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CB1 receptor deficiency decreases wheel-running activity: Consequences on emotional behaviours and hippocampal neurogenesis

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ABSTRACT

Chronic voluntary wheel-running activity has been reported to hypersensitise central CB1 receptors in mice. On the other hand, pharmacological findings suggest that the CB1 receptor could be involved in wheel-running behaviour and in running-induced neurogenesis in the hippocampus. We analysed wheel-running behaviour for 6 weeks and measured its consequences on hippocampal neurogenesis in CB1 knockout ($CB1^{-/-}$) animals, compared to wild-type ($CB1^{+/+}$) littermates. Because wheel running has been shown to affect locomotor reactivity in novel environments, memory for aversive events and depression-like behaviours, we also assessed these behaviours in control and running $CB1^{+/+}$ and $CB1^{-/-}$ mice. When compared with running $CB1^{+/+}$ mice, the distance covered weekly by $CB1^{-/-}$ mice was decreased by 30–40%, an observation accounted for by decreased time spent and maximal velocity on the wheels. Analyses of running distances with respect to the light/dark cycle revealed that mutant covered less distance throughout both the inactive and the active phases of that cycle. Locomotion in an activity cage, exploration in an open field, and immobility time in the forced swim test proved insensitive to chronic wheel running in either genotype. Wheel running, per se, did not influence the expression and extinction of cued fear memory but counteracted in a time-dependent manner the deficiency of extinction measured in $CB1^{-/-}$ mice. Hippocampal neurogenesis, assessed by doublecortin labelling of immature neurons in the dentate gyrus, was lowered by 40% in control $CB1^{-/-}$ mice, compared to control $CB1^{+/+}$ mice. Although $CB1^{-/-}$ mice ran less than their wild-type littermates, the 6-week running protocol increased neurogenesis to similar extents (37–39%) in both genotypes. This study suggests that mouse CB1 receptors control wheel running but not its neurogenic consequences in the hippocampus.

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Introduction

On the basis of the well documented antidepressant and anxiolytic effects of physical exercise in humans (Martinsen and Morgan, 1997; Raglin, 1997; Salmon, 2001), works initiated in the beginning of the 80's have tried to uncover the neurobiological mechanisms underlying these positive effects of exercise. The early analyses of the neurochemical effects of acute/chronic exercise, as performed with rodent treadmills and running wheels, have led to hypothesise that monoaminergic (Chaouloff, 1989; Meeusen et al., 2001) and endorphinergic (Hoffmann, 1997) systems play a key role in the psychotropic effects of exercise. The last decade of research has provided an additional hypothesis based on the findings that chronic exercise bears neurogenic and neurotrophic consequences in the CNS

(Cotman et al., 2007; Van Praag, 2009). This last hypothesis is appealing in that it also provides a framework to study the positive consequences of exercise on cognition processes (Hillman et al., 2008; Van Praag, 2009). More recently, another line of research has surged after the seminal observation that a single bout of exercise in trained humans increases blood levels of the endocannabinoid anandamide (Sparling et al., 2003). The parallels between the so-called “runner's high” (i.e. happiness, elation, peacefulness) and some psychotropic effects of the plant-derived cannabinoid Δ^9 -tetrahydrocannabinol (THC, one main component of marijuana) have strengthened the idea that the endocannabinoid system (ECS) might mediate, at least in part, the mental effects of physical exercise (Dietrich and McDaniel, 2004).

The ECS is formed by cannabinoid receptors, their endogenous lipid ligands (endocannabinoids) and the machinery for the synthesis and degradation of endocannabinoids (Piomelli, 2003). Most central functions of the ECS are mediated by the type-1 cannabinoid (CB1) receptor (Freund et al., 2003; Piomelli, 2003; Marsicano and Lutz, 2006; Pacher et al., 2006). Of note in the present context is the finding that CB1 receptors mediate THC psychotropic effects (Ledent et al., 1999; Monory et al., 2007). The CB1 receptor is a G-protein coupled

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receptor densely expressed in numerous brain regions, including the basal ganglia, the cerebral cortex, the amygdala, the hippocampus and the hypothalamus (Herkenham et al., 1990; Katona et al., 1999; Marsicano and Lutz, 1999). It is mainly located on the presynaptic terminals of numerous neuronal types and the retrograde action of endocannabinoids at the CB1 receptor results in the inhibition of transmitter release (Alger, 2002; Piomelli, 2003; Chevaleyre et al., 2006). In this context, it is noteworthy that wheel running for 2 weeks was found to sensitise CB1 receptor-mediated inhibition of striatal GABAergic transmission whilst leaving intact the control of glutamatergic transmission exerted by striatal CB1 receptors (De Chiara et al., 2009). Wheel running for 8 days was also reported to stimulate the hippocampal ECS as it increased CB1 receptor binding and intrinsic activity whilst rising the levels of anandamide (Hill et al., in press). Actually, these positive effects of wheel running on the ECS may be just one aspect of a more complex functional loop. Thus, acute (Zhou and Shearman, 2004), but not repeated (Hill et al., in press), administration of the CB1 receptor blocker AM251 has been found to increase the distance covered by wheel running (Zhou and Shearman, 2004). On the other hand, another study observed that the acute administration of a different CB1 receptor blocker, namely SR141716, actually decreased, albeit in a temporary manner, running wheel activity (Keeney et al., 2008). Lastly, on the basis of a negative impact of repeated AM251 administration on wheel-running-induced progenitor cell proliferation in the dentate gyrus, it has been proposed that the ECS may also exert a permissive influence on the neurogenic impact of exercise (Hill et al., in press).

The aforementioned studies indicate that the ECS might control wheel-running behaviour and its impact on neurogenesis. This suggestion relies however on the systemic use of CB1 receptor antagonists, indicating the crucial need to confirm these observations in other models of CB1 receptor inactivation. Among these, the genetic knockout of mouse CB1 receptors ($CB1^{-/-}$) allows investigating the consequences of the absence of CB1 receptors without the possible confounding variables linked to pharmacological approaches. Accordingly, the present study used $CB1^{-/-}$ mice and their wild-type littermates (Marsicano et al., 2002; Marsicano et al., 2003; Monory et al., 2007) with a three-fold aim. First, we compared wheel-running behaviour during a 6-week period in the two mouse genotypes. Second, because either wheel running or $CB1$ deficiency may affect locomotor reactivity to novel environments (Ledent et al., 1999; Urigüen et al., 2004; Duman et al., 2008; Fuss et al., in press; Salam et al., 2009), recall of fear memories (Marsicano et al., 2002; Cannich et al., 2004; Burghardt et al., 2006; Greenwood et al., 2003; Kamprath et al., 2006; Greenwood et al., 2009; Dubreucq et al., in press) and behavioural despair (Shearman et al., 2003; Duman et al., 2008; Steiner et al., 2008b; Dubreucq et al., in press), we assessed these emotional behaviours during the 6th week of running in mutant and wild-type mice. Third, in keeping with the aforementioned control of wheel-running hippocampal cell proliferation by CB1 receptors (Hill et al., in press), we analysed the impact of chronic wheel-running activity on hippocampal neurogenesis in $CB1^{+/+}$ and $CB1^{-/-}$ mice.

