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The effects of exercise on cocaine self-administration, food-maintained responding, and locomotor activity in female rats: Importance of the temporal relationship between physical activity and initial drug exposure

Mark A. Smith and Maryam A. Witte

Department of Psychology and Program in Neuroscience, Davidson College

Abstract

Previous studies have reported that exercise decreases cocaine self-administration in rats with long-term access (8+ weeks) to activity wheels in the home cage. The purpose of this study was to (1) examine the importance of the temporal relationship between physical activity and initial drug exposure, (2) determine the effects of exercise on responding maintained by a nondrug reinforcer (i.e., food), and (3) investigate the effects of exercise on cocaine-induced increases in locomotor activity. To this end, female rats were obtained at weaning and divided into four groups: (1) EXE-SED rats were housed in exercise cages for six weeks and then transferred to sedentary cages after the first day of behavioral testing; (2) SED-EXE rats were housed in sedentary cages for six weeks and then transferred to exercise cages after the first day of behavioral testing; (3) SED-SED rats remained in sedentary cages for the duration of the study; and (4) EXE-EXE rats remained in exercise cages for the duration of the study. Relative to the sedentary group (SED-SED), exercise reduced cocaine self-administration in both groups with access to activity wheels after initial drug exposure (EXE-EXE, SED-EXE), but did not reduce cocaine self-administration in the group with access to activity wheels only before drug exposure (EXE-SED). Exercise also decreased the effects of cocaine on locomotor activity, but did not reduce responding maintained by food. These data suggest that exercise may reduce cocaine use in drug-experienced individuals with no prior history of aerobic activity without decreasing other types of positively reinforced behaviors.

Keywords

cocaine; exercise; locomotor activity; physical activity; self-administration

Physical activity is negatively correlated with alcohol, tobacco, and drug use (Field, Diego, & Sanders, 2001; Iannotti, Kogan, Janssen, & Boyce, 2009; Kirkcaldy, Shephard, & Siefen, 2002; Ströhle et al., 2007) and is associated with positive outcomes in substance abuse treatment programs (Buchowski et al., 2011; Roessler, 2010; Weinstock, Barry, & Petry, 2008). Preclinical studies report that aerobic exercise serves as an alternative nondrug reinforcer to reduce drug-seeking behavior (Cosgrove, Hunter, & Carroll, 2002; Miller et al., 2012; Zlebnik, Anker, Gliddon, & Carroll, 2010), and a history of exercise for 8+ weeks in

Corresponding Author: Mark A. Smith, Ph.D., Department of Psychology, Box 7037, Davidson College, Davidson, NC 28035-7037, Phone: 704-894-2470, Fax: 704-894-2512, masmith@davidson.edu.

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the home cage reduces drug-seeking behavior during all phases of the addictive process: acquisition to use, maintenance of use, escalation of use, binge and compulsive use, and relapse and reinstatement (Smith & Lynch, 2011; Smith, Pennock, Walker, & Lang, 2012; Smith & Pitts, 2011; Smith, Walker, Cole, & Lang, 2011). These latter findings suggest that the effects of physical activity extend beyond a particular bout of exercise and have generalized effects on diverse measures of drug-maintained responding.

Many of the previous studies examining the effects of home-cage exercise, along with studies from our laboratory, assigned subjects to sedentary and exercise conditions at weaning and examined drug-seeking behavior several weeks later in young adulthood. Importantly, exercise was available both before and after initial drug exposure in these studies, so it wasn't clear when exercise was producing its protective effects. In one study, exercise output before initial drug exposure, but not after initial drug exposure, was inversely related to cocaine-maintained breakpoints on a progressive ratio (PR) schedule of reinforcement, suggesting there may be a critical period early in development during which exercise produces long-lasting effects on drug-seeking behavior (Smith, Schmidt, Iordanou, & Mustroph, 2008). In contrast, extensive exposure to an activity wheel prior to testing was not necessary for exercise to decrease measures of drug-seeking behavior under concurrent access conditions (Miller et al., 2012; Zlebnick et al., 2010). Knowledge of when exercise produces its protective effects will be necessary for designing activity-based interventions in at-risk and substance-abusing populations.

Any behavioral intervention for substance abuse would optimally reduce drug-seeking behavior but leave other forms of operant behavior, particularly those necessary for the health and well-being of the individual, unaffected. Very few studies have examined the effects of exercise on behavior maintained by nondrug rewards, especially under conditions of long-duration access to exercise in the home cage. Brief exposure to an activity wheel for 1 hr/day immediately before operant test sessions increases responding maintained by food (Belke, 2006), suggesting that exercise may increase the reinforcing efficacy of nondrug rewards. Although such findings suggest that exercise may have different and possibly opposite effects on behavior maintained by drug and nondrug rewards, no studies have examined the two forms of behavior under identical conditions in parallel experiments. Finally, interventions that specifically target cocaine and other psychomotor stimulants ideally would not be limited to decreasing their reinforcing effects. Cocaine and similar drugs produce a number of adverse effects, and both behavioral and physiological toxicity is observed when these drugs are administered repeatedly and at high doses (Bozarth & Wise, 1985; Morishima, Whittington, Iso, & Cooper, 1999).

