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Conditioned place preference for cocaine and methylphenidate in female mice from lines selectively bred for high voluntary wheel-running behavior

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Abstract

Behavioral addictions can come in many forms, including overeating, gambling and overexercising. All addictions share a common mechanism involving activation of the natural reward circuit and reinforcement learning, but the extent to which motivation for natural and drug rewards share similar neurogenetic mechanisms remains unknown. A unique mouse genetic model in which four replicate lines of female mice were selectively bred (>76 generations) for high voluntary wheel running (High Runner or HR lines) alongside four non-selected control (C) lines were used to test the hypothesis that high motivation for exercise is associated with greater reward for cocaine (20 mg/kg) and methylphenidate (10 mg/kg) using the conditioned place preference (CPP) test. HR mice run ~three times as many revolutions/day as C mice, but the extent to which they have increased motivation for other rewards is unknown. Both HR and C mice displayed significant CPP for cocaine and methylphenidate, but with no statistical difference between linetypes for either drug. Taken together, results suggest that selective breeding for increased voluntary running has modified the reward circuit in the brain in a way that increases motivation for running without affecting cocaine or methylphenidate reward.

KEYWORDS

addiction, artificial selection, behavior, cocaine, exercise physiology, experimental evolution, physical activity, reward, Ritalin, voluntary exercise, wheel running

1 | INTRODUCTION

Predisposition for addiction to drugs and other natural rewards has a strong genetic component, and a great deal of effort has been devoted to trying to find the genes, molecular mechanisms and specific neurological circuitry involved.[1-6] One of the central hypotheses in the recent literature is that genes that increase motivation for one reward also increase motivation for other rewards, that is, the neurobiological pathways are not reward-specific, but rather generalize across rewards of varying types. This hypothesis is related to the concept of the "addictive personality," which posits that the particular reward is less important than the general tendency to become addicted to whatever reward is available. Support for this hypothesis can be found in human twin studies where genetic risk for addiction

to one drug is strongly correlated with risk for addiction to other drugs.[7] Additional support comes from studies comparing different inbred strains of mice for behavioral responses to various drugs and natural reinforcers; these studies show strong genetic correlations.[8,9] The hypothesis that propensity for addiction generalizes across rewards is also supported by the idea that there is only one reward circuit in the brain, and both drugs of abuse and natural reinforces, such as wheel running and feeding, activate the same circuit, which results in similar neuroadaptations.[10]

On the other hand, individuals vary in their emotional reactions to different types of rewards. For example, some people like the feeling of being high on cocaine or methylphenidate (Ritalin), while others do not.[11] Likewise, some people and animals derive pleasure from running,[1,12-16] whereas others do not. In fact, exercise is even proposed to have addictive properties, as humans and rodents have shown signs of "withdrawal," including anxiety and depression, after being denied exercise.[1,17-22] The generality of neurogenetic predisposition for addiction across multiple types of drugs has been established for humans,[7] and for nonhuman animals,[8,23,24] and an extensive literature relates motivational circuits involved in drug and food rewards.[9,10] However, to the best of our knowledge, no studies have evaluated the extent to which genetic predisposition for exercise reward is associated with increased drug reward. Hence, the main purpose of the present study was to use a novel mouse model to test the hypothesis that selective breeding for a genetic predisposition for exercise results in a correlated response with respect to cocaine and methylphenidate rewards.

Over the past 26 years, we have maintained four replicate High Runner (HR) lines that have now been selectively bred for >90 generations for voluntary exercise on wheels, as compared with four nonselected Control (C) lines. Given a wheel attached to their home cage, HR mice run ~3x as many revolutions per day as C mice.[25,26] This type of artificial selection experiment offers a way to reliably alter phenotypes and provide results more consistent with the polygenic nature of complex traits (e.g., physical activity levels) than transgenic approaches targeting a single or a few genes.[27,28] Further, the 4-fold replication of both the HR and C lines reduces the chance of the experimental results being a consequence of random mutation and/or genetic drift.[29] Human ethnic and racial diversity in physical activity is also modeled to some extent by the use of multiple lines.[30,31]

The wheel-running literature suggests that exercise is a motivated or rewarding behavior for rodents and other animals, even in the wild.[15,32,33] Voluntary exercise by rodents on wheels may also serve as a preclinical model of human voluntary exercise.[14,34-37] In addition to changes in the physical ability for wheel running, several lines of evidence suggest that the HR mice have increased motivation to run on wheels compared with C mice.[14,38-42] Evidence that the reward circuit has been altered in HR mice relative to C mice includes differential sensitivity to the locomotor-activating effects of dopamine reuptake transporter blockers, cocaine, methylphenidate,[38,39,43] endocannabinoid agonist WIN 55,212–2 and antagonist rimonabant,[44,45] linetype differences in circulating endocannabinoid levels,[46] and notably, differential activation of the reward circuit during withdrawal from wheels.[40,47] However, changes in dopamine signaling and activation of the reward circuit are shared across all forms of motivation and reward. For the HR mice, the extent to which the reward circuit has been altered in such a way as to specifically increase motivation for running and not motivation for other potentially rewarding stimuli remains unknown.

