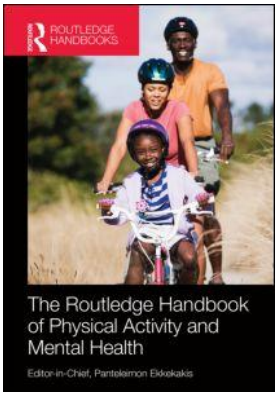

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Francis Chaouloff, Sarah Dubreucq, Isabelle Matias, Giovanni Marsicano
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3

PHYSICAL ACTIVITY FEEL-GOOD EFFECT

The role of endocannabinoids

Francis Chaouloff, Sarah Dubreucq, Isabelle Matias, and Giovanni Marsicano

It is recognized worldwide that physical exertion provides health benefits. This positive effect is linked to the improvement of numerous functions, including cardiovascular, immunological, metabolic, osteoarticular, and brain functions. The identification of the mechanisms through which physical exercise exerts these health-protective effects is considered essential as it paves the way for future fundamental and therapeutic knowledge (Powell & Paffenbarger, 1985). Among health benefits provided by exercise, those related to central functions have been well documented. In particular, the observations of positive consequences of acute and chronic exercise on emotional behaviors in humans, including through anti-stress properties (Salmon, 2001), have led clinicians to use exercise as a therapeutic tool against several psychopathologies, especially mild depression and anxiety (Martinsen & Morgan, 1997; Raglin, 1997; Salmon, 2001; Ströhle, 2009). Thus, exercise alone, or in combination with subeffective antidepressant/anxiolytic medications, is reported to trigger mood and emotional improvements similar to those elicited by classical antidepressants and anxiolytics (Martinsen & Morgan, 1997; Raglin, 1997).

In keeping with these psychological effects of physical exercise, it is now more than 30 years since scientists have tried to uncover the neurobiological bases for these positive effects of physical exercise. For obvious reasons, much of the work has been performed in laboratory animals using models of forced (treadmill running) and voluntary (wheel running) exercise. The first model requires a period of conditioning. During that phase, animals placed on a shock grid that is located at one end of the treadmill belt learn rapidly that running on that belt is the only means to escape from footshocks. Although the need for such a conditioning step is one disadvantage, the ability to modify the speed and the slope of the treadmill and the possibility to perform experiments during the inactive (i.e., light) phase of the diurnal cycle of the animals explain the success of this running paradigm. On the other hand, wheel running is a spontaneous and voluntary behavior which, as such, does not require conditioning. Plateau levels of endurance are generally reached after 1 week and animals can be provided limited or unlimited access to the wheels. As opposed to the treadmill, one limit of wheels is the lack of spontaneous running behavior during the inactive phase of the diurnal cycle, thereby requiring phase shifts in the diurnal cycle so as to allow a coincidence between the active (i.e., dark) phase of that cycle and the daily working period of the experimenters. Another limit is related to the most appropriate sedentary control to which the runners need to be compared. Thus, control animals are usually hosted either in standard cages or in cages with a locked wheel. As we will see below, choosing

between either control conditions is not easy as each of these conditions suffers its own limits, especially when dealing with the emotional impacts of running activity.

Works aimed at deciphering the neurobiological impacts of physical exercise have initially focused on brain monoamine neurochemistry (see reviews from Chaouloff, 1989, 1997; Dunn & Dishman, 1991; Meeusen, Piacentini, & De Meirleir, 2001). Indeed, such a deliberate choice was dictated by the growing interest scientists showed at that time for the monoaminergic theory of depression (Ransford, 1982). Exercise in treadmill-trained animals was reported to stimulate monoamine synthesis, turnover, and/or release rates in brain regions associated with the control of mood (e.g., frontal cortex, hippocampus, striatum, hypothalamus). Although these results brought some support for the "monoamine hypothesis" of the antidepressant effects of physical exercise, this direct link suffered a lack of compelling evidence for (1) functional changes in monoamine transmission, and (2) a causal relationship between these changes in monoamine synthesis/metabolism and the mood-elevating effects of exercise. Besides the monoaminergic systems, the opioidergic systems have also received much attention (see review by Hoffmann, 1997). This interest surged with the observation that exercise increased circulating endorphin levels while a pretreatment with the opioid receptor antagonist naloxone prevented the mood-elevating effects of acute exercise. These results led to the well-known "endorphin hypothesis." However, both the inability to reproduce these data in several laboratories and methodological issues have hampered that hypothesis (Dietrich & McDaniel, 2004). Among other neurobiological candidates for the positive effects of exercise on emotionality, much interest has been devoted to neurotrophins (Hillman, Ericksson, & Kramer, 2008; van Praag, 2009). Thus, exercise increases peripheral and central levels of trophic factors (such as brain-derived neurotrophic factor; BDNF), which are involved in both the mood-elevating properties of antidepressants – possibly through their regulation of hippocampal synaptic plasticity and neurogenesis – and in the exercise-induced facilitation of learning and memory processes. However, the mechanisms leading to the release of trophic factors during exercise are still under investigation.