The purpose of the present study was fourfold: (1) to replicate previous findings that a history (8+ weeks) of home-cage aerobic exercise reduces the positive reinforcing effects of cocaine, (2) to determine the importance of the temporal relationship between physical activity and initial drug exposure on cocaine-maintained responding, (3) to examine the effects of exercise on responding maintained by a nondrug reinforcer (i.e., food), and (4) to investigate the effects of exercise on cocaine-induced increases in locomotor activity. To this end, four groups of female rats were obtained at weaning and assigned to continuously sedentary conditions (SED-SED), sedentary then exercise conditions (SED-EXE), exercise then sedentary conditions (EXE-SED), or continuously exercise conditions (EXE-EXE). The effects of exercise on responding maintained by drug or food were examined after 6 weeks, at which time housing conditions were reversed for some animals. Following the conclusion of those tests, the effects of exercise on cocaine-induced increases in locomotor activity were examined in all animals.

Method

Animals and Apparatus

All major experimental events are depicted schematically in Figure 1. Female, Long-Evans rats were obtained at weaning (~21 days) from Charles River Laboratories (Raleigh, NC, USA) and randomly assigned to four conditions: SED-SED rats remained under sedentary conditions for the duration of the study; SED-EXE rats were housed under sedentary conditions for 6 weeks, then transferred to exercise cages on the first day of behavioral testing; EXE-SED rats were housed under exercise conditions for 6 weeks, then transferred to sedentary conditions on the first day of behavioral testing; EXE-EXE rats remained under exercise conditions for the duration of the study. Sedentary rats were housed in polycarbonate cages (interior dimensions: 50 × 28 × 20 cm) that permitted no exercise beyond normal cage ambulation. Exercising rats were housed in polycarbonate cages of equal dimensions but with an activity wheel (interior diameter: 35 cm) affixed to the interior of the cage. Wheel revolutions were counted with mechanical switches and recorded daily. Except during periods of experimenter-controlled food restriction (see below), food and drinking water were continuously available in the home cage. All cages were kept in a temperature- and humidity-controlled colony room maintained on a 12-hr light/dark cycle. All animals were treated in accordance with the regulations outlined in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animals Resources, 2011), and all procedures were approved by the Davidson College Animal Care and Use Committee. Tests of cocaine-maintained responding (n = 42 rats) and food-maintained responding (n = 48 rats) were conducted in separate groups of rats. All experimental sessions were conducted during the light phase of the light/dark cycle so as not to interfere with nocturnal running.

Tests of cocaine- and food-maintained responding were conducted in polycarbonate and aluminum operant conditioning chambers (interior dimensions: 31 × 24 × 21 cm) from Med Associates, Inc. (St Albans, VT, USA). Each chamber was equipped with two response levers on one wall, a food hopper located between the levers, a white stimulus light located above each lever, and a houselight located on the opposite wall. Drug infusions were delivered from an infusion pump mounted outside the chamber via Tygon tubing protected by a stainless steel spring and attached to a counter-balanced swivel suspended above the chamber. Food pellets were delivered via a pellet dispenser located behind the forward wall. The left lever was designated as the active lever in all experimental sessions. All experimental events were programmed and data were collected with software and interfacing from Med Associates, Inc.

Locomotor activity tests were conducted in open-field, locomotor-activity chambers (interior dimensions: 43 × 43 × 30 cm) obtained from Med Associates, Inc. The chamber consisted of a PVC floor and acrylic sidewalls with aluminum corner supports. Two circuit boards were located on opposite sidewalls 2.5 cm above the floor of the chamber. One board contained 16 infrared photocells spaced 2.5 cm apart; the opposite board contained 16 infrared detectors with identical spacing. All photocells and detectors were interfaced through a computer running a Microsoft Windows operating system and Med Associates software.

Lever-Press Training

Five weeks after arrival, all rats were lightly food restricted to no less than 90% of their free feeding body weight and trained to press a response lever on a fixed ratio (FR1) schedule of food reinforcement. Food restriction was used to facilitate acquisition of the lever press response. On the FR schedule, each response produced a single 45-mg grain pellet followed

by a 5-second timeout in which responding had no programmed consequences. Each session continued for 2 hr or until 40 reinforcers were delivered, whichever occurred first. If any rat had not acquired by the third day of training, one shaping session was conducted in which a technician shaped the lever press response using manually delivered food pellets. Training continued in individual rats until 40 reinforcers were earned in any three consecutive training sessions. All rats met the acquisition requirement within 7 days and no differences were observed between groups.

