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Research report

Parameters for abolishing conditioned place preference for cocaine from running and environmental enrichment in male C57BL/6J mice

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HIGHLIGHTS

- One week of running after conditioning was sufficient to reduce CPP.
- Running did not need to be contiguous to conditioning or testing to reduce CPP.
- Results suggest both exercise and enrichment likely contribute to reduced CPP.

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ABSTRACT

Rationale: Evidence suggests that 4 weeks of voluntary wheel running abolishes conditioned place preference (CPP) for cocaine in male C57BL/6J mice.
Objectives: To determine the duration and timing of exposure to running wheels necessary to reduce CPP, and the extent to which the running per se influences CPP as compared to environmental enrichment without running.
Methods: A total of 239 males were conditioned for 4 days twice daily with cocaine (10 mg/kg) and then split into 7 intervention groups prior to 4 days of CPP testing. Experiment 1 consisted of two groups housed as follows: short sedentary group (SS; $n = 20$) in normal cages for 1 week; the short running group (SR; $n = 20$) with running wheels for 1 week. Experiment 2 consisted of five groups housed as follows; short 1 week of running followed by a 3 week sedentary period (SRS; $n = 20$); a 3 week sedentary period followed by 1 week of running (SSR; $n = 20$); long sedentary group (LS; $n = 66$) in normal cages for 4 weeks; long running group (LR; $n = 66$) with running wheels for 4 weeks; and long environmental enrichment group (EE; $n = 27$) with toys for 4 weeks.
Results: Levels of running were similar in all running groups. Both running and environmental enrichment reduced CPP relative to sedentary groups.
Conclusions: Results suggest that the abolishment of cocaine CPP from running is robust and occurs with as low as 1 week of intervention but may be related to enrichment component of running rather than physical activity.

1. Introduction

Relapse is a prominent feature of drug addiction and a major obstacle to recovery [1]. Contextual cues associated with drug use (e.g., drug paraphernalia, places where drugs are taken, or people they are taken with) can serve as powerful triggers for relapse even after long periods of abstinence [2,3]. Therefore, finding interventions that weaken drug-to-context associations is critical for effective addiction treatment. Evidence from some drug addiction rehabilitation programs suggests that incorporating aerobic exercise into the life routine during abstinence can greatly improve long-term substance use outcomes [4–8]. In one study, a 12-week group exercise intervention decreased propensity for relapse compared to individuals who did not attend a majority of exercise sessions [4].

The conditioned place preference (CPP) paradigm models drug-context associations in mice [9–11]. Consistent with the human data which shows positive outcomes on substance from exercise interventions [4–8], running after conditioning reduces CPP in male C57BL/6J mice [12,13]. When mice were conditioned with cocaine and then housed for 30 days either in standard cages that allowed no exercise beyond normal cage ambulation or with a

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running wheel before CPP testing, runner mice exhibited significantly reduced CPP compared to sedentary control mice [12,13]. A considerable literature demonstrates that running is a powerful behavioral intervention for CPP in rodents [12–17]. In most, but not all of these studies [17], rodents were housed in cages with running wheels for several weeks [12–16]. It is currently unclear for how long running has to occur to weaken CPP. Therefore, the primary objective of this study was to determine the duration and timing of exposure to running hweels necessary to reduce CPP. To this end, we included a running treatment that lasted only 1 week between conditioning and testing. Because we anticipated that 1 week separation between the conditioning and testing would produce stronger preference than 1 month separation, we also included a group that experienced only 1 week of running but with 1 month still separating conditioning and testing. This was implemented in two patterns, having the 1 week of running immediately after the training or 1 week of running immediately before testing, with sedentary periods in the interim. These groups are described in more detail in the experimental design section of the methods.

Another purpose of the study was to examine the possibility that running reduces CPP via the sensory enrichment component of a running wheel, as opposed to the physical activity per se. The addition of a running wheel to a cage adds substantial sensory enrichment [18]. In all the studies of which we are aware that investigated the effect of environmental enrichment on CPP, the enrichment paradigm included running wheels [19–23], making it difficult to determine whether the reduction in CPP seen from environmental enrichment is due to running or enrichment per se. Sometimes a wheel that was prevented from rotating is used as a control for the non-aerobic, sensory component of a running wheel in behavioral studies [5,24–27]. However, the degree of sensory stimulation from running may not be well-matched to (i.e. may exceed) a stationary running wheel. A better control than a locked wheel might be a complex environment that provides more of a sensory experience than a stationary wheel but that does not allow physical exercise. To the best of our knowledge, no study has examined the effectiveness of environmental enrichment alone, i.e. devoid of running wheels, at accelerating extinction of CPP for cocaine, so no direct comparison of the effectiveness of running versus environmental enrichment on extinction of CPP has been attempted. Therefore, we aimed to determine the extent to which running per se influences CPP as compared to environmental enrichment without running by including an experimental group that only received environmental enrichment without running wheels.

