# Differential response to a selective cannabinoid receptor antagonist (SR141716: rimonabant) in female mice from lines selectively bred for high voluntary wheel-running behaviour

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**Exercise is a naturally rewarding behaviour in human beings and can be associated with feelings of euphoria and analgesia. The endocannabinoid system may play a role in the perception of neurobiological rewards during and after prolonged exercise. Mice from lines that have been selectively bred for high voluntary wheel running (high runner or HR lines) may have evolved neurobiological mechanisms that increase the incentive salience of endurance-type exercise. Here, we test the hypothesis that endocannabinoid signalling has been altered in the four replicate HR lines as compared with four nonselected control lines. After 18 days of acclimation to cages with attached wheels, we injected mice with rimonabant (SR141716), a selective cannabinoid CB1 receptor antagonist. During the time of normal peak running, each mouse received, in a randomized order, intraperitonial injection of rimonabant (0.1 or 3.0 mg/kg) or vehicle, over 9 days. Drug response was quantified as wheel revolutions, time and speed 10–70 min postinjection. Rimonabant decreased running in all mice; however, female HR mice differentially decreased running speed and distance (but not time) as compared with control females. We conclude that altered endocannabinoid signalling plays a role in the high wheel running of female HR mice. *Behavioural Pharmacology* 19:812–820 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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## Introduction

Voluntary wheel running is a classically self-rewarding behaviour in both rats and mice (Premack, 1964; Timberlake and Wozny, 1979; Sherwin, 1998). For example, Sherwin and Nicol, (1996) have demonstrated that mice are willing to cross an aversive water barrier, even as the barrier was increased in size, to receive a wheel-running reward. Moreover, several operant conditioning studies have shown that rats and mice are highly motivated to bar-press for a wheel-running reward (Belke and Heyman, 1994; Belke, 1996; Belke and Garland, 2007). However, our current understanding of the neurobiological basis of apparently high motivation for wheel running in rodents is limited.

The endocannabinoid system (ECS) is a complex endogenous signalling system made up of transmembrane cannabinoid receptors (CB receptors), their ligands (endocannabinoids), and proteins involved in the synthesis and modification of endocannabinoids (De Petrocellis *et al.*, 2004; Cota and Woods, 2005; Demuth and Molleman, 2006). There are two primary cannabinoid receptors: CB1 and CB2. The ECS is hypothesized to have a general modulatory effect on circuits of the reward system, and the perception of neurobiological rewards associated with such behaviours as voluntary locomotion and food consumption (Thornton-Jones *et al.*, 2005; Maldonado *et al.*, 2006). The ECS is also involved in aspects of energy balance, lipid metabolism, nociception and the stress response, among other factors, which are also relevant to the physiology of wheel running (Girard and Garland, 2002; Li *et al.*, 2004; Rhodes *et al.*, 2005; Pacher *et al.*, 2006; Pagotto *et al.*, 2006; Malisch *et al.*, 2008).

Endocannabinoid signalling is activated by aerobic exercise in human beings (Sparling *et al.*, 2003), and is associated with analgesia and the stimulation of locomotor activity in rodents (Lichtman *et al.*, 1996; Wiley, 2003; Hohmann and Suplita, 2006). Recent evidence indicates that CB1 signalling facilitates dopamine release in the shell of the nucleus accumbens, a neurochemical effect

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common to drugs of addiction (Kelley, 2004; Cheer *et al.*, 2007), and the expression of drug-seeking, conditioned behaviours (Cohen *et al.*, 2005; DeVries and Schoffelmeer, 2005; Laviolette and Grace, 2006; Xi *et al.*, 2006; Alvarez-Jaimes *et al.*, 2008). Dietrich and McDaniel (2004) recently suggested that CB1 signalling may account for exercise addiction that has been reported for human distance runners (Morgan, 1979). When injected directly into brain reward centres of the rat, low doses of cannabinoid agonists enhance locomotor behaviours and promote self-administration and conditioned place preference (Zangen *et al.*, 2006), consistent with the idea that motivational properties of the ECS may be physiologically coupled to behavioural activation. Accordingly, cocaine-induced hyperlocomotion can be blunted by either genetic deletion or pharmacological blockade of the CB1 receptor (Cheer *et al.*, 2007; Corbille *et al.*, 2007; Gerdeman *et al.*, 2008). Thus, CB1 signalling may 'motivate' running behaviours, similar to motivation and conditioning induced by drugs of abuse.

