < 0.0001$), and significant W X block of days ($F_{6,356} = 3.40$, $p = 0.003$) and sex X W X block of days ($F_{6,356} = 2.16$, $p = 0.0468$) interactions. Post hoc analyses showed a sex difference in the attenuating effect of W on extinction responding during the initial days of the extinction period. Females with access to W (FW) made fewer unreinforced responses during block 1 (days 1-2; $p < 0.01$), block 2 (days 3-4; $p < 0.01$), and block 6 (days 11-12; $p < 0.05$) compared to females with access to LW (FLW) (Fig. 1A); however, there were no differences in extinction responding among the male groups (MW and MLW) (Fig. 1B). Additionally during block 1, females with access to LW (FLW) had greater responding ($p < 0.01$) than males with access to LW (MLW), while females with access to W (FW) had lower responding ($p < 0.05$) than males with access to W (MW). However, while access to W decreased unreinforced responding in females but not males, daily wheel revolutions did not differ between males and females throughout the extinction period (Table 2).

Cue-primed reinstatement

Reinstatement responding elicited by cocaine-paired stimuli or cues is shown in Figure 3A. The effects of treatment with concurrent W, P, or their combination (W+P) on cue-primed reinstatement of cocaine seeking were analyzed by a 3-factor (sex X extinction wheel condition X treatment) ANOVA. Results indicated a significant main effect of treatment ($F_{4,214} = 8.33$, $p < 0.0001$) but otherwise nonsignificant main effects of sex and extinction conditions as well as nonsignificant interactions. Therefore, data were collapsed across sex and extinction wheel condition, and treatment effects were analyzed in a 1-factor repeated-measures ANOVA. Post hoc analyses following a significant F-test ($F_{4,249} = 7.34$, $p < 0.0001$) demonstrated a treatment effect by which animals made significantly fewer responses after treatment with W ($p < 0.05$) and W+P ($p < 0.01$) compared to the LW condition. Therefore, treatment with W alone and W+P significantly reduced cue-primed reinstatement responding in males and females.

Wheel revolutions during cue-primed reinstatement sessions (W vs. W+P only; Table 2) also were analyzed by a 3-factor (sex X extinction wheel condition X treatment) ANOVA. As with cue-primed reinstatement responses, there were no main effects of sex or extinction wheel condition. However, there was a significant main effect of treatment (W vs. W+P;

$F_{1,77} = 8.71$, $p = 0.0056$) and a significant extinction wheel condition X treatment interaction ($F_{1,77} = 7.96$, $p = 0.0078$). After collapsing across sex, a 2-factor ANOVA revealed a main effect of treatment ($F_{1,77} = 7.99$, $p = 0.0075$) and an extinction condition X treatment interaction ($F_{1,77} = 5.53$, $p = 0.0241$). Post hoc analyses showed greater wheel revolutions ($p < 0.01$) for the males and females that had access to W during extinction (MW + FW) when treated with P than with vehicle. Additionally, when treated with P, rats in MW + FW made significantly more revolutions ($p < 0.01$) than MLW + FLW.

Yohimbine-primed reinstatement

Yohimbine-primed reinstatement responding (Fig. 3B) also was analyzed with a 3-factor ANOVA, and results revealed only a significant main effect of treatment ($F_{4,249} = 13.31$, $p < 0.0001$). Therefore, the sex and extinction condition factors were collapsed, and treatment effects were analyzed with a 1-factor ANOVA. Post hoc tests after a significant F-test ($F_{4,249} = 12.87$, $p < 0.001$) confirmed a treatment effect of W alone (W; $p < 0.01$), P alone (LW+P; $p < 0.01$), and their combination (W+P; $p < 0.01$) as reinstatement responding under these conditions was significantly less than under LW conditions. These results indicate that all 3 treatments (e.g., W, P, W+P) effectively reduced yohimbine-primed reinstatement.

Analysis of wheel revolutions during the yohimbine-primed reinstatement period (Table 2) revealed significant main effects of extinction wheel condition ($F_{1,93} = 6.98$, $p = 0.0115$) and treatment ($F_{1,93} = 4.80$, $p = 0.0341$) but no significant interactions. Data were collapsed across sex, and a 2-factor repeated-measures ANOVA was conducted, and there were significant main effects of extinction wheel condition ($F_{1,93} = 6.13$, $p = 0.0171$) and treatment ($F_{1,93} = 4.27$, $p = 0.0445$) but no significant extinction wheel condition X treatment interaction.