Cocaine-Maintained Responding

Six weeks after arrival, rats used in the cocaine self-administration experiments were anesthetized with a combination of ketamine (100 mg/kg, ip) and xylazine (8.0 mg/kg, ip) and surgically implanted with intravenous catheters according to methods described previously (Smith et al. 2008; 2011). A solution of heparinized saline (200 units/ml, iv) and ticarcillin (20 mg/kg, iv) was infused through the catheter daily to prevent infection and maintain patency. After seven days, ticarcillin was discontinued and only heparinized saline was used to maintain catheter patency. Rats were given three days of recovery before beginning cocaine self-administration testing.

After recovery, rats were placed into the operant conditioning chambers and connected to infusion pumps via Tygon tubing. Each session began with illumination of the house light, illumination of the white stimulus light above the left response lever, and a priming infusion of the dose of cocaine available during that session. During the initial training sessions, lever presses were reinforced on an FR1 schedule of reinforcement. On this schedule, each lever press activated the infusion pump for 2.0 to 3.5 seconds (based on body weight) and delivered 0.5 mg/kg cocaine (Research Triangle Institute, Research Triangle Park, NC, USA). Coincident with each infusion, a tone was sounded for 5 seconds and the stimulus light turned off to signal a 20-sec timeout period during which cocaine was not available. Four training sessions were conducted in which responding was reinforced on an FR schedule of reinforcement. During the first two sessions, responding was reinforced on an FR1 schedule; during the subsequent two sessions, responding was reinforced on an FR2 schedule. After four days, training on the FR schedule was discontinued and testing immediately commenced the following day on a PR schedule of reinforcement (see below). Importantly, half of the sedentary rats and half of the exercising rats switched conditions immediately after the first training session. This was accomplished by switching sedentary home cage cages for exercise home cages (and vice versa) while rats were in the operant conditioning chambers during their first training session. Thus, cocaine-maintained responding was examined in four groups of rats (SED-SED, SED-EXE, EXE-SED, and EXE-EXE; see description above).

Throughout testing, responding was reinforced on a PR schedule of reinforcement. On this schedule, the number of responses required for reinforcement incremented progressively through the following ratio values: 1, 3, 6, 9, 12, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208, 268, 346, 445, and 573 (for complete algorithm, see Suto et al., 2002). Each session continued until a breakpoint was reached, with breakpoint defined as the number of infusions obtained before one hour elapsed with no infusions. Breakpoints were determined for 0.1, 0.3, and 1.0 mg/kg/infusion cocaine, as well as for saline. In the saline test, each reinforced response resulted in the delivery of the audiovisual stimulus associated with cocaine (tone on, stimulus light off, infusion pump on), but cocaine was not delivered. Doses were tested in an irregular order with the stipulation that no more than two ascending or descending doses could be tested in a row. Each dose was tested at least twice and no more than three times on nonconsecutive days. For individual rats, the average of all breakpoints at each dose was used in the statistical analysis.

Food-Maintained Responding

At the conclusion of lever-press training (see above), rats used in the food-maintained responding experiment were given a minimum three-day rest period in which they were placed on unrestricted feed and left undisturbed in their home cages. This rest period was included to keep all major experimental events temporally congruent with that of the cocaine-maintained rats. At the conclusion of the rest period, rats were again lightly food restricted to no less than 90% of their free feeding body weight. One day later, rats were placed into the operant conditioning chambers and tests of food-maintained responding were conducted. Each session began with illumination of the house light, illumination of the white stimulus light above the left response lever, and a noncontingent delivery of a food pellet. During the initial session, lever presses were reinforced on an FR2 schedule of reinforcement. On this schedule, every two lever presses activated the pellet dispenser and delivered a 45-mg Noyes grain pellet. Coincident with each delivery, a tone was sounded for 5 seconds and the stimulus light turned off to signal a 20-sec timeout period during which food was not available. After one day on the FR2 schedule, training on the FR schedule was discontinued and testing immediately commenced the following day on a PR schedule of reinforcement (see below). Training on the FR schedule was truncated in food-maintained rats relative to cocaine-maintained rats to keep major experimental events temporally congruent between the two groups (i.e., PR testing took four days longer in food-maintained rats) and because food-maintained rats already had extensive training with the reinforcing stimulus (i.e., they were trained with food during week 5). Importantly, half of the sedentary rats and half of the exercising rats switched conditions immediately after the first training session. This was accomplished by switching sedentary home cage cages for exercise home cages (and vice versa) while rats were in the operant conditioning chambers during their first training session. Thus, food-maintained responding was examined in four groups of rats (SED-SED, SED-EXE, EXE-SED, and EXE-EXE; see description above).