This study examined the possible links between endocannabinoids and voluntary exercise in mice from lines that have been selectively bred for high amounts of voluntary wheel running. A 15-year selection experiment to increase voluntary wheel running in laboratory house mice (*Mus domesticus*) provides a unique opportunity to understand how neural incentives may evolve in real time to affect motivation for exercise. After 10 generations of selection, mice from four replicate high runner (HR) lines, of both sexes, ran at least 70% more than four nonselected control lines (Swallow *et al.*, 1998). After 16 generations, HR mice ran on average 170% more than controls (Rhodes *et al.*, 2000). This differential response has been achieved primarily by an increase in running speed, as opposed to duration of running, particularly in female HR mice (Swallow *et al.*, 1998, 1999; Rhodes *et al.*, 2000; Girard *et al.*, 2001; Koteja and Garland, 2001; Garland, 2003). In general, over the course of selection, male and female HR mice have increased their total wheel revolutions in different ways: female HR mice increased speed, whereas male HR mice increased both speed and, to a lesser degree, the amount of time spent running per day. In addition to changes in locomotor behaviour, the selective breeding regimen has led to changes in many morphological, physiological and behavioural traits (Garland, 2003). For example, HR mice exhibit reduced body mass (Swallow *et al.*, 1999), reduced body fat (Swallow *et al.*, 1999), differences in open-field behaviour (Bronikowski *et al.*, 2001), differences in thermoregulatory nest-building behaviour (Carter *et al.*, 2000), increased predatory aggression (Gammie *et al.*, 2003) and higher plasma corticosterone (Girard and Garland, 2002; Malisch *et al.*, 2008) and adiponectin levels (Vaanholt *et al.*, 2007).

Rhodes *et al.* (2005) hypothesized that the motivation for voluntary endurance exercise has been altered in HR mice. Pharmacological studies with dopamine transporter blockers found differential effects on wheel running in HR and control mice (Rhodes *et al.*, 2001; Rhodes and Garland 2003). This differential effect was attributed to altered functionality in the D1 receptor system, although apparently not in the D2 receptor, serotonergic or opioidergic systems (Rhodes *et al.*, 2001, 2003, 2005; Li *et al.*, 2004). In addition, Fos immunohistochemistry studies show a greater increase in activity in several brain regions implicated in reward and motivation when wheel access is blocked, including the caudate–putamen complex, lateral hypothalamus, prefrontal cortex, medial frontal cortex, NAc, piriform cortex and sensory cortex (Rhodes *et al.*, 2003). These differences prompt numerous questions about how reward and salience mechanisms may have differentiated in HR mice over the course of selective breeding. However, because previous operant-conditioning, pharmacological and brain-imaging studies have involved only females, it is not known whether sex differences in high wheel running (i.e. longer-duration running only in males) are related to differences in reward or motivation.

The purpose of this study was to test the hypothesis that mice from the HR and control lines would respond differentially in voluntary wheel running when administered a selective cannabinoid receptor antagonist/inverse agonist. Moreover, we tested the hypothesis that the differential response would be sex specific. We used rimonabant (SR141716), a selective CB1 receptor antagonist (Carai *et al.*, 2005), that has previously been used to block both cannabinoid reward and conditioned drug-seeking or food-seeking behaviours (DeVries and Schoffelmeer, 2005; Thornton-Jones *et al.*, 2005; Zangen *et al.*, 2006; Ward *et al.*, 2007).

## Methods

### Subjects

We studied 48 female and 48 male mice from generation 48 of a long-term selection experiment for high voluntary wheel-running behaviour (Swallow *et al.*, 1998; Garland, 2003). The original progenitors were outbred Hsd: Institute for Cancer Research mice (*Mus domesticus*) purchased from Harlan Sprague Dawley (Indianapolis, Indiana, USA). Mice were randomly mated for two generations, then assigned to eight closed lines, four to be selectively bred for HR and four to be bred without regard to wheel running, hence serving as controls for founder effects and random genetic drift (control lines). In each subsequent generation, mice were paired within line, and offspring were toe clipped, weighed and weaned from dams at 21 days of age. Mice were then housed in same-sex groups of four until approximately 6–8 weeks of age, at which point they began a 6-day wheel-access trial. Mice were housed in standard cages with Wahman-type activity wheels (1.12 m circumference, 35.7 cm diameter, 10-cm-wide running surface of a 10-mm mesh enclosed

by clear Plexiglas and stainless steel) attached through a 5.5-cm-long stainless-steel tube inserted though a 7.7-cm-diameter hole in the side of the cage, allowing the mouse to continuously access the wheel. Wheel revolutions were recorded daily in 1-min intervals by a photocell counter attached to the wheel and compiled through customized software by San Diego Instruments (San Diego, California, USA). In the four replicate HR lines, the male and female from each family with the most total revolutions on days 5 and 6 of the 6-day test were chosen to propagate the lines to the next generation. In the four control lines, a male and a female were randomly chosen from each family. Within all lines, breeders were randomly paired, with the exception that sibling matings were not allowed. Throughout the selection experiment and for all studies described here, mice were routinely housed with free access to food and water, and maintained on a 12-h light-dark cycle.