Physical exercise and the endocannabinoid system

In 2003, it was reported that 50 minutes of moderate exercise through treadmill running or ergometer cycling increased the circulating levels of the endogenous cannabinoid (endocannabinoid) anandamide (AEA) in trained male college students (Sparling, Giuffrida, Piomelli, Rosskopf, & Dietrich, 2003). As AEA is one of the main peripheral and central endocannabinoids (Piomelli, 2003), this seminal observation suggested that exercise may indeed stimulate the endocannabinoid system (ECS). Because the ECS is one key modulator of numerous brain functions/processes including food intake, energy balance, pain sensitivity, emotionality, learning and memory, thermoregulation, and neuroinflammation (Freund, Katona, & Piomelli, 2003; Marsicano & Lutz, 2006; Pacher, Bàtkai, & Kunos, 2006; Piomelli, 2003), the observation of increased circulating AEA levels after exercise opened the promising hypothesis that some physiological effects of physical exercise might indeed be mediated by the ECS (Dietrich & McDaniel, 2004). Furthermore, because the ECS is the target of Δ^9-tetrahydrocannabinol (THC, the main psychoactive component of marijuana; Mechoulam & Hanus, 2000), the observation that several psychological effects of acute exercise – the so-called "runner's high" (well-being, happiness, elation, inner harmony, time distortion) – are similar to those often observed after marijuana consumption has led to the proposal that the ECS may play a crucial role in the feel-good properties of exercise (Dietrich & McDaniel, 2004).

The endocannabinoid system

The ECS is composed of endocannabinoids, the machinery for their synthesis/degradation, and the receptors, namely CB1 and CB2 receptors, through which endocannabinoids exert their functions (Freund et al., 2003; Marsicano & Lutz, 2006; Pacher et al., 2006; Piomelli, 2003). Endocannabinoids, which are lipid messengers synthesized "on demand" from membrane precursors, comprise numerous members. Among these, AEA and 2-arachidonoylglycerol (2-AG) are the best-studied molecules. In the brain, activation of voltage-gated Ca^{2+} channels with/without the concomitant activation of Gq/11-coupled receptors (such as the group 1 metabotropic glutamate receptors or the muscarinic 1/3 receptors) leads to AEA and/or 2-AG synthesis (Alger, 2002; Chevaleyre, Takahashi, & Castillo, 2006; Ohno-Shosaku, Tanimura, Hashimotodani, & Kano, 2011; Piomelli, 2003). Following their transport into the cells, the degradation of AEA and 2-AG is thought to occur respectively at the postsynapse and at the presynapse (Figure 3.1A). The effects of endocannabinoids are mainly mediated by their retrograde action at CB1 receptors located on presynaptic neuronal terminals and on glial cells. Besides CB1 receptors, endocannabinoids may also act on CB2 receptors, which are mainly located on microglial cells in the CNS. As the activation of CB1 receptors by endocannabinoids leads to an inhibition of cyclic AMP levels, an inhibition of voltage-gated Ca^{2+} channels, and an activation of K^+ channels, the result of the retrograde action of endocannabinoids on neurons is a net decrease in presynaptic excitability and thus neurotransmitter release (Figure 3.1B). Stimulation of CB1 receptors is thus a means to control for excessive presynaptic activity (Alger, 2002; Chevaleyre et al., 2006; Ohno-Shosaku et al., 2011). At GABAergic and glutamatergic synapses, such an inhibitory influence of endocannabinoids on transmitter release will bear consequences on synaptic plasticity, whether this plasticity is short lasting (as illustrated by the so-called depolarization-induced suppression of inhibition or suppression of excitation) or long lasting (Alger, 2002; Chevaleyre et al., 2006; Ohno-Shosaku et al., 2011). Recent results have indicated that 2-AG, rather than AEA, is the lipid-derived molecule involved in the inhibitory effects of endocannabinoids on short-lasting synaptic plasticity (Gao et al., 2010; Tanimura et al., 2010). In addition to the brain, the ECS is also present in the periphery, as illustrated by the presence of CB1 receptors in the heart, the liver, the muscles, the gastrointestinal tract, the endocrine pancreas, and the adipose tissue (Pacher et al., 2006; Pagotto, Marsicano, Cota, Lutz, & Pasquali, 2006). However, as opposed to the brain ECS (see below), there is still no information as to the specific relationships that may link physical exercise and peripheral CB1 receptors.

As indicated above, the ECS regulates many brain functions. Such a property is accounted for by the key inhibitory role of CB1 receptors on the release of different neurotransmitters (including the main inhibitory and excitatory neurotransmitters, namely GABA and glutamate), and by the location of this receptor in numerous brain regions (Herkenham et al., 1990; Marsicano & Lutz, 1999). This is illustrated by the observation of CB1 gene/protein expression in the cerebral cortex, the amygdala, the hippocampus, the ventral and dorsal striata, the basal ganglia, the nucleus accumbens, the midbrain (including the dorsal raphe nuclei and the ventral tegmental area), and the hypothalamus. Therein, CB1 receptors are involved in the control of essential brain functions, of which several are documented to be engaged or affected by physical exercise. For example, motor activity, food intake, energy expenditure, anxiety, learning/memory, and hedonia/motivation processes are all found to be controlled by CB1 receptors (El Manira & Kyriakatos, 2010; Lafenêtre, Chaouloff, & Marsicano, 2007; Maldonado, Valverde, & Berrendero, 2006; Pagotto et al., 2006).