To recapitulate, the primary objective of this study was to determine the duration and timing of exposure to running wheels necessary to reduce CPP, and the extent to which the running per se influences CPP as compared to environmental enrichment without running. Because certain physiological outcomes of exercise are relatively immediate [28–31], we hypothesized that all durations of running, including just one week of running, would be sufficient to reduce CPP for cocaine relative to sedentary mice. Because exercise impacts the brain in ways that environmental enrichment does not [32–39], we predicted that environmental enrichment alone would not be sufficient to reduce cocaine CPP.

2. Materials and methods

2.1. Animals

199 male C57BL/6J mice were obtained at 5 weeks of age and 40 male C57BL/6J mice at 8 weeks of age (The Jackson Laboratory, Bar Harbor, ME). Mice obtained at 5 weeks of age were housed 4 per cage in a climate-controlled environment on a 12 h light/dark cycle (lights off at 9:00 a.m.) for 1 week, and mice obtained at 8 weeks of age were housed 4 per cage in a climate-controlled environment on a 12 h light/dark cycle (lights off at 9:00 a.m.) for 4 weeks. Dimensions of cages without running wheels were 29 × 19 × 13 cm (L × W × H) (Harlan Tekland, Madison, WI). Mice were individually housed for 1 week before starting the experimental procedures and remained singly housed throughout the experiment. Single housing was needed to measure wheel running accurately for each animal. Note that animals in the enrichment group and control goups were also singly housed to keep this variable controlled. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee and adhered to NIH guidelines. All measures were taken to minimize the number of mice used as well as the pain and suffering of the animals.

2.2. Experimental design

A total of 239 males were conditioned for 4 days twice daily with cocaine (10 mg/kg) and were then split into 7 different intervention groups prior to 4 consecutive days of CPP testing (Fig. 1).

2.2.1. Experiment 1: short term: one week between conditioning and testing (2 groups)

The short sedentary group (SS; $n = 20$) were housed in normal cages for 1 week. The short running group (SR; $n = 20$) were housed with running wheels for 1 week.

2.2.2. Experiment 2: long term: four weeks between conditioning and testing (5 groups)

The short running group followed by a sedentary period (SRS; $n = 20$) were housed with running wheels for 1 week and then normal cages for 3 weeks. The sedentary group followed by a short running period (SSR; $n = 20$) were housed in normal cages for 3 weeks then 1 week with running wheels. The long sedentary group (LS; $n = 66$) were housed in normal cages for 4 weeks. The long running group (LR; $n = 66$) were housed with running wheels for 4 weeks. The long environmental enrichment group (EE; $n = 27$) were placed in cages with multiple novel toys that were rotated on a weekly basis for 4 weeks.

The reason why sample sizes are approximately three times as large in the LS and LR groups compared to the others, is that the experiment was completed in three batches and each batch included LS and LR alongside other groups. First batch included LR, LS, SS, SR. Second batch included LR, LS, SRS, SSR. Third batch included LR, LS, EE. This was done to facilitate direct comparison of the novel groups with the standard sedentary and runner groups previously demonstrated to display different CPP and a way to ensure results were consistent in all three batches.

Mice were 7 weeks of age when they underwent habituation, pretesting, and cocaine CPP conditioning, except for mice in the short running (SR) and short sedentary (SS) groups, which were 10 weeks of age so that all mice were the same age at testing (see Conditioned place preference section below and Fig. 1). Mice with running wheels in their cage at the start of testing had continuous access to wheels during the days of testing.

2.3. Running wheels and sedentary treatment

Dimensions of running wheel cages were 36 × 20 × 14 cm (L × W × H), with a 23 cm diameter wheel mounted in the cage top. Running wheel rotations were monitored continuously in 1 min increments throughout the experiment via magnetic switches interfaced to a computer. Mice assigned to the sedentary groups were deliberately not housed in cages with locked wheels because