Materials and methods

Animals

Constitutive $CB1$ mutant and wild-type mice, initially backcrossed to C57Bl/6NCrl for 7 generations (Marsicano et al., 2002; Marsicano et al., 2003; Monory et al., 2007), were generated by heterozygous breeding to avoid possible genetic differences in maternal care. $CB1^{-/-}$ mutant mice and their wild-type $CB1^{+/+}$ littermates were all genotyped at 1 week of age and regentyped at the end of the experiments by PCR as previously described (Marsicano et al., 2002). On arrival from the breeding facility, male wild-type and $CB1$ mutant mice were immediately housed individually in standard cages provided with running

wheels (diameter: 25 cm; Intellibio, Seichamps, France) set free (running mouse group) or blocked permanently (control mouse group). Twenty two of the 32 mice used in this study were aged 9–10 weeks, the others being aged 8, 11 or 12 weeks. In keeping with this heterogeneity, we matched age to genotype and housing condition ($CB1^{+/+}$ housed with locked and free wheels: 9–12 weeks and 8–10 weeks, respectively; $CB1^{-/-}$ housed with locked and free wheels: 8–12 weeks and 8–11 weeks, respectively). Mice were housed in a temperature-controlled room with food and water *ad libitum* under a regular 12-h light/dark cycle (lights on: 07:00 h).

Pain and discomfort of the animals were reduced at minimum in strict compliance with European directives and French laws on animal experimentation (authorization no. 06369).

Procedure

There were two independent series of experiments, with control and running $CB1^{-/-}$ mutant mice and wild-type $CB1^{+/+}$ mice included in each series (total number of mice/genotype/housing condition = 7–9). All running wheels were connected to a computer which allowed the on-line recording of all running variables (speed, distance covered, and number of running episodes) throughout the 6-week period (Dubreucq et al., in press). Mice were weighed twice, i.e. on the first day of their arrival and at the end of the 6th week. The amount of food eaten during this 6-week period was also monitored in the second series of experiments. To this aim, the food dispenser was weighed (with a 0.1 g precision level) without correction for spillage, if any. At the beginning of the 6th week, each mouse was exposed to behavioural tests (one test per day between 14:00 h and 17:00 h) for 7 consecutive days. Thus, mice were exposed to (i) an activity cage on day 1, (ii) an open field test on day 2, (iii) a fear conditioning protocol on day 3 followed by daily fear recall sessions on days 4 to 6, and (iv) a forced swim test on day 7. After each test, mice were returned to their respective home cages (with either a blocked or a free wheel; see above). In the first series of experiments, mice were sacrificed 30 min after the completion of the forced swim test. Their adrenals were rapidly dissected out and stored in dry ice. Mice from the second series of experiments were used for hippocampal neurogenesis quantification. To this end, mice were deeply anaesthetised with pentobarbital (3 days after the completion of the behavioural tests), their adrenals dissected out and stored in dry ice before mice underwent the perfusion procedure (see below). The fat surrounding the glands was visualised using an Olympus SZX10 Stereo microscope and removed for subsequent adrenal weight measurements.

Activity cages

Each mouse was placed for 5 min at the center of a plastic arena (length, 40 cm; width, 24 cm; height, 19 cm) bearing a floor divided into 15 equal squares (8×8 cm). Horizontal activity (number of squares crossed) was recorded under dim illumination (5 lx) by means of a videocamera (attached to the ceiling of the test room) connected to a computer placed outside the test room.

Open field

Each animal was placed for 5 min at the center of a white wooden square arena (60×60 cm; height 19 cm) bearing a floor divided into 36 equal squares (10×10 cm). Peripheral (number of crossings of the 20 squares adjacent to the walls) and central (number of crossings of the 16 central squares) horizontal activities were videorecorded under intense illumination (120 lx).

Fear conditioning and cued recall

A conditioning box, made of grey Perspex (length: 26 cm; width: 18 cm; height: 25 cm) with a metal grid floor, was located in a sound-proof chamber (length: 55 cm; width: 60 cm; height: 50 cm) in a room adjacent to the housing room. On the conditioning day, each mouse was placed in the conditioning box and left free to explore for 3 min. A sound (1.5 kHz, 60 dB) was then emitted for 20 s with the last second of tone emission being coupled to one single footshock (0.5 mA). The animal was left in the fear conditioning box for another minute without any stimulus before being housed back in its home cage.

On the next 3 days (recall tests), the top of each home cage (free/blocked running wheel, food and water dispensers) was removed to be covered by a grid allowing full observation of the mouse in its cage. The home cage was then placed into the sound-proof chamber. After a 3-min pre-tone period, the same tone used for conditioning was presented for a 3-min period. The mouse was then left for another minute in the chamber before removal of the home cage. The observation grid placed on the top of the cage was then removed, the running wheel and the food/water dispensers were fixed back to the home cage and the latter was returned back to the housing facility room.

Freezing time (i.e. lack of movements except those associated with breathing) was monitored during the 3 min exposure to sound on each of the three recall tests, and it was scored by means of a customised EVENTLOG program. Note that one mouse (wheel-running $CB1^{-/-}$ mouse) was then removed from the analysis due to an unusual behaviour (full immobility throughout the 3 days of recall).

Forced swim test

Each mouse was placed for 6 min in a glass beaker (diameter: 18 cm, height: 27 cm) filled with 3 l of water (height: 14 cm, temperature: $26 \pm 1^\circ\text{C}$) under a 100-lx illumination. Water was changed between mice. Immobility (floating) was recorded for the 6-min test duration and scored using a customised EVENTLOG program.

Doublecortin staining

Following deep anaesthesia (see above), mice were transcardially perfused with 40 ml of heparinised phosphate-buffered saline (PBS, pH = 7.3) followed by 30 ml of paraformaldehyde (4% in 0.1 M PBS pH = 7.3). Brains were postfixed in paraformaldehyde and sequential 40 μm coronal sections were cut on a vibratome. One mouse ($CB1^{-/-}$ housed with blocked wheels) was removed from the analysis at that stage due to a pentobarbital overdose. Immunoperoxidase labelling of doublecortin (DCX) was performed on one-in-ten free-floating sections as previously described (Lemaire et al., 2000; Koehl et al., 2008) using a rabbit polyclonal anti-DCX (1:6000; Sigma D9818). Staining was visualised with the biotin–streptavidin technique (ABCkit, DAKO) with 3,3-diaminobenzidine as chromogen. The number of immunoreactive (IR) cells in the DG was estimated using the optical fractionator method in which every tenth section through the dorso-ventral extent of the DG was examined (Lemaire et al., 2000; Koehl et al., 2008). All cells excluding those in the uppermost focal plane were counted under $\times 1000$ magnification. Results are expressed as the total number of cells in the whole DG (left and right hemispheres).

Statistics

All analyses were performed with the GB-Stat software (v10; Dynamic Microsystems Inc., Silver Spring, MD, USA). Genotype and housing condition comparisons were achieved through Student *t*-tests (parametric data) or Mann–Whitney U-test (nonparametric

data) when assessing two-group comparisons, and by means of ANOVAs with/without repeated factors. If interactions between main variables were significant, post hoc group comparisons were then performed using Tukey's multiple comparison test. When necessary, data were log-transformed to reach homogeneity of the variances.