As top runners were unavailable in the HR lines (used as breeders), we also excluded the lowest-running animals in HR-line families. Of the remaining mice, one male and female were chosen from each available family, except when only one sex was available from a given family. As a part of the routine selection protocol (see previous paragraph), mice were tested for voluntary wheel running over a 6-day period. Placement of mice in wheel cages was randomized with respect to linetype (HR vs. control) and sex, and experimenters were blind to line and linetype.

### Drug protocol and wheel running

Rimonabant (SR141716) was obtained from the NIDA Drug Supply Program (Baltimore, MD, USA). Several earlier pharmacological studies have used HR mice (Rhodes *et al.*, 2001; Rhodes and Garland, 2003; Li *et al.*, 2004). Following the design of Li *et al.* (2004), each individual mouse received vehicle injection, low-dose (0.1 mg/kg) rimonabant and high-dose (3.0 mg/kg) rimonabant in a randomly determined order over the course of 6 days, with 48 h between each injection to avoid carryover effects of the previous treatment. Vehicle injections were solution of 20% DMSO, 10% Tween-80 and 70% physiological saline. This vehicle solution has been previously described for the in-vivo delivery of cannabinoid compounds including rimonabant and does not by itself influence open-field locomotor behaviour in mice (Gerdeman *et al.*, 2008) or the firing of dopamine neurons (Wu and French, 2000). The doses of 0.1 and 3.0 mg/kg rimonabant have been previously reported to maintain a physiologically effective blockade of CB1 receptors in both mice and rats (Carai *et al.*, 2005). Injection solutions were prepared fresh each day, and injection volumes adjusted for dose and body mass of the animal. Mice received treatment at approximately the same time of day for each injection. Lights were turned off at 16.00 h, and injections began 2 h later, which was during the time of typical peak wheel-running activity (Girard *et al.*, 2001; Girard and Garland, 2002; Rhodes *et al.*, 2003; Malisch *et al.*, 2008). Mice were then split into three measurement batches for convenience, thus allowing injections to be completed between 18.00 and 20.00 h. Intraperitonial injections were administered as an experimenter held the scruff of the neck manually to restrain the mouse.

The acute locomotor response to treatment was measured as the total number of wheel revolutions in the period from 10 to 70 min postinjection (Coimbra, 2001). In addition, we analysed the number of 1-min intervals with at least one revolution (time spent running), the average running speed (revolutions/active intervals), and the maximum speed (revolutions in the single highest 1-min interval).

### Statistics

Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, North Carolina, USA). Analyses were first conducted separately by sex. The primary grouping factors were linetype (HR vs. C) and dose, and replicate line was a random effect nested within linetype. Individual was the factor for repeated measures, and we assumed compound symmetry in SAS Procedure Mixed. In this mixed-model analysis of covariance, the degrees of freedom for testing the effect of linetype, relative to line, is always 1 and 6. For dose and the dose $\times$ linetype interaction [tested relative to the dose $\times$ line (linetype) effect], degrees of freedom are 2 and 12, respectively. This interaction term is of prime interest because, if significant, it indicates a differential response of the HR and control lines to the drug dose. Wheel freeness (a measure of how easy it is to turn each wheel) was measured before each experiment and was included as a covariate in statistical analyses, as was age. During the course of the experiments, a total of three males and three females were eliminated because of death (one male), injection problems (one female), wheel malfunction (one male), or because they were observed to exhibit twirling behaviour (running in rapid, small, stereotypic circles) in their cages (two females and one male). Thus, 45 males and 45 females were analysed statistically.

Second, we performed combined analyses of both sexes using the difference between the running values of the female and male within each family. In other words, we analysed the sex difference within each family.

We also analysed wheel running of all of the mice from this generation that received the routine 6-day wheel test (i.e. including those used in this study), with age and wheel freeness as covariates.

## Results

### Baseline wheel running

Considering all of the mice from generation 48 ($n = 324$ females, 265 males) that received the 6-day wheel test, females from the HR lines (12 891 ± 285 revolutions/day; least-squares mean ± standard error) ran three-fold more than control females (4294 revolutions/day) on days 5 + 6 ($P < 0.001$). For males, HR ran 11 026 (± 492) as compared with 3529 (± 551) revolutions/day for control, yielding a 3.12-fold differential ($P < 0.001$). HR females ran faster than controls (HR = 24.8 ± 0.8, control = 9.3 ± 0.9 RPM, $P < 0.001$) but they did not run significantly more minutes per day (HR = 520 ± 27, control = 451 ± 28, $P = 0.13$). HR males ran both faster (HR = 21.7 ± 1.0, control = 9.2 ± 1.1 RPM, $P < 0.001$) and more minutes per day than control (HR = 503 ± 35, control = 371 ± 36, $P < 0.05$).