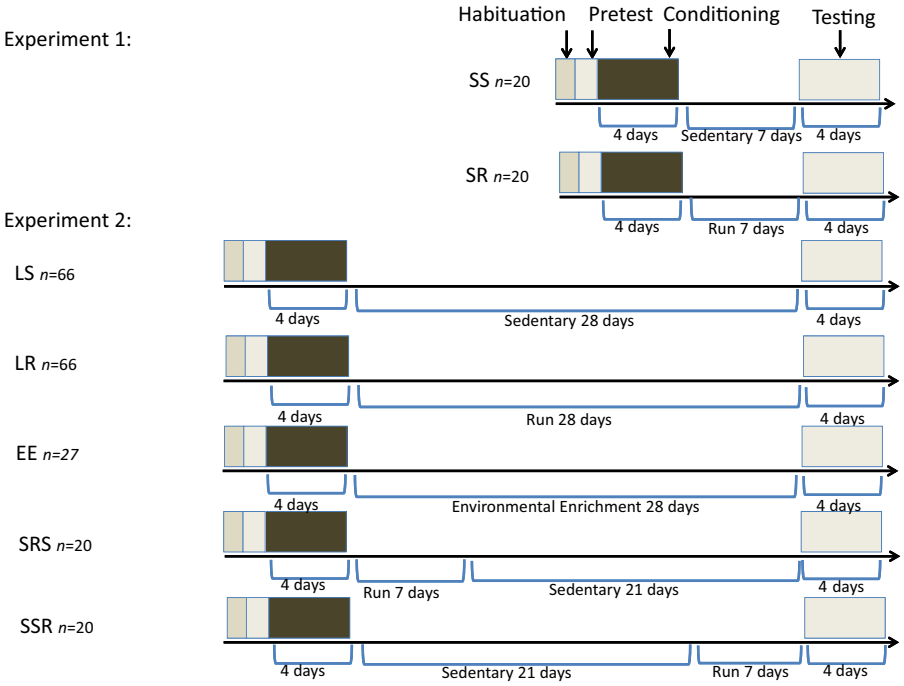

Fig. 1. Schematic diagram of the experimental design. The grey scale boxes indicate when CPP habituation, pretesting, and conditioning sessions were administered, respectively. The open boxes indicate when CPP testing took place. Over the 2 days immediately preceding the conditioning, mice experienced 1 day of habituation to reduce novelty effects and, subsequently, 1 day of CPP pretesting to establish baseline texture preferences. In Experiment 1, mice experienced 7 days of uninterrupted running/sedentary treatment between CPP conditioning and testing. In Experiment 2, mice experienced 28 days of uninterrupted running/sedentary/environmental enrichment treatment between CPP conditioning and testing. The labels on the left indicate the group assignments and sample sizes as follows: Experiment 1: short sedentary group (SS; $n = 20$), short running group (SR; $n = 20$); Experiment 2: long sedentary group (LS; $n = 66$), long running group (LR; $n = 66$), long environmental enrichment group (EE; $n = 27$), short running group followed by a sedentary period (SRS; $n = 20$), sedentary group followed by a short running period (SSR; $n = 20$).

mice climb in locked wheels [40–42] and we intended to keep physical activity to a minimum in the sedentary groups.

2.4. Environmental enrichment

Animals were housed in the same size cage as sedentary animals except that cages contained bedding and toys. As in a previous study from our laboratory [33], certain toys were always present and never rotated. They were 1 plastic igloo, 1 wooden gnaw stick, cotton nesting material, a plastic ball that contained a bell, and a handful of straw. In addition, two of the following toys were rotated into the cage every 4 days in an attempt to engage multiple sensory modalities: auditory (ticking plastic clock, squeeze toy, rattle), visual (mirror, small dome), vestibular (see-saw, smooth winding tunnel), and tactile (foam ball, small plastic hedgehog animal toy, towel piece, smooth tunnel, tunnel lined with bubble wrap, tunnel lined with Velcro material, and a tunnel lined with foam).

2.5. Drugs

Cocaine hydrochloride (Sigma Aldrich, St. Louis, MO) was dissolved in 0.9% saline and was administered at a dose of 10 mg/kg via *i.p.* injections in a volume of 10 ml/kg. Dose was chosen based on the literature and was prepared according to the salt not the base form [12,13,43,44].

2.6. Conditioned place preference

We used the same unbiased procedure as previously published by our lab [12,13,43] based on Cunningham's apparatus and experimental design [45] and as previously detailed in our other work [12,13,43]. Within each treatment group, mice were counterbalanced with respect to the conditioned stimulus (CS + GRID or CS + HOLE) and experienced cocaine on GRID (CS + GRID) or cocaine on HOLE (CS + HOLE) texture and saline on the alternate texture. During testing, animals explored the same size chamber as during conditioning except with the HOLE/GRID floor type. Hence the animals were forced to spend time on either HOLE or GRID side, and duration on HOLE is equivalent to the total duration of the test (30 min) minus duration on GRID. Conditioned place preference was determined by comparing the duration spent on HOLE (or GRID, statistics would be the same) between groups, CS + HOLE versus CS + GRID. CPP is defined as the difference in the mean duration spent on HOLE texture between CS + HOLE and CS + GRID groups [12,13,45]. The design ensures that any difference in duration spent on textures between groups (CS + GRID versus CS + HOLE) is due to drug-to-context learning, as this is the only variable that differs between the two groups. Biases in baseline preference for textures cannot produce false positives with this method because duration spent on one texture (HOLE or GRID) is compared between subgroups CS + GRID and CS + HOLE, both of which would be expected to display the bias if one developed. Hence, when the difference score is computed, any bias is subtracted out. The two groups are also matched for drug exposure, which is important because drug exposure itself could affect the development of biases in preference. Moreover, each group serves as the other group's learning control, because both groups learned to associate one texture with cocaine and the alternate texture with saline. This is important because as compared to using a control in which all animals receive saline on both textures, the experience of learning itself could bias preferences for the textures [12,13,43].

