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The Medial Prefrontal Cortex and Nucleus Accumbens Mediate the Motivation for Voluntary Wheel Running in the Rat

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Voluntary wheel running in rats provides a preclinical model of exercise motivation in humans. We hypothesized that rats run because this activity has positive incentive salience in both the acquisition and habitual stages of wheel running and that gender differences might be present. Additionally, we sought to determine which forebrain regions are essential for the motivational processes underlying wheel running in rats. The motivation for voluntary wheel running in male and female Sprague–Dawley rats was investigated during the acquisition (Days 1–7) and habitual phases (after Day 21) of running using conditioned place preference (CPP) and the reinstatement (rebound) response after forced abstinence, respectively. Both genders displayed a strong CPP for the acquisition phase and a strong rebound response to wheel deprivation during the habitual phase, suggesting that both phases of wheel running are rewarding for both sexes. Female rats showed a 1.5 times greater rebound response than males to wheel deprivation in the habitual phase of running, while during the acquisition phase, no gender differences in CPP were found. We transiently inactivated the medial prefrontal cortex (mPFC) or the nucleus accumbens (NA), hypothesizing that because these regions are involved in the acquisition and reinstatement of self-administration of both natural and pharmacological stimuli, they might also serve a role in the motivation to wheel run. Inactivation of either structure decreased the rebound response in the habitual phase of running, demonstrating that these structures are involved in the motivation for this behavior.

Keywords: motivation, medial prefrontal cortex, nucleus accumbens, wheel running, exercise

Aerobic exercise promotes both physical and mental well-being. Physically active individuals, for example, demonstrate better weight control and musculoskeletal health, decreased risk for diabetes and cardiovascular disease, increased energy and sleep quality, and lower levels of depression and anxiety (Centers for Disease Control and Prevention [CDC], 2011). Despite these benefits, 50% of the adult U.S. population do not get the recommended ~20 min per day (150 min per week) of moderate-intensity aerobic exercise, and 32% do not participate in any level of physical activity at all (American Heart Association, 2013; CDC, 2014; Department of Health and Human Services, 1996), suggesting that the lack of motivation to engage in physical activity is a critical problem.

Voluntary wheel running in laboratory rodents serves as a preclinical model of voluntary exercise in humans (Eikelboom, 1999). Rats engage with running wheels spontaneously and develop a regimen of running robust, stable daily distances over the course of 2 to 3 weeks, with females running on average 1.5 times farther and faster than males (Afonso & Eikelboom, 2003; Basso & Morrell, 2010; Greenwood et al., 2011; Richter, 1927; Sherwin, 1998; Shirley, 1929; Stewart, 1898). Hypotheses as to why rats engage in this behavior have suggested that voluntary wheel running is a measure of general locomotor activity; a means of exploring the environment for food, water, or other materials; an obsessive–compulsive or dependent behavior; a form of fictive migration or escape; and even play behavior (Barnett, 1958; Ferreira et al., 2006)—but data supporting those ideas are not robust (Albelda & Joel, 2012; Sherwin, 1998). Conversely, there is considerable evidence for the hypothesis that rats engage in wheel running because it has positive incentive salience for them.

Most notably, rodents perform operant responses for access to a wheel, develop a conditioned place preference (CPP) for the aftereffects of the wheel, and prefer an environment associated with running versus an environment with a variety of enrichment objects (Belke, 1997, 2006; Belke & Heyman, 1994; Belke & Pierce, 2009; Belke & Wagner, 2005; Collier & Hirsch, 1971; Greenwood et al., 2011; Hill, 1961; Iversen, 1993; Kagan & Berkun, 1954; Lett, Grant, Byrne, & Koh, 2000; Lett, Grant, & Koh, 2002; Pierce, Epling, & Boer, 1986; Premack, Schaeffer, & Hundt, 1964). Rats also demonstrate spontaneous recovery or increased running behavior after a period of forced wheel abstinence (Aoyama & McSweeney, 2001; Hill, 1956, 1961; Mueller, Herman, & Eikelboom, 1999; Mueller, Loft, & Eikelboom, 1997; Sugimoto, Shido, Sakurada, & Nagasaka, 1994), similar to the response after deprivation of other natural or pharmacological stimuli (McSweeney, Murphy, & Kowal, 2005). Recent work with entirely feral rodents in the wild has also demonstrated that both

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mice and rats voluntarily wheel run under their natural conditions in an environment complete with all natural stimuli, presumably including those with obvious high motivational salience, such as food, sexual partners, and offspring (Meijer & Robbers, 2014).

The mesocorticolimbic pathway consists of a series of regions including the ventral tegmental area, the nucleus accumbens (NA), and the medial prefrontal cortex (mPFC), that are necessary for responding to rewarding stimuli. Data indicate that voluntary wheel running, as well as forced treadmill running, has significant impact on this reward pathway, producing a variety of morphological and neurochemical alterations in these areas (de Castro & Duncan, 1985; Greenwood et al., 2011; Hattori, Naoi, & Nishino, 1994; Meeusen et al., 1997; Werme et al., 2002; Wilson & Marsden, 1995). Guided by the framework provided by these prior studies, we investigated the involvement of the NA and mPFC in the motivation to engage in voluntary wheel running.

This work examines the motivational basis for wheel running in its two phases, the acquisition phase (Days 1 to 7) and the stable, habitual phase (after Day 21; Basso & Morrell, 2010, 2012). We tested the motivation to wheel run in both periods and sought to determine whether the pattern of running greater distances and faster speeds in females versus males signified gender differences in motivation for running. For the acquisition phase experiments, we analyzed the conditioned response to the total experience of wheel running using a CPP model, which was modified from our prior protocols for CPP for pharmacological and natural stimuli (Mattson, Williams, Rosenblatt, & Morrell, 2001, 2003; Seip & Morrell, 2008; Wansaw, Pereira, & Morrell, 2008). For the habitual phase experiments, we examined the unconditioned response to the wheel after a period of forced wheel abstinence, which has been termed the "rebound effect" (Mueller et al., 1999). Through transient inactivation of distinct forebrain regions, we sought to investigate brain systems that mediate the motivation for voluntary wheel running. We hypothesized that the NA and mPFC are involved in motivational processes that generate the avid response to the wheel after a period of forced abstinence.

Method

Subjects

Sprague–Dawley rats (original stock from Charles River Laboratories, Kingston, NY) were bred in our colony at the Rutgers University Laboratory Animal Facility (RAF; Newark, NJ; accredited by the American Association for Accreditation of Laboratory Animal Care). All animals were kept on a 12-hr light–dark cycle (lights on at 7:00 a.m. unless otherwise noted) in a room at 22(± 1)°C and given ad libitum access to water and rat chow (Lab Diet 5008, PMI Nutrition International, LLC, Brentwood, MO). Daily checks were conducted for health and availability of food and water. Weight was measured once per week, and animal husbandry was performed 2–7 days a week, depending on the protocol. All animals remained healthy and retained normal body weight throughout the experiments. Animal care and experimental procedures performed in this protocol were in compliance with the National Institutes of Health (2011) *Guide for the Care and Use of Laboratory Animals* and were reviewed and approved by the Rutgers University Animal Care and Facilities Committee. Care was taken to minimize any suffering and limit the number of animals utilized.

Running Wheel Apparatuses

Running wheel apparatuses were either AccuScan Instruments (Columbus, OH) VersaMax Animal Activity Monitor (wheel: 25-cm diameter, stainless steel mesh floor; home cage: 40 cm long × 40 cm wide × 30 cm wide) or Med Associates Inc. (St. Albans, VT) ENV-046 Activity Wheel with Plastic Home Cage for Rats (wheel: 35.6-cm diameter, 4.8-mm stainless steel grid rods with a 1.6-cm spacing, 12 g freewheeling drag; home cage: 48.26 cm long × 26.67 wide cm × 20.32 cm high with a 7.2 cm wide × 10.2 cm high opening for the wheel). The resistance of both running wheels was low and equivalent, and no extra weight/resistance was placed on either wheel. Apparatuses were in chambers lined with woodchip bedding (Beta Chip, Northeaster Products Corp., Warrensburg, NY), and food and water were provided ad libitum. Data were captured automatically through Windows-based software. The AccuScan equipment also allowed measurement of open field activity of subjects to provide an additional measure of locomotor activity independent from wheel running activity. These measurements are as described in Smith and Morrell (2007).

Motivation During the Acquisition Phase of Voluntary Wheel Running Examined With Conditioned Place Preference

Informed by our prior CPP work, a two-chambered CPP apparatus was devised within the laboratory (Mattson et al., 2001; Seip & Morrell, 2008), consisting of two boxes of equal size (40 cm long × 40 cm wide × 30 cm wide; AccuScan Instruments, Columbus, OH), placed side by side and connected with a short opaque tunnel (4" diameter, 2" length). Each box was decorated with unique cues, which consisted of wallpaper in either horizontal or vertical black and white stripes and tactile flooring of small paper squares (ALPHA-dri, Shepherd Specialty Papers, Kalamazoo, MI) or small corn cobs (Bed-o'Cobs [1/4"] The Andersons, Maumee, OH). All boxes were covered with transparent lids and lit by overhead lights. Luminance (Konica Minolta Luminance Meter LS-100, Japan) was equal in all chambers (220 lumens). Two weeks before the preconditioning session, wheel naïve males (n = 14) and females (n = 24) were placed on a 12-hr light–dark cycle (lights on at 12:00 a.m., off at 12:00 p.m.). This change in light–dark cycle occurred so that the testing could be done at a reasonable time during the day (i.e., prior to lights off).

Preconditioning chamber preference baseline. At PND 65, subjects were exposed to the two-chambered apparatus for 60 min and allowed to roam freely between the chambers. This preconditioning session occurred around 10:30 a.m. so that the session ended before the lights turned off. Time spent in each chamber was manually recorded. Based on criteria discussed below, animals were then assigned to be placed with the wheel in one chamber decorated with unique cues and no wheel in the other uniquely decorated chamber.

Conditioning sessions. Preconditioning boxes were replaced with cue-decorated boxes without the entrance hole in the side, one of which had a running wheel and one of which did not, both

providing food and water ad libitum. Twenty-four hours after the preconditioning baseline session, animals were isolated in one of the uniquely decorated chambers for 23 hr (with or without a wheel). The 23 hr of conditioning occurred from ~11:30 a.m. to the following morning at 10:30 a.m. In the remaining hour of the 24-hr cycle, chambers were cleaned and animals were placed in the alternate chamber before the lights turned off at 12:00 p.m. During cleaning (1% Liquinox, 70% alcohol, in distilled water), animals were placed in a shoebox holding cage with food and water ad libitum. The next day, animals were placed in the alternate chamber environment for 23 hr. This cycle of chamber-environment changes continued for 14 days, such that each animal received 7 days of conditioning with the wheel and 7 days of conditioning without the wheel (i.e., alternate-day running). At the end of conditioning, each subject had received 7 days of wheel exposure, which we established to be the acquisition phase of wheel running behavior (Basso & Morrell, 2010, 2012). At the end of conditioning, animals were returned to shoebox cages with food and water, which served as temporary home cages until the postconditioning test.