Results were similar for the subset of males and females used in the present experiment. Females from the HR lines (13 201 ± 584 revolutions/day) ran 3.07-fold more than control females (4294 ± 547 revolutions/day) on days 5 + 6 ($P < 0.001$). For males, HR ran 11 930 ( ± 615) as compared with 3631 ( ± 589) revolutions/day for control, yielding a 3.29-fold differential ($P < 0.001$). HR females ran faster than controls (HR = 25.6 ± 1.2, control = 9.1 ± 1.1 RPM, $P < 0.001$), but they did not run significantly more minutes per day (HR = 518 ± 36, control = 442 ± 35, $P = 0.19$). HR males ran both faster (HR = 23.2 ± 1.1, control = 9.5 ± 1.0 RPM, $P < 0.001$) and more minutes per day than control (HR = 512 ± 40, control = 369 ± 39, $P < 0.05$).

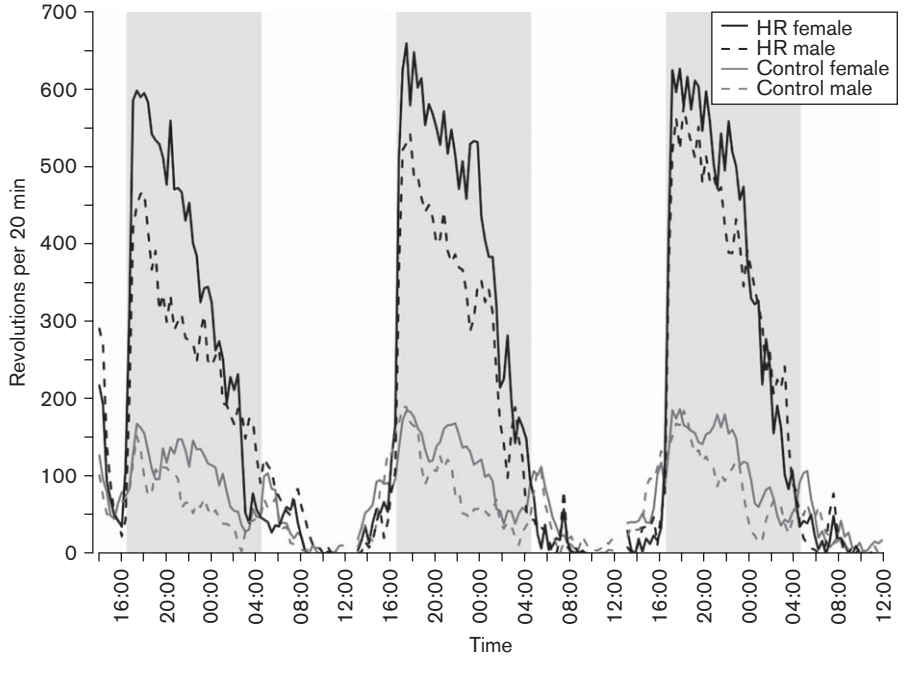
As expected, and as shown in Fig. 1 for the three days before injections, the 45 HR mice used in the drug trials ran significantly more total revolutions than did the 45 mice from the control lines. Figure 1 also shows that females ran more than males within both the HR and control lines.

### Drug response

Figure 2 shows the wheel running in 10-min bins of female (top) and male (bottom) HR and control mice during the 10–130 min postinjection, as well as average revolutions per 10 min during the 30 min before injection. Injections of both vehicle and rimonabant caused a reduction in wheel running in all mice. For females, the reduction depended on dose (repeated-measures analyses of covariance, Table 1, all $P < 0.001$), and the effect of dose depended on linetype for total revolutions, average speed, and maximum speed (all $P < 0.05$), but not for the amount of time spent running ($P = 0.7$). Adjusted means for females are shown in Table 2.