2.6.1. Habituation

To familiarize the mice with the place conditioning chambers, mice were placed on a flat surface without a texture in the conditioning chambers in the morning (1000 h; for 30 min) and in the

afternoon (1600 h; for 30 min) for one day without any injection treatment.

2.6.2. Pretesting

To determine individual biases in preference for the textures prior to drug pairing, mice were weighed, received a 10 ml/kg saline injection, and were immediately placed in the apparatus with HOLE/GRID floor in the morning (1000 h; for 30 min) and afternoon (1600 h; for 30 min). Mice had free access to both compartments.

2.6.3. Conditioning

Four conditioned stimulus (CS +) trials (i.e., cocaine paired with one floor texture: HOLE or GRID) and four CS- trials (i.e. vehicle paired with the alternate floor texture) were administered over four days. The assignment to HOLE or GRID was counterbalanced in each group. Each day, one CS+ trial and one CS- trial was administered in the morning and afternoon. The order of exposure to CS+ and CS- was counterbalanced within each group. Mice were weighed, received an injection of 10 mg/kg cocaine (CS+ trial) or vehicle (CS- trial), and were immediately placed on the appropriate floor texture in the morning (1000 h; for 30 min) and afternoon (1600 h; for 30 min).

2.6.4. Testing

Testing took place daily (morning and afternoon) for 30 min on days 29–32 (LS, LR, EE, SRS, SSR groups) or on days 8–11 (SS, SR groups) after the last conditioning session. Prior to each testing session, each mouse was weighed, injected *i.p.* with 10 ml/kg saline, and placed into the center of the HOLE/GRID conditioning chamber. Mice had free access to both compartments. All testing was conducted by experimenters blinded to the group assignment of the mice.

2.7. Statistical analysis

Data were analyzed using Proc Mixed module in SAS (version 9.3) statistical software. Proc Mixed uses restricted maximum likelihood to estimate parameters, not sums of squares, and hence results are not biased by uneven sample sizes between groups even if the sample sizes are very unequal, as they are in our experiments. In all analyses, $P < 0.05$ was considered statistically significant.

2.7.1. CPP

CPP is measured as the difference in duration spent on one side of the apparatus (HOLE or GRID) between CS + GRID versus CS + HOLE groups [12,13,45]. Note that duration spent on HOLE equals 30-duration spent on GRID, so the statistics are equivalent regardless of which side of the apparatus (HOLE or GRID) is analyzed. Note also that using this method, CPP is a between-subjects measure, not within-subjects. The pretest is conducted not to establish CPP but to confirm the apparatus is unbiased. The method has many advantages, including unbiased design and controlling for any biased preference that might emerge from learning the conditioned association or experiencing cocaine [45]. However, because CPP is measured between subjects, in order to identify a treatment effect on CPP, an interaction between the treatments (e.g., running, enrichment) and CPP (CS + HOLE versus CS + GRID) must be detected. A main effect without the interaction indicates a treatment effect on duration spent on one side of the apparatus, not CPP.

First, baseline duration spent on HOLE was compared between treatment groups (LS, LR, EE, SS, SR, SRS, SSR) using one-way ANOVA to make sure all groups displayed the same baseline preference before starting the experiment. Next, duration spent on the HOLE texture was analyzed by 4-way repeated measures ANOVA with CPP (CS + HOLE versus CS + GRID; between-subjects), intervention (2 levels; one for LR, EE, SR, SRS, SSR, and one for SS and LS, collapsed; between-subjects), interval between conditioning and testing (1 week or 4 weeks; between-subjects), day of testing (1–4; within-subjects), and all interactions entered as factors. Next, data were analyzed separately for 1 week versus 4 week intervals between conditioning and testing. For the 1 week groups, this consisted of 3-way ANOVA with CPP, intervention (runner or sedentary), and day as factors. For the 4 week interval groups, the same analysis was conducted except intervention was first entered as 2 levels (one level for LR, EE, SRS and SSR and one for LS). Finally, to determine whether certain running or EE interventions were relatively better at abolishing CPP than others, intervention groups were analyzed by 3-way ANOVA with CPP, treatment (LR, EE, SRS, SSR), day, and all interactions entered as factors. Testing session, whether at 10:00 h or 16:00 h, was also included as a factor in initial models but was never significant and therefore was removed from the final linear models. Posthoc comparisons of CPP were conducted within groups using unpaired *t*-tests comparing CS + HOLE versus CS + GRID.