Throughout testing, responding was reinforced on a PR schedule of reinforcement in a manner similar to that used in the cocaine self-administration experiment (see above). Each session continued until a breakpoint was reached, with breakpoint defined as the number of reinforcers obtained before one hour elapsed with no reinforcers. Breakpoints were determined for 45-mg Noyes grain pellets, 45-mg Noyes sucrose pellets, and the audiovisual stimulus associated with food delivery (tone on, stimulus light off, pellet dispenser on). For the audiovisual test, the pellet dispenser was empty and no pellet was delivered. Each stimulus was tested for three consecutive days, for a total of nine days. Food stimuli were tested on consecutive days rather than nonconsecutive days because switching food pellets required disassembly of the pellet dispenser between each session, which proved time limiting when multiple rats were tested consecutively in the same chamber each day. After the ninth day of PR testing, all rats were placed back on unrestricted feed. One day later, PR testing resumed under free feeding conditions for an additional nine days. Once again, breakpoints were determined for 45-mg Noyes grain pellets, 45-mg Noyes sucrose pellets, and the audiovisual stimulus associated with food delivery. Each stimulus was tested for three consecutive days, with the order of testing reversed relative to the sequence used under restricted food conditions. For individual rats, the average of the three breakpoints for each stimulus and for each food restriction condition was used in the statistical analysis. Tests were conducted under restricted feed and free feed conditions to test responding during high and low motivational states, respectively.

Locomotor Activity Testing

After tests of cocaine- and food-maintained responding were completed, rats were habituated to the locomotor activity chambers for one 60-min session. Over the next three consecutive days, locomotor activity tests were conducted with two doses of cocaine (3.0

and 10 mg/kg, ip) and saline. Doses were tested in an irregular order that varied across rats. In all three tests, cocaine or saline was administered immediately prior to the session via intraperitoneal injection, and the rat was placed immediately into a locomotor activity chamber. The session started immediately after placing the rat in the chamber, and locomotor activity (operationally defined as distance traveled in cm) was measured over the next 60 min. White noise was continuously present during testing. Locomotor activity data obtained in cocaine-maintained animals and food-maintained animals were analyzed separately.

Data Analysis

Exercise output data (measured as rev/day) and body weight data (measured as g) were analyzed via mixed-factor ANOVA, with group serving as a between-subjects factor and time (measured as weeks) serving as a within-subjects factor. Weeks were measured in 5- to 9-day intervals (rather than 7-day intervals) to ensure that all “weeks” contained the same experimental events across animals (e.g., surgery occurred as early as day 40 in some animals and as late as day 44 in other animals). Data from the cocaine-maintained responding tests, the food-maintained responding tests, and the locomotor activity tests were analyzed via mixed-factor ANOVA, with group serving as a between-subjects factor and dose (or food stimulus) serving as a within-subjects factor. As a secondary analysis, area under the curve (AUC) estimates were calculated for the dose-response data from the cocaine self-administration tests using the Trapezoidal Rule. These AUC estimates were then analyzed via one-way ANOVA using group as a factor. For the cocaine self-administration tests and the locomotor activity tests, data obtained with saline were analyzed separately via one-way ANOVA, using group as a between-subjects factor. In all analyses in which significant group effects were observed, post-hoc tests were conducted using Fisher’s LSD (Least Significant Difference) test for multiple comparisons. Pearson product moment correlations were used to examine the relationship between exercise output and cocaine self-administration. For the Pearson product moment correlations, AUC estimates were used (as opposed to individual breakpoints) because it allowed data from all doses to be included in the analysis without violating the assumption of independent observations.

Results

Tests of Cocaine-Maintained Responding

Exercise output and body weights—Exercise output increased significantly during the first six weeks of the study prior to the beginning of behavioral testing [main effect of week: $F(5, 95) = 110.286, p < .001$] (Figure 2). Importantly, no differences between the two exercise groups (EXE-SED, EXE-EXE) were observed. Exercise output decreased by approximately 50% after catheter implantation and the beginning of behavioral testing in the EXE-EXE group and remained stable thereafter. Exercise output was initially lower in the SED-EXE group when they were first exposed to the activity wheels (week 7), but increased rapidly and did not differ from the EXE-EXE group by the final week of testing (week 8) [week $\times$ group interaction: $F(1, 19) = 9.929, p = .005$]. Body weight increased steadily over the course of the study [main effect of week: $F(7, 259) = 582.715, p < .001$], but no differences were observed between the four groups.