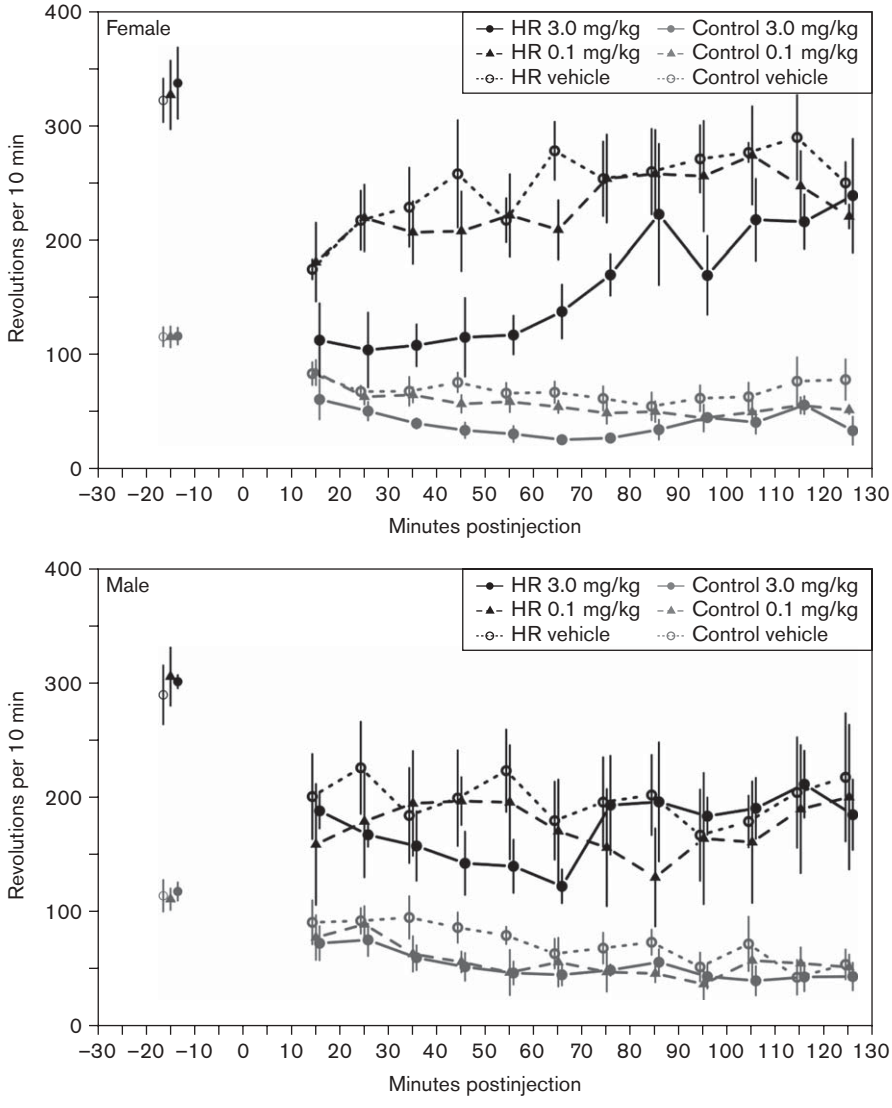
For males, the reduction in wheel running also depended on dose for revolutions and average speed (Table 1,

**Fig. 1**




Daily pattern of wheel running (revolutions in 20-min bins) for mice from high runner (HR)(selected) and control lines during 3 days before the start of rimonabant injections (28 April–1 May 2007). Note that females run more than males in both linetypes. Grey bars indicate lights off.

**Fig. 2**




Wheel running revolutions in 10-min bins during intraperitonial rimonabant injections (low dose = 0.1 mg/kg, high dose = 3.0 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). Rimonabant reduced wheel running acutely in all mice, but for females (top panel) the reduction was significantly greater for high runner (HR) lines than for control lines (see Table 1, $P$ for linetype × dose interaction = 0.0227).

$P < 0.05$), but not for duration (Table 1, $P = 0.10$). Moreover, the dose × linetype interaction was not statistically significant for any measure of wheel running (all $P > 0.5$). Adjusted means for males are shown in Table 3.

The foregoing separate analyses of males and females suggest a significant interactive effect of sex and linetype on the wheel-running response to rimonabant, and this is supported by analyses of the difference in running between females and males within families. For revolutions, this analysis indicated a significant effect of dose ($P < 0.05$), a nonsignificant effect of linetype ($P = 0.17$), but a significant dose × linetype interaction ($P < 0.05$). Thus, the magnitude of the sex difference depends on the dose of rimonabant, and this effect depends further on linetype. For the time spent running, the analysis indicated a significant effect of dose ($P < 0.05$), but a nonsignificant effect of linetype ($P < 0.63$), and dose × linetype interaction ($P = 0.28$). Thus, the magnitude of the sex difference in running time depends on the dose of rimonabant. For average speed, the analysis indicated no significant effect of dose ($P = 0.19$), linetype ($P = 0.14$) or dose × linetype ($P = 0.30$). Finally, for maximum speed, the analysis