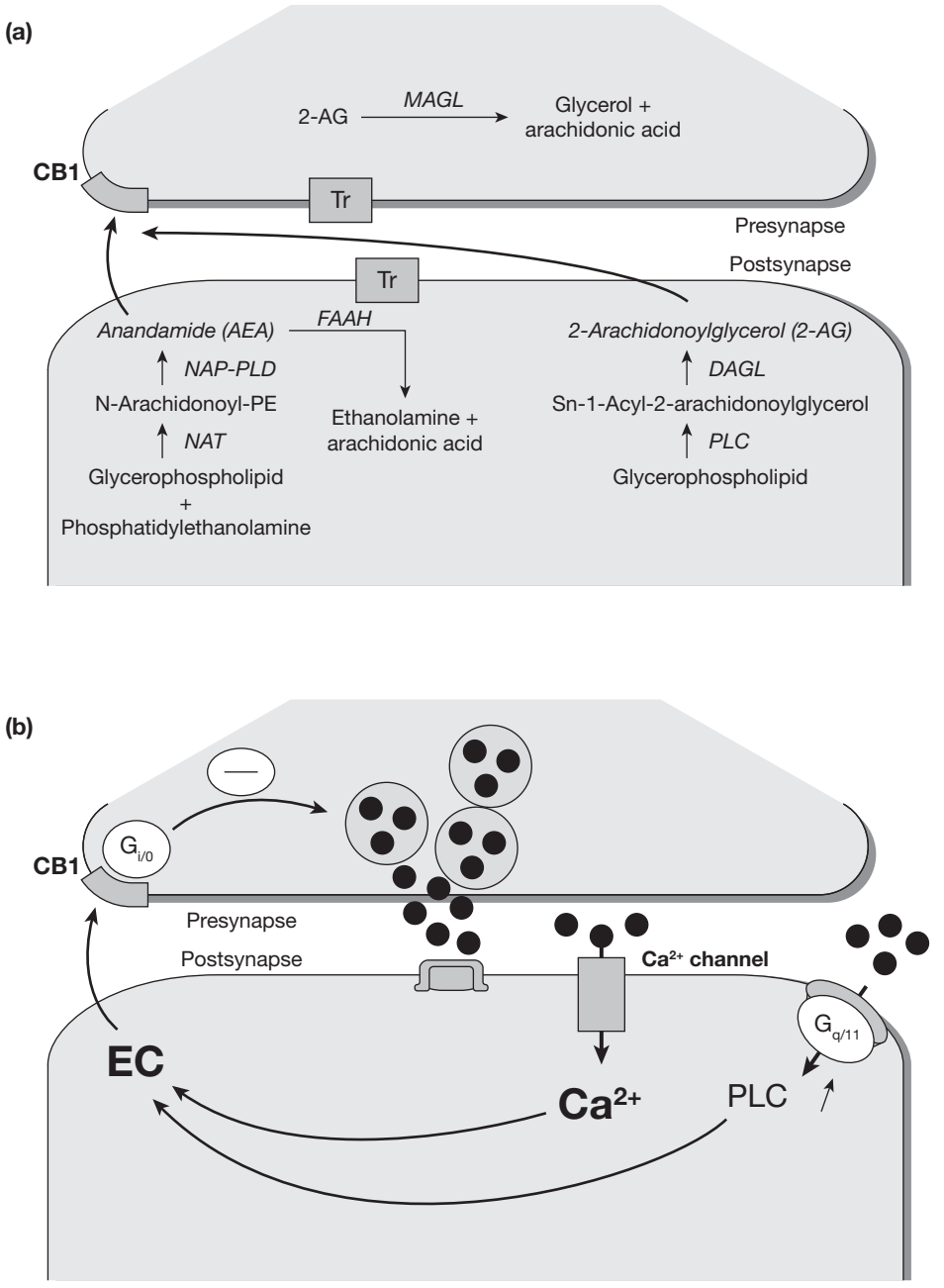

Figure 3.1 The endocannabinoid system. (a) Synthesis and degradation of the principal endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), in neurons. (b) Mechanisms underlying the retrograde action of endocannabinoids on transmitter release. DAGL: diacylglycerol lipase; FAAH: fatty acid amide hydrolase; MAGL: monoacylglycerol lipase; NAP-PLD: N-acylphosphatidylethanolamine-hydrolyzing phospholipase D; NAT: N-acyltransferase; PLC: phospholipase C; Tr: endocannabinoid transporter.