A widely used method to measure the rewarding (i.e. the attractive and motivational) value of a stimulus in animals is the conditioned place preference test (CPP). For reviews of the method, see References.[48-52] In brief, CPP is a form of classical conditioning that involves an animal receiving repeated access to an appetitive (or aversive) stimulus in a particular context.[48,53] Within the same experiment, animals are exposed to a second context but without the stimulus of interest. Following repeated conditioning trials, a choice test is administered in which animals receive unrestricted access to both contexts in the absence of the stimulus. An increase in time spent in the paired context relative to a control value is taken as evidence that the stimulus under investigation was rewarding.[49,53,54]

The goal of the present study was to determine whether HR mice display greater CPP than C mice to cocaine (experiment 1) and methylphenidate (experiment 2). Based on the hypothesis that selection for voluntary wheel running generally increased motivation for reward, we hypothesized that HR mice would display greater CPP for both cocaine and methylphenidate.

2 | MATERIALS AND METHODS

All procedures were approved by the University of California, Riverside, Institutional Animal Care and Use Committee, which follows the National Research Council Guide for the Care and Use of Laboratory Animals (revised 2011).

2.1 | Experimental animals

We used 64 adult female mice for each of three experiments (n = 8 per each of 8 mouse lines in each experiment; each mouse was from a different family). Females were used because they generally run more than males.[25,26,55,56] Mice were from generations 77 and 82 of an ongoing, replicated, selective breeding experiment for high voluntary wheel-running behavior, as previously described.[25,26] The original progenitors were 224 outbred, genetically variable laboratory house mice (*Mus domesticus*) of the Hsd:ICR strain. After two generations of random mating, mice were randomly paired and assigned to eight closed lines (10 pairs in each), with four replicated high-runner (HR) lines and four replicated control (C) lines. Beginning at ~6 weeks of age, each generation of mice are housed individually with access to a running wheel for 6 days. In the HR lines, the highest-running male and female from each family are selected as breeders based on the total number of revolutions run on days 5 and 6 of the 6-day test. In C lines, breeders are chosen without regard to wheel running. Within each line, the chosen breeders are randomly paired, avoiding sibling pairings.

2.2 | General methods

The overall experimental timeline is shown in Figure 1. Mice were weaned at postnatal day 21 and housed four per cage, separated by sex and line, in standard mouse cages ($27 \times 17 \times 12.5$ cm^3) with ad libitum food and water. Two weeks prior to experimental procedures (~10 weeks of age), mice were transferred to a reverse photoperiod. Rooms were controlled for temperature (21 ± 1°C) and photoperiod (12:12 L:D) with lights on at 20:00 hours and off at 08:00 hours Pacific Standard Time. Red incandescent lamps were utilized so that investigators could handle mice during the dark phase.[57] After a week of acclimation to the reversed photoperiod, mice were switched to individual housing in standard home cages. At ~12 weeks of age, all mice underwent a 5-day preconditioning phase (as in Reference 57) followed by conditioning to a particular texture (conditioned stimulus, or CS) with cocaine (20 mg/kg; experiment 1; generation 77) or methylphenidate (10 mg/kg; experiment 2; generation 82) as the unconditioned stimuli (US). None of the mice had any access to wheels prior to or during these experiments.

We used the CPP method established by Christopher Cunningham's group for laboratory mice.[48] The method uses a single chamber, with two different floor textures, referred to hereafter as GRID and HOLE to serve as the conditioned stimuli. No visual or olfactory cues were used. Mice are trained and tested during the dark phase of the light–dark cycle and in a dark room, as mice are nocturnal and more comfortable behaving in the dark. On the test day, the subjects are placed in the same size arena but with half the floor HOLE and half GRID texture. CPP is established by comparing percent time on the HOLE side (or GRID side, the math is equivalent since they sum to 1) between the HOLE-paired and GRID-paired mice. Exactly half the mice are conditioned to HOLE and half to GRID. Hence, it is a balanced, between-subjects design. Importantly, HOLE-paired and GRID-paired mice are treated identically with the only exception being which texture (HOLE or GRID) they received cocaine or saline on. Hence, whatever bias might occur at the time of testing, it cannot confound interpretation of CPP since both groups would experience that same bias. Although not necessary for interpretation of CPP, in the current study we also administered a pretest before conditioning, so that we could refine the CPP measure by adjusting for individual differences in pre-test preferences. However, it is important to note that this does not correct for possible biases that develop after the pre-test, which again illustrates the advantage of the between-subjects method in which possible biases that develop after the pre-test do not confound interpretation of CPP.

