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Sex differences in cannabinoid receptor-1 (CB1) pharmacology in mice selectively bred for high voluntary wheel-running behavior

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ABSTRACT

The endocannabinoid system (ECS) is involved in regulation of various physiological functions, including locomotion, antinociception, emotional states, and motivated behaviors. The ECS has been implicated in regulation of voluntary wheel running in mice via actions at the cannabinoid receptor-1 (CB1). Previously, we showed that four replicate lines of mice bred for high levels of voluntary wheel running (high-runner or HR lines) sex-specifically (females only) decreased running in response to antagonism of the CB1 receptor, as compared with four unselected Control lines. Here, we administered a CB1 receptor agonist, WIN 55,212-2 (WIN). We predicted that if CB1 activation is involved in the regulation of voluntary wheel running, then HR mice would show a greater response to CB1 agonism. Following our previous protocols, mice from generation 53 were acclimated to running wheels for 24 days, then received, in random order, either an intra-peritoneal injection of vehicle or a low (0.5 mg/kg), medium (1 mg/kg) or high dosage (3 mg/kg) of WIN. Each mouse received an injection and then experienced two nights without injections, for a total period of 12 days. Response to WIN was quantified as wheel revolutions, time spent running, and average running speed in the 10–120 min immediately following injection. Injection decreased wheel revolutions in all mice, but male HR mice decreased their running to a greater degree relative to Controls in response to the high dose of WIN over the entire period analyzed, whereas HR females showed a differential response relative to Controls only in the latter 70–120 min post-injection. These results, in conjunction with our previous study, show that (a) aspects of endocannabinoid signaling have diverged in four lines of mice bred for high levels of voluntary exercise and (b) male and female HR mice differ from one another in CB1 signaling as it relates to wheel running.

1. Introduction

Understanding the control of voluntary behavior is one of the greatest challenges for neurobiology. In particular, knowledge of how the brain motivates imperative, yet potentially costly behaviors – such as voluntary exercise – is of great relevance to an increasingly inactive human population (Garland et al., 2011a). The literature reflects an almost endless array of approaches to study the neurobiology of exercise; we have chosen to use a long-term selection experiment that targets high levels of voluntary wheel running in mice (Garland et al., 2011b; Rhodes et al., 2005; Swallow et al., 2009).

Within this experiment, four independent, genetically closed lines of house mice (*Mus domesticus*) have been selectively bred since 1993 (>60 generations) on the basis of their voluntary wheel running on days 5 and 6 of a 6-day trial (High Runner lines, HR), in parallel with four unselected Control lines. After 10 generations of selection, HR mice of both sexes ran at least 70% more revolutions/day than their Control counterparts (Swallow et al., 1998). The divergence between HR and Control lines eventually reached a plateau at a differential of approximately +170% (Kolb et al., 2010; Rhodes et al., 2000; Swallow et al., 2009). Concomitant with increases in voluntary wheel running, HR mice have also undergone a shift toward increased levels of spontaneous physical activity (SPA) in cages when wheels are absent (Malisch et al., 2009; Rhodes et al., 2001). Likewise, when running wheels were locked, HR mice spent more time climbing in the locked wheels, apparently trying to run (Koteja et al., 1999).

In addition to changes in locomotor behavior (see also Girard et al., 2001), the selective breeding regimen has led to changes in capacities for aerobic exercise (Kolb et al., 2010; Meek et al., 2009), and in various lower-level morphological and physiological traits that may affect endurance capacity (Garland, 2003). For example, HR mice exhibit reduced total body mass (Swallow et al., 1999), reduced body fat (Meek et al., 2010; Swallow et al., 2001; Vaanholt et al., 2008), more symmetrical hind limb bones (Garland and Freeman, 2005), higher circulating corticosterone (Girard and Garland, 2002; Malisch et al., 2008) and adiponectin levels (Vaanholt et al., 2007), as well as

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increased plasticity of some traits in response to wheel access (Gomes et al., 2009).

Although it is currently not well understood to what degree voluntary exercise can be considered a classical motivated behavior (Garland et al., 2011a), several studies in HR mice have documented divergences in the neural systems traditionally thought to regulate motivation and reward (see also Bronikowski et al., 2004; Belke and Garland, 2007). For instance, studies found linetype wheel-running differences in response to drugs that affect the D1 receptor system (but not in the D2 receptor, serotonergic or opioidergic systems) (Li et al., 2004; Rhodes et al., 2001, 2003, 2005; Rhodes et al., 2001; Rhodes and Garland, 2003). In addition, Fos immunohistochemistry showed HR mice to have a greater proportional increase in activity in some brain regions implicated in reward and motivation when wheel access is blocked, consistent with a state of withdrawal (Rhodes et al., 2003). Mathes et al. (2010) hypothesized that many of these differences reflect an overall dysregulation of dopaminergic signaling.

Alongside alterations in dopaminergic and reward signaling, we hypothesized that HR mice differ from Controls in their response to drugs that act upon one of the major receptors (cannabinoid receptor-1; CB1) of the endocannabinoid system (ECS) (Keeney et al., 2008). The ECS is a complex modulatory system, primarily composed of cannabinoid receptors, their endogenous ligands (endocannabinoids), and proteins involved in the synthesis and modification of endocannabinoids. Although the role of central cannabinoid signaling as mediated by the CB1 receptor is not fully understood, cannabinoids likely have a natural role in antinociception, memory, the perception of natural rewards, and the regulation of complex locomotor outputs (particularly those paired with rewarding stimuli) (De Chiara et al., 2010; Iversen, 2003).

Much evidence suggests a relationship between endocannabinoid signaling and physical activity (see Fuss and Gass, 2010 for review). Recent discussion has highlighted the tight involvement of central endocannabinoid activity with the expression of motor behavior (El Manira and Kyriakatos, 2010), and in particular, voluntary running (Chaouloff et al., 2011). Specific to rodent systems, it has been suggested that the ECS may regulate wheel-running behavior (Chaouloff et al., 2011; Dubreucq et al., 2010). To that end, Hill et al. (2010) have shown that wheel running, a form of voluntary exercise in rodents (Garland et al., 2011a), increases both CB1 signaling and the concentration of anandamide within the hippocampal formation of rats. Likewise, mouse synaptic responses to HU210, a selective cannabinoid CB1 receptor agonist, were greatly potentiated following 7 or 15 days of wheel access (De Chiara et al., 2010). Similarly, two weeks of voluntary wheel access was found to sensitize CB1 receptor-mediated inhibition of striatal GABAergic transmission in mice (Rossi et al., 2009). CB1 knockout mice showed less voluntary wheel running over a period of 6 weeks as compared with their wildtype counterparts (Dubreucq et al., 2010); however, they did not differ statistically in locomotion in an activity cage, exploration in an open field, or immobility time in the forced swim test. Similarly, Chaouloff et al. (2011) found that male mice lacking CB1 receptors display decreased voluntary running when housed with a running wheel for several weeks when compared to wild-type littermates (attributable to a decrease in the time spent running). In humans, parallels between the psychotropic effects of traditional cannabinoid drugs and the positive feelings associated with sustained, endurance-type exercise has led some to hypothesize that endocannabinoids may be involved, at least in part, with a so-called "runner's high" sensation that may help to motivate exercise behaviors (Dietrich and McDaniel, 2004). Consistent with this hypothesis, Sparling et al. (2003) showed that anandamide, the major endogenous ligand of the ECS, is increased in the circulation following exercise in trained male college students.

In the context of our system, we have previously shown that HR mice have a differential and sex-specific wheel-running response to intraperitoneal (i.p.) injection of a selective CB1 antagonist (SR141716; Rimonabant) (Keeney et al., 2008). When transmission at the CB1 receptor was blocked, female HR mice decreased running to a greater degree than male HR mice or those from Control lines. Although that study was the first to use pharmacology on both sexes of HR mice to demonstrate possible differences in neural correlates of wheel running, we have long known that male and female HR mice have responded differently to selective breeding for high wheel running (Garland et al., 2011b; Keeney et al., 2008). Specifically, female HR mice have evolved their higher daily running distances almost entirely by increasing the speed at which they run, whereas males have shown increases in both the speed and duration of wheel activity (Garland, 2003; Girard et al., 2001; Keeney et al., 2008; Koteja and Garland, 2001; Rezende et al., 2009; Rhodes et al., 2000; Swallow et al., 1998, 1999).

It is well known that males and females of many species may accomplish a given behavior in different ways, often as a result of the influence of either androgens or hormones of the estrous cycle. It is not definitively known to what extent such behaviors as voluntary exercise differ by sex, nor how these putative differences manifest in the brain (see Lightfoot, 2008 for review). The HR and their Control lines offer a unique system in which to explore the neurobiological underpinnings of an "exerciser" phenotype, as well as how these systems may differ between the sexes. Given the literature, and our previous results for this system, we believe that the CB1 receptor is important in the neural control of voluntary exercise in general, and specifically to the evolution of the HR phenotype. The role of CB1 transmission in sex-specific voluntary exercise is not yet clear; nor is it clear if stimulation of this receptor would have similar results by sex or by linetype (HR or Control). To further address these questions, we administered a CB1 receptor agonist (WIN 55,212-2) to HR and Control mice of both sexes and observed their subsequent wheel running (at the time of peak nightly activity). We predicted that if, as indicated by our previous study (Keeney et al., 2008), there are sex-specific differences in ECS physiology that underlie high levels of voluntary wheel running, then male and female HR mice will differ from their Control counterparts in their wheel-running response to CB1 agonism.