Wheel running activity and the endocannabinoid system

Except for the seminal study by Sparling et al. (2003) and for two recent studies confirming that exercise increases circulating AEA levels, albeit in an intensity-dependent manner (Heyman et al., 2012; Raichlen, Foster, Seillier, Giuffrida & Gerdeman, in press), all studies aimed at examining the relationships between physical exercise and the ECS have been conducted in laboratory animals provided with running wheels. The first issue that has been the focus of investigation relates to the impacts of wheel running on the synthesis/release of endocannabinoids. In rats housed with unlimited access to running wheels for 8 days, hippocampal, but not frontocortical, AEA was found to be increased in the morning of the ninth day (Hill et al., 2010). On the other hand, analyses of the other major endocannabinoid, namely 2-AG, revealed a lack of influence of wheel running in either brain region. It is worthy of mention that in this study control animals were housed with PVC tubing as a means to enrich the environment, thus leaving open the possibility that the results would have been different if controls were housed with locked wheels (see below). We have also recently addressed the question of wheel running effects on brain, but also blood, endocannabinoid levels. Besides the animal species (mice were used in the present study), our study differed from the former in several ways. Thus, our mice were allowed to run for 3h/day during 8 days and endocannabinoid levels were estimated after 30 minutes of running. These estimations were compared to those conducted in controls that were housed with locked wheels for a similar duration. As shown in Figure 3.2A, neither circulating levels of AEA nor brain concentrations of this endocannabinoid displayed changes with running activity. The former observation contrasts with that obtained in the blood samples from exercising human subjects, this contrast being likely accounted for by species differences in the physiological and psychological salience of each exercise paradigm. The analysis of 2-AG levels led to a similar picture to that observed for AEA levels, except for a significant decrease in the hippocampus of mice allowed to run that was associated with a similar trend in the frontal cortex (Figure 3.2B). Although the real significance of these observations is unknown, it is interesting to note that mice exposed for 7 days to a daily episode of social stress displayed an opposite pattern to that measured in exercising mice. Thus, 2-AG levels were found to be increased in the hippocampus and the frontal cortex when measured 40 minutes after the last stress episode (Dubreucq et al., 2012). Interestingly, such a brain region-dependent pattern of reactivity of 2-AG has been observed in rats submitted repeatedly to another type of stress, namely restraint (Patel & Hillard, 2008). These results raise the hypothesis that the respective patterns of reactivity of 2-AG after running and stress are indicative of the psychological salience of each experimental condition. Other issues that merit consideration are the possibilities that wheel running triggers major changes on rat and mouse endocannabinoid levels in discrete brain regions and/or the need for different timings than those used in our study.

In addition to endocannabinoid levels, several studies have explored the effects of wheel running on CB1 receptors. These analyses have focused on the protein itself (number and affinity of the receptor protein) and/or on its functional characteristics. In the above-mentioned study aimed at exploring the impact of wheel running on rat brain endocannabinoid levels, CB1 receptor binding capacities in controls and exercising animals were also investigated. It was found that the number of binding sites for a CB1 receptor agonist increased in the hippocampus, but not in the frontal cortex (Hill et al., 2010). However, such an increase was counterbalanced by a reduction in receptor affinity for the ligand (as revealed by an increased dissociation constant). Besides, one study reported that 10 days of wheel running increased the gene expression of the CB1 receptor in the hippocampus of female mice (Wolf et al., 2010). Concerning CB1 receptor function, 8 days of wheel running hypersensitized the activation of hippocampal