Cocaine self-administration—Breakpoints increased linearly across the three doses of cocaine [main effect of dose: $F(2, 74) = 87.019, p < .001$] and differed significantly across groups [main effect of group: $F(3, 37) = 4.714, p = .007$] (Figure 3). Post-hoc analyses revealed that both groups of rats that had access to activity wheels after initial drug exposure (SED-EXE, EXE-EXE) had lower breakpoints than the two groups of rats that were sedentary after initial drug exposure (EXE-SED, SED-SED). Similarly, an AUC analysis of

the dose-response data revealed a main effect of group [$F(3, 37) = 4.431, p = .009$], and post-hoc tests revealed that the two groups with access to activity wheels after initial drug exposure responded significantly less than the two groups that were sedentary after initial drug exposure. Interestingly, significant group differences were also observed during the saline substitution test [main effect of group: $F(3, 37) = 4.337, p = .010$]. Similar to that observed in the dose-response analysis, lower rates of responding were observed in the two groups with access to activity wheels after initial drug exposure than the two groups that were sedentary after initial drug exposure. Exercise output was determined prior to initial drug exposure (EXE-EXE, EXE-SED), after initial drug exposure (EXE-EXE, SED-EXE), and through the entire study (EXE-EXE) was not correlated with cocaine self-administration as determined via AUC estimates (data not shown).

Locomotor activity in cocaine-maintained rats—Cocaine produced dose-dependent increases in locomotor activity in all four groups of rats [main effect of dose: $F(1, 37) = 74.727, p < .001$] (Figure 4). The effects of cocaine differed significantly across the four groups [main effect of group: $F(3, 37) = 2.882, p = .049$], and post-hoc tests revealed that both group of rats with access to activity wheels after initial drug exposure (SED-EXE, EXE-EXE) were less sensitive to cocaine than the continuously sedentary group (SED-SED). Similar differences were observed following saline administration [main effect of group: $F(3, 37) = 7.017, p = .001$], and post hoc tests again revealed that the two groups with access to activity wheels after initial drug exposure traveled less than the continuously sedentary group.

Tests of Food Maintained Responding

Exercise output and body weights—Exercise output increased rapidly prior to the initiation of behavioral testing [main effect of week: $F(5, 110) = 65.642, p < .001$] (Figure 5), but no differences were observed between the two exercise groups (EXE-EXE, EXE-SED). Exercise output remained high during the first week of behavioral testing in the EXE-EXE group when the rats were food restricted (week 7), but declined by approximately 40% during the final week of testing when the rats returned to free feeding conditions (week 8). Exercise output was initially lower in the SED-EXE group when they were first exposed to the activity wheels, but increased rapidly during the final week of testing such that they did not differ from the EXE-EXE group [week $\times$ group interaction: $F(1, 22) = 24.688, p < .001$]. Body weights increased over the course of the study [$F(7, 308) = 1701.559, p < .001$], but no differences were observed between groups.

Food-maintained responding under food-restricted conditions—Under conditions of light food restriction, the three food stimuli maintained significantly different breakpoints [main effect of food stimulus: $F(2, 88) = 68.373, p < .001$] (Figure 6; top). Whereas breakpoints maintained by grain and sucrose were similar, breakpoints maintained by the audiovisual stimulus were markedly lower. Breakpoints also differed significantly across the four groups [main effect of group: $F(3, 44) = 3.189, p = .033$]. Post-hoc tests revealed that the pattern of group effects differed qualitatively from the pattern observed with cocaine-maintained responding. Specifically, breakpoints were greater in the group with continuous access to activity wheels (EXE-EXE) than in the continuously sedentary group (SED-SED) and the group with access to activity wheels only after initial drug exposure (SED-EXE).

Food-maintained responding under free feeding conditions—Similar to that observed under food-restricted conditions, the three food stimuli maintained significantly different breakpoints under free feeding conditions [main effect of food stimulus: $F(2, 88) = 47.078, p < .001$] (Figure 6; bottom). Again, breakpoints maintained by grain and sucrose

were similar and markedly greater than that maintained by the audiovisual stimulus. Importantly, breakpoints did not differ across the four groups. Responding maintained by each of the three food stimuli were very similar for all four groups, a pattern that differed markedly from that observed with cocaine.

Locomotor activity in food-maintained rats—Cocaine produced dose-dependent increases in locomotor activity in all groups of rats [main effect of dose: $F(1, 44) = 34.755$, $p < .001$] (Figure 7). Similar to that seen in cocaine-maintained rats, significant differences were observed across groups [main effect of group: $F(3, 44) = 3.406$, $p = .026$]. Also similar to that seen in cocaine-maintained rats, post-hoc tests revealed that both group of rats with access to activity wheels after initial drug exposure (SED-EXE, EXE-EXE) were less sensitive to cocaine than the continuously sedentary group (SED-SED). Similar differences were observed after saline administration [main effect of group: $F(3, 44) = 6.580$, $p = .001$], and the two groups with activity wheels after initial drug exposure traveled significantly less than the continuously sedentary group.

Discussion

The principal findings of the present study are (1) a history of continuous exercise decreases the positive-reinforcing effects of cocaine, (2) exercise is fully effective at decreasing cocaine self-administration even if exercise is not initiated until after initial drug exposure, (3) long-term exercise (6 weeks) immediately prior to drug exposure is not effective at decreasing cocaine self-administration if exercise is discontinued following drug exposure (4) exercise decreases the locomotor effects of cocaine in a manner similar to the way it decreases the reinforcing effects of cocaine, and (5) exercise does not decrease other types of positively reinforced behavior, such as responding maintained by food and a stimulus associated with food.