2. Methods

2.1. Animals

The subjects of study were male and female mice (*M. domesticus*), originally derived from Hsd:ICR stock (Harlan Sprague Dawley, Indianapolis, Indiana, USA). As discussed in detail elsewhere (Swallow et al., 1998), four lines were designated for selection for high voluntary wheel running on days 5 and 6 of 6-day period of wheel access (High Runner or HR lines), while four additional lines were maintained without selective breeding to serve as controls for random genetic effects, including drift (Control lines). In brief, the general selection protocol is as follows. Following birth, mice are weighed, toe-clipped for individual identification, and weaned at 21 days of age. Mice are then housed four/cage/sex by line. At ~6–8 weeks of age, mice are housed individually in cages with wheel (1.12 m circumference) access for a 6-day period. Wheel revolutions are recorded daily in 1-minute intervals by a photocell counter attached to the wheel. Revolutions are compiled via customized software (San Diego Instruments, San Diego, California, USA). Following wheel-testing, breeders are selected for the next generation. In the HR lines, a male and a female mouse from each family are selected for having the highest total revolutions during days 5 and 6 of the wheel-running trial. In the Control lines, a male and a female mouse from each family are chosen without regard to wheel revolutions. Breeders are then randomly paired within each line, with the exception that sibling pairs are not allowed. Throughout the selection process, and in all studies described here, mice are maintained on a standard 12-h light/dark cycle, with ad lib access to water and food.

Following our previous protocol (Keeney et al., 2008), mice for the current study were chosen from among those that underwent the routine 6-day wheel-running trial. Our sample excluded both the highest and lowest runners from each family. Of the remaining mice, one male or female was chosen from each family for a total sample size of 96 (48 males and 48 females), equally representing all 8 independently breeding lines (4 HR, 4 Control). Mice were allowed 24 days of acclimation to wheels prior to drug testing. While with wheel access, mice were maintained on a 12:12 photoperiod with lights on at 03:00 h and lights off at 15:00 h. Placement of mice with wheels was randomized with respect to sex and line, and experimenters were blind to sex, line, and linetype (HR or Control). Animal procedures were in accordance with University guidelines and with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. Drug protocol

WIN 55,212-2 (WIN) was obtained from BIOMOL International, LP (Enzo Life Sciences International, Inc., Plymouth Meeting, PA), and then dissolved in a vehicle solution of DMSO (20% final volume), Tween-80 (10% final volume), and physiological saline (70% final volume). Vehicle solutions were added in the order listed, with vigorous vortexing between steps. This vehicle has been used for the delivery of WIN in both rats (French, 1997; Hoffman et al., 2003) and mice (Kochman et al., 2006). This vehicle solution does not by itself influence open-field locomotor behavior in mice (Gerdeman et al., 2008), nor extinguish nightly wheel running in mice from the HR and Control lines (Keeney et al., 2008).

All drug solutions were prepared fresh immediately prior to use. On the 25th night of wheel-access, mice were divided into three batches to minimize the length of any disturbance during the active period. Batches were randomized by line and sex. At two hours after lights-off (17:00 h), during typical peak wheel-running activity (Girard et al., 2001; Girard and Garland, 2002; Malisch et al., 2008; Rhodes et al., 2003), a single batch received treatments. The total injection period for a batch was roughly two hours (17:00 h–19:00 h). Each mouse in a batch received one of four treatments (vehicle injection; low WIN [0.5 mg/kg]; medium WIN [1 mg/kg]; high WIN [3 mg/kg]) via i.p. injections. Doses were chosen based upon review of the literature, with specific attention to Patel and Hillard (2006). Injection volumes were adjusted for dose and body-mass of the animal. Over the entire experimentation period, injection volumes ranged from 0.104 to 0.242 mL for body masses that ranged from 20.7 g to 48.4 g.

Following the design of Li et al. (2004) and Keeney et al. (2008), each mouse received one treatment per three-night period until every individual had been injected with every treatment (12 nights total), with 72 h between each injection to avoid carryover effects. Six individuals from batch one were initially tested at a higher maximum dose level (10 mg/kg WIN) but did not display any wheel running (or activity at all) over the entire testing period. This dose was subsequently abandoned, and these individuals received an extra night of injections, such that they received all four doses comparable to the rest of the sample, making 13 total nights of injections for this subset.

Following Keeney et al. (2008), the acute locomotor response to treatment was measured as the total number of wheel revolutions in the period from 10 to 70 min, and also 70 to 120 min post-injection (not analyzed in our previous study). These time points are consistent with the known time course of WIN in mice (Spina et al., 1998), as well as injection effects on HR wheel-running (Keeney et al., 2008; Li et al., 2004). In addition to wheel revolutions, we also analyzed the number of 1-minute intervals with at least one revolution (time spent running), the average running speed (revolutions/minute), and the maximum running speed (revolutions in the single highest 1-minute interval) for the same time periods.

2.3. Statistical analysis

During the course of experimentation, a total of 4 males and 1 female were eliminated from the sample because of death before or during experimentation (3 males), or because they were observed to exhibit twirling behavior (running in rapid, small, stereotypic circles) in their cages (1 male and 1 female). Individuals with injection problems, wheel malfunction or injury were excluded from analysis on a night-by-night basis. Thus, 44 males and 47 females were statistically analyzed (total $N = 91$).

Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA). Analyses were first conducted separately by sex. The primary grouping factors were linetype (HR vs. Control) and dose, with replicate line as a random effect nested within linetype. Individual was the factor for repeated measures, and we assumed compound symmetry of the residual covariance matrix in SAS Procedure Mixed. In this mixed-model analysis of covariance, the degrees of freedom for testing the effect of linetype, relative to line, are always 1 and 6. For dose and the dose∗linetype interaction (tested relative to the dose∗line [linetype] effect), degrees of freedom are 2 and 12. This interaction term is of chief interest because, if statistically significant, it indicates a differential response of the HR and Control lines to the drug dose. Wheel-freeness (a measure of how long each wheel rotates following acceleration to a constant velocity) was recorded four days prior to injections and was included as a covariate in statistical analyses, as was individual age. After inspection of the residuals from the statistical models, all wheel-running traits were transformed by raising to the 0.6 power in order to reduce skewness. For analyses of proportional responses, all values were $\log_{10}$-transformed prior to analyses.

3. Results

3.1. Baseline wheel running and effects of vehicle injection

For generation 53, 553 mice representative of all eight lines underwent the standard 6-day wheel test ($N = 272$ females, 281 males). Females from the HR lines ($10{,}004 \pm 913$ rev/day; least-squares mean ± standard error) ran 3.01-fold more than Control females (3323 ± 387 rev/day) on days 5 + 6 ($p = 0.0005$). HR males (7126 ± 490 rev/day) ran 2.7-fold more than Control males (2636 ± 493 rev/day) on days 5 + 6 ($p = 0.0007$).

Results were similar for the subset of males and females used in the present experiment (47 males and 47 females from the present study underwent wheel testing; one male and female did not, but were included as replacements at a later date due to unexpected mortality). Females from the HR lines (8749 ± 843 rev/day) ran 2.92-fold more than Control females (2995 ± 490 rev/day) on days 5 + 6 ($p = 0.0011$). For males, HR ran 6599 (±549) as compared with 2680 (±392) revolutions/day for Control, yielding a 2.46-fold differential ($p = 0.0011$). As expected, Online Supplemental Figure A shows that HR mice ran significantly more than C mice in the three days prior to injections. Likewise, females of both linetypes (HR and C) ran more than males of both linetypes.

Fig. 1 shows the average wheel-running during the 30 min prior to injections (an hour and forty five minutes past lights off), as well as in each 10-minute interval during the first 10–130 min post-injection. Prior to injections, females from the HR lines (273 ± 18 rev/10 min) were running 2.95-fold more than Control females (94 ± 18) ($p = 0.022$). For males, HR ran 252 (±11 rev, $p = 0.0001$) as compared with 95 (±11 rev, $p < 0.0001$) revolutions/10 min for Control, yielding a 2.65-fold differential ($p < 0.0001$). Following vehicle injection, a repeated-measures ANCOVA for the difference between the average wheel-running during the 30 min prior to injection and the average wheel-running during the first 10–70 min following injection (with covariates of age and wheel-freeness) shows that both female HR