**Table 1 Repeated-measures analyses (SAS Procedure Mixed) of wheel running 10–70 min after injecting vehicle, low or high dose of rimonabant**

| Trait | *F* for dose | *P* for dose | *F* for linetype | *P* for linetype | *F* for interaction | *P* for interaction |
|---|---|---|---|---|---|---|
| Females | | | | | | |
| Revolutions | 16.04 | 0.0004 | 34.45 | 0.0011 | 5.28 | 0.0227 |
| Time | 19.90 | 0.0002 | 3.52 | 0.1096 | 0.35 | 0.7108 |
| Average speed | 21.68 | 0.0001 | 40.49 | 0.0007 | 5.58 | 0.0194 |
| Maximum speed | 21.39 | 0.0001 | 48.09 | 0.0004 | 3.99 | 0.0468 |
| Males | | | | | | |
| Revolutions | 6.32 | 0.0133 | 15.59 | 0.0076 | 0.72 | 0.5068 |
| Time | 2.85 | 0.0972 | 2.02 | 0.2053 | 0.24 | 0.7923 |
| Average speed | 4.68 | 0.0314 | 16.60 | 0.0065 | 0.46 | 0.6429 |
| Maximum speed | 2.09 | 0.1669 | 21.58 | 0.0035 | 0.29 | 0.7503 |

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

**Table 2 Least-squares (adjusted) means and standard errors from repeated-measures analyses of wheel running 10–70 min after injections, as reported in Table 1, for females**

| Trait | Control mean | Control SE | HR mean | HR SE | HR/control mean |
|---|---|---|---|---|---|
| Revolutions | | | | | |
| Vehicle | 72.0 | 18.0 | 226.8 | 18.8 | 3.15 |
| Low dose | 64.4 | 18.0 | 205.2 | 18.8 | 3.19 |
| High dose | 40.9 | 18.0 | 114.3 | 18.8 | 2.79 |
| Time | | | | | |
| Vehicle | 6.8 | 0.5 | 8.1 | 0.5 | 1.19 |
| Low dose | 6.3 | 0.5 | 7.6 | 0.5 | 1.20 |
| High dose | 4.8 | 0.5 | 5.6 | 0.5 | 1.16 |
| Average speed | | | | | |
| Vehicle | 9.1 | 1.7 | 25.1 | 1.8 | 2.77 |
| Low dose | 8.2 | 1.7 | 23.9 | 1.8 | 2.92 |
| High dose | 5.8 | 1.7 | 15.6 | 1.8 | 2.71 |
| Maximum speed | | | | | |
| Vehicle | 13.4 | 2.3 | 35.6 | 2.4 | 2.67 |
| Low dose | 12.0 | 2.3 | 34.9 | 2.4 | 2.90 |
| High dose | 8.8 | 2.3 | 25.3 | 2.4 | 2.89 |

All values are means per 10-min intervals. Time denotes number of 1-min intervals with at least one revolution; average speed is revolutions/time; maximum speed is revolutions in the single highest 1-min interval.
HR, high runner.

indicated no significant effect of dose ($P = 0.11$), linetype ($P = 0.07$) or dose × linetype ($P = 0.24$).

## Discussion

Females from four replicate lines of mice that have been selectively bred (48 generations) for high voluntary wheel-running behaviour (HR lines) showed altered responsiveness to a selective CB1 receptor antagonist as compared with females from four nonselected control lines. Males from the HR lines did not exhibit a differential response. Thus, aspects of the ECS, or a physiological system regulated by the ECS, seem to have evolved in a sex-specific manner in response to selective breeding for high activity levels.

**Table 3 Least-squares (adjusted) means and standard errors from repeated-measures analyses of wheel running 10–70 min after injections, as reported in Table 1, for males**

| Trait | Control mean | Control SE | HR mean | HR SE | HR/control mean |
|---|---|---|---|---|---|
| Revolutions | | | | | |
| Vehicle | 76.8 | 21.4 | 195.5 | 21.9 | 2.54 |
| Low dose | 57.2 | 21.4 | 176.6 | 21.9 | 3.09 |
| High dose | 50.9 | 21.4 | 148.7 | 21.9 | 2.92 |
| Time | | | | | |
| Vehicle | 6.7 | 0.8 | 7.8 | 0.8 | 1.17 |
| Low dose | 5.5 | 0.8 | 7.1 | 0.8 | 1.30 |
| High dose | 5.6 | 0.8 | 7.2 | 0.8 | 1.29 |
| Average speed | | | | | |
| Vehicle | 9.9 | 2.3 | 22.3 | 2.3 | 2.26 |
| Low dose | 7.2 | 2.3 | 20. 0 | 2.3 | 2.75 |
| High dose | 7.1 | 2.3 | 17. 7 | 2.3 | 2.48 |
| Maximum speed | | | | | |
| Vehicle | 14.9 | 2.9 | 30.6 | 2.9 | 2.05 |
| Low dose | 10.8 | 2.9 | 28.9 | 2.9 | 2.69 |
| High dose | 11.5 | 2.9 | 27.8 | 2.9 | 2.41 |

All values are means per 10-min intervals. Time denotes number of 1-min intervals with at least one revolution; average speed is revolutions/time; maximum speed is revolutions in the single highest 1-min interval.
HR, high runner.