The place conditioning chambers are black acrylic boxes ($33 \times 18 \times 16$ cm^3) with removable clear plastic tops to allow videotaping from above, following the general protocol of Zombeck et al,[57] who also studied outbred Hsd:ICR mice. The floors are interchangeable and consist of three types of distinct textures: stainless steel sheets with 6.4 mm round holes (HOLE), grids composed of parallel stainless steel rods mounted 6.4 mm apart (GRID), and combination half-hole/half-grid (HOLE/GRID) floors (Supplemental Figure S1). The CPP apparatuses were cleaned with warm, soapy water and dried between every usage. To absorb urine and collect feces, clean sheets of disposable paper or washable mats were placed underneath the chambers during each new test. Video recording was accomplished with overhead Logitech HD C525 Webcams, 1. m above the ground, with 720p resolution at 30 frames per second, and later analyzed with automated software (see below).

2.3 | CPP experiment 1 with cocaine reward

2.3.1 | Preconditioning phase

We first determined individual preferences for the floor textures before giving the mice any reward. Body mass was recorded just prior to the first preconditioning exposure. On preconditioning days, animals were individually placed in the combination HOLE/GRID chambers for 30 minutes twice daily. The first trial of the day took place at

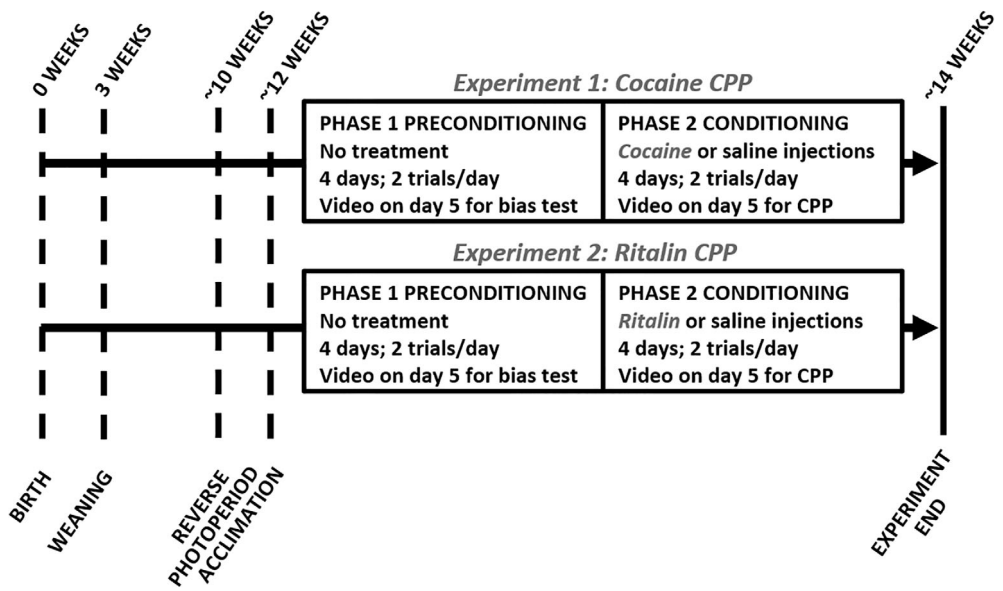

FIGURE 1 Simplified experimental timeline. Each experiment used 64 mice sampled from a distinct generation. For further detail, see text and online Supplemental Figures 2-4

~09:00 AM and ended before 13:00 PM, ~1–4 hour after lights off, when mice are most active.[58,59] The second trial of the day began at ~15:00 PM and ended before 19:00 PM, ~1 hour before lights came back on. This was done for five consecutive days without any treatment, always on combination HOLE/GRID chambers. For the last two trials on preconditioning day 5, animals were video recorded to test for preexisting bias for HOLE versus GRID, that is, in which side the animal spent more time (Supplemental Figure S2, left half).

2.3.2 | Conditioning phase with cocaine as US

After 2 days of rest, the conditioning phase began. Mice were weighed prior to the first conditioning trial. Twice daily for 4 days, each animal was removed from its home cage, given a saline or cocaine intraperitoneal (ip) injection, and individually placed into its assigned CS, a CPP chamber for 30 minutes with a HOLE- or GRID-textured floor. Cocaine hydrochloride (Sigma–Aldrich, St. Louis, MO) was administered via ip injection at a dose of 20 mg/kg in an injection volume of 5 mL/kg (as in Reference 57). Each mouse received conditioning to only one texture: either HOLE (n = 32) or GRID (n = 32). If mice were placed in a HOLE chamber in the morning, then they were placed in a GRID chamber in the afternoon. This order was reversed for the next day, in order to counterbalance the time of day in which each mouse experienced each condition. Thus, during the 4 days of conditioning, each mouse experienced each condition twice in the morning and twice in the afternoon. For the last two trials on conditioning day 5, all animals were injected with saline, placed into the combination HOLE/GRID chambers, and video recorded (Supplemental Figure S2, right half).