Postconditioning test of place preference. Twenty-four to 96 hr after the final conditioning session, animals were exposed to the two-chambered apparatus, which had been cleaned and decorated with the same cues used during the preconditioning and conditioning sessions, but without wheels present. The chambers were connected to allow exploration of both chambers. Animals were tested at most twice, that is, once with 24 or 48 hr of forced wheel abstinence and a second time with 72 or 96 hr of forced wheel abstinence. Previous data from our lab showed that repeated postconditioning testing (maximum of 2 times) provides robust results, so that protocol was adopted for the present work (Seip & Morrell, 2008). The subjects were allowed to roam freely between the chambers for 60 min, with time spent in each chamber and the number of times a subject changed from one chamber to the other recorded manually by three observers who were naïve to the stimulus–chamber associations learned by each animal during conditioning.

Analyses and chamber assignments. To understand the preference of each individual animal as well as the group preference as a whole, data were analyzed separately using individual chamber preference and group chamber time (Mattson et al., 2001, 2003). Data were analyzed at both the pre- and postconditioning sessions. In order to determine whether an individual animal showed a preference for a particular chamber, a stringent quantitative criterion was developed. To show a preference, the animal must have spent ≥30 min in one chamber, and this time also had to be ≥25% longer than the time spent in the other chamber. If these two criteria were not met, the animal was categorized as showing no preference. For the two-chambered apparatus, three preference categories were possible (squares, corn cobs, no preference). After individual chamber preference from the preconditioning session was established, animals were assigned to receive the wheel in their least-preferred side chamber. If an animal showed no preference, then the wheel was randomly assigned to one of the two chambers. Group chamber times were calculated by averaging the time spent in each chamber by all animals. There were no statistical differences in the time spent in the chamber associated with the wheel running experience as a function of the time between the last conditioning exposure and the postconditioning test (24 to 96 hr). Therefore, these data were pooled for graphical presentation and statistical comparison.

CPP data analyses. Time spent by the groups in each chamber (interval data, within groups, termed "group chamber time") during the pre- and postconditioning sessions were compared via two-way repeated-measures analysis of variance (ANOVA), whereas postconditioning times were compared via one-way repeated-measures ANOVA. Between-groups times were analyzed using independent-samples *t* tests. The individual chamber preference data (categorical data, within groups, termed "individual chamber preference") were analyzed using Fisher's exact test. Between-groups times were analyzed using a two-tailed test for significance of proportions.

Motivation During the Habitual Phase of Voluntary Wheel Running Examined With Reinstatement (Rebound) Running After Forced Abstinence

At PND 65, wheel-naïve males ($n = 12$) and females ($n = 34$), previously group housed in shoebox cages in the RAF, were individually placed in the AccuScan or Med Associates home cages with running wheels at ~1:00 p.m. (approximate midpoint during the light period; lights on at 7:00 a.m., off 7:00 p.m.). Animals remained in these home cages for 21 days, except for husbandry. After at least 21 days of ad libitum access to the wheels, subjects were in the stabilized or habitual phase of wheel running. Subjects were subsequently given two forced abstinence tests. One test was a 1-hr period of forced abstinence from the wheel, during which husbandry, weighing, and handling-based wellness checks were carried out. Conceptually this can be considered a test of what a cage disturbance might do to spontaneous wheel running, roughly equivalent to the impact of providing clean cage bedding and food and water. The second test was a longer forced wheel abstinence of 72 hr. During this period, routine husbandry occurred. Removing or returning wheel access always occurred around 1:00 p.m. (midpoint light phase of daily light cycle, i.e., the rat's normal resting time). In the AccuScan system, wheels were quietly removed or returned with minimal disturbance, and in the Med Associates system, manual sliding doors separating the wheels and the home cage were closed or opened.

Brain Region-Specific Inactivation Procedures in Either the Acquisition or the Habitual Phase of Running

To examine whether particular brain regions were required for the motivation to run at either the (a) acquisition phase or (b) the habitual phase of running, chronic indwelling cannula were implanted bilaterally in either the mPFC or the NA. After recovery, these brain regions were transiently inactivated and behavior was observed. Separate groups of subjects were examined at these two phases of running. To examine the effect of cannulation on running responses, two additional groups of subjects were prepared at each phase, with cannula in the mPFC or the NA.

1. To examine motivation during the *acquisition phase of wheel running (CPP)*, subjects ($n = 16$; eight of each gender) received cannula implants prior to any wheel running exposure. CPP pretesting and wheel running

exposure did not take place until at least 1 week postoperatively. For their posttest session, some animals were retested with at least 24 hr in between testing sessions. These retest sessions were performed with different infusion types (i.e., no infusion, saline infusion or inactivating infusion). These additional posttests were included in the analyses, and the numbers of animals ($n = 38$) reflect those repeated tests.

2. To examine motivation during the *habitual phase of wheel running (rebound test)*, habit running was first established by providing wheel access for 21 days. Subjects ($n = 51$) were then implanted with cannula and placed with their wheels immediately after surgery. Rebound testing did not take place until at least 1 week postoperatively.

3. To examine the *effect of cannulation on running responses*, two groups of subjects were analyzed. One group ($n = 8$) was cannulated seven days prior to any running experience and then provided with unlimited access to a running wheel for at least 28 days. These subjects had a cannula present at the start of the acquisition phase as well as subsequently. The second group ($n = 8$) was allowed to acquire habit running for 21 days and when full habit running was established, cannula were implanted and subjects were immediately provided with access to their homecage running wheels.

Cannula implantation via stereotaxic surgery. At approximately PND 65, subjects were anesthetized with 1 mL/kg of a solution containing ketamine HCl (75.0 mg/mL), xylazine (7.5 mg/mL), and acepromazine maleate (1.5 mg/mL). The incision site was shaved and injected subcutaneously with 0.5% bupivacaine hydrochloride + epinephrine. Animals were placed in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), and incisor bars were placed 3.2 mm below the interaural line so that the skull was flat and bregma and lambda were positioned at the same vertical coordinate. An incision was made and the scalp was carefully opened to reveal the skull. Bregma and lambda were identified, and for the mPFC, which included both the prelimbic and infralimbic components, a 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was implanted + 2.8 mm AP from bregma, ± 0.60 mm ML from the midline, and −2.0 to −3.0 mm DV from the skull surface. For the NA, which included both the core and shell components, a 22-gauge stainless steel guide cannula was implanted + 1.6 mm AP from bregma, ± 0.75 mm to ± 1.5 mm from the midline, and −6.0 mm DV from the skull surface. The guide cannula was secured to the skull using stainless steel screws and cranioplastic cement. To keep the guide cannula free of tissue or liquids, dummy stylets were inserted. After surgery, animals were placed individually in home cages, and overall health was checked daily.

Intracranial infusions. All animals were handled extensively for at least 1 week before all intracranial infusions. During this time, dummy cannula were removed periodically and cleaned to ensure that no biological debris accumulated. For infusion purposes, the dummy stylet was removed, and a 28-gauge stainless steel injector was inserted into the guide cannula. The injector extended 2.0 mm beyond the tip of the guide cannula and was connected by PE-10 tubing to 10-μl Hamilton syringes. Simultaneous bilateral infusions occurred via a two-syringe infusion pump (Harvard 22 syringe pump; Harvard Apparatus, Holliston, MA). Bupivacaine (2%), muscimol (50 ng/0.5 μl; Tocris, Ellisville, MO), or saline (0.9%) were injected at a rate of 0.5 μl per minute, with bupivacaine infusions receiving 1.0 μl per side and muscimol infusions receiving 0.5 μl per side. After the total amount was infused, the injectors were left in place for 1 min to allow diffusion of the substance.

To avoid a stress response, a possible confound to the infusion outcomes, cannulated subjects were exhaustively habituated to handling, including handling of their headsets. To even further decrease restriction disturbance, the subjects were allowed to freely roam around a holding cage during infusion. While gently restrained, subjects had the dummy cannula removed and the injector cannula inserted, which was attached to the PE tubing and the Hamilton microsyringes in the infusion pump apparatus. Then subjects were released to free roam in a holding cage (without a top) that was adjacent to the infusion pump during the infusion and diffusion processes. During infusions, animals explored the holding cage without any evidence of behavioral discomfort or immediate effect of the infusion process. After this 2- to 3-min process, animals were again handled with gentle restraint to remove the injector and replace the dummy cannula. When bupivacaine was administered, after infusions, animals were placed immediately into the postconditioning CPP apparatus or home cages with wheel access. In the case of muscimol, after infusions, animals were placed in a holding cage with food and water ad libitum for approximately 1 hr before being placed in the postconditioning CPP apparatus or home cages with wheel access. This difference in timing was due to the difference in the onset and duration of the drug and to allow the animals as much time as possible to settle before the CPP experiment began.

For the CPP experiments, groups of subjects were infused 1 or 2 days apart. In this way, all postconditioning testing was conducted with a maximum of 96 hr of wheel deprivation. Previous work indicates that uncannulated animals with postconditioning CPP sessions from 24–96 hr of wheel deprivation show similarly robust responses (Basso & Morrell, 2010). For the rebound response experiments, all injections were conducted at least 7 days apart. Our previous work indicated that repeated measures of the rebound response are similar (Basso & Morrell, 2010, 2012), so we adopted this protocol for repeated measures of the infusion experiments.

Surgical and intact controls. Surgical controls consisted of animals whose dummy stylets were immovable or whose cannula placements were off target (i.e., unilateral or dorsal placements). Prior to testing, these animals were handled in a manner similar to that of all surgical animals, as described above.

Histology. After all testing was complete, subjects were anesthetized with pentobarbitol (1 mL) and intracardially perfused with 4% formalin. Brains were then removed and placed in formalin for at least 1 day. They were then exposed to a 15% sucrose solution for an additional day. Brains were then blocked, and samples were mounted onto the cryostat's specimen holders with water and dry ice and placed in the −13 °C microtome. Brains were sectioned at 30 μm and mounted on chrom-alum coated slides. At least 1 week after slicing, all sections were stained with cresyl violet and

coverslipped. At least two investigators, naïve to the behavioral results, confirmed cannula locations using a microprojector and microscope. The most ventral portion of the cannula placement, together with the path of the cannula through the more dorsal tissue, were used as the indication of the anatomical location of the infusion site (based on Paxinos & Watson, 1998). Tissue surrounding the cannula tips did not show signs of lesion or any form of pathology or ischemia from the chronic indwelling cannula or the injected solutions. Animals that showed signs of lesion of the brain regions or overlying tissues, meninges, bone, or skin were removed from all analyses ($n = 1$).

Reinstatement (rebound) running data analyses. Running data were analyzed by examining wheel turns each minute of the day that the animal had access to the wheel. The computer software captured running wheel data in time bins of 1 min, and in this way, distance, time, and rate could be calculated. For these analyses, bupivacaine and muscimol conditions were pooled as no differences were found between these conditions in the percent decrease from saline in the percentage of total daily running (Figure 1, $p > .05$). For these experiments, the rebound response that occurred after 72 hr of wheel deprivation was divided by the total 24-hr running distance, resulting in the normalized rebound response as plotted in Figure 4A. This value can also be seen as the percentage of total daily running. This was done to control for the variability that occurred in running distances (both daily and rebound response) between genders and between individuals of the same gender, allowing us to include all subjects in the same analyses. This normalization procedure was also conducted for running time and rate. A one-way repeated-measures ANOVA was used to determine differences between running distances, times, and rates in the same group repeatedly measured at different times (e.g., saline vs. inactivating agent). An independent samples t test was used to determine statistically significant differences between one measure in two separate groups.

General analyses and statistics. All statistical analyses were conducted using the computer software IBM SPSS 21.0 or 22.0 (Chicago, IL). A significance value of $p \leq .05$ was used for all statistical analyses. Interval data met the tests of normalcy and homogeneity of variance and were analyzed with parametric tests. Categorical data were measured using nonparametric tests. If data did not meet normality (t test) or sphericity (repeated-measures ANOVA), corrections such as Greenhouse–Geisser (for repeated-measures ANOVA) were used.