Results

Wheel-running behaviour of $CB1$ receptor knockout mice

An analysis of the running distance covered each week revealed that $CB1^{-/-}$ mice ran less than their $CB1^{+/+}$ wild-type littermates during the whole course of the study ($F_{1,14} = 5.94$, $P = 0.029$; Fig. 1A). This difference was due to a decrease in both the time spent running by $CB1^{-/-}$ mice ($F_{1,14} = 7.86$, $P = 0.014$; Fig. 1B) and their maximal

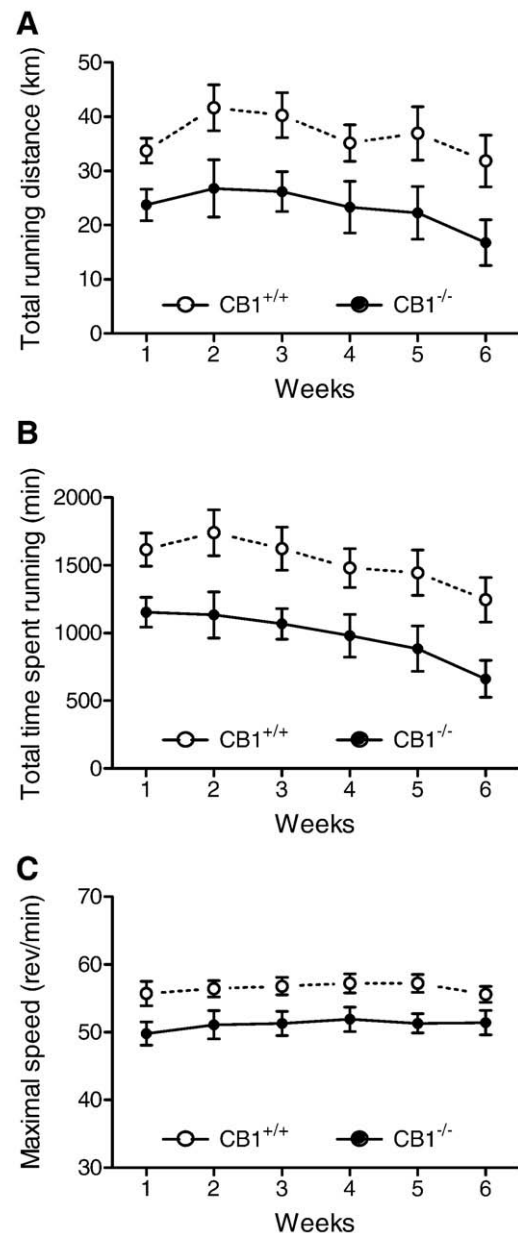


Fig. 1. Weekly wheel running profiles of $CB1^{+/+}$ and $CB1^{-/-}$ mice throughout the 6-week study. Total running distance (A), total time spent running (B), and maximal speed (C) were all found to be decreased in $CB1^{-/-}$ mice (see text for detailed statistics). Values are the means \pm SEM of 8 animals pooled from two series of experiments. Note that behavioural tests were performed throughout the 6th week.

velocities ($F_{1,14}=6.90$, $P=0.020$; Fig. 1C). The total distance covered by the mice ($F_{5,70}=8.17$, $P<0.0001$; Fig. 1A) and the time spent running ($F_{5,70}=14.77$, $P<0.0001$; Fig. 1B), but not the maximal velocities, varied throughout the 6 weeks of the study. This trend, however, proved identical in both genotypes, as illustrated by the nonsignificant influences of the genotype \times running week interactions. In keeping with these results, we next compared genotype-dependent wheel-running activities with respect to the light/dark cycle. As shown in Figs. 2A and B, $CB1^{-/-}$ mice ran less than their $CB1^{+/+}$ wild-type littermates ($F_{1,28}=5.73$, $P=0.024$) and wheel running activity throughout the dark phase (19 h00–07 h00) was much higher ($F_{1,28}=82.7$, $P<0.0001$) than that performed during the light phase (07 h00–19 h00). There was no statistical interaction between the genotype and the light/dark cycle phase considered, indicating that $CB1^{-/-}$ mice behaved as their $CB1^{+/+}$ wild-type littermates with respect to the light/dark cycle (Figs. 2A, B). Lastly, the distance covered during each phase of the light/dark cycle varied throughout the 6-week period ($F_{5,140}=7.75$, $P<0.0001$), with the genotype bearing no influence on that variation (Figs. 2A, B).

Metabolic and adrenocorticotrophic consequences of wheel running in $CB1$ receptor knockout mice

As expected (Cota et al., 2003), there was a significant body weight difference between genotypes at the onset of the study (29.9 ± 0.6 g and 25.7 ± 0.6 g in $CB1^{+/+}$ mice and $CB1^{-/-}$ mice, respectively: $t=4.84$, $P=0.0001$; $n=15$ –17). Therefore, wheel-running effects on body weight were examined as percent changes from initial body weight. Neither housing (blocked vs free wheels) nor the genotype had a significant influence on body weight growth (Table 1). On the other hand, food intake analyses over the 6-week period of

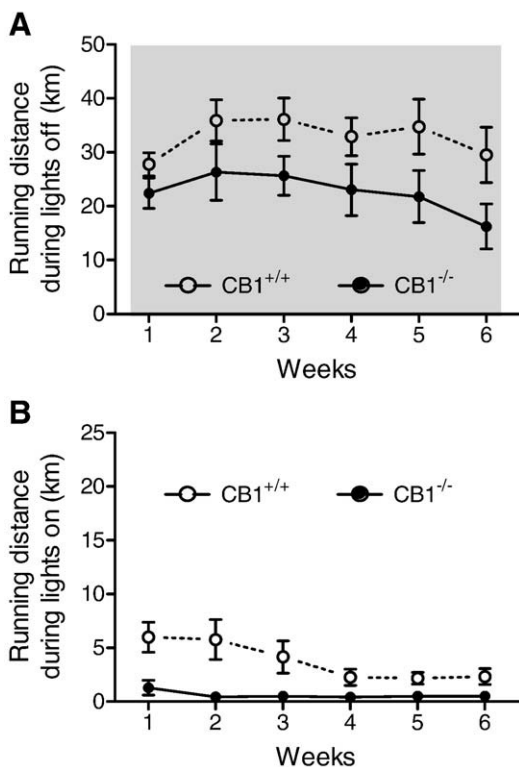


Fig. 2. Influence of the light/dark cycle on the weekly wheel running profiles of $CB1^{+/+}$ and $CB1^{-/-}$ mice. The total running distances, which are much higher during the dark (A) than during the light (B) phases of the light/dark cycle, are decreased in $CB1^{-/-}$ mice (see text for detailed statistics). Values are the means \pm SEM of 8 animals pooled from two series of experiments. Note that the scale of the Y axis is different between graphs and that behavioural tests were performed throughout the 6th week. The grey zone corresponds to the active phase (lights off) of the light/dark cycle.

Table 1
Metabolic, adrenocorticotrophic and emotional consequences of wheel running in $CB1^{+/+}$ and $CB1^{-/-}$ mice.