2.4 | CPP experiment 2 with methylphenidate reward

2.4.1 | Preconditioning phase

Preconditioning in experiment 2 followed the protocol from experiment 1, except that mice alternated mornings and afternoons in either the HOLE or the GRID chambers for the first 4 days instead of using the combination HOLE/GRID chambers. This change was made because it allowed more rapid testing of the fairly large number of animals involved. We counterbalanced these conditions so that each mouse experienced each condition twice in the morning and twice in the afternoon. On day 5, both trials used the combination HOLE/GRID chambers to test for preexisting bias (Supplemental Figure S3, left half).

2.4.2 | Conditioning phase with methylphenidate as US

Conditioning in experiment 2 was similar to experiment 1, except methylphenidate took the place of cocaine and was injected as 5 mL/kg methylphenidate hydrochloride (Sigma–Aldrich, St. Louis, MO) dissolved in 0.9% saline, administered at a dose of 10 mg/kg (as in Reference 60; Supplemental Figure S3, right half).

2.5 | Statistical analysis

We analyzed all recorded preconditioning and CPP videos in a semi-automated fashion with the TopScan LITE video tracking software (Clever Sys, Inc.). Whenever the tracking software failed to accurately follow the animal, the videos were manually analyzed in real-time with a stopwatch and tally system of the time spent in the HOLE versus GRID sides of the chamber. The video analyst was blind to conditioning treatment and linetype (HR vs C).

Following numerous previous studies on these lines of mice, data were analyzed using nested analysis of covariance (ANCOVA) in SAS Procedure Mixed with replicate line nested within linetype (C or HR) as a random effect. In such a model, the effect of linetype is tested relative to the variation among replicate lines with 1 and 6 degrees of freedom. The effects of texture and the texture * linetype interaction are also tested with 1 and 6 degrees of freesom. The CPP data were statistically analyzed following the general strategy of Mustroph et al.[61] First the average proportion time spent on the GRID side (or HOLE side; statistical results are equivalent because values sum to 1) during the CPP test was corrected for bias established during the pretest. Specifically, the proportion of time on the GRID side during the pretest (average of two trials) was subtracted from the proportion time spent on the GRID side during the CPP test (average of two trials). This adjusted proportion time on GRID (after subtracting preexisting bias) was used as the outcome variable for CPP. This outcome was compared between mice that received drug on GRID versus drug on HOLE. A significant difference between the GRID-paired group vs. the HOLE-paired group ("Texture" in Figure 2) for corrected proportion time spent on GRID (or HOLE) establishes CPP. This was implemented in a linear model that included the texture the mice were conditioned on along with linetype (and line nested within linetype). Hence, a main effect of texture indicates CPP collapsed across the two linetypes. A main effect of linetype indicates a difference in preference for GRID after correcting for pretest bias, collapsed across texture (HOLE and GRID). Hence, this effect is difficult to interpret because half the mice are conditioned to GRID and half to HOLE, so collapsing across them will display a large variance. Nevertheless, if the linetype effect is significant (in practice in never was), that means the linetypes differed in their bias for one texture that developed after the pretest and was unrelated to the drug conditioning. A significant interaction between linetype and texture is the key term that indicates whether one linetype conditioned more strongly to the reward than the other. Age and time of day had no effect when included as covariates, and thus were not included in the final models.

Previous authors have found that CPP is can be negatively related to distance traveled in the apparatus.[62] Because of the potential that the HR mice would move more in the apparatus (which did in fact occur, as shown in Results section *3.4*), we wanted to evaluate whether there was a difference in CPP between HR and C after