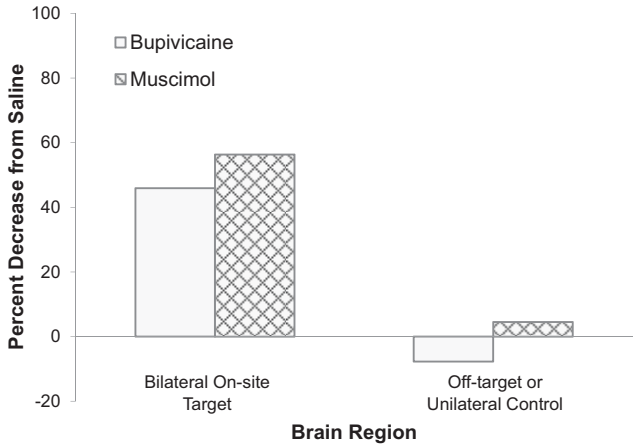

Figure 1. Percentage decrease from saline in the percentage of total daily running for both the bupivacaine and muscimol conditions in all bilateral on-site targets in the medial prefrontal cortex (mPFC) and nucleus accumbens (NA), and all other, off-target or unilateral control areas. Because bupivacaine and muscimol conditions did not differ from one another in any region, they were pooled for all further analyses.

Results

Running Behavior During Acquisition and Habitual Phases

All measures of spontaneous running—distance run, time spent running, and rate of running—increased significantly during the first 1–3 weeks of wheel availability (i.e., the acquisition phase) in both genders, after which running behavior stabilized, in what we refer to as the habitual phase, for up to the 15-week limit of these experiments (Figure 2A). During this 15-week period, there was no correlation between body weight and these parameters of running. Gender differences were found in both phases of running. The acquisition of stabilized habit running occurred more quickly in females, that is, by week 2 of wheel availability, week 1 vs. 2: $F(1, 27) = 21.472$, $p < .001$, whereas males did not achieve stabilized habit running until Week 3, Week 1 vs. 2, $F(1, 27) = 6.352$, $p = .018$; Week 2 vs. 3, $F(1, 27) = 26.401$, $p < .001$. Females ran significantly farther than males during the first and second weeks, Week × Gender, $F(2, 104) = 5.578$, $p = .005$, Week 1 $t(37.082) = 3.665$, $p = .001$; Week 2 $t(30.516) = 5.276$, $p < .001$. Similarly, females ran significantly faster and for a longer time than males (data not shown). By Day 21, which was the beginning of the habitual phase of running, females ran 1.5 times farther than males ($p > .05$). This difference most likely reflects the fact that females ran statistically farther (as well as faster and longer, data not shown) than males on the proestrus day of their estrus cycle (Figure 2B), $t(32) = 4.644$, $p < .001$.

Motivational Measures of Running During the Two Phases of Running

Acquisition phase—CPP. After the first 7 days of running, both males and females showed a statistically significant preference for the chamber associated with the total experience of wheel running (see Figure 3). Females showed a conditioning effect in terms of both individual preference ($p < .001$) and group chamber time, $F(1, 23) = 14.375$, $p = .001$, with 60% of females spending 75% of their time during the postconditioning session in the chamber associated with wheel running. Males also showed a conditioning effect in terms of both individual preference ($p < .001$) and group chamber time, $F(1, 13) = 8.739$, $p = .011$, with 64% of males spending 80% of their time at the postconditioning session in the chamber associated with the wheel running experience. For the acquisition phase, no statistically significant gender differences in running-experience CPP were found. Furthermore, there was no correlation between strength of the preference (time spent in the wheel-associated chamber at the postconditioning test) and distance run ($R^2 = 0.19$).

Habitual phase—rebound response. When subjects in the habitual phase were deprived of their running wheels for 1 or 72 hr, they all began running immediately upon return of the wheel (see Table 1). This

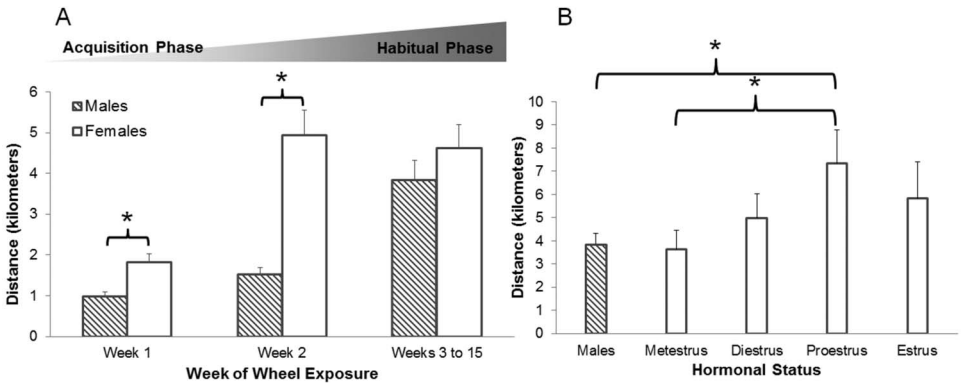

Figure 2. (A) Average (± *SEM*) daily distance (kilometers) run per week by male and female rats during the first to 15th week of wheel exposure. Females ran farther during the first and second week of wheel running and acquired the stable, habitual behavior faster than males, that is, by the second week compared with the third. (B) Average (± *SEM*) daily distance (kilometers) run in the habitual phase of running demonstrates that females ran significantly more on the proestrus day of their cycle compared with metestrus and significantly more than males only on proestrus. * $p < .05$.

sudden burst of rebound running is particularly impressive as the wheel was returned in the light period of their daily cycle when they would normally be resting (i.e., a period of low baseline running). This burst of running subsided within 1 hr after return of the wheel. Both genders showed a less intense burst of running after a period of 1 hr of forced wheel abstinence, such as might be caused by animal husbandry procedures (see Table 1). Both genders had a significantly greater response to the 72 hr of wheel deprivation compared with the 1-hr deprivation period, females: distance, $F(1, 33) = 22.155$, $p < .001$, time, $F(1, 25) = 53.826$, $p < .001$, and rate of running, $F(1, 25) = 49.615$, $p < .001$; males: distance, $F(1, 11) = 27.630$, $p < .001$, time, $F(1, 11) = 8.418$, $p = .014$, and rate of running, $p > .05$.

There was a gender difference in the response in that females ran farther, Distance × Gender, $F(1, 44) = 4.682$, $p = .036$; $t(44) = 2.151$, $p = .037$, and faster, Rate × Gender, $F(1, 36) = 14.296$, $p = .001$; $t(36)3.567$, $p = .001$, than males after 72 hr of forced wheel abstinence, regardless of the day of the estrus cycle. The rebound running responses after 72 hr of forced wheel abstinence are comparable to distances and times spent running during the first hour of the dark period on a day without any wheel deprivation, which is the time when rats ran the farthest distance and spent the most time running at the fastest pace. It is surprising that wheel deprivation did not alter the total daily distance run, only the pattern of the timing of the daily running.

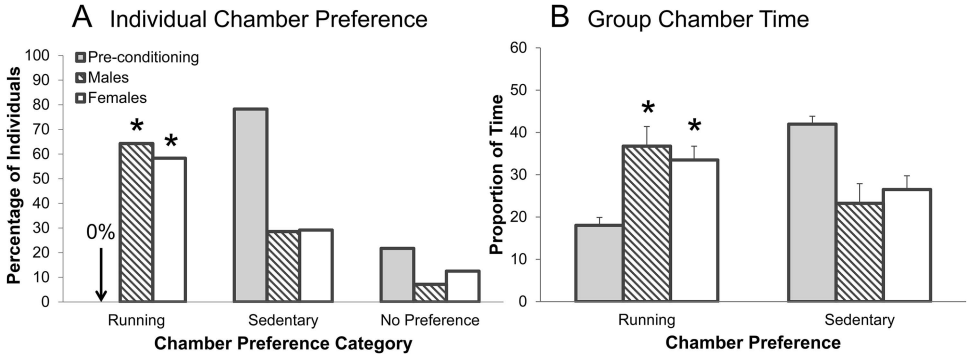

Figure 3. Conditioned place preference for the total experience of wheel running during the acquisition phase (i.e., first 7 days of running). Males and females showed an equally robust conditioned place preference both in terms of individual chamber preference and group chamber time. (A) Individual chamber preference or percentage of males and females that showed a preference for a chamber associated with a running experience, a sedentary experience, or no preference at the pre- and postconditioning test sessions. (B) Group chamber time or the proportion of time that males and females spent in the running- or sedentary-associated chamber at the pre- and postconditioning test sessions. * $p < .05$.

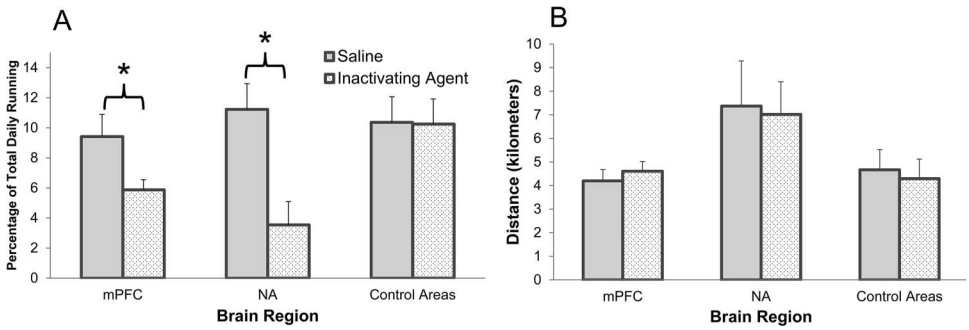

Figure 4. Rebound response, or the distance run (meters) in the first hour after wheel return, after 72 hr of wheel deprivation in animals that were infused with either saline or an inactivating agent in the medial prefrontal cortex (mPFC), nucleus accumbens (NA), or off-target, control areas. (A) Inactivation of the mPFC or NA, but not other control areas, significantly decreased the rebound response after 72 hr of wheel deprivation. Data are presented as average (± *SEM*) normalized distance (rebound response/total daily distance run) (B) Neither inactivation of mPFC, NA, nor other regions decreased the total daily distance run. Female data presented as an example. * $p < .05$.

Motivational Measures of Running After CNS Intervention

Habitual phase. Rats cannulated after habit running was established proved a helpful model to examine the brain regions mediating the motivation for voluntary wheel running.

Running responses are normal in the habitual phase after CNS intervention. Animals in the habitual phase of running (21 days of wheel exposure) quickly recovered their normal daily distance run after surgical implantation of guide cannula (see Figure 6), which was a reflection of their postoperative health. On the day of surgery, rats returned to substantial, although diminished, wheel running as soon as they awoke from anesthesia, Figure 6, top line; Day 21 = 8 km vs. surgical Day 22 = 5.3 km, $F(1, 7]) = 6.793$, + $p = .035$. They returned to normal running patterns (i.e., identical to uncannulated animals) on the day after surgery (Day 23) and retained those patterns for up to 4–8 weeks while chronically cannulated (all other comparisons after surgery, $p > .05$). Cannulation did not alter the rebound response to a 72-hr wheel deprivation. Although the saline-infused animals were highly habituated to the processes of handling, cleaning, insertion, and infusion by internal cannula, saline infusion alone transiently reduced the rebound response after 72 hr of wheel deprivation, $t(68) = 2.757$, $p = .007$. Therefore, the saline-infusion running response was a particularly salient baseline against which the effects of the inactivating agents were compared.