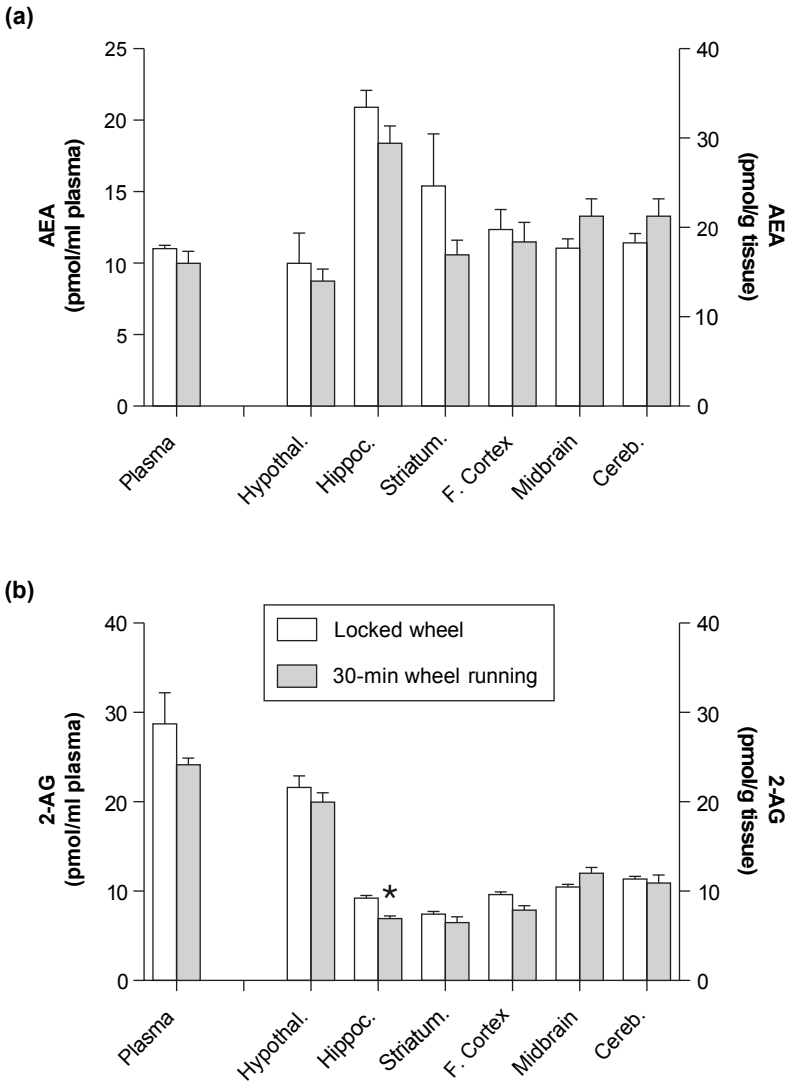

Figure 3.2 Endocannabinoids and wheel running. (a) Plasma and brain tissue anandamide (AEA), and (b) 2-arachidonoylglycerol (2-AG) levels 30 minutes after the initiation of wheel running in animals housed for 8 days with free wheels or with locked wheels. Values are the mean ± S.E.M. of 3 (blood) and 8 (brain regions) samples/group. ⋆ $P < 0.05$ for the difference between sedentary and running subjects.

CB1 receptor-associated G proteins by a CB1 receptor agonist, thus suggesting that wheel running bears stimulatory consequences on hippocampal CB1 receptor function (Hill et al., 2010). Indeed, such a conclusion may extend to another brain region, namely the striatum. Thus, the ability of a CB1 receptor agonist to decrease striatal GABA neurotransmission, as assessed by the reduction in the frequency of spontaneous and miniature inhibitory postsynaptic currents (IPSCs), was reinforced after 15 days (but not 7 days) of wheel running activity (de Chiara et al., 2010). Such a sensitizing effect of wheel running was selective for GABAergic neurons as the CB1 receptor-mediated decrease in striatal glutamatergic transmission was of similar amplitude

in control mice and in running mice. It is important to note here that providing control mice with a sweetened drinking solution led also to increased sensitivity of CB1 receptors on striatal GABAergic neurons (de Chiara et al., 2010). On the basis of this parallel, it has been proposed that it is the hedonic impact of running that may account for the above-mentioned electrophysiological results. It should be noted, however, that in this study the controls were housed in standard cages, leaving unaddressed the possibility that in addition to the hedonic impact of running, the enrichment of the environment contributed to the increased sensitivity of striatal CB1 receptors. This issue is particularly important in view of the promising observation that the desensitization of the inhibitory control exerted by CB1 receptors on striatal GABAergic transmission that is observed in animals exposed to three daily stress episodes was reported to be reversed by 1 day of housing with running wheels (Rossi et al., 2008).

Role of the endocannabinoid system during physical exercise

As illustrated above, the available data gathered so far strongly suggest that physical exercise impacts on the ECS in humans and possibly in laboratory rodents. However, it should be acknowledged that this relationship has been poorly documented, which is in keeping with the very recent surge of interest in this area of research. As shown in the present section, this paucity of results actually extends to the quest for the role exerted by the ECS during or immediately after exercise.

The tools to study the role of the endocannabinoid system in exercising subjects

The study of the functional role of any transmitter system involves classically the characterization of the responses elicited by direct agonists for the receptors of that transmitter. This strategy is often completed by the use of indirect receptor agonists, i.e., drugs acting respectively on the membrane transporters for that transmitter or on the synthesis/release of that transmitter. Such studies bring information on the potential impact of that transmitter system on a vast array of functions but do not inform on the tonic and permissive role that transmitter system may exert during particular situations. Pharmacological and genetic assays based respectively on the administration of antagonists or on the use of constitutive/conditional mutants for these targets are nowadays the routine procedures to gather that information. Studies on the relationships between the ECS and exercise have all focused on the tonic role the ECS may be endowed with during exercise, doing so by means of CB1 receptor antagonists, such as SR141716 (Rinaldi-Carmona et al., 1994) and AM251 (Gatley, Gifford, Volkow, Lan, & Makriyannis, 1996), or using constitutive mutants for the CB1 receptor wherein this receptor is absent from the whole body (Ledent et al., 1999; Marsicano et al., 2002; Zimmer, Zimmer, Hohmann, Herkenham, & Bonner, 1999). Because CB1 receptors are located on different neuronal (and glial) populations and expressed in numerous brain regions (see above), it is useful, however, to further define the population of CB1 receptors through which the ECS may play a role. This can be achieved using conditional mutants for CB1 receptors, and we will illustrate below the potential use of this strategy. The generation of these conditional mutations is based on the so-called "Cre/lox P" technique (Morozov, Kellendonk, Simpson, & Tronche, 2003). This technique is based on the ability of a recombinase protein (Cre) to excise any sequence of DNA flanked by short sequences called "loxP" sites. Given their small dimensions (34 base pairs), the introduction of loxP sites into the genome of mice flanking a given gene to generate "floxed" mutant mice does not change its expression. Therefore, "floxed" mice can be considered phenotypically wild-type mice. However, the presence of the Cre recombinase in specific cell types or tissues will lead to the