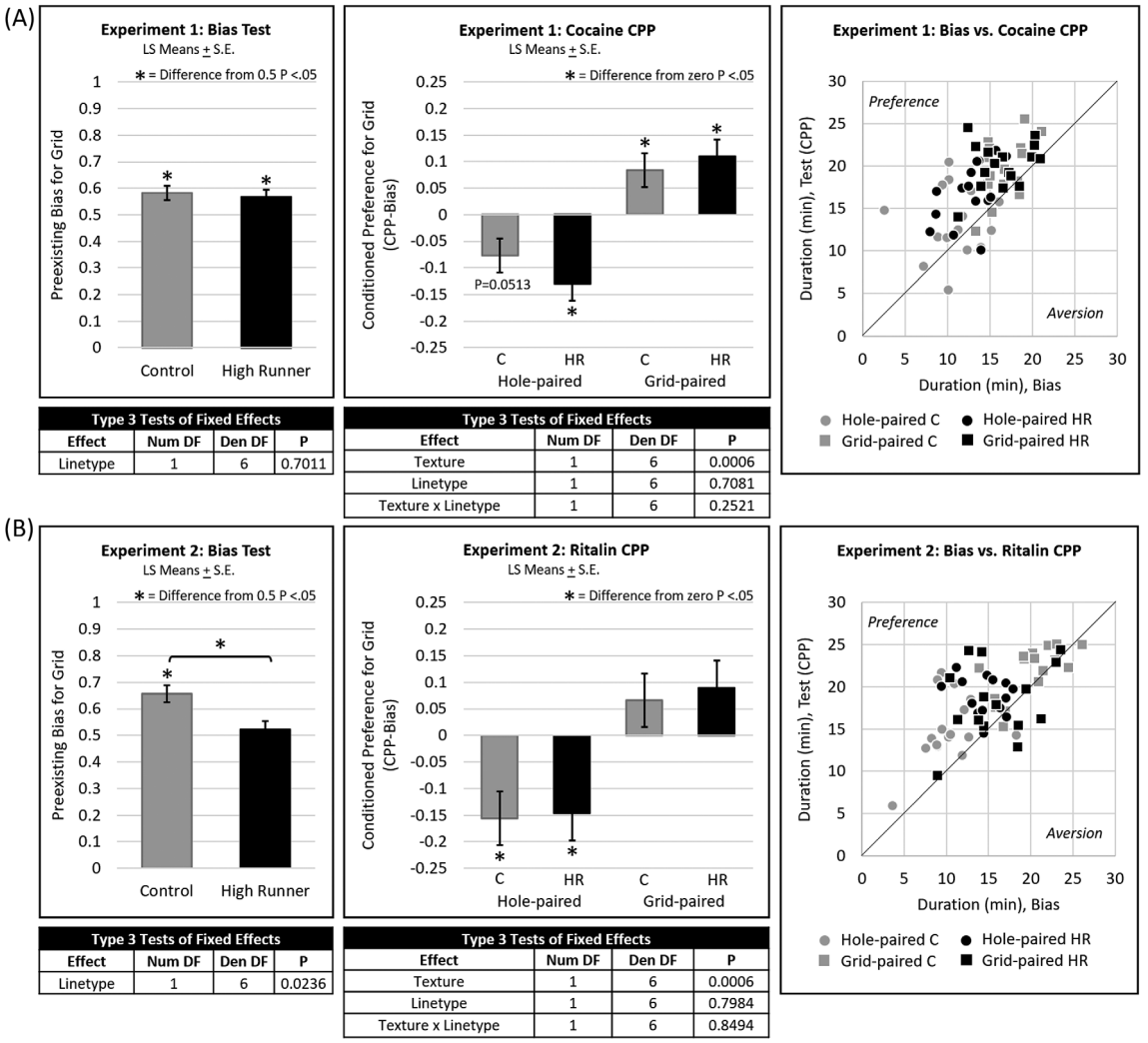

FIGURE 2 Preexisting bias and conditioned place preferences for two experiments. A, Experiment 1, cocaine. (left panel) Mice (generation 77, N = 63, 1 outlier removed) from both the selectively bred High Runner lines (*P* = .0482) and the nonselected Control lines (*P* = .0231) had a preexisting bias to spend more time on the GRID as compared with the HOLE. (center panel) Mice from both HR and C lines conditioned to the reward-paired floor texture with cocaine (*P* = .0006), with no effect of linetype (*P* = .7081) and no interaction between linetype and texture (*P* = .2521). (right panel) Duration (min) spent on HOLE floor (for HOLE-paired mice) or GRID floor (for GRID-paired mice) during cocaine CPP plotted against baseline duration (min) spent on those floor types during bias testing. The one-to-one line is shown. B, Experiment 2, methylphenidate. (Left panel) In this experiment (generation 82, N = 61), individuals from the C lines had a preexisting bias for GRID (*P* = .0025), but those from HR lines did not (*P* = .4972), resulting in a significant difference between the two linetypes (*P* = .0236). (Center panel) Mice from both HR and C lines conditioned to the reward-paired floor texture with methylphenidate (*P* = .0006), with stronger conditioning for the HOLE-paired texture, but no effect of linetype (*P* = .7984) and no interaction between linetype and texture (*P* = .8494). (Right panel) Same as A for the methylphenidate experiment

controlling for variation attributed to distance traveled. Therefore, we also analyzed models that included distance traveled and the interaction between distance and texture in the model to account for the possibility that CPP magnitude decreases with distance. These additional terms were never statistically significant (results not shown) and so the final models presented do not include them.