The present study replicates an earlier finding that aerobic exercise decreases breakpoints maintained by cocaine on a PR schedule of reinforcement (Smith et al., 2008). These data also support numerous other studies reporting that aerobic exercise decreases responding maintained by cocaine in procedures designed to model different transitional stages of the addictive process (Lynch, Piehl, Acosta, Peterson, & Hemby, 2010; Smith & Lynch, 2011; Smith et al., 2012; Smith & Pitts, 2011; Smith et al., 2011). In contrast to that reported in a previous study (Smith et al., 2008), exercise also decreased responding maintained by saline. It is difficult to reconcile the disparate findings between the two studies given that most of the major experimental parameters were identical. It should be emphasized, however, that the saline substitution test wasn't simply a test of extinction because the audiovisual stimulus previously associated with cocaine delivery (tone on, light off, infusion pump on) was still delivered contingently based on the parameters set by the PR schedule. Thus, the saline substitution test measured responding maintained by a cocaine-paired conditioned stimulus (CS), and may be viewed as a drug-seeking response (see Di Ciano & Everitt, 2004). Importantly, exercise decreased responding maintained by a cocaine-paired CS in a manner similar to the way it decreased cocaine-maintained responding.

One of the major aims of the present study was to examine the importance of the temporal relationship between physical activity and initial drug exposure. We previously reported that exercise output over 6 weeks prior to catheter implantation and behavioral testing was inversely related to cocaine-maintained breakpoints on at PR schedule of reinforcement (Smith et al., 2008), suggesting there may be a critical period during which exercise offers long-term protection on measures of drug-seeking behavior. Alternatively, studies in which an activity wheel was concurrently available during experimental sessions suggest that an extensive history of physical activity is not necessary for exercise to decrease drug self-

administration (e.g., Miller et al., 2012). In the present study, 6 weeks of aerobic exercise immediately prior to initial drug exposure offered no protection on drug-seeking behavior as measured by breakpoints on a PR schedule of reinforcement. Conversely, two weeks of exercise after initial drug exposure and overlapping the period of behavioral testing decreased cocaine-maintained breakpoints even in rats that remained sedentary for the first 6 weeks of the study. In fact, the two groups with access to activity wheels in the home cage after drug exposure responded in a nearly identical fashion in the self-administration procedure, suggesting that exercise prior to initial drug exposure may be irrelevant for measures of cocaine reinforcement. Interestingly, a recent study using the conditioned place preference (CPP) procedure, an assay in which a distinctive environment is paired with an interoceptive drug cue, reported similar findings (Mustroph, Stobaugh, Miller, DeYoung, & Rhodes, 2011). In that study, male mice were housed under sedentary or exercising conditions for 30 days either before or after the establishment of a cocaine-induced CPP. Exercise accelerated the extinction of cocaine-induced CPP (i.e., it decreased drug-seeking behavior) when exercise occurred entirely after initial drug exposure, but delayed extinction (i.e., it increased drug-seeking behavior) when it occurred before initial drug exposure. Collectively, these findings suggest that physical activity should occur after initial drug exposure if it is to offer protection on measures of drug-seeking behavior.

Another aim of the current study was to determine whether the effects of exercise extend to other behaviors influenced by cocaine. To this end, the effects of exercise on cocaine-induced locomotor activity were examined in all rats after completing the tests of cocaine- and food-maintained responding. The effects of exercise on cocaine-induced locomotor activity were very similar to the effects of exercise on cocaine self-administration. Specifically, both groups of rats with access to activity wheels after initial drug exposure were significantly less sensitive to cocaine than the continuously sedentary rats. As expected, cocaine-induced locomotor activity was markedly greater in cocaine-maintained rats than food-maintained rats (compare Figures 4 and 7), indicating that a history of repeated cocaine self-administration was sufficient to produce sensitization to its locomotor effects. Although we did not find evidence that exercise reduced sensitization *per se*, it is notable that exercise was similarly effective at reducing cocaine-induced locomotor activity in both sensitized and nonsensitized rats. One caveat about the effects of exercise on cocaine-induced locomotor activity is that baseline locomotor activity counts (as determined after saline administration) were also reduced in those rats with access to activity wheels after initial drug exposure. Thus, the effects of exercise on cocaine-induced locomotor activity may have been a consequence of its ability to reduce baseline rates of locomotor activity.