	Running wheels			
	Blocked		Free	
	$CB1^{+/+}$	$CB1^{-/-}$	$CB1^{+/+}$	$CB1^{-/-}$
Body weight (% change)	109 \pm 3	107 \pm 1	111 \pm 1	108 \pm 1
Food intake (g)	195 \pm 6	182 \pm 7*	239 \pm 8 ⁺	216 \pm 9* ⁺
Adrenals				
Weights (mg)	3.83 \pm 0.24	3.66 \pm 0.19	3.49 \pm 0.28	3.70 \pm 0.18
Weights (mg/10 g b.w.)	1.14 \pm 0.08	1.38 \pm 0.06*	1.15 \pm 0.05	1.30 \pm 0.07*
Activity cage				
Squares crossed	124 \pm 19	116 \pm 10	129 \pm 14	129 \pm 16
Open field test				
Peripheral squares crossed	143 \pm 20	170 \pm 20	155 \pm 16	149 \pm 33
Central squares crossed	55 \pm 9	74 \pm 10*	58 \pm 12	96 \pm 7*
Forced swim test				
Immobility (seconds)	162 \pm 8	135 \pm 14	159 \pm 12	141 \pm 20

Values are the mean \pm SEM of $n=7$ –9 animals, except for food intake ($n=4$). Statistics (see text) revealed main influences of genotype and/or housing condition (free vs blocked wheels), but no interaction between variables; * and ⁺ denote at least $P<0.05$ for the influences of genotype and wheel running, respectively.

investigation revealed that $CB1^{-/-}$ mice ate less than $CB1^{+/+}$ mice ($F_{1,12}=5.7$, $P=0.034$) whilst wheel running stimulated food intake ($F_{1,12}=27.7$, $P=0.0002$) (Table 1). There was no significant interaction between the genotype and the housing condition, indicating that wheel running-induced stimulation of feeding was similar in $CB1^{+/+}$ and $CB1^{-/-}$ mice (Table 1).

An analysis of adrenal weights in the respective groups revealed no significant influence of either genotype or running wheel activity (Table 1). However, when analysed as relative values (over body weights), $CB1^{-/-}$ mice displayed heavier adrenals than $CB1^{+/+}$ mice ($F_{1,26}=8.1$, $P=0.008$; Table 1).

Emotional consequences of wheel running in $CB1$ receptor knockout mice

Locomotion in activity cages exposed to a dim light was insensitive to either the mouse genotype or running wheel activity (Table 1). Central, but not peripheral, locomotion in a brightly lit open field was higher in $CB1^{-/-}$ mice ($F_{1,28}=8.3$, $P=0.008$) whilst none of these two exploratory activities proved sensitive to wheel running (Table 1). Lastly, forced swimming immobility was influenced neither by the mouse genotype (although a trend toward decreased immobility in mutants could be noted) nor by wheel running (Table 1).

Fig. 3 depicts the expression and extinction of conditioned fear in $CB1^{+/+}$ mice and $CB1^{-/-}$ mice housed with blocked or free running wheels. As indicated above, the extinction protocol began 24 h after the auditory fear conditioning session with one daily exposure to the auditory cue for 3 consecutive days. As summarised in Fig. 3A, $CB1^{-/-}$ mice froze more than $CB1^{+/+}$ mice throughout the 3 recall days ($F_{1,26}=18.2$, $P=0.002$) whilst wheel running proved without influence. Further, freezing intensity decreased with the number of recall tests ($F_{2,52}=37.7$, $P<0.0001$), and did so in a genotype-independent manner (Fig. 3A).

To complement this information, we then included a within-session analysis of extinction, analysing the freezing responses in 20-s bins for each experimental day (Fig. 3B,C). Because there were four variables to be analysed (genotype, running activity, 20-s analysis bins, recall days), two of which obeyed a repeated design (20-s analysis bins, recall days), we fractionated this analysis into two parts with respect to the housing condition.

Confirming the gross analysis mentioned above, an examination of the mice housed with blocked wheels showed that $CB1^{-/-}$ mice froze more than $CB1^{+/+}$ mice throughout the 3 recall days ($F_{1,14}=12.7$,

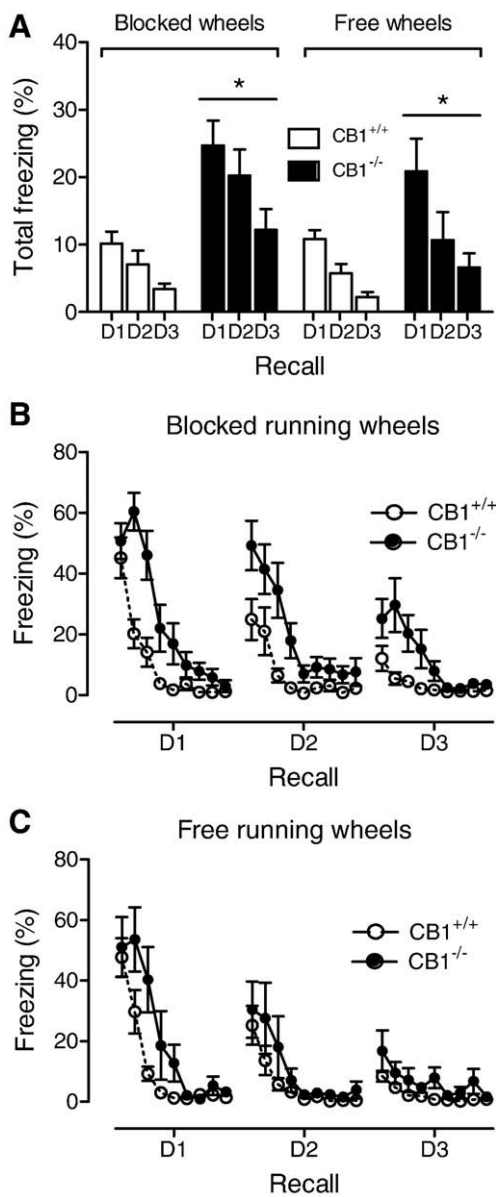


Fig. 3. Effects of wheel running on conditioned freezing behaviour of $CB1^{+/+}$ and $CB1^{-/-}$ mice during cued recall tests performed for 3 days after conditioning. The respective influences of wheel running, genotype and daily recall session (3 min) on freezing behaviour are shown in (A). Within-session variations (bin intervals: 20 s) in freezing behaviour in mice housed with blocked and free wheels are shown respectively in (B) and (C). Values are the means \pm SEM of 7–9 animals pooled from two series of experiments. D1–3 stands for days 1–3. * denotes at least $P < 0.05$ for the main influence of genotype (see text for detailed statistics).

$P = 0.003$; Fig. 3B). Freezing intensity decreased with time, whether examined within ($F_{8,112} = 55.8$, $P < 0.0001$) or between ($F_{2,28} = 11.4$, $P = 0.003$) sessions, reflecting effective fear extinction with time (Fig. 3B). However, the genotype was found to interact with the within-session fear extinction ($F_{8,112} = 8.2$, $P < 0.0001$ for the genotype \times 20-s bin interaction), illustrating that $CB1^{-/-}$ mice were slower than $CB1^{+/+}$ mice in extinguishing fear within each recall test (Fig. 3B).

The analysis of running mice confirmed that $CB1$ deletion increased freezing scores ($F_{1,12} = 5.5$, $P = 0.037$; Fig. 3C), although this was less marked than in mice with blocked wheels (Fig. 3B). Within-session ($F_{8,96} = 50.0$, $P < 0.0001$) and between-session ($F_{2,24} = 40.6$, $P < 0.0001$) fear extinction was observed, with the former being again weakened in $CB1^{-/-}$ mice, compared to $CB1^{+/+}$ mice ($F_{8,96} = 3.3$, $P = 0.002$; Fig. 3C). However, specific to running mice (Fig. 3C), compared to mice housed with blocked wheels (Fig. 3B), was the

finding that the within-session \times between-session interaction on freezing was influenced by the genotype ($F_{16,192} = 2.2$, $P = 0.006$). Thus, increasing the number of recall sessions allowed progressively wheel running $CB1^{-/-}$ mice to behave as wheel running $CB1^{+/+}$ mice during the first half of each session (i.e. the period during which the genotype difference was found to be maximal in mice housed with blocked wheels; Fig. 3B,C).