Data points with residual values >3 standard deviations above or below the mean were re-examined and excluded if deemed appropriate. For main effects and interactions, P-values below 0.05 were treated as statistically significant.

3 | RESULTS

3.1 | CPP experiment 1 with cocaine reward

3.1.1 | Preconditioning bias test

Mice from generation 77 (N = 63) had a preexisting bias to spend more time on the GRID texture in the conditioning chambers as compared with the HOLE texture in both the selectively-bred HR lines (*P* = .0482) and the non-selected C lines (*P* = .0231) (Figure 2A left

panel). Animals spent between 55% and 60% of their time on GRID and 40% to 45% of their time on HOLE.

3.1.2 | Conditioned place preference

Mice from both the HR and C lines conditioned to the reward-paired floor texture with cocaine (P = .0006), with no interaction between linetype and texture (P = .2521) (Figure 2A center panel). Figure 2A (right panel) shows the majority of individuals falling above the 1:1 line and thus are considered to have developed a preference for their reward-paired texture.

3.2 | CPP experiment 2 with methylphenidate reward

3.2.1 | Preconditioning bias test

Mice from generation 82 (N = 61) from the non-selected C lines had a preexisting bias for GRID (P = .0025), but those from selectively-bred HR lines did not (P = .4972), resulting in a significant difference between the two linetypes (P = .0236) (Figure 2B left panel). On average, C animals had a preference for GRID, spending approximately 65% of their time on GRID and 35% of their time on HOLE, while HR animals did not have this GRID preference (52% of time of GRID vs 48% on HOLE).

3.2.2 | Conditioned place preference

Both HR and C lines of mice conditioned to the reward-paired floor texture with methylphenidate (P = .0006), with stronger conditioning for mice paired with the HOLE texture, and no interaction between linetype and texture (P = .8494) (Figure 2B center panel). Figure 2B (right panel) shows the majority of individuals falling above the 1:1 line and thus are considered to have developed a preference for their reward-paired texture.

3.3 | Movement distances during the conditioned preference trials

During both studies, mice from the HR lines tended to move greater distances than C mice (P = .0773 and P = .0887 for cocaine and methylphenidate, respectively). Combining P values by Fisher's[63] method, the combined P = .0410. During the cocaine experiment, average distances moved per recorded CPP trial ($\log_{10}$ mm) were 4.695 ± 0.044 and 4.564 ± 0.044 for HR and C mice, respectively (SAS LS Means and standard errors). Values during the Ritalin trials were 4.622 ± 0.035 and 4.525 ± 0.033.

4 | DISCUSSION

The main finding of the study is that genetic predisposition for increased voluntary wheel-running behavior in HR mice is not associated with increased cocaine or methylphenidate CPP. This implies that selective breeding resulted in specific changes in the natural reward circuit to cause increased motivation for wheel running, or reward received from running, rather than general addictive tendencies for multiple types of rewards. The exact molecular genetic changes in the reward circuit are still being worked out. The cumulative data suggest that some aspect of dopamine signaling has been altered,[38-40,64-66] but dopamine is involved in all salient behaviors.[67,68] Future research is needed to disentangle the details of the changes in the dopamine reward circuit and other interacting circuits that have evolved in HR mice to produce specific increases in motivation for running without altering drug reward.

4.1 | Cocaine and methylphenidate CPP

Both cocaine and methylphenidate produced robust CPP in the HR and C lines, consistent with a large and well-tested experimental foundation, particularly in mice (e.g., see Reference 52). We used a relatively high dosage of cocaine (20 mg/kg), following a previous study that used the same outbred strain of mice as was used to begin the HR selection experiment (Hsd:ICR).[57] This dose was also chosen because it produced differing locomotor responses in HR and C mice, suggesting the psychoactive effects may have been perceived differently.[43] Similarly, at doses of 15 and 30 mg/kg, methylphenidate reduced running of HR mice but increased running of C mice ([39]). Moreover, 30 mg/kg methylphenidate caused a significantly higher activation of the c-fos gene in the medial frontal and sensory cortex of HR mice, indicating a greater amount of recent neuronal activity, as compared with C mice.[38] We used a lower dose of methylphenidate (10 mg/kg) herein, but we hypothesized these behavioral and neuronal activation patterns indicated HR mice perceived methylphenidate differently from C within a broad dose range. Nonetheless, we found no statistically significant difference in cocaine or methylphenidate CPP between HR and C mice, suggesting that they do not perceive the rewarding effects of these drugs differently, and that the locomotor sensitivity and neuronal activity differences are not related to the behavioral reward response.