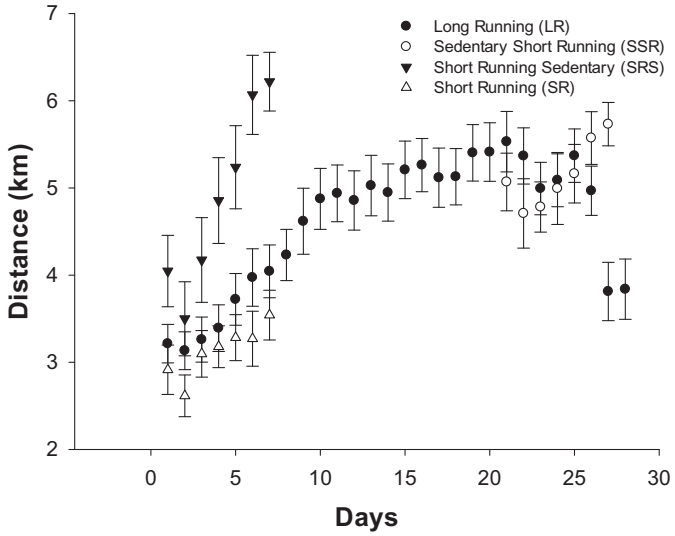

Fig. 2. Wheel running. Distance run (km/day) (±SE) shown separately for mice in the long running group ($n = 66$), short running group ($n = 20$), short running group followed by a sedentary period ($n = 20$), and sedentary group followed by a short running period ($n = 20$) groups. Levels of running were similar across groups. Increased running over the first 18 days is typical for mice.

2.7.2. Wheel running

Average distance traveled per day collapsed across all the days (1–28 for LR, 1–7 for the others) was analyzed by one-way ANOVA with treatment group (LR, SR, SRS, SSR) as the factor.

3. Results

3.1. Wheel running

No significant differences in average distance traveled were observed between any of the running groups. It is typical for mice to increase running levels during the first 2–3 weeks and to thereafter maintain a plateau (Fig. 2). Average distances traveled were 4.6 (±0.270 SE), 3.13 (±0.195 SE), 4.74 (±0.391 SE), and 5.15 km/day (±0.245 SE) for LR, SR, SRS and SSR groups, respectively.

3.2. CPP

3.2.1. Baseline preference

During the pretest, before the mice ever experienced cocaine, and before any of the mice ran on wheels or experienced enrichment, they spent an average of 53% (±0.0085 SE) of their time on the HOLE texture. Baseline duration spent on HOLE (or GRID) did not differ between groups.

3.2.2. Locomotor activity in CPP chambers

During testing, no significant differences in average distance moved per testing session were detected between groups.

3.3. CPP testing

The 4-way ANOVA revealed a significant effect of CPP (conditioned to HOLE versus GRID), indicating animals displayed CPP collapsed across all groups and all days ($F_{1,231} = 85.1$, $P < 0.0001$; Fig. 3). Magnitude of CPP was greater when conditioning and testing were separated by only 1 week (SS and SR) as compared to 4 weeks (LS, LR, EE, SRS, and SSR), as indicated by a significant interaction between interval (1 versus 4 weeks) and CPP ($F_{1,231} = 10.0$, $P = 0.002$). The interventions (LR, SR, EE, SRS, and SSR) significantly decreased CPP relative to the sedentary groups (SS and LS), as indicated by a significant interaction between intervention and CPP ($F_{1,231} = 5.4$, $P = 0.02$). No other interactions or main effects were significant.

3.3.1. Experiment 1: short term: one week between conditioning and testing

Mice that experienced one week between conditioning and testing displayed significant CPP on all 4 days, as indicated by main effect of CPP in the 3-way ANOVA ($F_{1,36} = 54.3 < 0.0001$). However, sedentary mice (SS) displayed slightly greater CPP than runners (SR), as indicated by a marginally non-significant interaction between CPP and intervention ($F_{1,36} = 2.8$, $P = 0.10$) (Fig. 3A). A main effect of day ($F_{3,108} = 3.5$, $P = 0.02$) was also detected. No other main effects or interactions were significant.