Consequences of wheel running on hippocampal neurogenesis in $CB1$ receptor knockout mice

To assess the impact of wheel running on hippocampal neurogenesis, the number of immature neurons expressing DCX were determined in the dentate gyrus of the hippocampus. Free wheel running ($F_{1,11} = 13.8$, $P = 0.003$) and $CB1$ deletion ($F_{1,11} = 35.8$, $P < 0.0001$) displayed respectively stimulatory and inhibitory influences on the number of DCX-IR cells (Fig. 4A, B). There was no interaction between genotypes and housing conditions, indicating that the effect of running on neurogenesis was independent of the presence/absence of $CB1$ receptors. Accordingly, if expressed as the percent changes of the number of DCX-IR cells counted in the controls (housed with blocked wheels), wheel running activity increased neurogenesis ($F_{1,11} = 13.8$, $P = 0.003$) to similar extents in $CB1^{+/+}$ mice and in $CB1^{-/-}$ mice (+37–39%; Fig. 4B).

Regression analyses of the number of DCX-IR cells over the running distance covered throughout the 6 weeks in either $CB1^{+/+}$ mice or $CB1^{-/-}$ mice revealed no significant relationships between variables, which is in keeping with the low number of animals in each group ($n = 4$). As the percent increases in wheel running-induced neurogenesis did not differ with the genotype (see above), we next performed a single regression analysis combining both mouse groups. Doing so, a significant relationship between the total amount of running and the level of neurogenesis ($r^2 = 0.6$; $F_{1,6} = 9.1$, $P = 0.023$) was observed (Fig. 4B).

Discussion

This study shows that $CB1$ deletion is associated with decreased wheel running activity. However, despite this negative relationship and the observation that $CB1$ deletion leads to a reduced hippocampal neurogenesis (present results; Jin et al., 2004; Aguado et al., 2006), we report that wheel running-induced neurogenesis is independent from $CB1$ receptors. Furthermore, wheel running did not affect locomotor reactivity and behavioural despair (as assessed by the forced swim test) but interacted with fear extinction processes in $CB1^{-/-}$ mice.

The finding that acute physical exercise in trained volunteers stimulates the ECS, as assessed by blood anandamide levels (Sparling et al., 2003), has raised much interest on the relationships between physical exercise and $CB1$ receptors. The link between $CB1$ receptors and the control of wheel running has been investigated in three studies. The first reported that an acute pretreatment (1 h before the active period of the light/dark cycle) with the $CB1$ receptor antagonist AM251 (1–10 mg/kg p.o.) doubled the wheel running distance covered overnight by C57Bl/6J male mice (Zhou and Shearman, 2004). In a second study, it was observed that repeated AM251 injections (1 mg/kg i.p. daily for 8 days), performed at the beginning of the inactive (light) phase, did not influence the running wheel activity of rats (Hill et al., in press). The third study showed that pretreatment (2 h after the onset of the active period of the light/dark cycle) with the $CB1$ receptor antagonist SR141716 decreased in a dose-dependent manner the running distances both in a control mouse line and in a mouse line selected for high wheel running activity (Keeney et al., 2008). Such an inhibitory effect of SR141716 was indeed accounted for by decreases in the time spent running and velocities (Keeney et al., 2008). In keeping with the limits of these pharmacological protocols and the contradictory findings they did

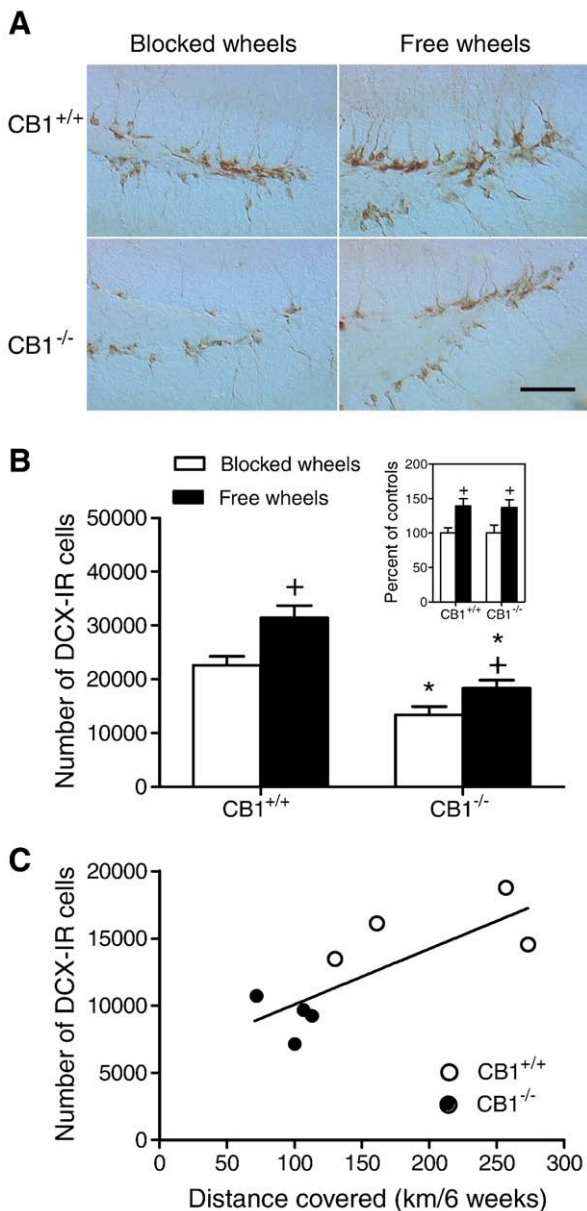


Fig. 4. Effects of wheel running on hippocampal neurogenesis in $CB1^{+/+}$ and $CB1^{-/-}$ mice. Illustrations of DCX staining in the dentate gyrus of $CB1^{+/+}$ and $CB1^{-/-}$ mice housed with blocked or free running wheels. Scale bar is 50 μ M (A). Wheel running and $CB1$ knockout respectively increased and decreased the number of DCX-IR cells (B). Values are the mean \pm SEM of 4 animals, except for $CB1^{-/-}$ mice housed with blocked wheels ($n = 3$). Relative running-induced increases in neurogenesis are similar in both genotypes (B, inset). Relationship between the number of DCX-IR cells and the total distance ran during the 6-week period in 4 $CB1^{+/+}$ and 4 $CB1^{-/-}$ mice (C). * and + denote at least $P < 0.05$ for the main influences of genotype and wheel running, respectively (see text for detailed statistics).

generate, we thought to examine wheel running behaviour in male $CB1^{-/-}$ mice and in their wild-type littermates. Our results indicate that $CB1$ deficiency is associated with a permanent reduction in the distance covered by mice given access to free running wheels. Further, we show that a reduction both in the time spent running and in the maximal running velocity contribute to this genotypic difference. Because nocturnal species such as mice display most of their wheel running activity during the dark phase (see Koteja et al., 1999 and de Visser et al., 2005), one likely explanation could be that $CB1^{-/-}$ mice display dysregulations in their diurnal rhythms of activity. The analysis of the respective running distances performed by $CB1^{-/-}$ mice, compared to $CB1^{+/+}$ mice, during each phase of the

light/dark cycle suggests a general (i.e. activity phase-independent) negative influence of $CB1$ deletion.