It is important to emphasize that this interpretation is based on statistical analyses of the actual wheel-running traits measured in control line versus HR females. If posttreatment running responses are analysed as proportional values, relative to baseline running behaviours within each group, then differences between groups lose statistical significance. For example, an analysis of the ratio of revolutions after high-dose/sham injection indicates no significant effect of linetype for either females ($P = 0.62$) or males ($P = 0.83$). Thus, the proportional response to rimonabant does not differ between HR and control lines. However, analysis of proportional responses can be quite misleading when the groups being compared differ greatly in baseline values, as is true in the present case, where HR mice run approximately three-fold more than controls (see Figs 1 and 2). We believe that the repeated-measures analysis of the actual values – not ratios to sham-injection values – is the most statistically sound way to analyse these data because of physiological differences in wheel running between the HR and control lines. The HR mice may run voluntarily near their maximal aerobic speed (i.e. almost at their maximal rate of oxygen consumption), whereas mice from the control lines do not (Girard *et al.*, 2001; Rezende *et al.*, 2005). This means that HR and control mice are operating under different physiological regimens during the times of peak running every night, when this study was conducted. Thus, an increase or decrease of, say, 10%, in wheel running would not mean the same thing physiologically to an HR and control mouse.

### Sex differences

Earlier studies of these lines of mice have documented substantial sex differences in wheel running between HR

and control lines. In particular, female HR mice have evolved higher daily running distances almost entirely by an increase in average (and maximum) running speed, whereas males have shown increases in both speed and duration of wheel activity (Swallow *et al.*, 1998, 1999; Rhodes *et al.*, 2000; Girard *et al.*, 2001; Koteja and Garland, 2001; Garland, 2003; this study). Baseline wheel-running of mice in this study (Fig. 1) is consistent with the activity profiles of mice from previous generations and studies. However, this is the first study to use pharmacology as a means of understanding the neurobiological correlates of the sex differences. After injecting high dose of rimonabant, female HR mice differentially decreased total revolutions, and this was done through a reduction in running speed, with no statistical reduction in the amount of time spent running (Table 1).

One possible partial explanation for these results is that the effects of rimonabant are stronger in females – at least those prone to high activity – compared with males. However, there is little indication that the effects of rimonabant are sex dependent in other taxa. For example, Foltin and Haney (2007) found few sex differences in the effects of rimonabant on appetite in baboons. In addition, human trials of rimonabant for weight loss report no sex differences in efficacy (Isoldi and Aronne, 2008).

Therefore, the simplest interpretation of these results is that selection for high voluntary activity has altered some aspect of CB1 functionality in female HR mice, but apparently not in males. At a high dose of rimonabant (3.0 mg/kg), HR females had the greatest proportional response in terms of voluntary wheel running, which indicates that they are more sensitive to that dosage than male HR mice or controls in general. These results suggest that female HR mice may differentially utilize CB1 signalling during wheel running.

We suggest two hypotheses to explain such enhanced CB1 signalling in female HR mice. First, CB1 signalling modulates pain perception and likely plays an important role in exercise-induced analgesia (Richardson, 2000; Sparling *et al.*, 2003; Hohmann and Suplita, 2006). Heightened exercise-induced analgesia could allow female HR mice to increase running intensity and, therefore, run longer distances than controls. A recent study indicates that exercise-induced analgesia is intensity dependent (Hoffman *et al.*, 2004), suggesting that exercise-induced increases in CB1 signalling may be intensity dependent, and are able to influence neural systems of nociception. Although we have evidence that female HR mice do not differ in one measure of pain sensitivity (thermal tail-flick test: Li *et al.*, 2004), it is possible that exercise-induced analgesia is under at least partly separate control. Second, CB1 signalling is thought to mimic the action of drugs of abuse, producing a rewarding sensation that can condition behaviours (De Vries and Schoffelmeer, 2005; Maldonado *et al.*, 2006), and may therefore motivate increased high-speed running in females. If high-speed wheel running increases CB1 signalling, then it could lead to conditioning through neurobiological rewards. Although these possibilities require further testing, they suggest that the evolution of high-speed (i.e. high-intensity) wheel running in female mice may be linked to CB1 signalling, whereas the increased running duration observed only in HR males may have other causes.