The medial prefrontal cortex and nucleus accumbens are necessary for the motivation to engage in the habitual phase of voluntary wheel running. After 72 hr of forced wheel abstinence, the rebound running response (running distances and rates during the first hour after wheel return) was significantly decreased by inactivation of the mPFC or NA compared with saline infusion, Figure 4A, mPFC: percentage of total daily running, $F(1, 26) = 6.999$, $p = .014$, normalized rate, $F(1, 26) = 11.567$, $p = .002$; NA: percentage of total daily running, $F(1, 6) = 6.953$, $p =$

Table 1
Rebound Response After Various Deprivation Periods

	No deprivation	1-hr deprivation	72-hr deprivation
Distance (meters)			
Females	0.07 (±0.05)	260.57 (±62.12)*	666.16 (±60.77)[a,b]
Males	1.23 (±1.16)	111.00 (±32.16)*	435.06 (±52.26)[a,b]
Time (minutes)			
Females	0.09 (±0.06)	14.88 (±2.08)*	40.08 (2.83)[a]
Males	0.50 (±0.42)	20.75 (±5.08)*	40.50 (±2.97)[a]
Rate (meters/minute)			
Females	0.04 (±0.03)	7.5 (±1.02)*	15.46 (±0.82)[a,b]
Males	0.30 (±0.24)	8.32 (±1.79)*	10.67 (±0.85)[a,b]

Note. Larger rebound responses occurred with longer wheel deprivation. Average (±*SEM*) distance (meters), time (minutes), and rate (meters/minute) run during the first hour after wheel return after no deprivation, 1 hr of wheel deprivation, or 72 hours of wheel deprivation. Wheels were returned in the light part of their light–dark cycle (1:00 pm). Females ran 1.5 times farther and faster than males in the first hour after wheel return after 72 hr of wheel deprivation.
[a] $p < .05$ compared with no deprivation and 1 hr of deprivation. [b] $p < .05$ between genders.
* $p < .05$ compared with no deprivation.

.039, normalized rate, $F(1, 6) = 7.750$, $p = .032$). Inactivation of off-target controls did not result in significant alteration in the rebound running response (Figure 4A). The location of the cannula in target and off-target controls are as depicted in Figure 5.

Inactivation of the regions of interest did not affect rebound running after 1 hr of wheel abstinence. The expected short burst of running (~185 m), identical to that seen in uncannulated noninfused controls, occurred in all subjects in the habitual phase of wheel running when they were returned to their wheels after being disturbed by the 1-hr wheel deprivation. As with uncannulated animals, cannulated, infused subjects showed no gender differences in this 1-hr disturbance response, so these data were pooled. Brain region inactivation did not affect the distance, time, or rate of disturbance-response running regardless of whether target or nontarget comparator regions were infused (data not shown, $p > .05$).

Inactivation of regions of interest or off-target sites did not affect total daily distance run. Inactivation did not affect the total daily distance, time, or rate of running regardless of whether target or nontarget comparator regions were infused (Figure 4B, $p > .05$).

Acquisition Phase. Rats cannulated prior to wheel exposure revealed that the acquisition phase of running is a fragile behavioral state.

Cannulation impairs the acquisition of running. Subjects naïve to the wheel underwent surgical cannulation and were allowed 1 week of recovery before being exposed to the wheel for 28 or more days. Their running data over this time period were compared with a group of age-matched, uncannulated wheel naïve subjects that were also given exposure to a running wheel for 21 days and subsequently cannulated on Day 21. It is surprising that cannulation, regardless of the anatomical location (mPFC or NA), dramatically affected the acquisition of running, such that the daily distances run were significantly lower in cannulated compared with uncannulated subjects over the 21 days of running, Figure 6: Time × Group effect, $F(20, 280) = 2.752$, $p < .001$. Although these subjects initially had a slower rate of running than their uncannulated counterparts, they steadily gained in the amount they ran over the days of wheel exposure (see Figure 6). Although their running was still somewhat lower than that of subjects cannulated after habit running was established, by the time these subjects reached 23 days of running, the amount of running was not statistically different across these two groups (Day 23, $p > .05$).

Careful review of the cannula locations verified that these cannulas were found within the distribution represented in Figure 5 for the rebound subjects, and that no animals were spuriously cannulated in the motor cortex. Furthermore, no health problems

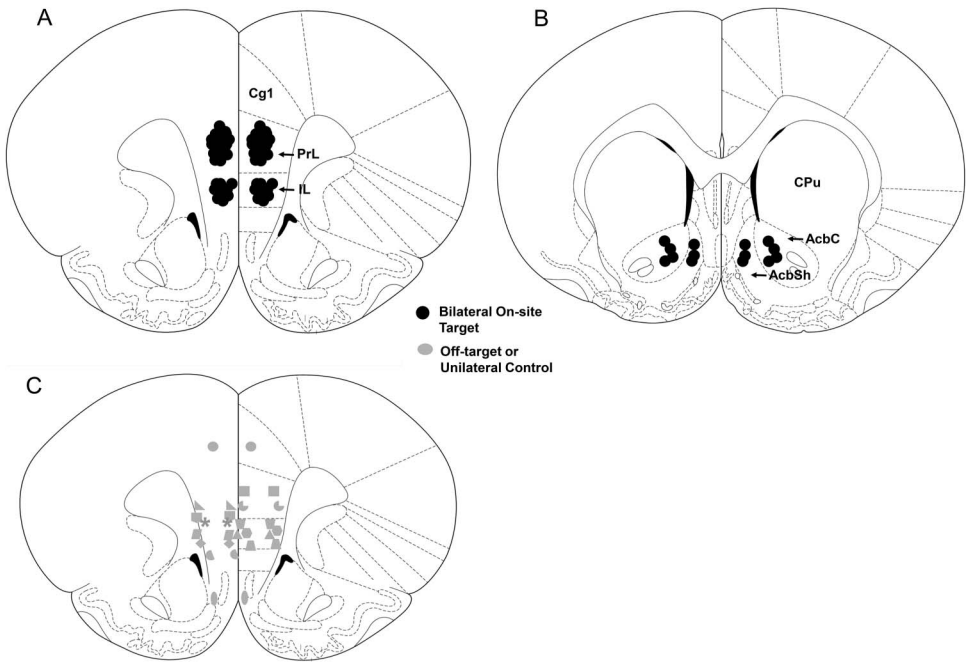

Figure 5. Schematic representation, based on the microscopic analysis of cresyl violet-stained sections, of subregion-specific sites for all rats receiving infusion treatments. (A) On-target bilateral mPFC infusion sites ($n = 27$), (B) On-target bilateral NA infusion sites ($n = 7$), (C) All controls, $n = 17$, 1 of which was a bilateral placement in the cingulate cortex, 3 of which were unilateral placements in the prelimbic cortex, 9 of which were unilateral placements in the infralimbic cortex, 1 of which was a unilateral placement in the NA shell, and 3 in which the cannula had fallen out and no infusion took place. Cg1 = Cingulate cortex; PrL = Prelimbic cortex; IL = Infralimbic cortex; CPu = Caudate putamen; AcbC = Accumbens core; AcbSh = Accumbens shell. Adapted from G. Paxinos & C. Waston, 1998, *The Rat Brain in Stereotaxic Coordinates* (6th ed.), Figure 9 and Figure 12. Copyright 1998 by Academic Press. Copyright, 1998 by Elsevier Academic Press, San Diego, CA. Adapted with permission.

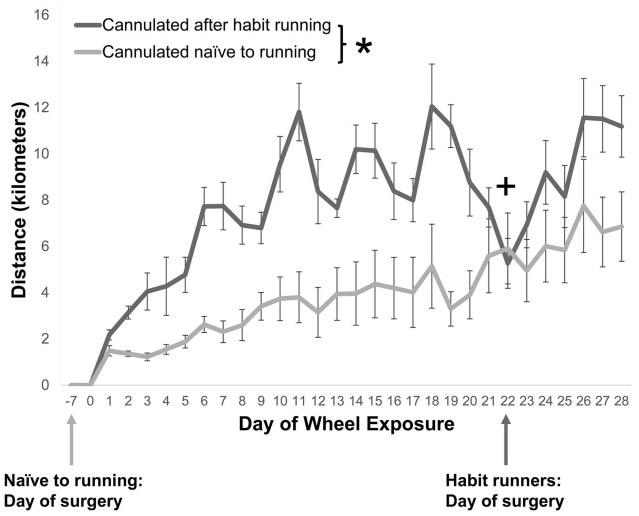

Figure 6. Acquisition running is impaired by cannulation prior to wheel exposure. Average (± *SEM*) daily distance run (kilometers) by rats that were either cannulated prior to wheel running exposure or after habit running was established. Rats cannulated naive to running ran significantly less than uncannulated subjects over the first 21 days of running (* $p < .05$), but eventually (Day 23) reached similar distances to rats cannulated after habit running. In rats cannulated after habit running (top line), rats showed a significant decrease only on the day of surgery, although still at substantial distances († $p < .05$).

or postmortem lesions of brain or surgical sites were found in any of these subjects.

Impaired wheel running in acquisition phase co-occurs with impaired CPP for the wheel running experience. The process of cannulation and vehicle infusion also disrupted CPP for the chamber associated with the wheel during the acquisition phase of wheel running, regardless of the location of the cannula. This is in stark contrast to a different treatment group demonstrating that uncannulated subjects can readily acquire a CPP for a chamber associated with the wheel (see Figure 3).

Statistically fewer subjects that were cannulated and then saline infused (36%) preferred the chamber associated with the wheel running experience compared with uncannulated subjects (60%), z test $p < .05$; group chamber time $t(58) = 1.922$, $p = .059$. No significant decrease below the saline infused state of the response occurred with specific target-region inactivation.

Retesting in the habitual phase of wheel running with rebound test. After these cannulated subjects were CPP tested in the acquisition phase of running, they were allowed to survive with continuous access to their running wheels in their home cages. Once these subjects had reached the habitual phase of running (Day 23), 11 of the original 16 subjects were then retested for motivation for wheel access in this habitual phase using the rebound test. These subjects had a rebound response virtually identical to that of the subjects implanted with cannula after acquisition of habit running and tested only in the habitual phase of running ($p > .05$). That is, the rebound response of both groups of cannulated animals without infusion was virtually identical to that seen in the uncannulated animals (Table 1, Figure 7). After 72 hr of forced wheel abstinence, the rebound response was decreased by inactivation of the mPFC or NA compared with saline infusion, similar to those subjects seen in Figure 4A.

Cannulation does not interfere with general locomotor activity. As can be seen in Figure 8A, unlike wheel running behavior, cannulation prior to wheel exposure did not interfere with general locomotor activity. These data represent daily locomotor activity in the home cage that is separate from wheel running activity. Subjects that were cannulated prior to their running wheel experience had identical locomotor activity as subjects that were uncannulated during their first 21 days of wheel running exposure.

Furthermore, cannulation did not impair postconditioning exploration in the CPP test (Figure 8B). That is, the number of times a subject switched from one chamber to the next during the postcondition test session was equivalent for uncannulated and cannulated subjects. This was also the case for saline and inactivation infusions.