4.2 | Strengths and limitations

One strength of our study is the CPP method we used, in which interpretation of CPP is not confounded by pre-existing biases in preference because of the balanced, between-subjects design (see Methods). Another major strength is the selective breeding model, which includes a total of eight reproductively isolated lines (or strains), four of which were bred for increased running, while four unselected lines serve as multiple controls, maintained at the same time for more than 77 generations. The replicate lines allow us to empirically test the extent to which selection as opposed to random genetic drift contributes to phenotypic variation.[27-29]

The limitations of our study include that we only examined one dose of cocaine and one for methylphenidate. It is possible that we could have observed a different result had we explored other doses. On the other hand, cocaine CPP does not display a strong dose–response[69]; hence, it is unlikely we would have seen different results had we used other doses. Moreover, we explored two different drugs with similar pharmacological actions, and saw the same result—no difference between genetic linetypes—which increases the likelihood it would generalize to other doses. Another limitation is the possibility that a ceiling effect for CPP prevented us from observing genetic differences. This seems unlikely given that stronger CPP has been observed in inbred strains of mice for doses similar to those used here.[70]

An additional limitation is our use of only one sex. Given the large number of animals to be tested (because we needed to measure mice from eight separate lines), we chose only one sex. We chose females because they generally run more than males in both the HR and control lines.[25,26,55,56] Use of females raises the possibility that the estrus cycle may have affected results. However, we note that mice from both HR and C lines did indeed condition to both drugs. We are aware of only one study that has specifically addressed estrus-cycle effects on CPP. In rats, Walker et al.[71] found that vaginal lavage performed immediately prior to the conditioning session induced a significant preference. Given that vaginal lavage is generally required to score estrus stage, as we have done in previous studies (e.g. see[72]), we were concerned that doing so in the present studies could affect results. Additionally, Korol et al.[73] reported that learning strategy (place vs. response) varied across the estrous cycle in female rats. In any case, it would be of interest to include males in future studies of CPP in these lines of mice.

4.3 | Conclusions

Overall, we conclude that selective breeding for increased voluntary wheel-running behavior does not alter perception of cocaine or methylphenidate reward in mice. This suggests that the neurogenetic underpinnings of high motivation for exercise are somewhat specific to exercise because they do not transfer to these drug rewards. Of course, it may be the case that the HR mice find other drugs more rewarding than C mice, such as opioid, serotoninergic, or cannabinoid drugs. Future studies are needed to evaluate HR and C perceptions of other drug rewards as well as using gold standard operant conditioning methods in addition to CPP for measuring drug reward and motivation.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Online Supplemental Figure 1

Figure S1. Conditioned place preference (CPP) chambers used in all experiments. Chambers are same from Zombeck (2008). The interchangeable stainless-steel bottoms have holes or a grid of rods for conditioning, or a combination of hole/grid for preference testing. The walls are black, and the lids are clear plexiglass to allow for videotaping from above.