3.3.2. Experiment 2: long term: four weeks between conditioning and testing

Mice that experienced four weeks between conditioning and testing displayed significant CPP, but the magnitude of CPP depended on the group. The long sedentary (LS) group displayed greater CPP than the other groups (EE, SRS, SSR, and LR). This was indicated by a significant interaction between texture and intervention in the 3-way ANOVA ($F_{1,195} = 4.3$, $P = 0.04$). Texture ($F_{1,195} = 48.3$, $P < 0.0001$), intervention ($F_{1,195} = 7.4$, $P = 0.007$), and the interaction between day and texture ($F_{3,581} = 4.2$, $P = 0.006$) were also significant. No other interactions were significant, including the three-way interaction between day, intervention, and CPP, which would have indicated differential extinction between the groups. However, CPP extinguished (as evidenced by the significant interaction between day and texture cited above, and non-significant CPP, by unpaired *t*-tests) in all groups as the days progressed except in LS.

All four intervention groups (EE, SRS, SSR, and LR) displayed similar levels of CPP as indicated by non-significant interaction between intervention and CPP when only the four intervention groups were included in the analysis (i.e., no sedentary group included). In this analysis without the sedentary group, main effects of CPP ($F_{1,125} = 13.3$, $P = 0.0004$) and day ($F_{3,374} = 5.2$, $P = 0.002$) were detected, but no other main effects or interactions were significant. While CPP in LS was significant on all 4 days, mice in the enriched (EE) group showed significant CPP only on days 1 and 2. Mice in the short running groups (SRS, SSR) displayed significant CPP only on day 1, and mice in the long running group (LR) only on days 1 and 3 (as indicated by unpaired *t*-tests in Fig. 3B).

4. Discussion

The main finding of this study is that a group of mice with access to running wheels or environmental enrichment displayed significantly reduced CPP relative to sedentary mice (Fig. 3). We offer two possible interpretations of this finding. The first of these interpretations revolves around the fact that the addition of a running wheel to an otherwise barren cage is a form of sensory stimulation. The fact that environmental enrichment and exercise both reduced CPP could be taken to mean that sensory stimulation underlies the CPP-reducing effect of running of this and previous studies [12–17]. Because we did not use locked wheels as controls for running wheels, we cannot distinguish between effects of exercise and sensory enrichment from a wheel *in our running group* [18]. The implication is that reduction in CPP from running and environmental enrichment may not be due to an exercise-specific effect. In line with the theory that CPP reduction from running is not attributable to an exercise-specific effect, a recent study from our lab showed that increased hippocampal neurogenesis from running is not required for running to reduce CPP for cocaine [12]. One of the strongest exercise-specific effects in the brain is increased adult hippocampal neurogenesis [32–35]. A comprehensive meta-analysis of the literature found that new neurons are not required for running to cause behavioral performance improvement on a variety of hippocampus-involved tasks [46]. Our finding that environmental enrichment has the capacity to reduce CPP to levels approaching the reduction seen from running eliminates hippocampal neurogenesis, which is not seen from environmental enrichment to appreciable levels [32,33], as a candidate mechanism by which exercise reduces CPP but leaves a myriad of other mechanisms by which exercise reduces CPP. Among these mechanisms that environmental enrichment and running share are BDNF upregulation, spine morphology, dendritic branching, and synaptogenesis [29,47–55].

A second, alternative interpretation of our finding that both exercise and environmental enrichment reduced CPP is that mice in the enriched environment actively engaged with the toys to a large enough extent that it constituted physical activity. This phenomenon has previously been documented to occur with locked running wheels, as mice climb in locked wheels [40–42]. A prior study from our lab showed that when mice are housed in a cage with one side enriched with toys and the other with a running wheel, mice spent the majority (68%) of their time on the enriched side [33]. Monitoring the activity of mice in the enriched environment in future experiments will reveal whether mice exercised to an appreciable extent. If we find that our enrichment condition does not offer the same opportunities for physical activity as a running wheel, then we would have strong support for the interpretation that environmental enrichment alone and not just incidental exercise in an enriched environment reduces CPP.

Presumably the interventions used here (either exercise or environmental enrichment) reduced CPP proactively by producing some (still unknown) change in the brain that facilitated the formation of a new inhibitory memory during CPP testing and extinction. There are several ways in which the treatments could have exerted such an inhibitory effect on CPP, none of which are mutually exclusive. First, the interventions could have impaired consolidation of the drug-to-context association. However, we do not favor this interpretation because if it were true, we would have expected SRS to display a greater reduction in CPP than SSR, but they displayed similar reductions in CPP. In the SRS group, running was administered immediately after conditioning, whereas in