Cocaine-primed reinstatement

Analysis of cocaine-primed reinstatement responding (Fig. 3C) revealed a significant main effect of treatment ($F_{4,219} = 19.84$, $p < 0.0001$) and a sex X treatment interaction ($F_{4,219} = 3.19$, $p = 0.015$), but there were no other significant main effects or interactions. Data were collapsed across extinction wheel condition, and results of the 2-factor (sex X treatment) ANOVA showed a significant main effect of treatment ($F_{4,219} = 13.47$, $p < 0.0001$) and a significant sex X treatment interaction ($F_{4,219} = 2.60$, $p = 0.0379$). Post hoc comparisons indicated a differential effect of W in females (FW+FLW) compared to males (MW+MLW): cocaine-primed reinstatement responding under W conditions was significantly lower ($p < 0.01$) than under LW conditions in females but not in males. No other differences were seen between males and females. Among females, in addition to the treatment effect of W, treatment with W+P significantly decreased reinstatement responding ($p < 0.05$) compared to LW. Among males, P ($p < 0.05$) and W+P ($p < 0.01$) both decreased responding compared to LW. Further, since W+P conditions had significantly lower responding than either W ($p < 0.01$) or P ($p < 0.05$) alone, an additive treatment effect was found for the W +P treatment combination in males. Overall, W attenuated cocaine-primed reinstatement in females but not males, and the combination W+P was significantly more effective than either W or P alone in males.

Wheel revolutions during cocaine-primed reinstatement sessions with access to W (both W and W+P; Table 2) were analyzed by 3-factor ANOVA. There were significant main effects of sex ($F_{1,87} = 8.83$, $p = 0.005$) and treatment ($F_{1,87} = 4.43$, $p = 0.0416$) but no interactions; thus data were collapsed across extinction wheel condition for analysis in a 2-factor ANOVA. This analysis resulted in a significant main effect of sex ($F_{1,87} = 6.61$, $p = 0.0138$), indicating that females completed more revolutions than males, and a significant main effect of treatment ($F_{1,87} = 4.57$, $p = 0.0384$), indicating that rats made fewer revolutions when treated with P vs. vehicle. The sex X treatment interaction was not significant.

Cocaine + cocaine-paired cue-primed reinstatement

Reinstatement responding precipitated by the combination of cocaine and cocaine-paired cues or stimuli (Fig. 3D) was analyzed by 3-factor ANOVA. As for the reinstatement conditions described above, results indicated a significant main effect of treatment ($F_{4,229} = 22.45$, $p < 0.0001$) but otherwise nonsignificant main effects of sex and extinction conditions. Further there were no significant interactions, so sex and extinction wheel condition factors were collapsed to utilize a 1-factor repeated-measures ANOVA. Following a significant F-test ($F_{4,249} = 20.70$, $p < 0.0001$), post hoc comparisons revealed significant attenuation of responding under W ($p < 0.01$), P ($p < 0.01$), and W+P ($p < 0.01$) conditions compared to LW conditions. Further, W+P decreased reinstatement responding relative to P alone ($p < 0.01$). While these results indicate significant attenuation of cocaine + cocaine-paired cue-primed reinstatement by W, P, and W+P, they also suggest a greater treatment effect with W+P vs. P alone.

Again, wheel revolutions during the cocaine + cocaine-paired cue-primed reinstatement period (Table 2) were also analyzed by 3-factor ANOVA. There was a significant main effect of sex ($F_{1,91} = 13.92$, $p = 0.0006$) but no other significant main effects or interactions. Subsequently, the extinction wheel condition and treatment factors were collapsed, and a 2-tailed Student's t-test was used strictly to compare male vs. female mean revolutions. Females had significantly greater wheel revolutions than males during this reinstatement condition ($t_{88} = 4.12$, $p = 0.0001$).

Yohimbine + cocaine-paired cue-primed reinstatement

Like for other reinstatement priming conditions, yohimbine + cocaine-paired cue-primed reinstatement responding (Fig. 4) was analyzed by 3-factor ANOVA, and there was no significant main effect of sex or extinction wheel condition. However, there was a main effect of treatment ($F_{4,244} = 55.78$, $p < 0.0001$) and significant sex X extinction wheel condition ($F_{1,244} = 4.87$, $p = 0.0325$) and sex X extinction wheel condition X treatment ($F_{4,244} = 5.93$, $p = 0.0002$) interactions. Post hoc comparisons revealed significant differences among all 4 groups under LW conditions, likely contributing to factorial interactions. The FW group made fewer responses than both FLW ($p < 0.01$) and MW ($p < 0.01$), and FLW made more responses than MLW ($p < 0.01$). However, interestingly MW made more responses than MLW ($p < 0.01$). There were no other significant differences among the groups. Comparing treatment conditions, all groups showed an attenuation of reinstatement responding under W, P, and W+P conditions relative to LW conditions.