The mechanisms underlying the decrease in wheel running activity in $CB1$ deficient mice are unknown at the present time. One may exclude differences in basal locomotion because $CB1^{-/-}$ mice do not differ from $CB1^{+/+}$ mice with regard to locomotor activity when this is maximal (i.e. during the dark period: Jacob et al., 2009). On the other hand, one could speculate that these genotypic differences in running wheel activity are to some extent linked to the interactions between the ECS and rewarding centers. Thus, voluntary wheel running is often considered a self-reinforcing and rewarding behaviour (Sherwin, 1998; Lett et al., 2000; Werme et al., 2002; De Chiara et al., 2009), and $CB1$ signalling is well-known to impact onto central reward networks (Maldonado et al., 2006). Indeed, both $CB1$ receptor blockade and $CB1$ deletion promote reduced reward-driven behaviours (Maldonado et al., 2006). Our results thus support a reward-based hypothesis for the differences in wheel running between $CB1^{+/+}$ and $CB1^{-/-}$ mice. However, because the causalities and functions of voluntary wheel running are still unclear (Sherwin, 1998), the possibility that different mechanisms, including peripheral ones, might partly/totally underlie wheel running hypoactivity in $CB1^{-/-}$ mice cannot be excluded. One such mechanism could be metabolic in nature as $CB1$ deletion leads to decreased body weights due to a reduction in food intake and energy storage (Cota et al., 2003). Because wheel running is an energy-consuming task, we measured whether the genotypic differences in wheel running behaviour were associated with differences in body weight and food intake. Wheel running did not reduce body weight in either mouse genotype whilst the percent increase in food intake due to wheel running was similar in all mice. Further, correlation analyses of individual total distances over individual body weights did not reveal significant relationships whether $CB1^{+/+}$ mice ($r^2 = 0.07$), $CB1^{-/-}$ mice ($r^2 = 0.11$) or both mouse genotypes ($r^2 = 0.04$) were considered.

A survey of the consequences of $CB1$ deletion on the hypothalamo-pituitary-adrenal (HPA) axis suggests that such a mutation leads to increased basal and/or stress-induced elevations in circulating adrenocorticotropic hormone (ACTH) and corticosterone levels (Steiner and Wotjak, 2008). Wheel running may increase circulating corticosterone levels (Droste et al., 2003; Fediuc et al., 2006; Fuss et al., in press). Except from one study which reported increased adrenal weights in wheel running animals (Droste et al., 2003), exercise-elicited increases in circulating corticosterone levels may well occur without changes in adrenal weight (Fediuc et al., 2006; Fuss et al., in press). In our hands, wheel running proved ineffective on adrenal weights, whether absolute or relative weights were considered. On the other hand, the genotype influenced the relative adrenal weights with mutants bearing heavier glands, which could reflect hyperactivity of the corticotropic axis (Steiner and Wotjak, 2008).

The impact of chronic wheel running activity on locomotor activity/reactivity to novel environments is not clear (Duman et al., 2008; Fuss et al., in press; Salam et al., 2009). In the present study, we measured locomotor activity on the one hand, and locomotor reactivity to a highly aversive environment on the other hand. To assess locomotor activity, mice were first exposed under dim light to an environment of limited size. This setting, by minimising the aversiveness of the environment allows to capture the activity dimension of emotionality even if animals are tested during the light phase of the light/dark cycle (Ramos et al., 1997; Ramos and Mormède, 1998). On the other hand, the analysis of the exploration of the periphery of a brightly lit open field allows to measure the locomotor reactivity to aversive situations (Ramos et al., 1997; Ramos and Mormède, 1998). Through these means, we found that neither locomotor activity nor locomotor reactivity to an aversive setting were affected by prior wheel running. On the other hand, we observed that $CB1^{-/-}$ mice displayed increased central locomotion of the open field, in line with a trend observed in animals housed under standard conditions

(Moustié et al., unpublished data). In keeping with the antidepressant properties of physical exercise in humans (see above), numerous studies have investigated the impact of wheel running on so-called depression-like behaviours (but see: Holmes, 2003) in the forced swim and learned helplessness tests. To date, such a quest has provided contradictory results (Yoo et al., 2000; Greenwood et al., 2003; Duman et al., 2008; Fuss et al., in press). Herein, neither wheel running nor genotype bore a significant influence on immobility time in the forced swim test. We only noticed a trend toward decreased immobility in $CB1^{-/-}$ mice, in line with two previous studies (Shearman et al., 2003; Steiner et al., 2008a) whilst a third one reported that this trend was significant (Steiner et al., 2008b).

Wheel-running animals tested in a contextual fear conditioning paradigm display increased recall memory without alteration in extinction processes (Burghardt et al., 2006; Greenwood et al., 2009). However, it is still unknown whether this facilitatory impact of wheel running on context fear, which relies on hippocampal networks, extends to cued fear, i.e. an amygdala-dependent process (Phillips and LeDoux, 1992; Maren and Quirk, 2004; but see Maren, 2008). In our hands, freezing responses to the recall cue as well as the profiles of extinction of these freezing responses were insensitive to wheel running in $CB1^{+/+}$ mice. As concerns $CB1^{-/-}$ mice, we first observed that these mice displayed delayed intra- and inter-session extinction of fear, compared to $CB1^{+/+}$ mice. This result confirms previous reports (Marsicano et al., 2002; Cannich et al., 2004; Kamprath et al., 2006) and may reflect a dysregulation in habituation processes in mutant mice (Kamprath et al., 2006). Second, we observed that the condition under which $CB1^{-/-}$ mice were housed affected their profiles of fear extinction. Thus, the genotypic differences in freezing along the three recall sessions were stronger in mice housed with blocked wheels, as compared to wheel runners. Interestingly, whereas a clear impairment in extinction was present in $CB1^{-/-}$ mice housed with blocked wheels over the course of the three recall days, this phenotype progressively vanished in animals that had access to free running wheels so that freezing profiles were undistinguishable on the last recall session. This preliminary observation may be indicative of a genotype-dependent positive influence of running on extinction processes. This issue, which requires a more detailed investigation than the present one, will be addressed in the near future.

Since the seminal observation of van Praag et al. (1999), there has been extensive evidence that voluntary wheel running increases hippocampal progenitor cell proliferation and neurogenesis (van Praag, 2009). Because physical exercise increases circulating anandamide levels in humans (Sparling et al., 2003), and repeated CB1 receptor activation stimulate hippocampal neurogenesis (Jiang et al., 2005), a recent study addressed whether the ECS mediates wheel-running progenitor cell proliferation (Hill et al., in press). It was observed that one daily administration of the CB1 receptor antagonist AM251 (at the onset of the inactive period of the light/dark cycle) was sufficient to prevent the stimulatory effect of an 8-day wheel running paradigm on hippocampal cell proliferation in rats. In the present study, the relative increase in hippocampal neurogenesis elicited by wheel running was indeed similar (37–39%) between $CB1^{+/+}$ mice and $CB1^{-/-}$ mice. The reasons for this discrepancy may lie in the species used (rats vs mice), the approach (pharmacological vs genetic), and/or the duration of running (8 days vs 6 weeks). Interestingly, in the present study, the genotype-independent effect of wheel running occurred although (i) $CB1^{-/-}$ mice displayed decreased hippocampal neurogenesis, compared to their wild-type littermates (confirming previous observations: Jin et al., 2004; Aguado et al., 2006), and (ii) wheel running activity was lower in the former mouse group. This suggests that the decreased wheel running activity of $CB1^{-/-}$ mice was however sufficient to trigger neurogenesis independently of CB1 receptor signalling. To further analyse this possibility, we examined the relationships between the distance covered throughout the 6-week period and the number of DCX+ cells but the low number

of animals per group impeded any conclusion. Only if all animals were grouped did a significant relationship between the two variables emerge, in line with previous observations (van Praag, 2009). Taken together, the results from the present study indicate that wheel running-induced neurogenesis is independent from CB1 receptors. It remains to be explored whether the respective influences of wheel running and genotype on neurogenesis reported herein extend to cell proliferation processes.