Discussion

These behavioral findings demonstrate that the total experience of voluntary wheel running is a stimulus with positive incentive salience (i.e., a rewarding stimulus) to both male and female rats during both the acquisition and habitual phases of running. Using these behavioral measures together with transient inactivation of specific brain regions, we found that the mPFC and NA are necessary for regulating the motivation for voluntary wheel running in habit runners. We also conclude that the acquisition phase of running is a fragile phase of running behavior, with cannulation prior to wheel exposure decreasing running distances and impairing the incentive salience of acquisition phase wheel running, even in healthy animals with full locomotor capacity. Despite these detriments, these subjects still acquire habit running, although less robust, and report that in the habitual phase, running still has incentive salience. To the best of our knowledge, this is the first report of particular subregions of the CNS being required for the motivational processes of voluntary wheel running.

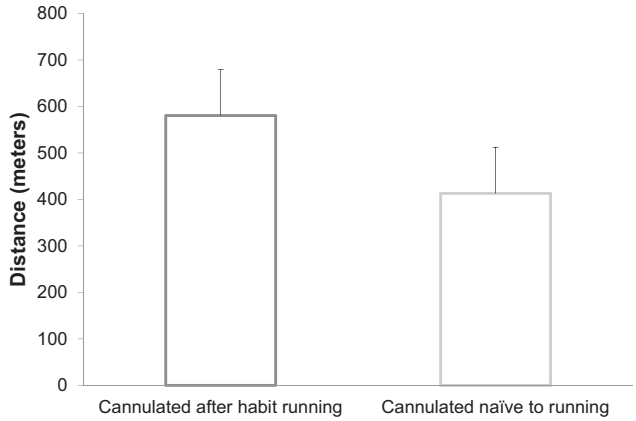

Figure 7. Rebound response, or the average (± *SEM*) distance run (meters) in the first hour after wheel return, after 72 hr of wheel deprivation in two groups of subjects, those cannulated after habit running was established and those cannulated before wheel exposure. In habitual phase running, both groups report similar levels of incentive salience for wheel running.

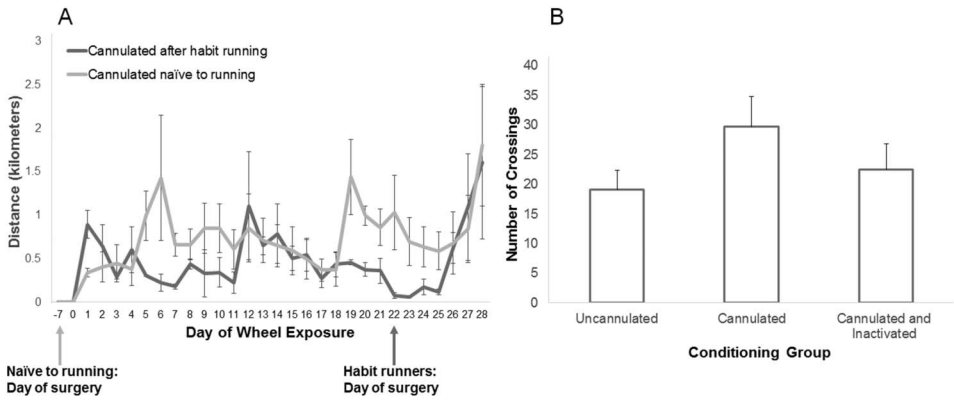

Figure 8. General locomotor activity is not affected by cannulation. (A) Average (± *SEM*) daily distance locomoted by rats that were either cannulated prior to wheel running exposure or after habit running was established. Daily locomotor distance of both groups was equivalent. (B) Average (± *SEM*) number of crossings between chambers during the conditioned place preference test for uncannulated, cannulated (no infusion or saline infusion) or cannulated (infusion with bupivacaine or muscimol) subjects.

Behavioral Conclusions

The detailed analysis of the distance, time and rate of running in both the acquisition and habitual phases of running in male and female rats provided the basis for the motivational tests and CNS interventions utilized here. This examination of running behavior provides an updated and complete analysis of running that accords with and adds to much earlier work of others that sometimes lacks both genders, an analysis of the acquisition of running, and/or equipment and housing that may not meet current standards (Eikelboom & Mills, 1988; Sherwin, 1998). In accordance with the literature, we posit that in both the unconditioned (rebound after deprivation) and conditioned response test (CPP), three components of the wheel running experience have potential salience—interaction with the wheel as an enrichment object, the wheel running itself, and the aftereffects of running, that is, the neurophysiological state after the animal has completed the running.

Motivation During the Acquisition Phase

Our findings demonstrate that rats find the experience of the acquisition of ad libitum voluntary wheel running rewarding and that both genders find it equally salient. The only prior demonstration of the salience of the acquisition phase of wheel running in any rodent was carried out in male hamsters that were allowed only 30 min of wheel running per day for the first 4 days of wheel running experience (Antoniadis, Ko, Ralph, & McDonald, 2000; Ralph et al., 2002). Our work is in agreement with these findings, adding to the literature that rats also find this time period of running rewarding and that females find it equally rewarding as males.

Although CPP is a widely used technique to examine reward-related responses, like any other technique, limitations exist to this paradigm. CPP is fundamentally different than other classically conditioned responses because it involves the act of approaching the conditioned stimulus rather than increasing the probability of a certain behavior. One limitation of CPP is the potentially confounding influence of the novel chamber on test day (Bardo & Bevins, 2000), as rodents are known to prefer a novel context to a familiar one (Bardo et al., 1993). In our case, pairing the wheel with one context rendered it more novel relative to the nonwheel chamber on the wheel-free test day. However, both chambers were novel on test day as they were also food and water free. Another limitation of CPP regards the tendency for animals to favor one chamber over the other prior to conditioning (Bardo & Bevins, 2000). This then forces the experimenter to pair the stimulus with the initially nonpreferred chamber, which may produce a CPP by decreasing aversion to this environment rather than increasing preference to the stimulus. A way to reduce this effect is to utilize a three-chambered CPP, which we did not utilize in this study because of the large nature of the chambers needed to fit the running wheel. Although previously, CPP was not an established protocol in humans, interesting recent work has shown that it is an applicable and useful paradigm in humans as well as rodents (Astur, Carew, & Deaton, 2014; Molet, Billiet, & Bardo, 2013; Napier, Herrold, & de Wit, 2013). Therefore, both in the laboratory situation and human condition, CPP can be a very valuable tool for studying reward-related processes, and we found it to be so for examining the appetitive nature of the acquisition of wheel running in rodents.

Motivation During the Habitual Phase

After 72 hr of forced wheel abstinence, both male and female rats showed a robust rebound response, with running distances, times, and rates similar and proportional to those run during their most vigorous running period (first hour of the dark cycle). Although the subjects also displayed a rebound response upon removal of the wheel for only 1 hr, this 1-hr deprivation response was significantly less than the 72-hr response in every quantitative parameter and was not different in males and females. We consider this 1-hr deprivation to be a disruption period similar to that needed for animal husbandry and possibly overlooked as a deprivation period in many wheel-running paradigms. Our data suggest that the longer the forced abstinence from a stimulus with positive incentive salience, the greater the rewarding properties of the stimulus, as suggested by the elevated rebound running response after 72 hr of deprivation. All previous work using an uncondi-

tioned response to a wheel returned after forced abstinence was carried out using males, and we now additionally show that females also exhibit a robust effect, with a greater distance and faster running rate after a 72-hr forced abstinence.

Rebound running after forced abstinence can be seen as a process of wheel-running binge behavior, and we, as well as others, posit that this behavior is an unconditioned indicator of the incentive salience of this stimulus. Hill (1956, 1961) conducted the earliest test of wheel running behavior, but it may have been confounded by the stressful nature of the confined cage during the wheel deprivation phase (McGlone et al., 2004). Our approach was based on that of Mueller et al. (1999) who conducted an in-depth investigation of the effects of short-term (a few hours) wheel deprivation on running response after return of the wheel. Their data showed a marked rebound running effect upon wheel return, with a longer deprivation period resulting in a larger rebound effect. Further, they found that the increase in wheel running was proportional to the amount of running that would have occurred if animals had ad libitum access to the wheel.

The rebound effect has also been called "spontaneous recovery of response to a stimulus" or "reinstatement of stimulus response," and it occurs for other natural and pharmacological stimuli with positive incentive salience (see McSweeney et al., 2005 for review), suggesting that voluntary wheel running shares similar characteristics with other stimuli having positive incentive salience for rats. For example, after a period of forced abstinence, upon reexposure to drugs of abuse, such as alcohol, cocaine, or heroin, rats have been shown to binge or increase usage of the drug (Lê & Shaham, 2002; Shalev, Grimm, & Shaham, 2002). We now show that both male and female rats also binge on wheel running after a period of forced abstinence.

Running Farther or Faster Does Not Indicate Greater Incentive Salience

It is interesting that we found that rats that run longer distances do not have an increased preference for the total experience of wheel running during the acquisition phase. Our work accords with other studies that have shown that neither distance run nor running rate correlates with preference for the total experience or aftereffects of wheel running (Antoniadis et al., 2000; Belke & Wagner, 2005; Greenwood et al., 2011; Lett et al., 2002). Other studies have also reported that neither lever-pressing rates for access to a running wheel nor postreinforcement pauses showed a correlation with preference for the aftereffects of the wheel (Belke & Wagner, 2005). To those data, we add that gender does not influence the preference for voluntary wheel running. Thus, it is not the case that because females run on average 1.5 farther and faster than males (these data and Basso & Morrell, 2010), they prefer the running experience more.

Rats Have a Set Point for the Amount of Voluntary Wheel Running They Conduct

The facts that (a) during the habitual phase of running, rodents run relatively stable distances from day to day and (b) there is no correlation between the distance run and preference for running might indicate that there is a motivational set point for running that is unique to each subject. If we posit that rats run because it is rewarding, then we can imagine that a variety of reward-related brain processes are activated, including the dopamine, endogenous opioid, and/or endocannabinoid systems (Basso, Callahan, Farrar, Abercrombie, & Morrell, 2011; Boer, Epling, Pierce, & Russell, 1990; de Castro & Duncan, 1985; Fuss & Gass, 2010; Hoffmann, Terenius, & Thorén, 1990; Knab & Lightfoot, 2010; Lett, Grant, & Koh 2001; Monroe, Holmes, Koch, Britton, & Dishman, 2014; Rhodes & Garland, 2003; Sisti & Lewis, 2001; Tantimonaco et al., 2014; Werme, Thorén, Olson, & Brené, 2000). Rats may use running as way to acquire a set amount of neuronal stimulation or neurotransmitter/neuromodulator accumulation within the brain, which can be achieved by a certain amount of running per individual (i.e., a neural set point of running sufficiency). This set point appears to be altered by ovarian hormones, which have been implicated in regulating other types of rewards (Dreher et al., 2007; Parada, Vargas, Kyres, Burnside, & Pfaus, 2012; Russo et al., 2003; Seip & Morrell, 2008).

Brain Regions Important for Motivation to Wheel Run During the Habitual Phase of Wheel Running

We demonstrated that specific brain regions, namely the mPFC and NA, are required for the motivational processes that result in habitual-phase wheel running behavior. Inactivation of the mPFC and NA, but not other brain regions, significantly decreased distances and rates of rebound running during the first hour after 72 hr of forced wheel abstinence. The fact that the running response after only 1 hr of wheel deprivation was not decreased by inactivation of any brain region suggests that this rebound response is likely to be a more general disturbance response and is not regulated by the motivational system. Together, these data suggest that the prelimbic and infralimbic mPFC and NA core and shell might regulate the reinstatement response that occurs after significant wheel deprivation. The reinstatement response reflects the motivation for a behavior and occurs after periods of either food or drug abstinence, and just as the mPFC and NA are necessary for these motivated behaviors, this work indicates that it is also needed for the motivation to engage in voluntary wheel running.