Further, for FLW and MW, responding under the W+P condition was significantly lower ($p < 0.05$) than under the P alone condition.

Results of the 3-factor ANOVA analyzing wheel revolutions during the yohimbine + cocaine-paired cue-primed reinstatement period (Table 2) revealed no significant main effects of sex, extinction wheel condition, or treatment, but there was a significant sex X extinction wheel condition X treatment interaction ($F_{1,89} = 4.20$, $p = 0.0469$). Subsequent comparisons demonstrated that FW had higher wheel revolutions than MW when treated with P (W+P; $p < 0.01$), and MLW males had more revolutions when treated with P vs. vehicle (W+P vs. W; $p < 0.05$).

Discussion

This experiment used a novel treatment approach to attenuate cocaine-seeking behavior in a rodent model of relapse. Wheel running and P, highly effective treatments, were examined separately and in combination for their ability to decrease reinstatement of cocaine seeking by singular priming conditions such as cocaine; a stress-inducing agent, yohimbine; and cocaine-paired cues; and also by compound priming conditions such as cocaine + cocaine-paired cues and yohimbine + cocaine-paired cues. Results confirmed earlier work demonstrating greater treatment effects of W on cocaine seeking in female vs. male rats (Cosgrove et al. 2002). Further, this investigation found that the combination of W and P was more effective in reducing reinstatement of responding than either treatment alone in rats that were less responsive to each individual treatment (e.g., males) and more effective than P alone following the most challenging priming conditions (e.g., cocaine + cocaine-paired cues, yohimbine + cocaine-paired cues). These results suggest that combining both behavioral (W) and pharmacological (P) treatment strategies may be a highly successful approach to reducing addiction-related behaviors.

The present experiment extended findings from earlier work on sex differences in cocaine self-administration and in the individual treatment effects of W and P on cocaine-seeking behavior. Rats were given long-access (6-h) sessions to cocaine self-administration under a fixed ratio 1 (FR 1) schedule at the 0.4 mg/kg unit dose during the maintenance phase of the reinstatement procedure, and both males and females escalated their responding for cocaine over this period. In accordance with previously published work (Roth and Carroll 2004), females had significantly more responding for cocaine than males over a period as short as 10 days. These differential rates of responding were maintained during the beginning of the extinction phase as females (FLW) had significantly higher rates of extinction responding than males (MLW) during initial sessions following discontinuation of cocaine self-administration. However, all groups reached low levels of responding by the end of the extinction period; and despite having initially greater extinction responding, females were more receptive to treatment with W than males. Although wheel revolutions did not differ among the groups, W significantly attenuated extinction in the FW group but not the MW group. Prior work has demonstrated a reduction in extinction for females with access to W (Zlebnik et al. 2010) and for both males and females with chronic access to W in the home cage (Smith et al. 2012). Although Smith et al. (2012) showed that W decreased extinction responding in both males and females, their results indicated a stronger effect in females

than males with a suppression of responding for a greater period of time (i.e., over more days). Together, the results of the present experiment and those of previous studies demonstrate greater cocaine-motivated responding and greater treatment effects of W in female than male rats.

While there were few differences among male and female rats in the magnitude of reinstatement of cocaine-seeking behavior under control treatment (LW) conditions across all 5 reinstatement priming conditions, there was a difference in their response to treatment with W. Consistent with results during the extinction phase, W significantly reduced cocaine-primed reinstatement of cocaine seeking in female but not male rats. During this reinstatement priming condition and the cocaine + cocaine-paired cue priming condition, females ran more than males (Table 2). However, W had equivalent treatment effects on cocaine + cocaine-paired cue-induced responding in males and females, suggesting that the greater treatment effect for cocaine-primed reinstatement in females was not necessarily due to their engagement in greater levels of W than the males. For other priming conditions (e.g., yohimbine, cocaine-paired cues, cocaine + cocaine-paired cues, yohimbine + cocaine-paired cues) in the present experiment, W reduced reinstatement equally well in both sexes. These results suggest that sex differences in the efficacy of exercise to decrease cocaine seeking may be influenced by the conditions provoking reinstatement.