In conclusion, the present study (i) shows that $CB1$ deficiency is associated with reduced wheel running activity, (ii) opens the promising possibility that wheel running reverses the deficit in extinction of fear memories in $CB1$ knockout animals, and (iii) indicates that although the mutant animals ran less and displayed reduced neurogenesis, compared to wild-type controls, the relative impact of wheel running on neurogenesis did not differ between genotypes. These results shed new light on the relationships between the ECS and voluntary wheel running but their relevance to human physical exercise is difficult to draw at the present time. Wheel running, as opposed to treadmill running, is voluntary but the time spent exercising (mostly during the dark phase) differs from humans where voluntary training sessions consist in acute, often single, daily bouts of exercise, as illustrated in the study of Sparling et al. (2003). One means to circumvent the limit of the wheel running model would be to restrict the duration during which mice are given the opportunity to run daily. In this context, it is noteworthy that mice given daily a 3-h access to wheel running activity display increased cell proliferation and neurogenesis in the dentate gyrus (Holmes et al., 2004). Interestingly, this statement holds true only if such an access is allowed in the middle of the dark phase (Holmes et al., 2004). The application of such a restricted wheel running protocol to our $CB1^{+/+}$ mice and $CB1^{-/-}$ mice should help to further dissect the relationships between the ECS and physical activity.

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References

- Aguado, T., Palazuelos, J., Monory, K., Stella, N., Cravatt, B., Lutz, B., Marsicano, G., Kokaia, Z., Guzmán, M., Galve-Roperh, I., 2006. The endocannabinoid system promotes astroglial differentiation by acting on neural progenitor cells. *J. Neurosci.* 26, 1551–1561.
- Alger, E., 2002. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog. Neurobiol.* 68, 247–286.
- Burghardt, P.R., Pasumarthi, R.K., Wilson, M.A., Fadel, J., 2006. Alterations in fear conditioning and amygdalar activation following chronic wheel running. *Pharmacol. Biochem. Behav.* 84, 306–312.
- Cannich, A., Wotjak, C.T., Kamprath, K., Hermann, H., Lutz, B., Marsicano, G., 2004. CB1 cannabinoid receptors modulate kinase and phosphatase activity during extinction of conditioned fear in mice. *Learn. Mem.* 11, 625–632.
- Chaouloff, F., 1989. Physical exercise and brain monoamines: a review. *Acta Physiol. Scand.* 137, 1–13.
- Chevalyere, V., Takahashi, K.A., Castillo, P.E., 2006. Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu. Rev. Neurosci.* 29, 37–76.
- Cota, D., Marsicano, G., Tschöp, M., Grübler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thöne-Reineke, C., Ortmann, S., Tomassoni, F., Cervino, C., Nisoli, E., Linthorst, A.C., Pasquali, R., Lutz, B., Stalla, G.K., Pagotto, U., 2003. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J. Clin. Invest.* 112, 423–431.
- Cotman, C.W., Berchtold, N.C., Christie, L.A., 2007. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci.* 30, 464–472.
- De Chiara, V., Errico, F., Musella, A., Rossi, S., Mataluni, G., Sacchetti, L., Siracusanò, A., Castelli, M., Cavasinni, F., Bernardi, G., Usiello, A., Centonze, D., 2009. Voluntary