Online Supplemental Figure 2

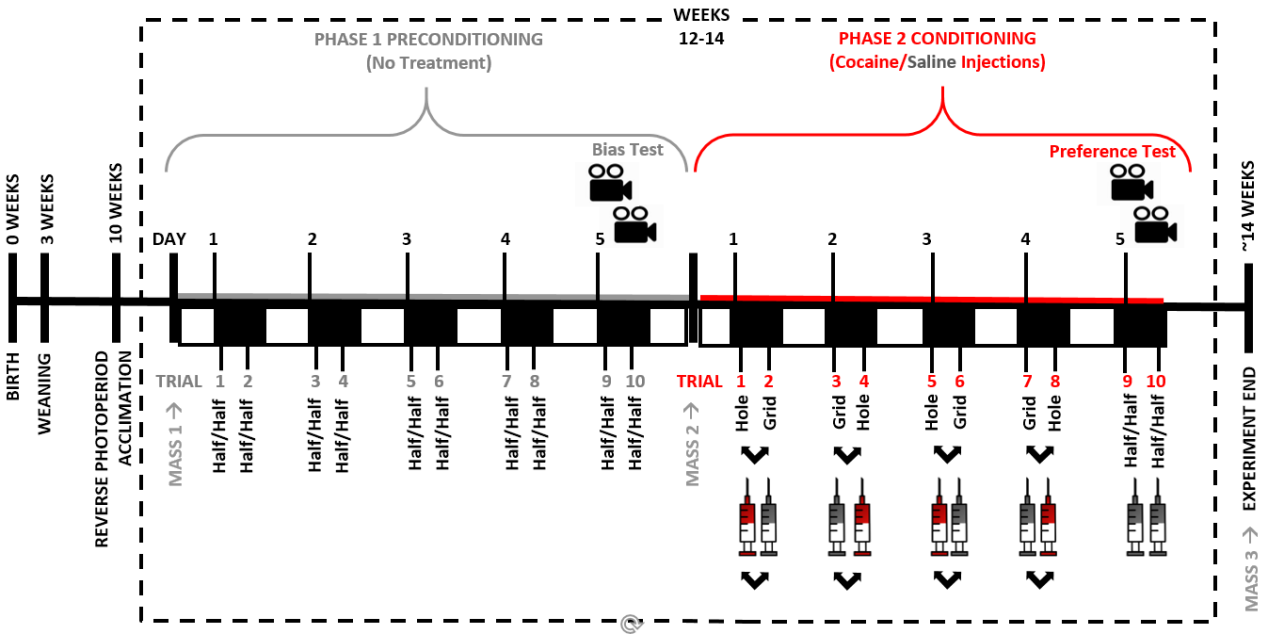

Figure S2. Timeline for conditioned place preference (CPP) with cocaine as the US. Phase 1 began at approximately postnatal week 12 and lasted 5 days. Mice were placed individually into combination hole/grid (Half/Half) CPP chambers twice per day for 30 min, for 4 days and video recorded in combination hole/grid chambers twice on day 5. Next, Phase 2 conditioning lasted 4 days. Mice were placed individually into CPP chambers twice per day for 30 min, with half of the subjects injected with cocaine and paired with a hole or grid floor, followed by the opposite for the next trial and injected with saline. Mice were given saline and video recorded twice on combination hole/grid floors on day 5.

Online Supplemental Figure 3

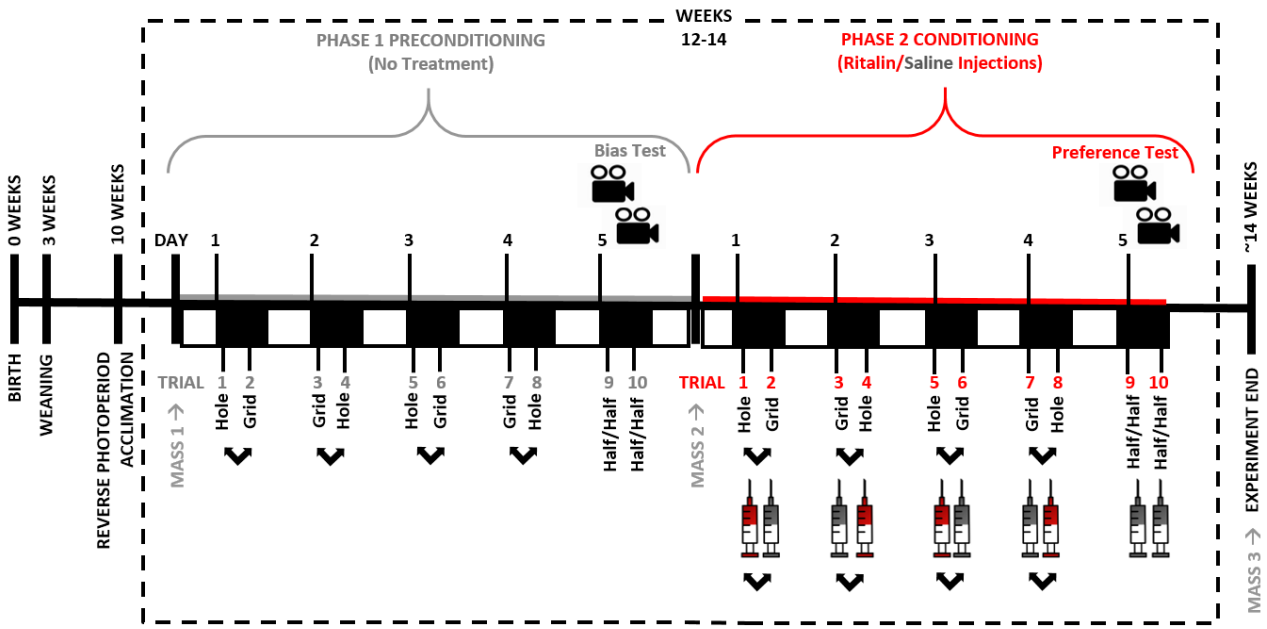

Figure S3. Timeline for conditioned place preference (CPP) with Ritalin as the US. Phase 1 began at approximately postnatal week 12 and lasted 5 days. Mice were placed individually into combination hole or grid CPP chambers twice per day for 30 min, for 4 days and video recorded in combination hole/grid chambers twice on day 5. Next, Phase 2 conditioning lasted 4 days. Mice were placed individually into CPP chambers twice per day for 30 min, with half of the subjects injected with Ritalin and paired with a hole or grid floor, followed by the opposite for the next trial and injected with saline. Mice were given saline and video recorded twice on combination hole/grid floors on day 5.