Treatment with P reduced cocaine seeking precipitated by yohimbine, cocaine, cocaine + cocaine-paired cues, and yohimbine + cocaine-paired cues. That this investigation found that exogenously-administered P did not decrease cocaine seeking in response to cocaine-paired cues in rats was in contrast to clinical reports showing reduced cocaine-paired cue-induced craving during phases of high endogenous P (Sinha et al. 2007); however, under control conditions, responding precipitated by cues was relatively low compared to other work on cue-induced reinstatement collected in the same laboratory (Anker and Carroll 2010, 2011). These results may be explained by the multicomponent reinstatement procedure whereby cues were presented many times over the course of 20 sessions, facilitating extinction to these stimuli.

While most prior work has indicated a significant treatment effect of P on cocaine seeking in female vs. male rats (Carroll and Anker 2010, 2011), P significantly decreased cocaine-primed reinstatement in males, but results demonstrated only a nonsignificant trend for the attenuation of cocaine-primed reinstatement in females. The length and nature of the reinstatement procedure used in the present investigation may have resulted in diminished responding under control treatment (LW) conditions, masking treatment effects in the female groups. In humans, reports regarding the treatment efficacy of P in males have been mixed. While one study found a significant attenuation of positive subjective effects and cardiovascular responses in men (Sofuoglu et al. 2004), another found little to no effect on cocaine's subjective effects (Evans and Foltin 2006) and cocaine use (Sofuoglu et al. 2007) and only dose-dependent decreases in cardiovascular responses to cocaine (Evans and Foltin 2006) in males. These equivocal findings are in accordance with rodent studies demonstrating P treatment as both effective (Romieu et al. 2003) and ineffective (Russo et al. 2010) in decreasing cocaine conditioned place preference in males. Overall, few investigations have examined the role of P to reduce cocaine-motivated behavior in males,

and the results of this experiment suggest that treatment with P under the present conditions reduces cocaine seeking in male rats.

In contrast to treatment effects seen with concurrent unlocked wheel access and P separately, treatment with W+P significantly attenuated cocaine seeking compared to control treatment (LW) across all 5 priming conditions, indicating efficacy over a broader range of relapse-provoking stimuli than individual treatments alone. For male rats under cocaine priming conditions, the combination of W+P was more effective at reducing cocaine seeking than either W or P treatment alone. Additionally, compound priming conditions such as cocaine + cocaine-paired cues and yohimbine + cocaine-paired cues elicited higher rates of responding than singular priming conditions (Feltenstein and See 2006), and the combination of W+P decreased cocaine seeking in response to these stimuli more effectively than P treatment alone. Together, all of these data suggest that the combination of concurrent W+P may be more effective at reducing cocaine seeking than each individual treatment, and this is in accordance with clinical work demonstrating additive or synergistic treatment effects of methadone maintenance (McLellan et al. 1993, Peirce et al. 2006) and tricyclic antidepressants (Kosten et al. 2003, Poling et al. 2006) with behavioral therapies. The present study also extends findings demonstrating greater treatment outcomes for addiction when cognitive (Martin et al. 1997) or motivational (Weinstock et al. 2008) behavioral strategies are combined with exercise. Together, the results of the current experiment and prior work demonstrate greater efficacy from combined behavioral + pharmacological treatments vs. singular treatments.

For individuals in recovery, the combination of behavioral and pharmacological treatments may help overcome frequent challenges to maintaining abstinence. Threats to recovery include drug cravings precipitated by both external (e.g., people, places, things) and internal (e.g., stress) cues, and former addicts must learn to tolerate and resist craving as well as develop healthier patterns of behavior that replace drug reinforcement with alternative nondrug reinforcement (Potenza et al. 2011). The present findings suggest that access to exercise may decrease drug seeking during initial abstinence and that, compared to either treatment alone, the combination of exercise and P may prevent relapse to cocaine-seeking behavior over a broader range of relapse-provoking stimuli, including both external (e.g., cocaine-paired stimuli) and internal (e.g., cocaine, yohimbine) drug-related cues. Additionally, the incorporation of exercise in a dual treatment plan could accelerate adoption of healthier behaviors and augment attempts to sustain abstinence (Ussher et al. 2012). These treatment effects may be especially effective in individuals with certain vulnerability factors for addiction (i.e., females vs. males), suggesting a customized approach using treatment combinations in order to achieve optimal treatment outcomes. Overall, the present investigation demonstrated that, under certain conditions, combined behavioral (W) and pharmacological (P) interventions were more successful at reducing cocaine-seeking behavior than either intervention alone, and these results may have implications for the treatment of substance abuse in clinical settings.

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Figure 1.

Mean ($\pm$ SEM) daily responses for cocaine (0.4 mg/kg/inf) made by males and females during the 10-day maintenance period. Overall, females made more responses than males (** $p < 0.01$), and both females and males significantly increased their responding over the 10-day period (days 1-2 vs. days 9-10, # $p < 0.05$).