Cannulated animals showed similarly robust rebound responses as well as daily distances run to their uncannulated counterparts. These data suggest that the lesions from cannulation did not impair either the motoric or motivational components of voluntary wheel running. However, saline infusion transiently blunted the rebound response as well as the daily distance run. We posit that this nonregion specific phenomenon may be a stress effect due to transfer to the infusion room, noise from the infusion pump, and insertion of the infusers. Although others have hypothesized that stressed rats run more, for example, for the purpose of fictive escape (see Sherwin, 1998 for review), here we see a situation where stress actually leads to a decrease in wheel running. If one reason that rats run is for fictive escape due to stress, this type of running might occur in short bursts (i.e., directly after the stressful incident), with the overall long-term effect being a decrease in running. Regardless of the reason for this effect, we realized that this response was an essential control or "new baseline," and thus our results from all inactivation infusions had to be directly compared with running responses after these saline infusions and not to noninfused animals.

Although we argue that the marked inhibition of the rebound response by inactivation of the mPFC or NA be taken as evidence that these regions are necessary for the motivation to wheel run, it remains a formal possibility that inactivation of these regions might result in a "higher" level motor skill impairment, which contributed to reduced rebound running. Thus, we recognize that in the future, including motivational tests that do not depend upon the running response or additional high-level motor skill testing would strengthen our conclusions.

Although no previous studies have examined the direct involvement of these brain regions in the motivation for voluntary wheel running, other evidence supports our finding that the mPFC and NA are involved in physical activity. For example, 30 days of ad libitum running increases levels of ΔFosB in the NA core, specifically in the dynorphin-containing neurons (Werme et al., 2002). ΔFosB is a transcription factor that accumulates in areas that have undergone chronic perturbation or stimulation, such as the striatum after repeated administration of drugs of abuse (Nestler, Kelz, & Chen, 1999). Additionally, rats that are well habituated to wheel running (6 weeks of ad libitum access) show lower levels of D2 dopamine receptor mRNA and higher levels of kappa opioid receptor mRNA in the NA core than their sedentary counterparts (Greenwood et al., 2011). Finally, in a study that used a methodology similar to that of the present work, suppressing or enhancing NA dopamine in rats selectively bred for high levels of voluntary wheel running causes decreased nightly running distances (Roberts et al., 2012). Collectively, these studies highlight the involvement of the dopaminergic and endogenous opioid systems of the NA in the motivation for voluntary wheel running.

Although little has been done to examine the effects of voluntary exercise on the mPFC in rodents, in humans, both cross-sectional as well as randomized controlled studies show that exercise causes improvements in a variety of PFC-dependent tasks including the Stroop task, Erickson Flanker task, and the N-back task (Dustman et al., 1984; Colcombe et al., 2004; Hillman et al., 2006; Smiley-Oyen, Lowry, Francois, Kohut, & Ekkekakis, 2008; Prakash et al., 2010; Guiney & Machado, 2013). These exercise-induced behavioral improvements in executive function are also accompanied by increased gray matter volume in the frontal lobe (Colcombe et al., 2006) and increased blood-oxygen-level-dependent activity in the PFC during task performance (Colcombe et al., 2004). Two hours of endurance running has also been associated with decreased opioid receptor availability in the orbitofrontal cortices as measured through positron emission tomography (Boecker et al., 2008), another indicator that the endogenous opioid system may be at play in the motivation for physical activity.

Acquisition of Running Is Impaired by Brain Cannulation in Healthy Subjects With Intact Locomotor Capacity

In contrast to the lack of behavioral impact of cannulation on habitual runners, cannulation during the acquisition period dramatically impaired daily distances run. Although running in these subjects remained lower than uncannulated subjects throughout wheel availability, it climbed steadily closer to subjects cannulated in the habitual phase of running, and eventually was not statistically different (Day 23 and on) from the latter group. Furthermore, although CPP tests in uncannulated subjects demonstrate that wheel availability during the acquisition phase of running had positive incentive salience, CPP tests after cannulation did not demonstrate this finding. The intervention of cannulation alone was sufficient to impair the CPP for wheel availability in the acquisition phase. Because the cannula surgery/headcap by itself impaired wheel running in acquisition, it is reasonable to suggest and explore in future experiments that this intervention consequently prevented any CPP being established in this initial stage of running acquisition when running was particularly low.

This change in running acquisition pattern and CPP response occurred regardless of the location of the cannula and hence was not brain-region dependent. It is important to note that none of the implant locations were in or near the motor cortex. Because these subjects and those cannulated during habitual phases of running were equally healthy, and at postmortem analysis, all brain tissue was healthy and without obvious lesions outside of cannula placement, health concerns were ruled out as a source of impairment. Furthermore, locomotor capacity of these subjects was normal (see Figure 8) as demonstrated by two data sets additional to the wheel running data. General locomotor activity measured by daily distance covered in home cage activity and locomotor activity within the CPP apparatus (i.e., the number of times they moved from one chamber to another in the exploration of the CPP apparatus) were both virtually identical to uncannulated animals. Our locomotor tests in the CPP apparatus and homecage demonstrate that the capacity to carry out the motor aspects of a CPP response as well as the normal activities of daily living were not impaired after the cannula surgery/headcap. Although we hypothesize a motivational impairment was the source of the CPP impairment, further tests will be needed to rule out the possibility of a higher level motor impairment as a source of the impaired wheel running and as a correlation to the impaired CPP formation.

The blunting of the acquisition of running by the presence of bilateral cannula and the altered CPP response to the wheel was surprising, as we have a considerable body of published work showing that the expression of maternal care or CPP measures of the incentive salience for pups or cocaine is not affected by cannulation of the mPFC, NA, or other forebrain targets (for review see Pereira & Morrell, 2011). Although the size and shape of the cannula/headset do not interfere with wheel running, it is possible that animals first exposed to the wheel with cannulation find this wheel interaction including climbing, swinging, walking, or slow running, more difficult or awkward, which prevents them from establishing the behavior in as robust a manner as uncannulated animals. This is in contrast to the fact that when animals are cannulated after running has reached its maximal daily pattern, their running patterns are no different than uncannulated subjects, and they reenter the wheel the moment they recover from anesthesia. Although it is very unusual for the presence of a fairly minor intrusion on brain tissue such as induced by a cannula to have such measurable effects, the literature does have other such examples, particularly in the case of the onset of maternal behaviors (Corodimas, Rosenblatt, & Morrell, 1992).

We were able to retest the motivation to run in the habitual phase of a significant subset of these subjects. After their acquisition phase CPP test, these subjects continued in the experiment, eventually acquiring habitual phase running. For the motivational test in these subjects, we used the rebound response test for two

reasons. First, we wanted to make a direct comparison to other subjects that had been assessed for their motivation only in the habitual phase. Second, we wanted to avoid confounded CPP results from a second CPP procedure, as our findings from past work (Seip et al., 2008) show that CPP training results in outcomes that remain stable upon retest even after long intervals, thus not demonstrating current incentive salience but training for a prior incentive salience.

The rebound response data showed that subjects cannulated prior to wheel access had in the habitual phase acquired an incentive salience for wheel running, and that they responded just as uncannulated or habitual phase cannulated subjects. Further, inactivation of mPFC or NA also inhibited rebound in these subjects. Thus, these subjects appeared similar in every way to those cannulated in the habitual phase.

We conclude that the acquisition phase of wheel running behavior is particularly fragile. We postulate that even minor changes in the animal's initial experience with running behavior are sufficient to impair the positive salience of the running, and hence impair the incentive salience of the experience as manifested in altered amounts of running as well as the CPP responses. It is also reasonable to postulate that once the long-term habit of running is established, the incentive salience of the experience is more than sufficient to overcome any postcannulation experience difficulties with the wheel running activity, hence the habit runner gets right back on the wheel, returning to their set daily running patterns.

A Recognition of the Limitations of Using a Rat Model and a Final Speculation About the Rewarding Nature of Exercise in Humans

When using an animal model to study a phenomenon in humans, one must consider the extent of similarities or differences between the behavior, anatomy, and physiology between humans and rodents. Rodents are used most frequently to study running behavior because rodents quickly and avidly utilize voluntary running wheels, which are a cheap and easy addition to any standard home cage. This model has been useful in the understanding of how running affects the brain in both healthy and preclinical models of diseases such as Parkinson's and Alzheimer's. However, the current animal model falls short in several ways and results may not directly translate to humans. For example, rodents given voluntary access to a running wheel spend approximately 1/3 of their active time (i.e., 4 hr of the 12-hr dark period) running. Therefore, the behavioral and brain effects that this and other research have examined are on a background of maximal exercise. Unless one is a competitive athlete, the average time people spend exercising per day is approximately 30 min, with only 5% of the American populace obtaining this amount of physical activity (U.S. Department of Agriculture, 2010). Therefore, it is difficult to predict whether this relatively minimal amount of exercise in humans causes the significant behavioral and brain changes seen with maximal exercise in rodents. Additionally, humans exist in a world abundant with enriching objects and experiences. In the rodent's world, the running wheel is one of the only enriched experiences that are offered to them in their lifetime. Perhaps it is not surprising that rodents find anything in addition to their normally deprived state rewarding, and the standard cage with running wheel may not be a directly comparable paradigm to the day-to-day experience of the human.

Despite these limitations, our findings from this preclinical model suggest that exercise in humans can be a rewarding experience, both during an initial acquisition period and after the exercise routine becomes habitual. Considering that 50% of the U.S. population does not attain the recommended daily level of aerobic exercise (American Heart Association, 2013; CDC, 2014) and obesity is an epidemic (34.9% of the U.S. population is obese), the lack of motivation to exercise is an obvious issue for the American populace. Participation in a daily physical activity regimen is one way to combat a variety of health-related issues; however, understanding the long-term physical and mental health benefits of exercise does not seem to be enough to get Americans to exercise. This work suggests that physical activity has immediate, short-term effects that can be robustly rewarding for both genders and that this rewarding experience may be a motivator to keep people exercising. The results also indicate that the acquisition period of the exercise regimen may be a more sensitive or critical time period than habitual phases of exercise. Therefore, an awareness of this in individuals who are just starting an exercise routine may be helpful in keeping them exercising until it becomes a habit. This is one example where habits are actually beneficial.

Finally, the present work demonstrates that the mPFC and NA might directly regulate the motivation for voluntary wheel running. Based on these preclinical studies, we speculate that the functional homologies of these regions might also be involved in the motivation for exercise in humans. The mPFC and NA are certainly involved in other disorders for which exercise has shown to have a positive benefit, such as depression, anxiety, obsessive–compulsive disorder, and addiction. This work suggests that these reward centers might also be dysregulated in people who have a severe lack of motivation for voluntary physical activity, such as individuals with obesity.