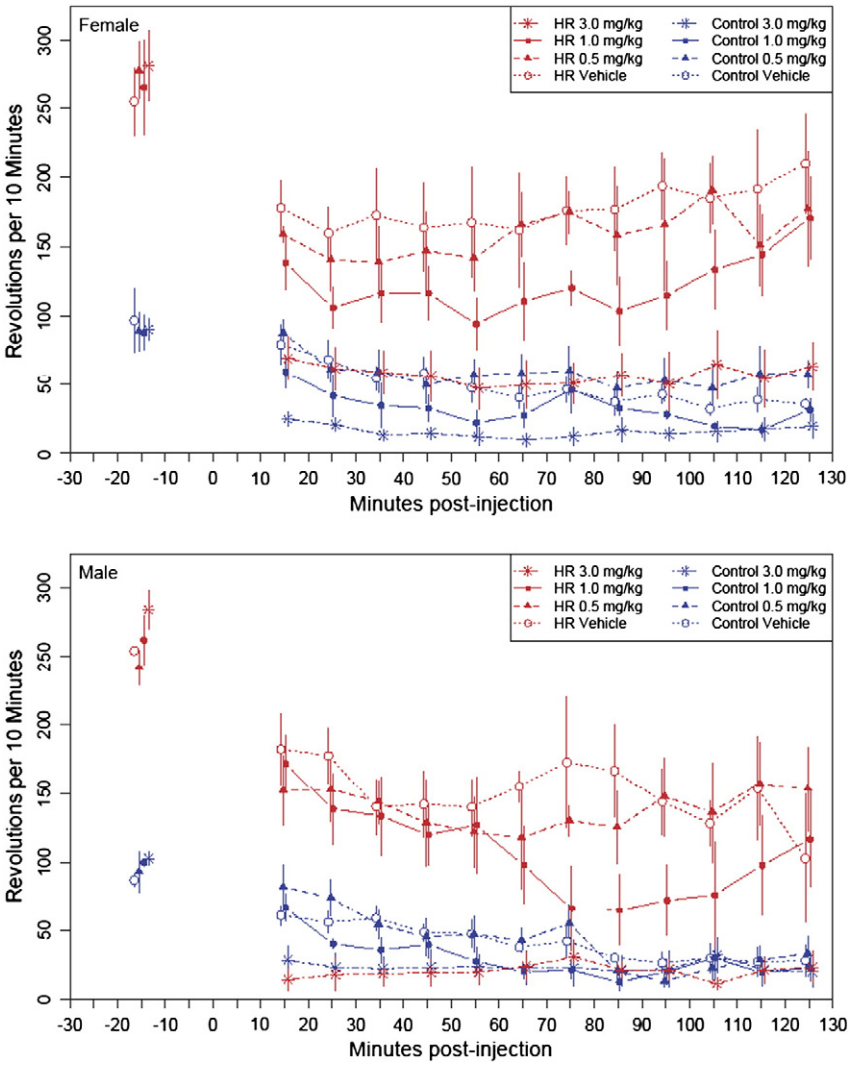

Fig. 1. Wheel running revolutions in 10-min bins during intraperitoneal WIN 55,212-2 injections (high WIN (3 mg/kg); medium WIN (1 mg/kg); low WIN (0.5 mg/kg)). (First 10-min period after injection is omitted.) Values at −15 min are pooled revolutions in the 30-min period before injections. Values are simple means and standard errors. Points are centered on the 5-min mid-point (i.e. the point for the 11–20 min bin is located at 15 min), but have been offset slightly for clarity. WIN 55,212-2 reduced wheel running acutely in all mice, but for males (lower panel) the reduction was significantly greater for High Runner (HR) lines than for Control lines at the highest dose (Table 1, p for linetype × dose interaction = 0.0004; Online Supplemental Table B).

(−86 ± 13 rev/10 min, $p = 0.0007$) and Control mice (−36 ± 14, $p = 0.0420$) significantly decrease their wheel running, with the decrease being significantly greater for HR females ($p = 0.0424$). The trends were similar for males, with HR decreasing by 86 rev/10 min (±17, $p = 0.0025$) and Controls decreasing by 32 rev/10 min (±17 rev, $p = 0.0975$), and the effect of linetype marginally nonsignificant ($p = 0.0674$). These decreases may be the result of a natural trend for decreasing wheel running over the course of the night (peak running is typically reached around 2 h following lights out, e.g., see Girard and Garland, 2002; Malisch et al., 2009; Rhodes et al., 2001) and/or an effect of the vehicle injections per se. Without a control group that received no injections, we cannot separate these possibilities. For proportional responses (average running 10–70 min following injection divided by average running during the 30 min prior to injections), we observed no difference between HR and Control lines for either females ($p = 0.5013$) or males ($p = 0.3212$).

3.2. Drug response

Table 1 shows results of the repeated-measures ANCOVAs for the first 10–70 (corresponding to our previous study: Keeney et al., 2008) and also for 70–120 min post-injection (which was not analyzed in our previous study). For female mice in the first 10–70 min post-injection, the reduction in wheel revolutions depended on both dose and linetype. Likewise, there was a significant effect of both dose and linetype on the average and maximum speed, but only a statistically significant effect of dose for the amount of time spent running. Results were similar 70–120 min post-injection for females, in that there was a significant effect of both dose and linetype on total revolutions run, as well as the average and maximum running speed. However, unlike the first 10–70 min, in this period there was a significant effect of both dose and linetype on the time spent running, as well as a dose by linetype interaction for both the total revolutions and average running speed. Thus, for up to 70 min, the wheel running of females from both linetypes is depressed in a similar fashion for all doses. However, inspection of Fig. 1 indicates that during the final 70–120 min HR females receiving the 1 mg/kg dose gradually resumed running to values near vehicle levels, while wheel running of Control females given this dose remained depressed.

For males, wheel revolutions, as well as average and maximum speed, depended on dose and linetype ($p < 0.05$ for all) for all time periods studied (Fig. 2), with higher doses generally depressing wheel running more for both linetypes. Similar to females, only dose had a significant effect on the time spent running, both in the

Table 1
Repeated-measures analyses (SAS procedure mixed) of wheel running (binned in 10-min intervals) during 10–70 and 70–120 min following injections with vehicle, low, medium or high dose of WIN 55,212-2 in males and females.

Trait and transform used	*F* for dose	*P* for dose	*F* for linetype	*P* for linetype	*F* for interaction	*P* for interaction
Females (10–70 min)						
Revolutions^0.6	17.27	<.0001	27.84	0.0019	1.10	0.3730
Time^0.6	24.26	<.0001	4.44	0.0796	0.25	0.8623
Average speed (rpm)^0.6	19.80	<.0001	37.61	0.0009	1.69	0.2052
Maximum speed (rpm)^0.6	22.62	<.0001	37.43	0.0009	1.48	0.2529
Females (70–120 min)						
Revolutions^0.6	15.98	<.0001	22.23	0.0033	4.04	0.0233
Time^0.6	9.34	0.0006	10.09	0.0191	0.89	0.4664
Average speed (rpm)^0.6	11.70	0.0002	24.80	0.0025	3.52	0.0363
Maximum speed (rpm)^0.6	11.02	0.0002	25.75	0.0023	2.80	0.0693
Males (10–70 min)						
Revolutions^0.6	35.85	<.0001	10.66	0.0171	10.27	0.0004
Time^0.6	30.34	<.0001	1.25	0.3062	5.07	0.0102
Average speed (rpm)^0.6	39.95	<.0001	10.95	0.0162	11.18	0.0002
Maximum speed (rpm)^0.6	43.08	<.0001	11.48	0.0147	10.15	0.0004
Males (70–120 min)						
Revolutions^0.6	18.70	<.0001	10.41	0.018	9.74	0.0005
Time^0.6	10.63	0.0003	4.99	0.0669	2.79	0.0702
Average speed (rpm)^0.6	16.30	<.0001	10.36	0.0182	7.83	0.0015
Maximum speed (rpm)^0.6	15.84	<.0001	9.98	0.0196	7.25	0.0022

Time denotes number of 1-minute intervals with at least one revolution; average speed is revolutions/time; maximum speed is revolutions in the single highest 1-minute interval. Degrees of freedom are 2 and 12 for dose, 1 and 6 for linetype, and 2 and 12 for the dose*linetype interaction. All p-values are for 2-tailed tests. All analyses also included age and wheel freeness as covariates (results not shown).

first 10–70 min and in the latter 70–120 min post-injection. Unlike results for females, the reduced wheel running in males was significantly greater for HR lines than for Controls for total revolutions run, time spent running, as well as average and maximum speed ($p<0.05$ for all dose by linetype interactions) in the first 10–70 min post-injection. In addition, males also showed a significant dose by linetype interaction for the total revolutions run and for all measures of speed in the 70–120 min post-injection ($p<0.05$ for all).

4. Discussion

Results from the present study show that agonism of the CB1 receptor (via i.p. injection of WIN) decreases wheel running in all groups analyzed. However, male mice from the HR lines decreased their wheel running to a greater degree in response to WIN, as compared with males from the non-selected Control lines, for both time periods studied (10–70 and 70–120 min post-injection) (Table 1, Fig. 2). In contrast, female HR mice had a differential decrease in wheel running in response to WIN only during the latter time period (70–120 min post-injection). These results complement a prior study, in which we found that female HR mice from generation 48 showed altered responsiveness to a selective CB1 receptor antagonist (SR141716; Rimonabant) as compared with females from the four non-selected Control lines, while HR males did not differ from their Control counterparts (Keeney et al., 2008). Both the previous and current studies show that HR mice differ from Control mice in the magnitude of the wheel-running response of one sex or the other to the highest dose of CB1 antagonist or agonist, respectively. In both studies (and sexes), this reduction in wheel-running is primarily caused by a decrease in the speed of running, with only HR males during the first 10–70 min of WIN injection showing a statistical reduction in the amount of time spent running.