Studies of rats suggest that sex-based differences in CB1 signalling are common. For example, male and female rats show differential CB1 receptor expression in the brain (Rodriguez de Fonseca *et al.*, 1994; Gonzalez *et al.*, 2000), and several cannabinoids are known to be more potent, or show greater effects, in females than males (Cohn, 1972; Tseng and Craft, 2001; Craft, 2005; Fattore *et al.*, 2007). It is also possible that the expression or function of the CB1 receptor itself is not directly related to the promotion of voluntary wheel running in female HR mice. For example, HR females could be more sensitive to potential negative effects of rimonabant administration (Pacher *et al.*, 2006), or have alterations upstream of the CB1 receptor that affect its functionality, such as in the synthesis, release or degradation of endocannabinoids.

Finally, it is important to note that our results seem to differ from a study by Compton *et al.* (1996), in which intravenous injection of rimonabant in doses upwards of 3 mg/kg is shown to produce locomotor stimulation for up to 4 h postinjection in male Institute for Cancer Research mice. However, their measure of locomotion was taken in a standard (novel) cage, during the day, 5–15 min postinjection. It is likely that this sort of locomotion has little to do with voluntary wheel running, which occurs at much higher speeds and over much longer time periods. Of course, it is possible that if we had included a higher dose then we may have observed locomotor stimulation in our mice, given that our highest dosage level (3 mg/kg) is at the lower bound of the range of doses used by Compton *et al.* (1996). Our maximum dosage level was chosen because it has been shown to be fully effective in blocking many cannabinoid, or presumed endocannabinoid effects, specific to CB1 receptors (Carai *et al.*, 2005; DeVries and Schoffelmeer, 2005; Pacher *et al.*, 2006). Multiple studies have found this dosage of rimonabant to have no effect on baseline exploratory behaviours in mice of the C57Bl6 strain (Tzavara *et al.*, 2003; Patel and Hillard, 2006; Gerdeman *et al.*, 2008). Thus, rather than stimulating behaviour, several studies have now found CB1 receptor blockade to reduce locomotor activity that is thought to reflect motivation (Introduction, and DeVries and Schoffelmeer, 2005) or emotional affect (e.g. swimming in the forced swim test: Tzavara *et al.*, 2003; Steiner *et al.*, 2007), suggesting in both cases a blunting of internal reward or positive emotional mechanisms.

### Conclusion

Our results suggest an important link between the ECS and the evolution of increased voluntary wheel-running behaviour in house mice. Knowledge of the relation between CB1 receptor signalling and voluntary exercise can increase the understanding of the role of cannabinoid signalling in exercise, and how the neurobiological correlates of reward incentive may change with selective breeding for a voluntary behaviour. In addition, because the ECS is an important neuromodulator of neural systems implicated in the perception of reward (Lupica and Riegel, 2005; Maldonado *et al.*, 2006; Cheer *et al.*, 2007; Mahler, 2007; Pillolla *et al.*, 2007), our results can help to elucidate aspects of the neurobiology of motivated behaviours in general.

Our results also supplement the growing body of work on sex differences in the behavioural effects of cannabinoids and their receptors. Although the field is primarily built on studies of rats and mice (Craft, 2005; McGregor and Arnold, 2007), these sex differences extend to human beings. Studies show that women seem to be more sensitive to cannabinoid-induced hypotension than men (Mathew *et al.*, 2003) and have greater expression of CB1 receptor protein in leucocytes (Onaivi *et al.*, 1999), and show age-related changes in CB1 receptor binding that are distinct from men, including in brain areas related to reward and emotion (Van Laere *et al.*, 2008). Makela *et al.* (2006) found that women were more prone than men to exhibit deficits in a spatial span memory task after a low sublingual dose of the cannabinoid, tetrahydrocannabinol. Interestingly, because the tetrahydrocannabinol treatment also enhanced a spatial working memory task facilitated by dopamine, the authors speculated that both effects might be secondary to cannabinoid-enhanced dopamine signalling (Makela *et al.*, 2006). These observations augment clinical studies that suggest, in general, women are more vulnerable than men during the transition period between opportunity to use and drug abuse (Brady *et al.*, 1999), and that women are more responsive to the rewarding effects of addictive drugs (Lynch, 2006). Given the therapeutic potential of cannabinoid-related treatments for a variety of disorders (Russo, 2004; Pacher *et al.*, 2006), including many that occur disproportionately in women, further examination of sex differences in the effect of cannabinoids are clearly warranted.

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