specific excision of the "floxed" gene, generating conditional (i.e., spatially and/or temporally restricted) mutant mice. Thus, crossing mice bearing a Cre recombinase specific for a given cell population with mice hosting a CB1 gene sequence flanked by loxP sites ("floxed CB1") generates two types of subjects in the progeny: (a) those bearing just the floxed CB1 gene sequence (phenotypically wild-type animals) and (b) those lacking the CB1 sequence in the cell populations expressing the Cre recombinase (conditional CB1 mutant animals). As an illustration, crossing mice that either carry or do not carry the Cre recombinase Nex with mice hosting a floxed CB1 gene generates wild-type controls (termed below Glu-CB1$^{+/+}$) and mice lacking CB1 receptors from glutamatergic neurons (termed below Glu-CB1$^{-/-}$; Monory et al., 2006). Using other mutant mice expressing a Cre recombinase specific to a cell population (Monory et al., 2007) allows further dissecting the functional role of CB1 receptors with respect to the cell population where these receptors are expressed. For example, the respective roles of CB1 receptors located on GABAergic neurons (the brain cells in which CB1 receptors are the most abundantly expressed; Marsicano & Lutz, 1999; Monory et al., 2006) and on glutamatergic cells (where CB1 are expressed to a low level; Marsicano & Lutz, 1999; Monory et al., 2006) have been recently documented with respect to the control of fasting-induced food intake (Bellochio et al., 2010) and long-term memory deficits triggered by THC (Puighermanal et al., 2009). As indicated above, investigations on the relationships between the ECS and exercise have used CB1 receptor antagonists and constitutive/conditional CB1 receptor mutants (see below). Undoubtedly, the use of conditional mutants for the CB1 receptor will expand in the near future to further define the role of the ECS during exercise. This is also true for other promising tools that have been generated recently, among which (a) selective inhibitors of the enzymes degrading the endocannabinoids or of the transporter that allows the cellular uptake of these lipids (Long et al., 2009; Piomelli, 2003; Schlosburg et al., 2010), and (b) mouse mutants for FAAH (Cravatt et al., 2001), DAGL (Gao et al., 2010; Tanimura et al., 2010), and MAGL (Chanda et al., 2010).

The endocannabinoid system and running performance

In 2004, it was reported that an acute pretreatment with the CB1 receptor antagonist AM251 before the active phase of the diurnal cycle increased in a dose-dependent manner the wheel running distance covered overnight by lean mice while decreasing that distance for the highest doses in obese mice (Zhou & Shearman, 2004). However, several years later, a study using the same antagonist reported that this drug, even if provided repeatedly, did not influence wheel running in rats (Hill et al., 2010). It should be noted here, however, that the latter study relied on the administration of the CB1 receptor antagonist at the beginning of the inactive phase of the diurnal cycle, i.e., 10 hours before the normal onset of the running episode. This opens the possibility that the antagonist was devoid of activity when the animals started their running activities. In a third work, it was documented that the administration of the CB1 receptor antagonist SR141716 2 hours after the onset of wheel running activity decreased the running distances in a dose-dependent manner (Keeney et al., 2008). This decrease, which was observed both in a control mouse line and in a mouse line selected for high wheel running activity, was accounted for by sex-dependent decreases in running duration and/or speed (Keeney et al., 2008). These three pharmacological studies thus lead to different conclusions, that heterogeneity being accounted for by their respective experimental settings (acute/repeated treatment, delay between treatment and running activity). Besides these experimental differences, one additional variable that differed between the studies was the duration of time provided to the animals to habituate to the wheels before pharmacological treatments. In the first and third studies, mice were provided with a 5- to 7-day training period beforehand, whereas in the second study rats received their