These results suggest that over the course of selective breeding, HR mice have evolved to utilize CB1 signaling in a different way than Control-line mice during the performance of voluntary wheel running (i.e., voluntary exercise: Garland et al., 2011a). Furthermore, HR mice have done this in a sex-specific manner. Put differently, our results show that male and female HR mice have evolved to similar behavioral endpoints (i.e., similar factorial increase in voluntary wheel running) via at least partially separate mechanisms (Garland et al., 2011b).

It is important to note that, as expected from numerous previous studies (e.g., Garland et al., 2011b; Keeney et al., 2008), HR mice of both sexes differ substantially from Control-line mice in baseline running. In the present study, HR mice ran approximately three-fold more than Control under baseline conditions (e.g., see Fig. 1), a typical differential. Given this large difference in baseline running, it is possible that HR and Control mice are operating under considerably different physiological regimens during times of peak nightly running, when the present study was conducted. For example, in a given night of wheel running, HR mice could voluntarily approach performances at or near their maximal aerobic speed (i.e., almost at their maximal rate of oxygen consumption), unlike the nightly running of typical Control mice (Girard et al., 2001; Rezende et al., 2005, 2009). Therefore, even nominal increases or decreases in total wheel running during the active period could have different physiological consequences for an HR mouse as compared with a Control mouse. These and other differences between the HR and Control lines (e.g., differences in body fat: Meek et al., 2010; Swallow et al., 2001; Vaanholt et al., 2008) may also influence the pharmacokinetics of WIN. In many cases, exercise is known to alter the metabolism, absorption, and excretion of drugs (van Baak, 1990), and it is certainly possible that the elevated activity levels of HR mice (or of females relative to males) can affect the dynamics of WIN injection.

It is of interest that seemingly opposing treatments (Rimonabant blocks CB1 receptors, while WIN stimulates them) had negative effects on wheel running in both HR and Control mice. This is consistent with other studies of HR mice, which show that to date there has not been a pharmacological agent that has significantly increased wheel running in HR mice (Rhodes et al., 2001; Rhodes et al., 2005; Rhodes and Garland, 2003; Li et al., 2004). Indeed, the only substance that has significantly increased wheel running in HR mice has been a high-fat (Western) diet (Meek et al., 2010), which may have had effects on both exercise abilities and motivational aspects of wheel running. Despite the direction of the effect of pharmacological manipulation of the CB1 receptor, there is a sex-specific differential in wheel running

between HR and Control mice. Similar to Rhodes and Garland (2003), we believe that this differential is of prime interest. If the CB1 receptor is important to the performance of HR wheel running, then we might predict that pharmacological manipulations that perturb the specific function of the receptor, regardless of the type of perturbation (stimulation or blockade), could potentially interfere with such a specific, and high-performance behavior.

4.1. Sex differences

Several studies have documented robust sex differences in the evolution of high levels of voluntary wheel running over the course of selection. In general, the increased daily running distance of HR mice is accomplished mainly by speed in female HR mice, but by both speed and duration of running (to a lesser degree than females) in HR males (Garland, 2003; Girard et al., 2001; Keeney et al., 2008; Koteja and Garland, 2001; Rezende et al., 2009; Rhodes et al., 2000; Swallow et al., 1998, 1999). In recent generations HR males (seemingly at a selection limit) can run for as many minutes per day as HR females (Garland et al., 2011b; Rezende et al., 2009). The rodent literature supports a generalized trend for higher levels of locomotor activity in female rodents. This trend seems particularly true for wheel running activity (see Lightfoot, 2008 for review). For instance, Eikelboom and Mills (1988) find that female rats run more than

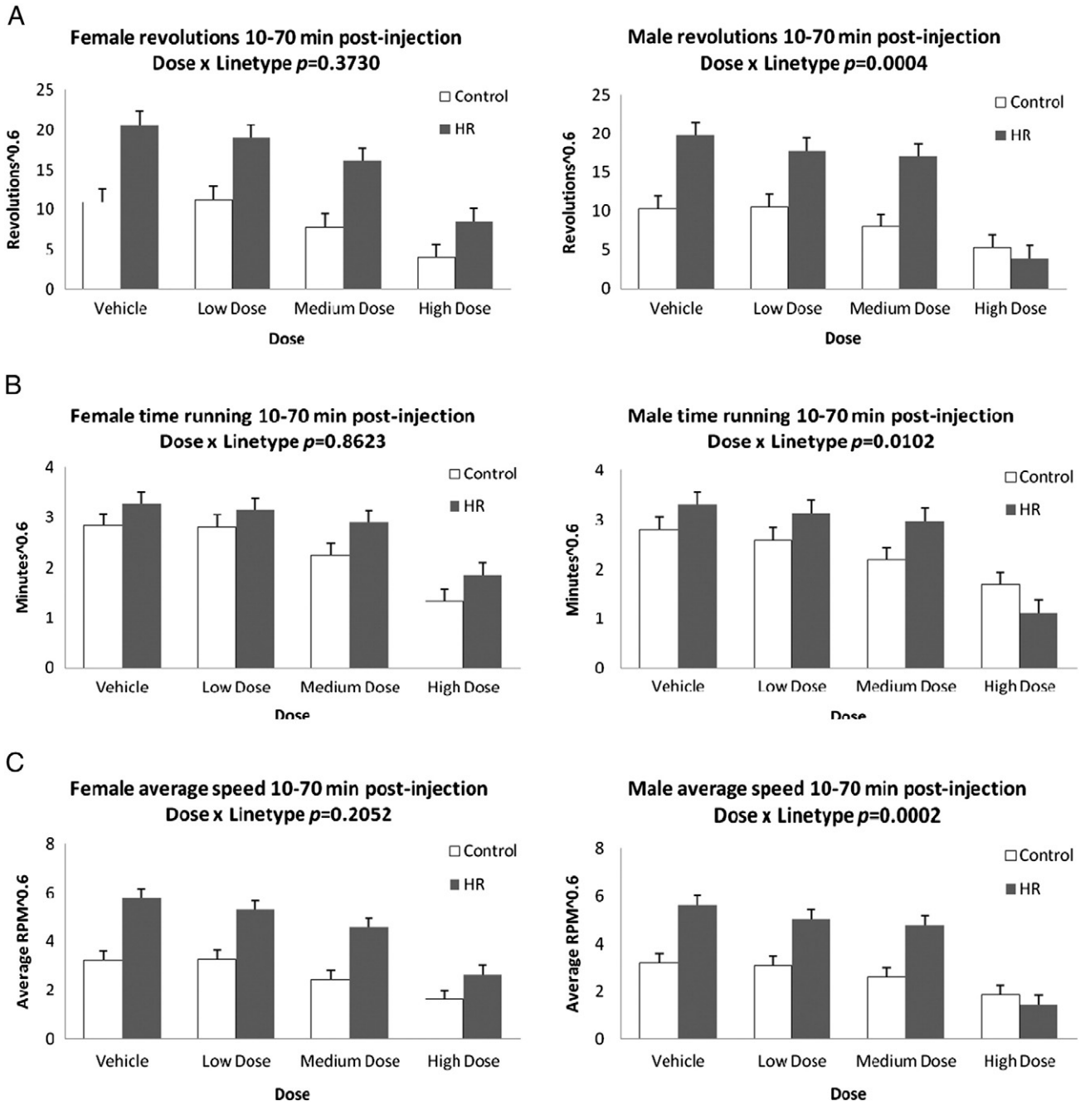

Fig. 2. Least squares means and standard errors from repeated-measures analyses of revolutions, minutes spent running, and revolutions per minute during 10–70 min following injection (A,B,C) and 70–120 min following injection (D,E,F) for males and females after injection of the CB1 receptor agonist WIN 55,212-2. Results show a dose by linetype interaction in the first 10–70 min for total revolutions, time spent running, and average speed of running for males only. During the latter 70–120 min, there is a dose by linetype interaction for males and females for total revolutions, as well as average speed of running. Significance levels are presented in Table 1; least squares means and standard errors are presented in Online Supplemental Table A.