References

Afonso, V. M., & Eikelboom, R. (2003). Relationship between wheel running, feeding, drinking, and body weight in male rats. *Physiology & Behavior, 80,* 19–26. http://dx.doi.org/10.1016/S0031-9384(03)00216-6

Albelda, N., & Joel, D. (2012). Current animal models of obsessive compulsive disorder: An update. *Neuroscience, 211,* 83–106. http://dx.doi.org/10.1016/j.neuroscience.2011.08.070

American Heart Association. (2013). Physical inactivity. Retrieved from http://www.heart.org/idc/groups/heart-public/@wcm/@sop/@smd/documents/downloadable/ucm_319589.pdf

Antoniadis, E. A., Ko, C. H., Ralph, M. R., & McDonald, R. J. (2000). Circadian rhythms, aging and memory. *Behavioural Brain Research, 114,* 221–233. http://dx.doi.org/10.1016/S0166-4328(00)00290-4

Aoyama, K., & McSweeney, F. K. (2001). Habituation contributes to within-session changes in free wheel running. *Journal of the Experimental Analysis of Behavior, 76,* 289–302. http://dx.doi.org/10.1901/jeab.2001.76-289

Astur, R. S., Carew, A. W., & Deaton, B. E. (2014). Conditioned place preferences in humans using virtual reality. *Behavioural Brain Research, 267,* 173–177. http://dx.doi.org/10.1016/j.bbr.2014.03.018

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: What does it add to our preclinical understanding of drug reward? *Psychopharmacology, 153,* 31–43. http://dx.doi.org/10.1007/s002130000569

Bardo, M. T., Bowling, S. L., Robinet, P. M., Rowlett, J. K., Lacy, M., & Mattingly, B. A. (1993). Role of D_1 and D_2 receptors in novelty-

maintained place preference. *Experimental and Clinical Psychopharmacology, 1,* 101–109. http://dx.doi.org/10.1037/1064-1297.1.1-4.101

Barnett, S. A. (1958). Exploratory behaviour. *British Journal of Psychology, 49,* 289–310. http://dx.doi.org/10.1111/j.2044-8295.1958.tb00667.x

Basso, J. C., Callahan, J. W., Farrar, A. M., Abercrombie, E. D., & Morrell, J. I. (2011, November). *Voluntary wheel running throughout rearing in the rat alters baseline monoamine content and content levels in response to cocaine in brain regions that mediate motivation*. Poster session presented at the annual meeting of the Society for Neuroscience Conference, Washington, DC.

Basso, J. C., & Morrell, J. I. (2010, November). *Motivation for voluntary wheel running is indicated by analysis of this behavior across gender, wheel exposure history, and time availability of the wheel*. Poster session presented at the annual meeting of the Society for Neuroscience Conference, San Diego, CA.

Basso, J. C., & Morrell, J. I. (2012, October). *Emerging systems-level understanding of the motivation to engage in voluntary wheel running in the rat*. Poster session presented at the annual meeting of the Society for Neuroscience Conference, New Orleans, LA.

Belke, T. W. (1997). Running and responding reinforced by the opportunity to run: Effect of reinforcer duration. *Journal of the Experimental Analysis of Behavior, 67,* 337–351. http://dx.doi.org/10.1901/jeab.1997.67-337

Belke, T. W. (2006). Responding for sucrose and wheel-running reinforcement: Effect of pre-running. *Behavioural Processes, 71,* 1–7. http://dx.doi.org/10.1016/j.beproc.2005.08.003

Belke, T. W., & Heyman, G. M. (1994). The matching law: Analysis of the reinforcing efficacy of wheel running in rats. *Animal Learning & Behavior, 22,* 267–274. http://dx.doi.org/10.3758/BF03209834

Belke, T. W., & Pierce, W. D. (2009). Body weight manipulation, reinforcement value and choice between sucrose and wheel running: A behavioral economic analysis. *Behavioural Processes, 80,* 147–156. http://dx.doi.org/10.1016/j.beproc.2008.11.006

Belke, T. W., & Wagner, J. P. (2005). The reinforcing property and the rewarding aftereffect of wheel running in rats: A combination of two paradigms. *Behavioural Processes, 68,* 165–172. http://dx.doi.org/10.1016/j.beproc.2004.12.006

Boecker, H., Sprenger, T., Spilker, M. E., Henriksen, G., Koppenhoefer, M., Wagner, K. J., . . . Tolle, T. R. (2008). The runner's high: Opioidergic mechanisms in the human brain. *Cerebral Cortex, 18,* 2523–2531. http://dx.doi.org/10.1093/cercor/bhn013

Boer, D. P., Epling, W. F., Pierce, W. D., & Russell, J. C. (1990). Suppression of food deprivation-induced high-rate wheel running in rats. *Physiology & Behavior, 48,* 339–342. http://dx.doi.org/10.1016/0031-9384(90)90324-W

Centers for Disease Control and Prevention (CDC). (2014). *Facts about physical activity*. Retrieved from http://www.cdc.gov/physicalactivity/data/facts.html

Centers for Disease Control and Prevention (CDC). (2011). *Physical activity*. Retrieved from http://www.cdc.gov/physicalactivity/everyone/health/index.html?s_cid=cs_284

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., . . . Kramer, A. F. (2006). Aerobic exercise training increases brain volume in aging humans. *The Journals of Gerontology: Series A*. Biological Sciences and Medical Sciences, *61,* 1166–1170. http://dx.doi.org/10.1093/gerona/61.11.1166

Colcombe, S. J., Kramer, A. F., Erickson, K. I., Scalf, P., McAuley, E., Cohen, N. J., . . . Elavsky, S. (2004). Cardiovascular fitness, cortical plasticity, and aging. *Proceedings of the National Academy of Sciences of the United States of America, 101,* 3316–3321. http://dx.doi.org/10.1073/pnas.0400266101

Collier, G., & Hirsch, E. (1971). Reinforcing properties of spontaneous activity in the rat. *Journal of Comparative and Physiological Psychology, 77,* 155–160. http://dx.doi.org/10.1037/h0031588

Corodimas, K. P., Rosenblatt, J. S., & Morrell, J. I. (1992). The habenular complex mediates hormonal stimulation of maternal behavior in rats. *Behavioral Neuroscience, 106,* 853–865. http://dx.doi.org/10.1037/0735-7044.106.5.853

de Castro, J. M., & Duncan, G. (1985). Operantly conditioned running: Effects on brain catecholamine concentrations and receptor densities in the rat. *Pharmacology, Biochemistry, and Behavior, 23,* 495–500. http://dx.doi.org/10.1016/0091-3057(85)90407-1

Department of Health and Human Services. (1996). *Physical activity and health, a report of the Surgeon General*. Retrieved from http://www.cdc.gov/nccdphp/sgr/pdf/sgrfull.pdf

Dreher, J. C., Schmidt, P. J., Kohn, P., Furman, D., Rubinow, D., & Berman, K. F. (2007). Menstrual cycle phase modulates reward-related neural function in women. *Proceedings of the National Academy of Sciences of the United States of America, 104,* 2465–2470. http://dx.doi.org/10.1073/pnas.0605569104

Dustman, R. E., Ruhling, R. O., Russell, E. M., Shearer, D. E., Bonekat, H. W., Shigeoka, J. W., . . . Bradford, D. C. (1984). Aerobic exercise training and improved neuropsychological function of older individuals. *Neurobiology of Aging, 5,* 35–42. http://dx.doi.org/10.1016/0197-4580(84)90083-6

Eikelboom, R. (1999). Human parallel to voluntary wheel running: Exercise. *Animal Behaviour, 57,* F11–F12. http://dx.doi.org/10.1006/anbe.1998.1045

Eikelboom, R., & Mills, R. (1988). A microanalysis of wheel running in male and female rats. *Physiology & Behavior, 43,* 625–630. http://dx.doi.org/10.1016/0031-9384(88)90217-X

Ferreira, A., Lamarque, S., Boyer, P., Perez-Diaz, F., Jouvent, R., & Cohen-Salmon, C. (2006). Spontaneous appetence for wheel-running: A model of dependency on physical activity in rat. *European Psychiatry, 21,* 580–588. http://dx.doi.org/10.1016/j.eurpsy.2005.02.003

Fuss, J., & Gass, P. (2010). Endocannabinoids and voluntary activity in mice: Runner's high and long-term consequences in emotional behaviors. *Experimental Neurology, 224,* 103–105. http://dx.doi.org/10.1016/j.expneurol.2010.03.016

Greenwood, B. N., Foley, T. E., Le, T. V., Strong, P. V., Loughridge, A. B., Day, H. E. W., & Fleshner, M. (2011). Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behavioural Brain Research, 217,* 354–362. http://dx.doi.org/10.1016/j.bbr.2010.11.005

Guiney, H., & Machado, L. (2013). Benefits of regular aerobic exercise for executive functioning in healthy populations. *Psychonomic Bulletin & Review, 20,* 73–86. http://dx.doi.org/10.3758/s13423-012-0345-4

Hattori, S., Naoi, M., & Nishino, H. (1994). Striatal dopamine turnover during treadmill running in the rat: Relation to the speed of running. *Brain Research Bulletin, 35,* 41–49. http://dx.doi.org/10.1016/0361-9230(94)90214-3

Hill, W. F. (1956). Activity as an autonomous drive. *Journal of Comparative and Physiological Psychology, 49,* 15–19. http://dx.doi.org/10.1037/h0044786

Hill, W. F. (1961). Effects of activity deprivation on choice of an activity incentive. *Journal of Comparative and Physiological Psychology, 54,* 78–82. http://dx.doi.org/10.1037/h0043817

Hillman, C. H., Motl, R. W., Pontifex, M. B., Posthuma, D., Stubbe, J. H., Boomsma, D. I., & de Geus, E. J. (2006). Physical activity and cognitive function in a cross-section of younger and older community-dwelling individuals. *Health Psychology, 25,* 678–687. http://dx.doi.org/10.1037/0278-6133.25.6.678

Hoffmann, P., Terenius, L., & Thorén, P. (1990). Cerebrospinal fluid immunoreactive beta-endorphin concentration is increased by voluntary

exercise in the spontaneously hypertensive rat. *Regulatory Peptides, 28,* 233–239. http://dx.doi.org/10.1016/0167-0115(90)90021-N

Iversen, I. H. (1993). Techniques for establishing schedules with wheel running as reinforcement in rats. *Journal of the Experimental Analysis of Behavior, 60,* 219–238. http://dx.doi.org/10.1901/jeab.1993.60-219

Kagan, J., & Berkun, M. (1954). The reward value of running activity. *Journal of Comparative and Physiological Psychology, 47,* 108. http://dx.doi.org/10.1037/h0058877

Knab, A. M., & Lightfoot, J. T. (2010). Does the difference between physically active and couch potato lie in the dopamine system? *International Journal of Biological Sciences, 6,* 133–150. http://dx.doi.org/10.7150/ijbs.6.133

Lê, A., & Shaham, Y. (2002). Neurobiology of relapse to alcohol in rats. *Pharmacology & Therapeutics, 94,* 137–156. http://dx.doi.org/10.1016/S0163-7258(02)00200-0

Lett, B. T., Grant, V. L., Byrne, M. J., & Koh, M. T. (2000). Pairings of a distinctive chamber with the aftereffect of wheel running produce conditioned place preference. *Appetite, 34,* 87–94. http://dx.doi.org/10.1006/appe.1999.0274

Lett, B. T., Grant, V. L., & Koh, M. T. (2001). Naloxone attenuates the conditioned place preference induced by wheel running in rats. *Physiology & Behavior, 72,* 355–358. http://dx.doi.org/10.1016/S0031-9384(00)00427-3

Lett, B. T., Grant, V. L., & Koh, M. T. (2002). Delayed backward conditioning of place preference induced by wheel running in rats. *Learning and Motivation, 33,* 347–357. http://dx.doi.org/10.1016/S0023-9690(02)00002-4

Mattson, B. J., Williams, S., Rosenblatt, J. S., & Morrell, J. I. (2001). Comparison of two positive reinforcing stimuli: Pups and cocaine throughout the postpartum period. *Behavioral Neuroscience, 115,* 683–694. http://dx.doi.org/10.1037/0735-7044.115.3.683

Mattson, B. J., Williams, S. E., Rosenblatt, J. S., & Morrell, J. I. (2003). Preferences for cocaine- or pup-associated chambers differentiates otherwise behaviorally identical postpartum maternal rats. *Psychopharmacology, 167,* 1–8.