A final aim of the current investigation was to determine whether the ability of exercise to reduce cocaine-maintained responding is specific to the cocaine stimulus or whether it reflects a more global effect that generalizes to all forms of positively reinforced behavior. To this end, a parallel study was conducted in food-maintained rats using parameters as close as possible to those used in the cocaine self-administration tests. Tests were conducted under conditions of both restricted and unrestricted food access, and tests were conducted with grain (i.e., a stimulus similar to the normal food ration), sucrose (i.e., a stimulus similar to cocaine in that it was novel, preferred, and only available during the experimental sessions), and an audiovisual stimulus that was previously paired with food (i.e., tone on, light off, pellet dispenser on). Under food-restricted conditions, exercise influenced food-maintained responding, but in a manner very different from that observed for cocaine-maintained responding. Responding was greatest in rats with continuous access to activity wheels, which was opposite of that observed in the cocaine self-administration tests. This effect is consistent with a previous study reporting greater food-maintained responding in rats with access to activity wheels (Belke, 2006, but see McMaster & Carney, 1985). Under

free feeding conditions, no differences were observed across the four groups of rats. In fact, responding was very similar for all groups for all three stimuli, including the audiovisual stimulus associated with food delivery. This is also in contrast to that observed for cocaine-maintained responding, where exercise reduced responding maintained by both cocaine and a stimulus associated with cocaine. Such findings indicate that exercise selectively decreases responding maintained by cocaine and does not extend to all forms of positively reinforced behavior.

Females are often preferred in studies of aerobic exercise because they run significantly more than males when given free access to activity wheels (Boakes, Mills, & Single, 1999; Eikelboom & Mills, 1988; Smith et al., 2011; 2012). Only a few studies have specifically examined sex differences in the effects of exercise on measures of drug-seeking behavior, and mixed results have been reported. For instance, an early study reported that concurrent access to activity wheels decreased cocaine self-administration in females but not males (Cosgrove et al., 2002), but several recent studies reported that the effects of exercise did not differ between males and females when activity wheels were available in the home cage (Smith et al., 2011; 2012). Because only females were examined in the present study, we can't state definitively whether similar effects would be observed with males. It is also important to note that we did not monitor or manipulate the estrous cycle. Both cocaine-maintained breakpoints (Roberts, Bennett, & Vickers, 1989) and wheel running (Kent, Hurd, & Satinoff, 1991; Steiner, Katz, & Carroll, 1982) reach their highest levels during estrous. The gonadal hormone estrogen increases both cocaine self-administration and physical activity (Hu & Becker, 2008; Ribeiro, Pfaff, & Devidze, 2009), and it is reasonable to ask whether hormonal fluctuations during the estrous cycle impacted the present results. We believe this is unlikely because the experimental design and data analysis were selected, in part, to reduce the impact of variability caused by the estrous cycle. Breakpoints maintained by each dose of cocaine and each food stimulus were determined on at least two (and in most cases three) occasions, and all data used in the statistical analysis reflected the mean of these observations. Furthermore, all wheel running data were averaged over 5 to 9 days, thus capturing every day of the 4–5 day estrous cycle. Finally, each cohort tested had equal numbers of rats in each of the four groups, and each cohort arrived on the same day at the same age. Given that female rats housed together in the same colony have a tendency to cycle together (McClintock, 1978), hormonal fluctuations due to the estrous cycle were likely “matched” across the four groups.

Long-term aerobic exercise produces functional changes in a number of neurotransmitter and second-messenger molecules that are linked to drug-seeking behavior. The effects of exercise on dopamine and dopamine binding proteins are perhaps most important for cocaine, given the critical role this neurotransmitter plays in cocaine's locomotor and reinforcing effects (see reviews by Tzschentke, 2001; Wise, 1987). Acute bouts of exercise increase central concentrations of dopamine (Hattori, Naoi, & Nishino, 1994; Meeusen et al., 1997; Petzinger et al., 2007), and these effects are positively correlated with exercise output (Freed & Yamamoto, 1985). Animals selectively bred for high rates of wheel running have higher basal and exercise-induced concentrations of dopamine and dopamine metabolites, and lower dopamine turnover in the striatum as compared to controls (Mathes et al., 2010). Exercise also normalizes dopamine signaling in the striatum of animals that have abnormally low levels of dopamine by facilitating calcium/calmodulin-dependent dopamine synthesis (Sutoo & Akiyama, 2003). Chronic, long-term exercise downregulates dopamine D2 receptor mRNA in the nucleus accumbens (Greenwood et al., 2011) and reduces dopamine transporter binding in the striatum (Fisher et al., 2004). Similarly, transcripts encoding both D1 and D2 receptor genes in the striatum are downregulated in mice bred for high levels of running as compared to controls (Mathes et al., 2010). Chronic exercise also blocks 3,4-methylenedioxymethamphetamine-induced dopamine release in the

nucleus accumbens (Chen et al., 2008) and amphetamine-induced dopamine release in the striatum (Marques et al., 2008). Such reductions in cocaine-induced increases in dopamine would account for the ability of exercise to reduce cocaine's locomotor and reinforcing effects in the present study.