daily antagonist administration on the first day of housing with wheels. In our hands, pretreatment with the CB1 receptor antagonist SR 141716 30 minutes before a 3-hour daily wheel running episode that started at the onset of the dark phase of the diurnal cycle actually decreased running activity in 7-day trained mice, a finding extended to mice given the opportunity to run for the first time (Dubreucq et al., 2013). Taken together with the data from the study from Keeney et al. (2008), our data strongly suggest that the ECS acts as a tonic stimulatory control of running activity, including when running activity has already been engaged. The use of CB1 mutant mice provides definitive evidence for this suggestion. Thus, in a study in which mice were given unrestricted access to running wheels, constitutive CB1 mutant mice displayed a 30–40% decrease in wheel running activity compared to their wild-type littermates (Dubreucq, Koehl, Abrous, Marsicano, & Chaouloff, 2010). A detailed analysis of the behaviors of CB1 mutant mice revealed that the negative consequence of CB1 receptor deletion on running activity was accounted for by decreased time spent running and decreased maximal velocity. Furthermore, wheel running differences between CB1 receptor mutants and wild-type littermates were already observed when mice began to engage in their running activity, i.e., during the dark phase of the diurnal cycle (Chaouloff, Dubreucq, Bellocchio, & Marsicano, 2011). In the present context, the following observations are noteworthy. First, this inhibitory consequence of CB1 receptor deletion on running performance is unlikely to be accounted for by an intrinsic impact on locomotor activity as CB1 receptor knock-out mice do not differ from their wild-type littermates when housed for 1 week in cages provided with horizontal and vertical actimeters (Chaouloff et al., 2011). Second, the inhibitory impact of CB1 receptor deletion on wheel running activity was also observed in mice provided with a restricted (3-h) daily access to the running wheels (Dubreucq et al., 2013).

Whenever a reduction in wheel running activity in SR141716-pretreated/treated animals is observed (see above), the amplitude of that reduction ranges between 30 and 60% of the activity measured in vehicle-injected animals. As already mentioned above, CB1 knock-out mice display a 30 to 40% decrease in wheel running activity, compared to wild-type animals. Taken together, these observations suggest that the ECS exerts only a partial tonic stimulatory control of wheel running activity. Where and how such a control is exerted is a question we have recently tried to address although (1) wheel running is a complex behavior (Sherwin, 1998), and (2) the ECS controls many functions in the CNS as well as in the periphery. Thus, in the CNS, the ECS exerts a tight stimulatory control of motoric behavior through the CB1 receptor-dependent modulation of the excitability of neuronal networks in the neocortex, the striatum, the cerebellum, and the spinal cord (El Manira & Kyriakatos, 2010). Accordingly, pharmacological antagonism or genetic silencing of CB1 receptors in either of these networks could have inhibitory consequences on running activity. The same holds true for the impact that the ECS exerts over metabolism through actions on food intake and energy expenditure (Pacher et al., 2006; Pagotto et al., 2006). Another hypothesis, which does not exclude the former ones, lies in the observation that rodents will lever-press to get access to running wheels (Belke & Wagner, 2005) and display conditioned place preference for brief and sustained wheel access (Greenwood et al., 2011; Lett, Grant, & Koh, 2001). These results are in keeping with the proposal that although wheel running is an extremely complex behavior, it can be considered a natural reward with high motivation roots (Sherwin, 1998). Because the ECS, through CB1 receptors, modulates in a positive manner rewarding and motivational processes (Maldonado et al., 2006), we recently suggested that the ECS might control running activity by acting on the naturally rewarding properties of running. Indeed, our most recent experiments using conditional CB1 mutant lines wherein CB1 receptors are deleted from selective neuronal or glial populations indicated that (1) the tonic control exerted by CB1 receptors on running performance is fully accounted for

by CB1 receptors located on GABAergic neurons, (2) these receptors are located on GABAergic nerve terminals lying in the ventral tegmental area (VTA), a key area involved in reward-based motivation processes, and (3) the presence of CB1 receptors on GABAergic neurons prevents the negative after-effects of exercise on VTA dopaminergic activity (Dubreucq et al., 2013).

The endocannabinoid system and the emotional consequences of running

We have indicated above the importance of laboratory animal studies in the quest for the neurobiological mechanisms underlying the positive emotional effects of physical exercise in humans. However, this quest lies on a prerequisite, which is that these animal models of human physical activity have positive emotional effects. The literature on that particular aspect of exercise has yielded numerous, albeit contradictory, data. Indeed, part of that heterogeneity is accounted for by the respective experimental settings among which the conditions under which the sedentary/control animals are housed (see below).

Tools to measure anxiety and fear memory in laboratory animals

Emotionality is the behavioral repertoire that any individual expresses when confronted by novel situations. Accordingly, it is made of different dimensions, including anxiety, fear, curiosity, joy, sadness, . . . etc. (Lazarus, 1991), several of which may be modeled in laboratory rodents (File, 1987; Ramos & Mormède, 1998). Among these dimensions, anxiety and fear memory have received a great deal of attention, including in studies aimed at measuring the impact of voluntary/forced exercise.

Anxiety tests are classically divided into two categories, the first one grouping unconditioned tests where the innate behavioral reactions of the animal are measured when placed in a novel environment (File, 1987; Treit, 1985). Anxiety is measured as the conflict between the curiosity for that novel environment and the reticence for the same environment due to its potential danger. Typically, this conflict is assessed by, e.g., measuring the number of transits from the closed/protected arms to the open/unprotected arms of an elevated plus-maze, the number of visits from the dark compartment to the highly lit compartment of a light/dark box, or the exploration of the highly illuminated center, as opposed to the less illuminated periphery, of an open field. In the second group of tests, conditioned anxiety, which is also based on conflict, is assessed by measuring vital behaviors, such as feeding or drinking, under conditions wherein these consumptions are punished by mild footshocks.