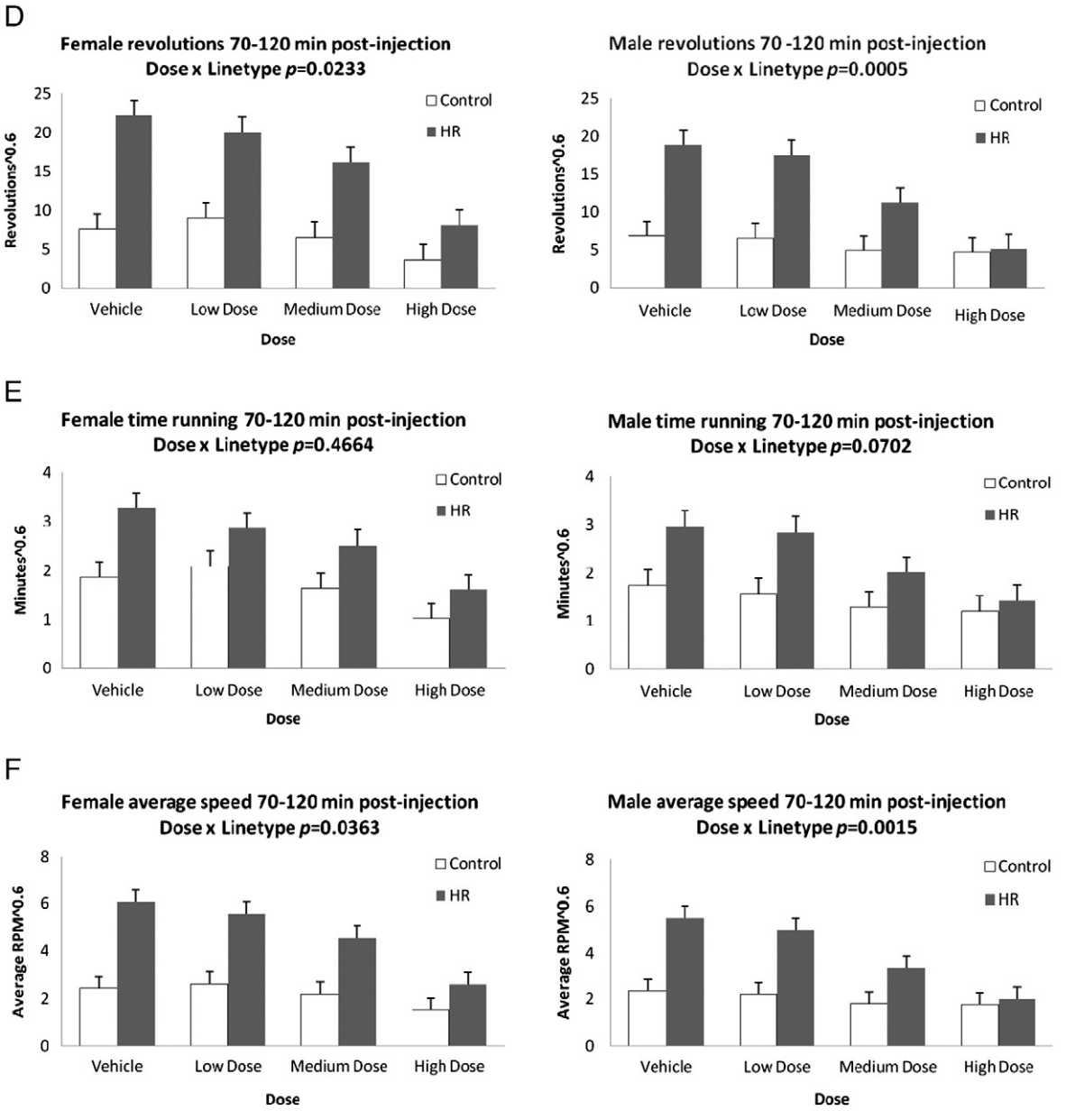

Fig. 2 (*continued*).

males, at a higher speed. Likewise, Konhilas et al. (2004) found that female mice of two different strains ran more than male mice of their respective strain, both at higher speeds and for a longer duration. Indeed, Field and Pellis (2008) find significant, overarching differences in the ways male and female rats organize their movements across a wide variety of motor tasks, and suggest that these sex differences in movement are not a byproduct of dimorphisms in body size or shape, but rather a result of neural differences.

In the context of our understanding of the voluntary wheel running of HR mice, it is likely that some of these neural sex differences are associated with the ECS (or factors related to downstream or upstream ECS activity). Several studies suggest that sex-based differences in CB1 signaling are common. For example, Fattore et al. (2007) found that ovarian hormones play a crucial role in the behavioral response of rats to cannabinoids. Similarly, it has been shown in rats that estrogen can affect cannabinoid receptor density (Rodriguez de Fonseca et al., 1994), transcription (González et al., 2000), and signal transduction (Mize and Alper, 2000). Likewise, Hill et al. (2007) reported that the antidepressant effect of estrogen in a rat model of anxiety was prevented by pharmacologically blocking CB1 receptors, thus implicating a modulatory role of estrogen on the ECS. Similarly, although THC and other cannabinoid agonists are antinociceptive regardless of sex, these effects seem to be stronger in female rats than males (Cohn et al., 1972; for review and other species, see Craft, 2005; Fattore et al., 2008; Tseng and Craft, 2001).

Although it seems evident that female sex hormones can affect aspects of ECS functioning, there is also evidence that suggests male-specific ECS dynamics. For example, Reich et al. (2009) found that male rats have higher basal levels of CB1 receptors than females. Likewise, Miller et al. (2004) showed that CP 55,940, a full agonist at both the CB1 and CB2 receptors, increased intake of a highly palatable food reward to a greater degree in male than female rats. In line with these results, Diaz et al. (2009) found that administration of WIN produced a greater degree of hyperphagia in male than female guinea pigs. It is not yet clear if these dimorphisms represent a clear sex-bias in ECS-influenced behaviors (see Fattore and Fratta, 2010 for review).

Given these known sexual dimorphisms in ECS physiology, we suggest that perhaps the psychotropic effects of ECS activity may

play a role in our observations following CB1 receptor agonism and antagonism in male and female HR mice. As previously mentioned, it has long been hypothesized that the ECS may contribute to a pleasurable "runner's high" sensation associated with prolonged endurance-type exercise (Dietrich and McDaniel, 2004). It has been shown that CB1 signaling can mimic the action of drugs of abuse, thus producing a rewarding sensation that is capable of conditioning behaviors (De Vries and Schoffelmeer, 2005; Maldonado et al., 2006). It is possible that this neurobiological "reward" may motivate, or be stimulated by, high-intensity (high-speed) running. If it were true that CB1 activity (at least in areas of the brain relevant to the performance of wheel running) is intensity-dependent, then we can predict that relatively high-speed running would be conditioned by the neural "pay off" of CB1 activation. Given that HR females tend to run at higher speeds than males, we would expect females to decrease their wheel running to a greater degree when CB1 transmission is blocked, and to a lesser degree (perhaps influenced by the injection itself) when transmission is stimulated (as this could approach normal CB1 activity during running). This is indeed what we observed (see Table 1), although without actual quantification of CB1 dynamics, behavioral observations alone are not sufficient evidence of any particular mechanism.

Of course, it is also possible that the expression or function of the CB1 receptor itself is not directly related to promoting voluntary wheel running in HR mice. It has long been understood that receptor agonist dynamics can affect either a compensatory downregulation or upregulation of a target receptor protein (Meyer and Quenzer, 2005). Male or female HR mice could have any number of alterations upstream of the CB1 receptors that affect its functionality, including those that interrupt the synthesis, release or degradation of endocannabinoids. If the ECS is important to network-level neural mechanisms, such as those that may control overall "motivation" to run, even mutations in indirectly-related genes (e.g. those affecting COX-2, which would in turn act on 2-arachidonoylglycerol, a common endocannabinoid) could have an influence on such a complex phenotype.

Alternately, HR females could be more sensitive to potential negative effects of Rimonabant administration (Pacher et al., 2006), while males are more sensitive to the catalepsy-inducing effects of cannabinoid agonists such as WIN. It is also possible that some or all of these observed sex differences in the effects of cannabinoids could be due to patterns of drug deposition, as related to differences in body fat. WIN, THC, and other cannabinoids are highly lipophilic, and can be readily absorbed by fat cells (Nahas et al., 1981). Cortright et al. (1997) found that male rats have a higher percentage of body fat than females, which led Tseng et al. (2004) to hypothesize that perhaps the behavioral effects of cannabinoids are less apparent in male rodents due to their body fat levels. Contrary to this idea, however, male and female HR mice do not significantly differ in their percentage body fat (Swallow et al., 2001), which is very low compared to other common laboratory strains of mice (Nehrenberg et al., 2009), suggesting that sex differences in the behavioral response to CB1 manipulation are resultant of more than just levels of body fat.

4.2. Conclusions

In summation, our results strongly implicate involvement of the ECS in the performance of wheel running, a type of voluntary exercise in rodents (Garland et al., 2011a). When we deconstruct how voluntary locomotion is accomplished, obviously there is a large contribution of physical ability; however, of potentially equal importance is an individual's intrinsic motivation for a potentially taxing and relatively energetically expensive behavior. After more than 50 generations of selective breeding for high levels of voluntary wheel running, we have observed correlated differences in both the ability and seeming "desire" of HR mice to run to on wheels (Garland, 2003; Rhodes et al., 2005; Belke and Garland, 2007; Swallow et al., 2009; Garland et al., 2011a). It is interesting that both the dopaminergic system and the ECS seem to have been involved in the development of the HR phenotype. Dopamine and endocannabinoids interact (Laviolette and Grace, 2006; Lupica and Riegel, 2005; Maldonado et al., 2006; Pillolla et al., 2007), and in some cases, both the ECS and the dopaminergic system have been shown to influence the performance of locomotor behaviors (Beltramo et al., 2000; Giuffrida et al., 1999; Gorriti et al., 2005). Equally interesting, but perhaps less expected, is the fact that ECS activity via both activation and suppression of CB1 transmission results in unique sex differences in running behavior in HR mice. A logical next step following both selective CB1 agonism and antagonism would be to administer mice of both sexes and linetypes an indirect CB1 agonist (such as an uptake blocker or FAAH inhibitor). On a more general level, it is possible that over the course of selective breeding male and female HR mice have evolved large-scale differences in how the brain motivates and/or rewards relatively high-speed wheel running.