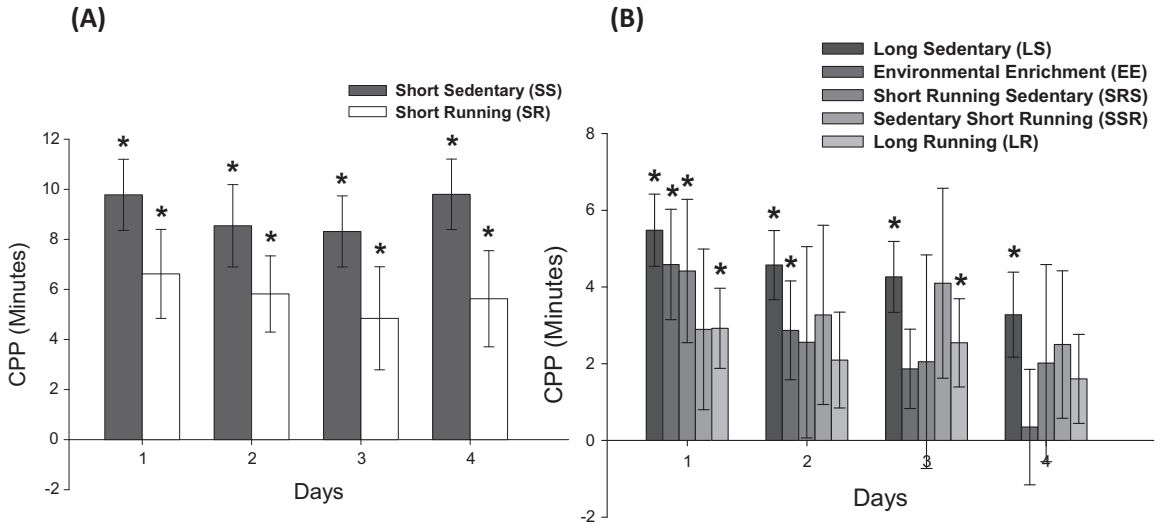

Fig. 3. Conditioned place preference for cocaine. A. Mean difference in duration (min) ± SE spent on the HOLE texture between mice receiving cocaine on HOLE texture (CS + HOLE) and mice receiving cocaine on GRID texture (CS + GRID) plotted separately for mice in the two experimental groups over the 4 days of testing in experiment 1, 1 week interval between conditioning and testing. The standard error of the difference between the two means is equal to the pooled standard error across both groups, assuming equal variance between groups, i.e., the denominator of the unpaired t-statistic. Each bar represents data for the following mice: short sedentary (SS) group $n = 10$ CS + HOLE mice and $n = 10$ CS+ GRID mice, short running (SR) group $n = 10$ CS + HOLE mice and $n = 10$ CS + GRID mice. The stars indicate significant place preference at $P < 0.05$. B. Same as A for experiment 2, 4 week interval between conditioning and testing. Each bar represents data for the following mice: long sedentary (LS) group $n = 33$ CS + HOLE mice and $n = 33$ CS + GRID mice, long running (LR) group $n = 33$ CS + HOLE mice and $n = 33$ CS + GRID mice, long environmental enrichment (EE) group $n = 16$ CS + HOLE mice and $n = 11$ CS + GRID mice, short running (SRS) group followed by a sedentary period $n = 10$ CS + HOLE mice and $n = 10$ CS + GRID mice, sedentary group (SSR) followed by a short running period $n = 10$ CS + HOLE mice and $n = 10$ CS + GRID mice. The stars indicate significant place preference at $P < 0.05$.

SSR, it was administered 3 weeks after conditioning. This is crucial because consolidation of memory occurs within minutes or hours, not weeks after training [56–61]. Therefore, if the interventions reduced CPP by interfering with consolidation of the drug-context association, we would have expected mice in the SSR group to show normal CPP, but they showed similar reduction of CPP as mice in the SRS group. An alternative possibility is that the interventions impaired reconsolidation of the memory during testing. If this is true, our data suggest it does not matter when the intervention is administered; when implemented immediately after training or before testing, or when continuously administered, the effect on reconsolidation is the same. The data are consistent with this hypothesis. A third possibility is that the interventions do not affect consolidation, reconsolidation, learning, or memory of the drug-context associations *per se* but instead affect the perception of the unconditioned reward value of the drug [12]. This interpretation posits that the intervention decreases the perception of the reward value of the drug. In other words, lower CPP was displayed, not because the animals forgot about the drug-context association or learned that the context was no longer associated with subjective effects of the drug, but rather because they attributed less value to the drug after the intervention, and hence displayed less preference for the drug-paired side. However, we do not favor this hypothesis because if it were true, we would have expected CPP to have been stronger for SSR than SRS because of the closer proximity of the intervention to the CPP testing, which was not what was observed. Future studies are needed to determine which of many different interpretations explain why CPP is reduced from exercise or enrichment.