McGlone, J. J., Von Borell, E. H., Deen, J., Johnson, A. K., Levis, D. G., Meunier-Salaun, M., . . . Sundberg, P. L. (2004). Review: Compilation of the scientific literature comparing housing systems for gestating sows and gilts using measures of physiology, behavior, performance and health. *The Professional Animal Scientist, 20,* 105–117.

McSweeney, F. K., Murphy, E. S., & Kowal, B. P. (2005). Regulation of drug taking by sensitization and habituation. *Experimental and Clinical Psychopharmacology, 13,* 163–184. http://dx.doi.org/10.1037/1064-1297.13.3.163

Meeusen, R., Smolders, I., Sarre, S., de Meirleir, K., Keizer, H., Serneels, M., . . . Michotte, Y. (1997). Endurance training effects on neurotransmitter release in rat striatum: An in vivo microdialysis study. *Acta Physiologica Scandinavica, 159,* 335–341. http://dx.doi.org/10.1046/j.1365-201X.1997.00118.x

Meijer, J. H., & Robbers, Y. (2014). Wheel running in the wild. *Proceedings of the Royal Society: B*. Biological Sciences, *281,* 1–5. http://dx.doi.org/10.1098/rspb.2014.0210

Molet, M., Billiet, G., & Bardo, M. T. (2013). Conditioned place preference and aversion for music in a virtual reality environment. *Behavioural Processes, 92,* 31–35. http://dx.doi.org/10.1016/j.beproc.2012.10.001

Monroe, D. C., Holmes, P. V., Koch, L. G., Britton, S. L., & Dishman, R. K. (2014). Striatal enkephalinergic differences in rats selectively bred for intrinsic running capacity. *Brain Research, 1572,* 11–17. http://dx.doi.org/10.1016/j.brainres.2014.05.014

Mueller, D. T., Herman, G., & Eikelboom, R. (1999). Effects of short- and long-term wheel deprivation on running. *Physiology & Behavior, 66,* 101–107. http://dx.doi.org/10.1016/S0031-9384(98)00278-9

Mueller, D. T., Loft, A., & Eikelboom, R. (1997). Alternate-day wheel access: Effects on feeding, body weight, and running. *Physiology & Behavior, 62,* 905–908. http://dx.doi.org/10.1016/S0031-9384(97)00266-7

Napier, T. C., Herrold, A. A., & de Wit, H. (2013). Using conditioned place preference to identify relapse prevention medications. *Neuroscience and Biobehavioral Reviews, 37* (9 Pt. A), 2081–2086. http://dx.doi.org/10.1016/j.neubiorev.2013.05.002

National Institutes of Health. (2011). *Guide for the care and use of laboratory animals* (8th ed.) (NIH Publication No. 80-23, revised 1996). Bethesda, MD: Author. Retrieved from https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf

Nestler, E. J., Kelz, M. B., & Chen, J. (1999). ΔFosB: A molecular mediator of long-term neural and behavioral plasticity. *Brain Research, 835,* 10–17. http://dx.doi.org/10.1016/S0006-8993(98)01191-3

Parada, M., Vargas, E. B., Kyres, M., Burnside, K., & Pfaus, J. G. (2012). The role of ovarian hormones in sexual reward states of the female rat. *Hormones and Behavior, 62,* 442–447. http://dx.doi.org/10.1016/j.yhbeh.2012.07.012

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (6th ed.). San Diego, CA: Academic Press.

Pereira, M., & Morrell, J. I. (2011). Functional mapping of the neural circuitry of rat maternal motivation: Effects of site-specific transient neural inactivation. *Journal of Neuroendocrinology, 23,* 1020–1035. http://dx.doi.org/10.1111/j.1365-2826.2011.02200.x

Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learning & Memory, 16,* 279–288. http://dx.doi.org/10.1101/lm.1041309

Peters, J., LaLumiere, R. T., & Kalivas, P. W. (2008). Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *The Journal of Neuroscience, 28,* 6046–6053. http://dx.doi.org/10.1523/JNEUROSCI.1045-08.2008

Pierce, W. D., Epling, W. F., & Boer, D. P. (1986). Deprivation and satiation: The interrelations between food and wheel running. *Journal of the Experimental Analysis of Behavior, 46,* 199–210. http://dx.doi.org/10.1901/jeab.1986.46-199

Prakash, R. S., Voss, M. W., Erickson, K. I., Lewis, J. M., Chaddock, L., Malkowski, E., . . . Kramer, A. F. (2010). Cardiorespiratory fitness and attentional control in the aging brain. *Frontiers in Human Neuroscience, 4,* 229. http://dx.doi.org/10.3389/fnhum.2010.00229

Premack, D., Schaeffer, R. W., & Hundt, A. (1964). Reinforcement of drinking by running: Effects of fixed ratio and reinforcement time. *Journal of the Experimental Analysis of Behavior, 7,* 91–96. http://dx.doi.org/10.1901/jeab.1964.7-91

Quirk, G. J., Garcia, R., & González-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biological Psychiatry, 60,* 337–343. http://dx.doi.org/10.1016/j.biopsych.2006.03.010

Ralph, M. R., Ko, C. H., Antoniadis, E. A., Seco, P., Irani, F., Presta, C., & McDonald, R. J. (2002). The significance of circadian phase for performance on a reward-based learning task in hamsters. *Behavioural Brain Research, 136,* 179–184. http://dx.doi.org/10.1016/S0166-4328(02)00131-6

Rhodes, J. S., & Garland, T. (2003). Differential sensitivity to acute administration of Ritalin, apomorphine, SCH 23390, but not raclopride in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology, 167,* 242–250.

Richter, C. P. (1927). Animal behavior and internal drives. *Quarterly Review of Biology, 2,* 307–343. http://dx.doi.org/10.1086/394279

Roberts, M. D., Gilpin, L., Parker, K. E., Childs, T. E., Will, M. J., & Booth, F. W. (2012). Dopamine D1 receptor modulation in nucleus accumbens lowers voluntary wheel running in rats bred to run high distances. *Physiology & Behavior, 105,* 661–668. http://dx.doi.org/10.1016/j.physbeh.2011.09.024

Rocha, A., & Kalivas, P. W. (2010). Role of the prefrontal cortex and nucleus accumbens in reinstating methamphetamine seeking. *European Journal of Neuroscience, 31,* 903–909. http://dx.doi.org/10.1111/j.1460-9568.2010.07134.x

Russo, S. J., Festa, E. D., Fabian, S. J., Gazi, F. M., Kraish, M., Jenab, S., & Quiñones-Jenab, V. (2003). Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. *Neuroscience, 120,* 523–533. http://dx.doi.org/10.1016/S0306-4522(03)00317-8

Seip, K. M., & Morrell, J. I. (2008). Exposure to pups influences the strength of maternal motivation in virgin female rats. *Physiology & Behavior, 95,* 599–608. http://dx.doi.org/10.1016/j.physbeh.2008.09.003

Seip, K. M., Pereira, M., Wansaw, M. P., Reiss, J. I., Dziopa, E. I., & Morrell, J. I. (2008). Incentive salience of cocaine across the postpartum period of the female rat. *Psychopharmacology, 199,* 119–130. http://dx.doi.org/10.1007/s00213-008-1140-9

Shalev, U., Grimm, J. W., & Shaham, Y. (2002). Neurobiology of relapse to heroin and cocaine seeking: A review. *Pharmacological Reviews, 54,* 1–42. http://dx.doi.org/10.1124/pr.54.1.1

Sherwin, C. M. (1998). Voluntary wheel running: A review and novel interpretation. *Animal Behaviour, 56,* 11–27. http://dx.doi.org/10.1006/anbe.1998.0836

Shirley, M. (1929). Spontaneous activity. *Psychological Bulletin, 26,* 341–365. http://dx.doi.org/10.1037/h0073516

Sisti, H. M., & Lewis, M. J. (2001). Naloxone suppression and morphine enhancement of voluntary wheel-running activity in rats. *Pharmacology Biochemistry and Behavior, 70,* 359–365. http://dx.doi.org/10.1016/S0091-3057(01)00624-4

Smiley-Oyen, A. L., Lowry, K. A., Francois, S. J., Kohut, M. L., & Ekkekakis, P. (2008). Exercise, fitness, and neurocognitive function in older adults: The "selective improvement" and "cardiovascular fitness" hypotheses. *Annals of Behavioral Medicine, 36,* 280–291. http://dx.doi.org/10.1007/s12160-008-9064-5

Smith, K. S., & Morrell, J. I. (2007). Comparison of infant and adult rats in exploratory activity, diurnal patterns, and responses to novel and anxiety-provoking environments. *Behavioral Neuroscience, 121,* 449–461. http://dx.doi.org/10.1037/0735-7044.121.3.449

Smith, K. S., & Morrell, J. I. (2011). Behavioral differences between late preweanling and adult female Sprague–Dawley rat exploration of animate and inanimate stimuli and food. *Behavioural Brain Research, 217,* 326–336. http://dx.doi.org/10.1016/j.bbr.2010.10.038

Stewart, C. C. (1898). Variations in daily activity produced by alcohol, by changes in barometric pressure and diet, with a description of recording methods. *The American Journal of Physiology, 1,* 39–56.

Sugimoto, N., Shido, O., Sakurada, S., & Nagasaka, T. (1994). Persisting changes in the 24-hour profile of locomotor activity by daily activity restriction in rats. *Japanese Journal of Physiology, 44,* 735–742. http://dx.doi.org/10.2170/jjphysiol.44.735

Tantimonaco, M., Ceci, R., Sabatini, S., Catani, M. V., Rossi, A., Gasperi, V., & Maccarrone, M. (2014). Physical activity and the endocannabinoid system: An overview. *Cellular and Molecular Life Sciences, 71,* 2681–2698. http://dx.doi.org/10.1007/s00018-014-1575-6

U.S. Department of Agriculture. *Dietary guidelines for Americans, 2010.* Retrieved from http://www.cnpp.usda.gov/dietaryguidelines.htm

Wansaw, M. P., Pereira, M., & Morrell, J. I. (2008). Characterization of maternal motivation in the lactating rat: Contrasts between early and late postpartum responses. *Hormones and Behavior, 54,* 294–301. http://dx.doi.org/10.1016/j.yhbeh.2008.03.005

Werme, M., Messer, C., Olson, L., Gilden, L., Thorén, P., Nestler, E. J., & Brené, S. (2002). Δ FosB regulates wheel running. *The Journal of Neuroscience, 22,* 8133–8138.

Werme, M., Thorén, P., Olson, L., & Brené, S. (2000). Running and cocaine both upregulate dynorphin mRNA in medial caudate putamen. *European Journal of Neuroscience, 12,* 2967–2974. http://dx.doi.org/10.1046/j.1460-9568.2000.00147.x

Wilson, W. M., & Marsden, C. A. (1995). Extracellular dopamine in the nucleus accumbens of the rat during treadmill running. *Acta Physiologica Scandinavica, 155,* 465–466. http://dx.doi.org/10.1111/j.1748-1716.1995.tb09997.x

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