Likewise, although pharmacology can be a useful tool in detecting behavioral correspondences to neural activity, it does not allow us to make quantitative statements about how male and female HR mice might differ with respect to CB1 distribution, regulation or activation in various behavioral contexts. Our two pharmacological tools (receptor antagonism and agonism) do not fully illuminate the role of the CB1 receptor during HR running. Therefore, additional studies aim to better characterize the involvement of the ECS in voluntary wheel running. The genetic basis of sex differences in running by HR mice is now under study (Hannon et al., 2011; Kelly et al., 2010), and future studies may aim to characterize the extent and nature of how both sexes of HR mice organize and utilize mechanisms of neural reward, with a special emphasis on an understanding of the dynamics of ECS activity in vivo.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.pbb.2012.02.017.

References

Belke TW, Garland Jr T. A brief opportunity to run does not function as a reinforcer for mice selected for high daily wheel-running rates. J Exp Anal Behav 2007;88:199–213.
Beltramo M, de Fonseca FR, Navarro M, Calignano A, Gorriti MA, Grammatikopoulos G, et al. Reversal of dopamine D(2) receptor responses by an anandamide transport inhibitor. J Neurosci 2000;20:3401–7.
Bronikowski AM, Rhodes JS, Garland Jr T, Prolla TA, Awad T, Gammie SC. The evolution of gene expression in the hippocampus in response to selective breeding for increased locomotor activity. Evolution 2004;5:2079–86.
Chaouloff F, Dubreucq S, Bellocchio L, Marsicano G. Endocannabinoids and motor behavior: CB1 receptors also control running activity. Physiology 2011;26(2):76–7.
Cohn RA, Barratt E, Pirch JH. Differences in behavioral responses of male and female rats to marijuana. Proc Soc Exp Biol Med 1972;140:1136–9.
Cortright RN, Chandler MP, Lemon PW, DiCarlo SE. Daily exercise reduces fat, protein and body mass in male but not female rats. Physiol Behav 1997;62:105–11.
Craft RM. Sex differences in behavioral effects of cannabinoids. Life Sci 2005;77:2471–8.
De Chiara V, Errico F, Musella A, Rossi S, Mataluni G, Sacchetti L, et al. Voluntary exercise and sucrose consumption enhance cannabinoid CB1 receptor sensitivity in the striatum. Neuropsychopharmacology 2010;35:374–87.
De Vries TJ, Schoffelmeer ANM. Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci 2005;26:420–6.
Diaz S, Farhang B, Hoien J, Stahlman M, Adatia N, Cox JM, et al. Sex differences in the cannabinoid modulation of appetite, body temperature and neurotransmission at POMC synapses. Neuroendocrinology 2009;89:424–40.
Dietrich A, McDaniel WF. Endocannabinoids and exercise. Br J Sports Med 2004;38:536–41.
Dubreucq S, Koehl M, Abrous DN, Marsicano G, Chaouloff F. CB1 receptor deficiency decreases wheel-running activity: consequences on emotional behaviours and hippocampal neurogenesis. Exp Neurol 2010;224:106–13.
Eikelboom R, Mills R. A microanalysis of wheel running in male and female rats. Physiol Behav 1988;43:625–30.

El Manira A, Kyriakatos A. The role of endocannabinoid signaling in motor control. Physiology 2010;25:230–8.
Fattore L, Fratta W. How important are sex differences in cannabinoid action? Brit J Pharmacol 2010;160:544–8.
Fattore L, Spano MS, Altea S, Angius F, Fadda P, Fratta W. Cannabinoid self-administration in rats: sex differences and the influence of ovarian function. Brit J Pharmacol 2007;152:795–804.
Fattore L, Altea S, Fratta W. Sex differences in drug addiction: a review of animal and human studies. Womens Health 2008;4:51–65.
Field EF, Pellis SM. The brain as the engine of sex differences in the organization of movement in rats. Arch Sex Behav 2008;37:30–42.
French ED. Δ9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. Neurosci Lett 1997;226:159–62.
Fuss J, Gass P. Endocannabinoids and voluntary activity in mice: runner's high and long-term consequences in emotional behaviors. Exp Neurol 2010;224:103–5.
Garland Jr T. Selection experiments: an under-utilized tool in biomechanics and organismal biology. In: Bels VL, Gasc J-P, Casinos A, editors. Vertebrate biomechanics and evolution. Oxford, UK: BIOS Scientific Publishers; 2003. p. 23–56.
Garland Jr T, Freeman PA. Selective breeding for high endurance running increases hindlimb symmetry. Evolution 2005;59:1851–4.
Garland Jr T, Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, et al. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. J Exp Biol 2011a;214:206–29.
Garland Jr T, Kelly SA, Malisch JL, Kolb EM, Hannon RM, Keeney BK, et al. How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. Proc R Soc B 2011b;278:574–81.
Gerdeman GL, Schechter JB, French ED. Context-specific reversal of cocaine sensitization by the CB(1) cannabinoid receptor antagonist rimonabant. Neuropsychopharmacology 2008;33:2747–59.
Girard I, Garland Jr T. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. J Appl Physiol 2002;92:1553–61.
Girard I, McAleer MW, Rhodes JS, Garland Jr T. Selection for high voluntary wheel running increases intermittency in house mice (*Mus domesticus*). J Exp Biol 2001;204:4311–20.
Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 1999;2:358–63.
Gomes FR, Rezende EL, Malisch JL, Lee SK, Rivas DA, Kelly SA, et al. Glycogen storage and muscle glucose transporters (GLUT-4) of mice selectively bred for high voluntary wheel running. J Exp Biol 2009;212:238–48.
González S, Bisogno T, Wenger T, Manzanares J, Milone A, Berrendero F, et al. Sex steroid influence on cannabinoid CB(1) receptor mRNA and endocannabinoid levels in the anterior pituitary gland. Biochem Biophys Res Commun 2000;270:260–6.
Gorriti MA, Ferrer B, del Arco I, Bermúdez-Silva FJ, de Diego Y, Fernandez-Espejo E, et al. Acute delta-9-tetrahydrocannabinol exposure facilitates quinpirole-induced hyperlocomotion. Pharmacol Biochem Behav 2005;81:71–7.
Hannon RM, Meek TH, Acosta W, Maciel RC, Schutz H, Garland Jr T. Sex-specific heterosis in line crosses of mice selectively bred for high locomotor activity. Behav Genet 2011;41:615–24.
Hill MN, Karacabeyli ES, Gorzalka BB. Estrogen recruits the endocannabinoid system to modulate emotionality. Psychoneuroendocrinology 2007;32:350–7.
Hill MN, Titterness AK, Morrish AC, Carrier EJ, Lee TT, Gil-Mohapel J, et al. Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. Hippocampus 2010;20:513–23.
Hoffman AF, Riegel AC, Lupica CR. Functional localization of cannabinoid receptors and endogenous cannabinoid production in distinct neuron populations of the hippocampus. Eur J Neurosci 2003;8:524–34.
Iversen L. Cannabis and the brain. Brain 2003;126:1252–70.
Keeney BK, Raichlen DA, Meek TH, Wijeratne RS, Middleton KM, Gerdeman GL, et al. Differential response to a selective cannabinoid receptor antagonist (SR141716: rimonabant) in female mice from lines selectively bred for high voluntary wheel-running behavior. Behav Pharmacol 2008;19:812–20.
Kelly SA, Nehrenberg DL, Peirce JL, Hua K, Steffy BM, Wiltshire T, et al. Genetic architecture of voluntary exercise in an advanced intercross line of mice. Physiol Genomics 2010;42:190–200.
Kochman LJ, dos Santos AA, Fornal CA, Jacobs BL. Despite strong behavioral disruption, Delta 9-tetrahydrocannabinol does not affect cell proliferation in the adult mouse dentate gyrus. Brain Res 2006;1113:86–93.
Kolb EM, Kelly SA, Middleton KM, Sermsakdi LS, Chappell MA, Garland Jr T. Erythropoietin elevates $\dot{V}O_{2,max}$ but not voluntary wheel running in mice. J Exp Biol 2010;213:510–9.
Konhilas JP, Maass AH, Luckey SW, Ikeda K, Stauffer BL, Olson EN, et al. Sex modifies exercise and cardiac adaptation in the mouse. Am J Physiol Heart Circ 2004;287:H2768–76.
Koteja P, Garland Jr T. Forum: response to R. Eikelboom. Anim Behav 2001;61:F25–6.
Koteja P, Garland Jr T, Sax JK, Swallow JG, Carter PA. Behaviour of house mice artificially selected for high levels of voluntary wheel running. Anim Behav 1999;58:1307–18.
Laviolette SR, Grace AA. The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. Cell Mol Life Sci 2006;63:1597–613.
Li G, Rhodes JS, Girard I, Gammie SC, Garland Jr T. Opioid-mediated pain sensitivity in mice bred for high voluntary wheel running. Physiol Behav 2004;83:515–24.
Lightfoot JT. Sex hormones' regulation of rodent physical activity: a review. Int J Biol Sci 2008;4:126–32.
Lupica CR, Riegel AC. Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. Neuropharmacology 2005;48:1105–16.
Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. Trends Neurosci 2006;29:225–32.
Malisch JL, Breuner CW, Gomes FR, Chappell MA, Garland Jr T. Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. Gen Comp Endocrinol 2008;156:210–7.
Malisch JL, Breuner CW, Kolb EM, Wada H, Hannon RM, Chappell MA, et al. Behavioral despair and home-cage activity in mice with chronically elevated baseline corticosterone concentrations. Behav Genet 2009;39:192–201.
Mathes WF, Nehrenberg DL, Gordon R, Hua K, Garland Jr T, Pomp D. Dopaminergic dysregulation in mice selectively bred for excessive exercise or obesity. Behav Brain Res 2010;210:155–63.
Meek TH, Lonquich BP, Hannon RM, Garland Jr T. Endurance capacity of mice selectively bred for high voluntary wheel running. J Exp Biol 2009;212:2908–17.
Meek TH, Eisenmann JC, Garland Jr T. Western diet increases wheel running in mice selectively bred for high voluntary wheel running. Int J Obes 2010;34:960–9.
Meyer JS, Quenzer LF. Schizophrenia. In: Meyer JS, Quenzer LF, editors. Psychopharmacology: drugs, the brain and behavior. Sunderland: Sinaver Associates Inc.; 2005. p. 441–67.
Miller CC, Murray TF, Freeman KG, Edwards GL. Cannabinoid agonist, CP 55,940, facilitates intake of palatable foods when injected into the hindbrain. Physiol Behav 2004;80:611–6.
Mize AL, Alper RH. Acute and long-term effects of 17-beta estradiol on G(i/o) coupled neurotransmitter receptor function in the female rat brain as assessed by agonist-stimulated [35S] GTP gamma S binding. Brain Res 2000;859:326–33.
Nahas G, Leger C, Tocque B, Hoellinger H. The kinetics of cannabinoid distribution and storage with special reference to the brain and testes. J Clin Pharmacol 1981;21:208S–14S.
Nehrenberg DL, Hua K, Estrada-Smith D, Garland Jr T, Pomp D. Voluntary exercise and its effects on body composition depend on genetic selection history. Obesity 2009;17:1402–9.
Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006;58:389–462.
Patel S, Hillard CJ. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther 2006;318:304–11.
Pillolla G, Melis M, Perra S, Muntoni AL, Gessa GL, Pistis M. Medial forebrain bundle stimulation evokes endocannabinoid-mediated modulation of ventral tegmental area dopamine neuron firing in vivo. Psychopharmacology 2007;191:843–53.
Reich CG, Taylor ME, McCarthy MM. Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats. Behav Brain Res 2009;203:264–9.
Rezende EL, Chappell MA, Gomes FR, Malisch JL, Garland Jr T. Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. J Exp Biol 2005;208:2447–58.
Rezende EL, Gomes FR, Chappell MA, Garland Jr T. Running behavior and its energy cost in mice selectively bred for high voluntary locomotor activity. Physiol Biochem Zool 2009;82:662–79.
Rhodes JS, Garland Jr T. Differential sensitivity to acute administration of Ritalin, apormorphine, SCH 23390, but not raclopride in mice selectively bred for hyperactive wheel-running behavior. Psychopharmacology 2003;167:242–50.
Rhodes JS, Koteja P, Swallow JG, Carter PA, Garland Jr T. Body temperatures of house mice artificially selected for high voluntary wheel-running behavior: repeatability and effect of genetic selection. J Therm Biol 2000;25:391–400.
Rhodes JS, Hosack GR, Girard I, Kelley AE, Mitchell GS, Garland Jr T. Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. Psychopharmacology 2001;158:120–31.
Rhodes JS, Garland Jr T, Gammie SC. Patterns of brain activity associated with variation in voluntary wheel-running behavior. Behav Neurosci 2003;117:1243–56.
Rhodes JS, Gammie SC, Garland Jr T. Neurobiology of mice selected for high voluntary wheel-running activity. Integr Comp Biol 2005;45:438–55.
Rodriguez de Fonseca F, Cebeira M, Ramos JA, Martin M, Fernandez-Ruiz JJ. Cannabinoid receptors in rat brain areas: sexual differences, fluctuations during the estrous cycle and changes after gonadectomy and sex steroid replacement. Life Sci 1994;54:159–70.
Rossi S, De Chiara V, Musella A, Mataluni G, Sacchetti L, Bernardi G, et al. Adaptations of striatal endocannabinoid system during stress. Mol Neurobiol 2009;39:178–84.
Sparling PB, Giuffrida A, Piomelli D, Rosskopf L, Dietrich A. Exercise activates the endocannabinoid system. Neuroreport 2003;14:2209–11.
Spina E, Trovati A, Parolaro D, Giagnoni G. A role of nitric oxide in WIN 55,212-2 tolerance in mice. Eur J Pharmacol 1998;343:157–63.
Swallow JG, Carter PA, Garland Jr T. Artificial selection for increased wheel-running behavior in house mice. Behav Genet 1998;28:227–37.
Swallow JG, Koteja P, Carter PA, Garland Jr T. Artificial selection for increased wheel-running activity in house mice results in decreased body mass at maturity. J Exp Biol 1999;202:2513–20.
Swallow JG, Koteja P, Carter PA, Garland Jr T. Food consumption and body composition in mice selected for high wheel-running activity. J Comp Physiol B 2001;171:651–9.
Swallow JG, Hayes JP, Koteja P, Garland Jr T. Selection experiments and experimental evolution of performance and physiology. In: Garland Jr T, Rose MR, editors. Experimental evolution: concepts, methods, and applications of selection experiments. California: University of California Press; 2009. p. 301–51.
Tseng AH, Craft RM. Sex differences in antinociceptive and motoric effects of cannabinoids. Eur J Pharmacol 2001;430:41–7.