Another important consideration is the generality of the effect of the running/enrichment interventions on reducing CPP. We believe exercise/enrichment interventions will not only reduce CPP to drug rewards, but would also reduce CPP for other positive and negative reinforcers. There is evidence that running/enrichment when administered between conditioning and testing reduce contextual fear conditioning [62]. We favor the hypothesis that exercise/enrichment aided in the acquisition of the new drug-context association presented during testing. Evidence from a prior study that fits with a large literature on the pro-cognitive effects of exercise [33,36–39] supports the theory that exercise reduces CPP by helping runner animals to acquire the new association, first presented during testing, that the floor texture previously associated with cocaine is no longer paired with the drug [13]. The large literature on the pro-cognitive effects of exercise [33,36–39] also suggests that this effect of exercise is not unique to cocaine-related associations or to extinction learning, and that any subsequent associative learning is enhanced. The suggestion that exercise or enrichment reduce cocaine-induced CPP by aiding in the acquisition of extinction learning supports consideration of such interventions during treatment for drug addiction, but it also raises the possibility that individuals who exercise regularly before their initial drug exposure might develop stronger drug-context associations (compared to sedentary individuals), possibly increasing their initial risk for developing drug addiction. Indeed, data from our prior study demonstrated that mice that exercised before conditioning showed enhanced CPP relative to mice that had not exercised prior to conditioning, whereas mice that exercised after conditioning but before testing showed weakened CPP relative to mice that had not exercised, which suggests that the timing of exercise implementation is critical [13]. As long as exercise is implemented after drug exposure, it appears to weaken CPP.

The findings of our study may be relevant for designing interventions with appropriate durations of exercise for drug addiction. One week of running was sufficient to reduce CPP, and it did not matter whether the week of running was preceded or followed by a three week sedentary period (Fig. 3B). Results suggest the abolishment of cocaine CPP from running is robust and occurs with as low as one week of intervention and that both increased physical activity and enrichment likely contribute to the phenomenon. The fact that one week of running was sufficient to reduce CPP means that even short bouts of exercise are therapeutically helpful and weaken drug-to-context associations. When the last drug-context pairing occurred four weeks in the past, exercise also reduced CPP (Fig. 3B). Our data add to a growing body of evidence suggesting that exercise

protects against cue-induced reinstatement of drug seeking even after cessation of drug use has been achieved [18]. Although the variations in delay and duration of exercise studied here appeared to have little impact, the effect of exercise presumably depends on some minimum duration and will eventually dissipate over time. The longevity of the exercise effect across time and the importance of the exercise duration warrant further exploration.

In addition, we demonstrated that environmental enrichment was as effective as exercise at reducing CPP (Fig. 3B). While exercise appears to extinguish the salience of drug-paired cues in people [4–8], it will not be a viable option for everyone. Individuals in poor physical shape or with low motivation to exercise will not be good candidates for an intervention centering around exercise. Environmental enrichment might be a useful alternative intervention to exercise because compliance to exercise is notoriously low [4]. Compliance to an environmental enrichment intervention may be much higher. In addition, an environmental enrichment intervention can be tailored to an individual's preference. For instance, if an individual enjoys socializing, an intervention could heavily employ this form of environmental enrichment in the intervention.

The parameters for abolishing CPP from environmental enrichment and exercise need to be further worked out. A study recently gave rats access to running wheels for three weeks before cocaine reinstatement testing. They then either removed running wheels for 24 h before cocaine reinstatement testing or left the animals with access to wheels up to reinstatement testing [18]. Removing the running wheels for 24 h before cocaine reinstatement had no effect when compared to the reinstatement of rats that had run for three weeks without having the running wheels removed before reinstatement testing [18]. This suggests that chronic not acute effects of exercise likely mediate CPP abolishment from exercise. Chronic effects of exercise include gross structural or chemical changes such as increased hippocampal volume [63–66]. Further characterizing the time course of exercise and environmental enrichment effects on CPP will reveal more about the mechanism by which exercise and environmental enrichment reduce CPP for cocaine. The minimum duration of running and environmental enrichment required to reduce CPP remains to be determined. If the minimum duration required to reduce CPP parallels the minimum duration required for one of these gross structural or chemical changes in the brain to occur, this would narrow down which gross structural or chemical change in the brain from chronic exercise mediates the abolishment of CPP. Another avenue for future research would be to determine the intensity of running necessary to reduce CPP, so that specific recommendations about optimal exercise intensity can be made to recovering individuals. Insights gained into which parameters constitute effective interventions for drug-to-context associations will not only refine our understanding of the mechanisms mediating their behavioral effects, but this knowledge will be helpful to individuals trying to achieve abstinence from drug use.

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