The present findings support clinical studies reporting that physical activity is associated with positive outcomes in substance abuse treatment programs (Buchowski et al., 2011; Roessler, 2010; Weinstock et al., 2008). Importantly, the present data suggest that exercise is effective at reducing drug-seeking behavior in populations without a history of physical activity, and that the protective effects seen in such populations are equivalent to that seen in populations that have exercised continuously since early adolescence. Such findings are particularly relevant considering that 55% of U.S. adults never engage in vigorous activity (Pleis, Ward, & Lucas, 2010). It is also notable that we did not see any significant correlations between exercise output and cocaine-maintained responding. In other words, rats that ran the least were just as likely to receive a beneficial effect of exercise as those that ran the most. This is important because the affective states produce by exercise vary with exercise intensity, with greater negative affective states associated with greater degrees of exercise output (Ekkekakis, Hall, & Petruzzello, 2008; Hall, Ekkekakis, & Petruzzello, 2002; Lind, Ekkekakis, & Vazou, 2008). Because such negative affective states are associated with lower levels of exercise compliance (Ekkekakis, Parfitt, & Petruzzello, 2011), low levels of exercise may lead to greater compliance while maintaining full efficacy to decrease compulsive patterns of drug use. Finally, the present findings suggest that the effects of exercise are selective for drug-seeking behavior. Continuous exercise increased responding maintained by food when the rats were food restricted, but under free feeding conditions (conditions that are more analogous to human populations) exercise did not alter responding maintained by a familiar food, a novel and preferred food, and a conditioned stimulus associated with food. In other words, exercise did not exhibit any evidence of behavioral toxicity, and thus should have a good side-effect profile in clinical populations.

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Figure 1.

Schematic diagram showing timeline of major events for the four groups of rats. In weeks 1–4, all groups of rats were left undisturbed in the home cage for acclimation and maturation. In week 5, all groups were trained to lever press using food reinforcement. In week 6, rats used in the cocaine self-administration experiment were implanted with intravenous catheters; rats used in the food-maintained responding experiment were simply given a rest period (minimum 3 days). After the conclusion of the cocaine- and food-maintained responding experiments, locomotor activity tests (LM) were conducted in all rats over four consecutive days. Sedentary-sedentary (SED-SED) rats remained in sedentary rats for the duration of the study; sedentary-exercise (SED-EXE) rats were housed in sedentary cages for the first 6 weeks of the study and transferred to exercise cages for the remainder of the study; exercise-sedentary (EXE-SED) rats were housed in exercise cages for the first 6 weeks of the study and transferred to sedentary cages for the remainder of the study; exercise-exercise (EXE-EXE) rats were housed in exercise cages for the duration of the study.

Figure 2.

Top Panel: Wheel running in cocaine-maintained rats. Left axis depicts exercise output expressed as the mean number of wheel revolutions per day (rev/day); right axis depicts exercise output expressed as the mean number of kilometers per day (km/day). Bottom panel: Body weights in cocaine-maintained rats. Vertical axis depicts body weight expressed in grams (g). For both panels, horizontal axis depicts time expressed in “weeks” of 5- to 9-day intervals. Reference line after week 6 (vertical broken line extending from abscissa) indicates the beginning of behavioral testing. Vertical lines surrounding data points represent the SE; where not indicated, the SE fell within the data point. *Note:* Rats were food restricted for a portion of Week 5.

Figure 3.

Left Panel: Breakpoints maintained by cocaine on a PR schedule of reinforcement. Vertical axis depicts breakpoints expressed as number of infusions obtained; horizontal axis depicts dose of cocaine in mg/kg/infusion. Points above 0.0 depict the effects of saline. Right Panel: Area under the curve (AUC) estimates for cocaine in rats responding on a PR schedule of reinforcement. Significant differences between groups are denoted by horizontal lines. For both panels, vertical lines surrounding data points represent the SE; where not indicated, the SE fell within the data point.

Figure 4.

Locomotor activity in cocaine-maintained rats. Vertical axis depicts distance traveled expressed in cm ($\times 1000$); horizontal axis depicts dose of cocaine in mg/kg/infusion. Points above 0.0 depict the effects of saline. Vertical lines surrounding data points represent the SE; where not indicated, the SE fell within the data point.

Figure 5.

Top Panel: Wheel running in food-maintained rats. Bottom panel: Body weights in cocaine-maintained rats. See Figure 2 for details. *Note:* Rats were food restricted for a portion of Week 5 and for all of Week 7.

Figure 6.

Breakpoints maintained by grain, sucrose, and an AV stimulus on a PR schedule of reinforcement under food-restricted conditions (top panel) and under free feeding conditions (bottom panel). Vertical axis depicts breakpoints expressed as number of reinforcers obtained. Significant differences between groups are denoted by horizontal lines. Vertical lines represent the SE.

Figure 7.
Locomotor activity in food-maintained rats. See Figure 4 for details.