Tseng AH, Harding JW, Craft RM. Pharmacokinetic factors in sex differences in D9-tetrahydrocannibinol-induced behavioral effects in rats. Behav Brain Res 2004;154:77–83.

Vaanholt LM, Meerlo P, Garland Jr T, Visser GH, van Dijk G. Plasma adiponectin is increased in mice selectively bred for high wheel-running activity, but not by wheel running per se. Horm Metab Res 2007;39:377–83.

Vaanholt LM, Jonas I, Doornbos M, Schubert KA, Nyakas C, Garland Jr T, et al. Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity. Int J Obes 2008;32:1566–75.

van Baak MA. Influence of exercise on the pharmacokinetics of drugs. Clin Pharmacokinet 1990;698:68–78.

Online Supplemental Table A. Least squares means (all traits transformed by raising to the 0.6 power) and standard errors from repeated-measures analyses (Table 1) of wheel running 10-70 and 70-120 minutes following injections, for females and males.

	Control Mean	Control SE	HR Mean	HR SE	Control Mean	Control SE	HR Mean	HR SE
	Females 10-70 mins				**Females 70-120 mins**			
Revolutions^0.6								
Vehicle	10.887	1.660	20.655	1.610	7.599	1.991	22.202	1.932
Low Dose	11.211	1.656	19.014	1.610	8.980	1.989	20.041	1.932
Medium Dose	7.810	1.657	16.067	1.631	6.554	1.991	16.198	1.957
High Dose	3.978	1.657	8.524	1.610	3.633	1.991	8.147	1.932
Time^0.6								
Vehicle	2.842	0.238	3.275	0.230	1.861	0.313	3.278	0.304
Low Dose	2.817	0.238	3.150	0.230	2.081	0.312	2.875	0.304
Medium Dose	2.249	0.238	2.900	0.234	1.627	0.312	2.520	0.308
High Dose	1.327	0.238	1.865	0.230	1.016	0.312	1.598	0.304
Average Speed^0.6								
Vehicle	3.254	0.378	5.798	0.365	2.415	0.513	6.088	0.498
Low Dose	3.286	0.377	5.299	0.365	2.614	0.512	5.585	0.498
Medium Dose	2.439	0.378	4.583	0.371	2.195	0.513	4.539	0.505
High Dose	1.605	0.378	2.660	0.365	1.515	0.513	2.603	0.498
Maximum Speed^0.6								
Vehicle	4.246	0.448	7.149	0.432	3.052	0.619	7.374	0.601
Low Dose	4.344	0.447	6.690	0.432	3.306	0.618	6.729	0.601
Medium Dose	3.268	0.448	5.969	0.439	2.791	0.619	5.598	0.609
High Dose	2.066	0.448	3.404	0.432	1.848	0.619	3.265	0.601
	Males 10-70 mins				**Males 70-120 mins**			
Revolutions^0.6								
Vehicle	10.296	1.603	19.795	1.671	6.843	1.899	18.876	1.962
Low Dose	10.485	1.622	17.730	1.671	6.552	1.917	17.526	1.964
Medium Dose	7.983	1.603	17.013	1.649	4.924	1.899	11.284	1.942
High Dose	5.291	1.603	3.898	1.671	4.737	1.899	5.118	1.962
Time^0.6								
Vehicle	2.804	0.252	3.310	0.263	1.746	0.320	2.961	0.330
Low Dose	2.588	0.255	3.133	0.263	1.565	0.323	2.844	0.331
Medium Dose	2.194	0.252	2.972	0.259	1.294	0.320	2.000	0.327
High Dose	1.690	0.252	1.114	0.263	1.205	0.320	1.415	0.330
Average Speed^0.6								
Vehicle	3.230	0.390	5.602	0.406	2.363	0.501	5.494	0.518
Low Dose	3.114	0.395	5.024	0.406	2.220	0.506	4.987	0.518
Medium Dose	2.626	0.390	4.767	0.401	1.795	0.501	3.340	0.512
High Dose	1.890	0.390	1.464	0.406	1.759	0.501	2.010	0.518
Maximum Speed^0.6								
Vehicle	4.367	0.478	7.127	0.498	3.007	0.630	6.838	0.650
Low Dose	4.028	0.483	6.536	0.498	2.804	0.636	6.225	0.651
Medium Dose	3.468	0.478	6.070	0.491	2.336	0.630	4.193	0.644
High Dose	2.380	0.478	1.865	0.498	2.171	0.630	2.488	0.650