- exercise and sucrose consumption enhance cannabinoid CB1 receptor sensitivity in the striatum. *Neuropsychopharmacology* 35, 374–387.
- Dietrich, A., McDaniel, W.F., 2004. Endocannabinoids and exercise. *Br. J. Sports Med.* 38, 536–541.
- Droste, S.K., Gesing, A., Ulbricht, S., Müller, M.B., Linthorst, A.C., Reul, J.M., 2003. Effects of long-term voluntary exercise on the mouse hypothalamic–pituitary–adrenocortical axis. *Endocrinology* 144, 3012–3023.
- Dubreucq, S., Marsicano, G., Chaouloff, F., 2010. Emotional consequences of wheel running in mice: which is the appropriate control? *Hippocampus* (in press).
- Duman, C., Schlesinger, L., Russell, D.S., Duman, R.S., 2008. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Res.* 199, 148–158.
- Fediuc, S., Campbell, J.E., Riddell, M.C., 2006. Effect of voluntary wheel running on circadian corticosterone release and on HPA axis responsiveness to restraint stress in Sprague–Dawley rats. *J. Appl. Physiol.* 100, 1867–1875.
- Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83, 1017–1066.
- Fuss, J., Ben Abdallah, N.M.B., Vogt, M.A., Touma, C., Pacifici, P.G., Palme, R., Witzemann, V., Hellweg, R., Gass, P., 2009. Voluntary exercise induces anxiety-like behavior in adult C57BL/6 mice correlating with hippocampal neurogenesis. *Hippocampus* (in press).
- Greenwood, B.N., Foley, T.E., Day, H.E.W., Campisi, J., Hammack, S.H., Campeau, S., Maier, S.F., Fleshner, M., 2003. Free-wheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. *J. Neurosci.* 23, 2889–2898.
- Greenwood, B.N., Strong, P.V., Foley, T.E., Fleshner, M., 2009. A behavioral analysis of the impact of voluntary physical activity on hippocampus-dependent contextual conditioning. *Hippocampus* 19, 988–1001.
- Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U. S. A.* 87, 1932–1936.
- Hill, M.N., Titterness, A.K., Morrish, A.C., Carrier, E.J., Lee, T.T., Gil-Mohapel, J., Gorzalka, B.B., Hillard, C.J., Christie, B.R., 2009. Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. *Hippocampus* (in press).
- Hillman, C.H., Erickson, K.I., Kramer, A.F., 2008. Be smart, exercise your heart: exercise effects on brain and cognition. *Nat. Rev. Neurosci.* 9, 58–65.
- Hoffmann, P., 1997. The endorphin hypothesis. In: Morgan, W.P. (Ed.), *Physical Activity & Mental Health*. Taylor & Francis, Washington, pp. 163–177.
- Holmes, P.V., 2003. Rodent models of depression: reexamining validity without anthropomorphic inference. *Crit. Rev. Neurobiol.* 15, 143–174.
- Holmes, M.M., Galea, L.A.M., Mistlberger, R.E., Kempermann, G., 2004. Adult hippocampal neurogenesis and voluntary wheel running activity: circadian and dose-dependent effects. *J. Neurosci. Res.* 76, 216–222.
- Jin, K., Xie, L., Kim, S.H., Parmentier-Batteur, S., Sun, Y., Mao, X.O., Childs, J., Greenberg, D.A., 2004. Defective adult neurogenesis in CB1 cannabinoid receptor knockout mice. *Mol. Pharmacol.* 66, 204–208.
- Katona, I., Sperlagh, B., Ski, A., Kálfalvi, A., Vizi, E.S., Mackie, K., Freund, T.F., 1999. Presynaptically located CB1 receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* 19, 4544–4558.
- Jacob, W., Yassouridis, A., Marsicano, G., Monory, K., Lutz, B., Wotjak, C.T., 2009. Endocannabinoids render exploratory behaviour largely independent of the test aversiveness: role of glutamatergic transmission. *Genes Brain Behav.* 8, 685–698.
- Jiang, W., Zhang, Y., Xiao, L., Van Cleemput, J., Ji, S.P., Bai, G., Zhang, X., 2005. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *J. Clin. Invest.* 115, 3104–3116.
- Kamprath, K., Marsicano, G., Tang, J., Monory, K., Bisogno, T., Di Marzo, V., Lutz, B., Wotjak, C.T., 2006. Cannabinoid CB1 receptor mediates fear extinction via habituation-like processes. *J. Neurosci.* 26, 6677–6686.
- Keeney, B.K., Raichlen, D.A., Meek, T.H., Wijeratne, R.S., Middleton, K.M., Gerdeman, G.L., Garland Jr., T., 2008. Differential response to a selective cannabinoid receptor antagonist (SR141716: rimonabant) in female mice from lines selectively bred for high voluntary wheel-running behaviour. *Behav. Pharmacol.* 19, 812–820.
- Koehl, M., Meerlo, P., Gonzales, D., Rontal, A., Turek, F.W., Abrous, D.N., 2008. Exercise-induced promotion of hippocampal cell proliferation requires beta-endorphin. *FASEB J.* 22, 2253–2262.
- Koteja, P., Garland Jr., T., Sax, J.K., Swallow, J.G., Carter, P.A., 1999. Behaviour of house mice artificially selected for high levels of voluntary wheel running. *Anim. Behav.* 58, 1307–1318.
- Ledent, C., Valverde, O., Cossu, G., Petitot, F., Aubert, J.-F., Beslot, F., Böhme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M., 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* 283, 401–404.
- Lemaire, V., Koehl, M., Le Moal, M., Abrous, D.N., 2000. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11032–11037.
- Lett, B.T., Grant, V.L., Byrne, M.J., Koh, M.T., 2000. Pairings of a distinctive chamber with the aftereffect of wheel running produce conditioned place preference. *Appetite* 34, 87–94.
- Maldonado, R., Valverde, O., Berrero, F., 2006. Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci.* 29, 225–232.
- Maren, S., 2008. Pavlovian fear conditioning as a behavioral assay for hippocampus and amygdala function: cautions and caveats. *Eur. J. Neurosci.* 28, 1661–1666.
- Maren, S., Quirk, G.J., 2004. Neuronal signalling of fear memory. *Nat. Rev. Neurosci.* 5, 844–852.
- Marsicano, G., Lutz, B., 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* 11, 4213–4225.
- Marsicano, G., Lutz, B., 2006. Neuromodulatory functions of the endocannabinoid system. *J. Endocrinol. Invest.* 29, 27–46.
- Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutierrez, S.O., van der Stelt, M., López-Rodríguez, M.L., Casanova, E., Schütz, G., Ziegler, W., Di Marzo, V., Behl, C., Lutz, B., 2003. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302, 84–88.
- Marsicano, G., Wotjak, C.T., Azad, S., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Ziegler, W., Di Marzo, V., Lutz, B., 2002. The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418, 530–534.
- Martinez, E.W., Morgan, W.P., 1997. Antidepressant effects of physical activity. In: Morgan, W.P. (Ed.), *Physical Activity & Mental Health*. Taylor & Francis, Washington, pp. 93–106.
- Meeusen, R., Piacentini, M.F., De Meirleir, K., 2001. Brain microdialysis in exercise research. *Sports Med.* 31, 965–983.
- Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schütz, G., Wotjak, C.T., Lutz, B., Marsicano, G., 2007. Genetic dissection of behavioural and autonomic effects of delta⁹-tetrahydrocannabinol in mice. *PLoS-Biol.* 5, e269.
- Pacher, P., Bátkai, S., Kunos, G., 2006. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* 58, 389–462.
- Phillips, R.G., LeDoux, J.E., 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* 106, 274–285.
- Piomelli, D., 2003. The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.* 4, 873–884.
- Raglin, J.S., 1997. Anxiolytic effects of physical activity. In: Morgan, W.P. (Ed.), *Physical Activity & Mental Health*. Taylor & Francis, Washington, pp. 107–126.
- Ramos, A., Berton, A., Mormède, P., Chaouloff, F., 1997. A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behav. Brain Res.* 85, 57–69.
- Ramos, A., Mormède, P., 1998. Stress and emotionality: a multidimensional and genetic approach. *Neurosci. Biobehav. Rev.* 22, 33–57.
- Salam, J.N., Fox, J.H., Detroy, E.M., Guignon, M.H., Wohl, D.F., Falls, W.A., 2009. Voluntary exercise in C57 mice is anxiolytic across several measures of anxiety. *Behav. Brain Res.* 197, 31–40.
- Salmon, P., 2001. Effects of physical exercise on anxiety, depression, and sensitivity to stress: a unifying theory. *Clin. Psychol. Rev.* 21, 33–61.
- Shearman, L.P., Rosko, K.M., Fleischer, R., Wang, J., Xu, S., Tong, X.S., Rocha, B.A., 2003. Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. *Behav. Pharmacol.* 14, 573–582.
- Sherwin, C.M., 1998. Voluntary wheel running: a review and novel interpretation. *Anim. Behav.* 56, 11–27.
- Sparling, P.B., Giuffrida, A., Piomelli, D., Rosskopf, L., Dietrich, A., 2003. Exercise activates the endocannabinoid system. *NeuroReport* 14, 2209–2211.
- Steiner, M.A., Wotjak, C.T., 2008. Role of the endocannabinoid system in regulation of the hypothalamic–pituitary–adrenocortical axis. *Prog. Brain Res.* 170, 397–432.
- Steiner, M.A., Marsicano, G., Nestler, E.J., Holsboer, F., Lutz, B., Wotjak, C.T., 2008a. Antidepressant-like behavioral effects of impaired cannabinoid receptor type 1 signaling coincide with exaggerated corticosterone secretion in mice. *Psychoneuroendocrinology* 33, 54–67.
- Steiner, M.A., Wanisch, K., Monory, K., Marsicano, G., Borroni, E., Bächli, H., Holsboer, F., Lutz, B., Wotjak, C.T., 2008b. Impaired cannabinoid receptor type 1 signaling interferes with stress-coping behavior in mice. *Pharmacogenomics J.* 8, 196–208.
- Urigüen, L., Pérez-Rial, S., Ledent, C., Palomo, T., Manzanares, J., 2004. Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. *Neuropharmacology* 46, 966–973.
- van Praag, H., 2009. Exercise and the brain: something to chew on. *Trends Neurosci.* 32, 283–290.
- van Praag, H., Kempermann, G., Gage, F.H., 1999. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* 2, 266–270.
- de Visser, L., van den Bos, R., Spruijt, B.M., 2005. Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. *Behav. Brain Res.* 160, 382–388.
- Werme, M., Messer, C., Olson, L., Gilden, L., Thorén, P., Nestler, E.J., Brene, S., 2002. Delta FosB regulates wheel running. *J. Neurosci.* 22, 8133–8138.
- Yoo, H.S., Bunnell, B.N., Crabbe, J.B., Kalish, L.R., Dishman, R.K., 2000. Failure of neonatal clomipramine treatment to alter forced swim immobility: chronic treadmill or activity-wheel running and imipramine. *Physiol. Behav.* 70, 407–411.
- Zhou, D., Shearman, L.P., 2004. Voluntary exercise augments acute effects of CB1-receptor inverse agonist on body weight loss in obese and lean mice. *Pharmacol. Biochem. Behav.* 77, 117–125.