Figure 2.

Mean ($\pm$ SEM) daily unreinforced responses during the 14-day extinction period. **A.)**

Females with access to W (FW) made significantly fewer responses than females with concurrent access LW (FLW) during days 1-2, 3-4, and 10-11 (** $p < 0.01$, * $p < 0.05$).

Further, FW had less responding than MW, while FLW had more responding than MLW during days 1-2 (# $p < 0.05$).

B.) There were no differences in responses made by the male groups (MW and MLW).

Figure 3.

Mean ($\pm$ SEM) responses per treatment session over 4 separate reinstatement priming conditions (e.g., cocaine-paired cues or stimuli, yohimbine, cocaine, cocaine + cocaine-paired cues). As there were no significant main effects of sex or extinction wheel condition (W vs. LW), in most cases data were collapsed across groups to focus on treatment effects.

A.) Cocaine-paired cue-primed reinstatement responding was attenuated by W + vehicle treatment (W; * $p < 0.05$) and W+P (** $p < 0.01$) compared to control conditions LW + vehicle treatment (LW). **B.)** Yohimbine-primed reinstatement responding was reduced by W, P, and W+P (** $p < 0.01$) compared to LW. **C.)** Due to a sex X treatment interaction, males and females were not combined for the cocaine-primed reinstatement condition. Unlocked wheel running access W significantly attenuated responding compared to LW conditions in females but not males (# $p < 0.01$). Among females, W+P also reduced cocaine-primed reinstatement (* $p < 0.05$); however, among males, P (* $p < 0.05$) and W+P (** $p < 0.01$) both decreased responding compared to LW. Further, an additive treatment effect was seen for the W+P treatment combination in males as responding under this condition was significantly lower than under either W (** $p < 0.01$) or P (* $p < 0.05$) conditions alone. **D.)** Reinstatement responding precipitated by the combination of cocaine priming injection and cocaine-paired cues was reduced by W, P, and W+P (** $p < 0.01$). Further, reinstatement responding under W+P conditions was significantly lower than under P alone conditions, suggesting a greater treatment effect of W+P (** $p < 0.01$).

Figure 4.

Mean ($\pm$ SEM) reinstatement responses per treatment session for the yohimbine + cocaine-paired cue condition. There were significant differences among all 4 groups under LW conditions: FW made fewer responses than both FLW (** $p < 0.01$) and MW, and FLW made more responses than MLW; however, MW made more responses than MLW. There were no other significant differences among the groups. Comparing treatment conditions, all groups showed an attenuation of reinstatement responding under W, P, and W+P vs. LW (# $p < 0.05$). Further, for FLW and MW, there was an enhanced treatment effect of W+P, as responding under that condition was significantly lower (@ $p < 0.05$) than under the P alone condition.

Table 1

Experimental timeline

<i>Phase</i>	<i>Wheel training</i>	<i>Maintenance</i>	<i>Extinction</i>	<i>Reinstatement conditions given in random order</i>			
				<i>Yoh</i>	<i>Yoh+ Cues</i>	<i>Cues</i>	<i>Coc Coc+ Cues</i>
<i>Days</i>	3	10	14	20 (4 session/condition)			
<i>Sessions</i>			6 h/day, 9 am – 3 pm				

Yoh = yohimbine, 2.5 mg/kg, ip; Coc = cocaine, 10 mg/kg, ip; Cues = house light, lever lights, infusion pump

Table 2

Mean (SEM) wheel revolutions during extinction and reinstatement periods

Extinction			Reinstatement									
			Cues		Yohimbine		Cocaine		Cocaine + Cues		Yohimbine + Cues	
			W	W+P	W	W+P	W	W+P	W	W+P	W	W+P
Females												
<i>FLW</i>	271 (177)	233 (72)	435 (100)	654 (155)#	883 (214)&†	934 (252)f	712 (110)†	674 (125)†	683 (188)	665 (218)		
<i>FW</i>	421 (72)	889 (286)*#	800 (171)@	842 (224)#@	918 (281)&†	642 (157)f	643 (99)†	715 (140)†	834 (236)	928 (258)†		
Males												
<i>MLW</i>	--	273 (86)	324 (85)	348 (50)	496 (100)&	331 (64)	355 (69)	411 (91)	491 (79)	725 (141)#		
<i>MW</i>	245 (84)	199 (72)	513 (154)*#	709 (158)@	525 (141)&	314 (121)	322 (115)	376 (130)	637 (181)	515 (149)		

W+P > W; @ MW, FW > MLW, FLW; & W > W+P; f F > M

* MW+FW > MLW+FLW