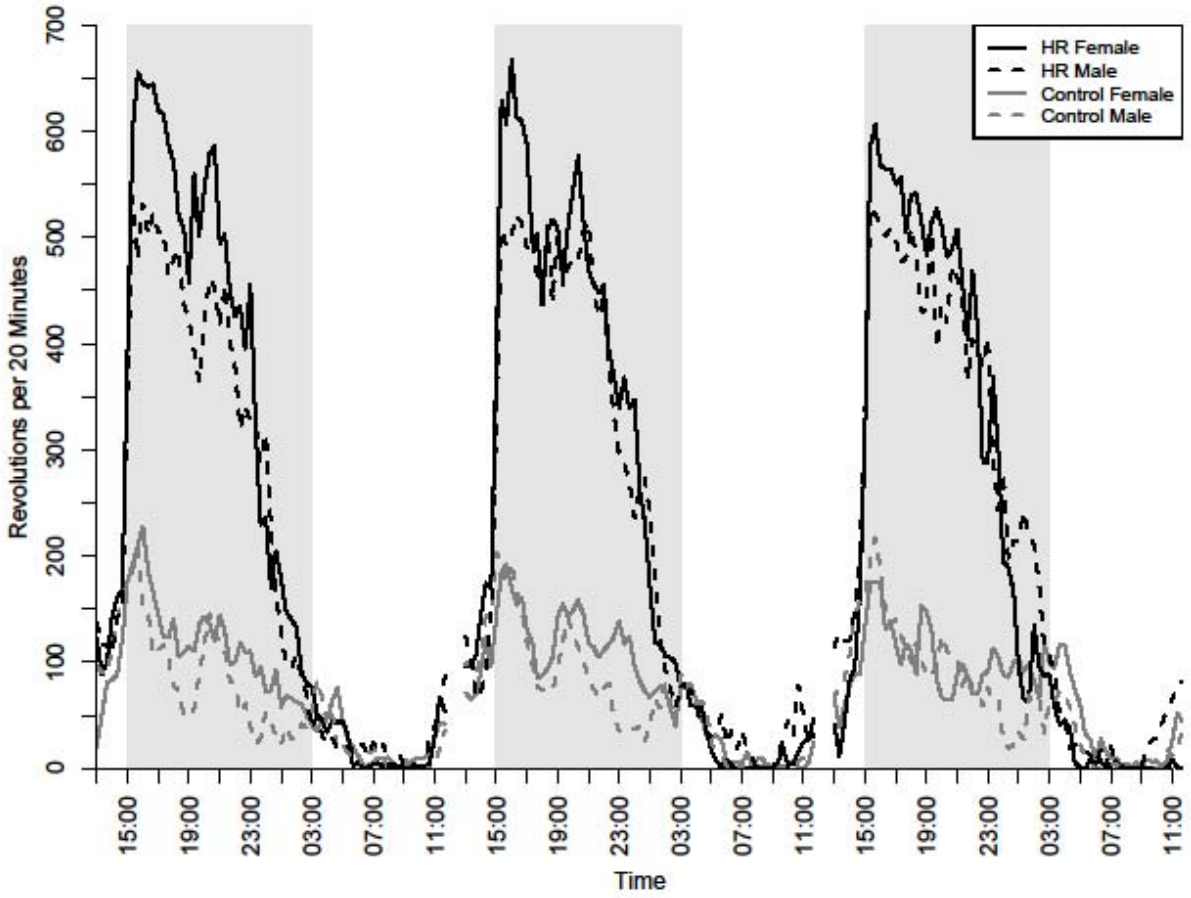
All values are means per 10-minute intervals. Time denotes number of 1-minute intervals with at least one revolution; Average Speed is revolutions/time; Maximum Speed is revolutions in the single highest 1-minute interval.

Online Supplemental Table B. Proportional response (dose/vehicle) to WIN 55,212-2 in the first 10-70 minutes post-injection in females (log_{10} transformed) and males (log_{10} transformed). Values are least squares means from SAS Procedure Mixed. Covariates are wheel freeness and age at time of injection (results not shown).

Females 10-70 mins after injection	Dose (mg/kg)	Control	Standard Error	High Runner	Standard Error	F 1,6	P for Linetype
Revolutions	3.0	-0.560	0.234	-0.674	0.219	0.130	0.734
	1.0	0.083	0.266	0.063	0.260	0.000	0.958
	0.5	0.451	0.143	0.192	0.135	1.730	0.236
Time	3.0	-0.329	0.097	-0.310	0.091	0.020	0.893
	1.0	-0.034	0.114	-0.018	0.112	0.010	0.924
	0.5	0.143	0.053	0.034	0.050	2.270	0.183
Average Speed	3.0	-0.308	0.130	-0.464	0.122	0.760	0.417
	1.0	-0.013	0.151	-0.060	0.148	0.050	0.829
	0.5	0.187	0.083	0.063	0.079	1.180	0.319
Maximum Speed	3.0	-0.341	0.144	-0.471	0.135	0.430	0.536
	1.0	0.019	0.165	-0.007	0.162	0.010	0.916
	0.5	0.256	0.084	0.097	0.079	1.900	0.217
Males 10-70 mins after injection							
Revolutions	3.0	-0.343	0.202	-1.083	0.214	6.250	0.047
	1.0	0.032	0.168	0.060	0.181	0.010	0.914
	0.5	0.177	0.140	0.059	0.151	0.320	0.590
Time	3.0	-0.211	0.095	-0.479	0.100	3.750	0.101
	1.0	-0.056	0.072	-0.016	0.078	0.140	0.721
	0.5	0.017	0.058	0.009	0.062	0.010	0.928
Average Speed	3.0	-0.234	0.102	-0.730	0.109	10.950	0.016
	1.0	-0.029	0.090	-0.042	0.096	0.010	0.926
	0.5	0.061	0.078	-0.027	0.084	0.580	0.474
Maximum Speed	3.0	-0.284	0.125	-0.774	0.133	7.090	0.037
	1.0	-0.026	0.105	-0.029	0.112	0.000	0.986
	0.5	0.055	0.096	-0.002	0.104	0.160	0.703

Online Supplemental Table C. Proportional response (dose/vehicle) to WIN 55,212-2 in the latter 70-120 minutes post-injection in females (log_{10} transformed) and males (log_{10} transformed). Values are least squares means from SAS Procedure Mixed. Covariates are wheel freeness and age at time of injection (results not shown).

Females 70-120 mins after injection	Dose (mg/kg)	Control	Standard Error	High Runner	Standard Error	F 1,6	P for Linetype
Revolutions	3.0	-0.031	0.235	-0.690	0.222	4.110	0.089
	1.0	0.400	0.248	-0.111	0.239	2.200	0.188
	0.5	0.446	0.202	0.222	0.191	0.640	0.453
Time	3.0	-0.102	0.101	-0.360	0.095	3.460	0.112
	1.0	0.073	0.097	-0.098	0.093	1.600	0.253
	0.5	0.166	0.088	0.004	0.084	1.770	0.231
Average Speed	3.0	-0.016	0.148	-0.486	0.142	5.210	0.063
	1.0	0.167	0.136	-0.144	0.131	2.690	0.152
	0.5	0.207	0.111	0.079	0.105	0.700	0.434
Maximum Speed	3.0	-0.010	0.174	-0.484	0.167	3.850	0.097
	1.0	0.219	0.156	-0.116	0.150	2.370	0.175
	0.5	0.250	0.129	0.101	0.123	0.700	0.436
Males 70-120 mins after injection							
Revolutions	3.0	0.214	0.195	-0.408	0.205	4.770	0.072
	1.0	0.338	0.226	0.068	0.240	0.660	0.447
	0.5	0.535	0.243	0.486	0.253	0.020	0.894
Time	3.0	-0.002	0.082	-0.241	0.086	4.000	0.093
	1.0	0.055	0.091	-0.067	0.097	0.840	0.396
	0.5	0.142	0.096	0.114	0.100	0.040	0.847
Average Speed	3.0	0.084	0.115	-0.358	0.120	7.000	0.038
	1.0	0.115	0.140	-0.100	0.147	1.110	0.333
	0.5	0.219	0.113	0.197	0.120	0.020	0.897
Maximum Speed	3.0	0.118	0.133	-0.356	0.140	5.930	0.051
	1.0	0.191	0.166	-0.077	0.175	1.220	0.312
	0.5	0.275	0.133	0.241	0.140	0.030	0.870



Online Supplemental Figure A. Daily pattern of wheel running (revolutions in 20-min bins) for mice from selectively bred High Runner (HR) and Control lines during 3 days before the start of WIN 55,212-2 injections (22 Oct- 24 Oct 2008). Note that females run more than males in both linetypes. Grey bars indicate lights off.