Another dimension that has received some particular attention is conditioned fear memory. In the classical fear conditioning protocol, a neutral cue (conditioned stimulus), which can be an environmental context – a light, a sound, or an odor – is associated with an aversive stimulus (unconditioned stimulus), usually an electric footshock. The pairing between both stimuli elicits a fear response to the presentation of the conditioned stimulus alone (Davis & Whalen, 2001; LeDoux, 2000). Such a fear response is assessed by means of the so-called "freezing" behavior, characterized by the absence of movements except those necessary for breathing (Blanchard & Blanchard, 1969).

The key role of the housing conditions when assessing the effects of wheel running on unconditioned anxiety and fear memory

As indicated above, the literature on the consequences of voluntary running on unconditioned anxiety and, to a lesser extent, on fear memory is somewhat contradictory. Thus, several studies have reported that wheel running exerts anxiolytic effects while others reported that such an activity either had no effect on anxiety or proved to be anxiogenic (Binder, Droste, Ohl, & Reul,

2004; Burghardt, Fulk, Hand, & Wilson, 2004; Duman, Schlesinger, Russell, & Duman, 2008; Fuss et al., 2010). Among the issues that have been highlighted to explain such discrepancies, we have recently focused on the housing condition of the control animals to which the exercising subjects are compared (Dubreucq, Marsicano, & Chaouloff, 2011). Thus, several studies used control animals housed under standard conditions while others used control animals housed with a locked wheel. The main argument for housing animals under the former condition stems from the observation that a wheel, even if locked, may promote behavioral activities (e.g., wheel hanging) that may bias the results. However, besides the fact that a locked wheel may enrich the environment, and thus bear important consequences (e.g., on hippocampal neurogenesis and neuroplasticity; Lledo, Alonso, & Grubb, 2006), such an argument is contradicted by the observation of lid hanging in animals housed in standard cages as well as the importance of wheel hanging in animals housed with free wheels (Koteja, Garland, Sax, Swallow, & Carter, 1999). To explore the intrinsic impacts of the two aforementioned control housing conditions (standard housing without any wheel, housing with a locked wheel) when compared with housing with a free wheel, we have recently used mice housed under any of these three conditions for more than 3 weeks. Thereafter, all the mice were tested for several emotional behaviors, including anxiety in a light/dark box and contextual fear memory expression.The results show that the effects of wheel running on anxiety and fear memory depend on the experimental condition under which the controls are housed (Dubreucq et al., 2011). As an illustration, wheel running proved anxiolytic in the light/dark box when compared with the standard housing condition but not when compared with housing with a locked wheel.

Role of the endocannabinoid system in the emotional profile displayed by wheel running subjects

To our knowledge, only one study has explored the respective influences of wheel running and CB1 receptors on some aspects of emotionality (Dubreucq et al., 2010). In that study, male CB1 mutant mice were compared with their wild-type littermates for their behavioral responses in an open field and their cued fear memory after a 6-week housing with locked or free wheels. It was observed that neither the exploration of the center of the open field, an index of anxiety (see above), nor the peripheral locomotion in that test were sensitive to wheel running in the two genotypes (Dubreucq et al., 2010). This result confirms the above-mentioned difficulty of revealing the anxiolytic impact of wheel running, if any. On the other hand, examination of cued fear memory expression, which is an amygdala-dependent process (as opposed to contextual fear memory, which is hippocampus-dependent; Maren & Quirk, 2004), revealed a significant interaction between wheel running and CB1 receptor expression. Confirming previous observations, CB1 receptor deletion *per se* increased cued fear expression and delayed fear extinction during recall sessions, as assessed from freezing reactions to a tone previously associated with a shock during the conditioning session (Marsicano et al., 2002). Interestingly, wheel running, which was ineffective in wild-type animals, counteracted both the increased fear expression and the delayed extinction that are observed in sedentary CB1 mutant mice (Dubreucq et al., 2010). The observation that wheel running was ineffective on cued fear memory in wild-type animals contrasts with previous findings showing that wheel running increases contextual fear memory (Burghardt, Pasumarthi, Wilson, & Fadel, 2006; Greenwood, Strong, Foley, & Fleshner, 2009). This may indicate that wheel running bears differential effects on hippocampal- and amygdala-dependent fear memories. In keeping with the inhibitory impact of wheel running on the hypersensitized fear expression displayed by CB1 receptor mutants, we recently wondered whether such an interaction between wheel running and CB1 receptor deletion would still be observed if CB1 receptors were selectively deleted from selective neuronal populations. As

indicated above, the Cre/lox P technique has allowed the generation of CB1 receptor mutants wherein CB1 receptors are missing from selected neuronal populations, including cortical glutamatergic neurons (Bellocchio et al., 2010; Monory et al., 2006, 2007; Puighermanal et al., 2009). We thus housed male Glu-CB1$^{-/-}$ and male Glu-CB1$^{+/+}$ mice for 3 weeks with either locked or free wheels, and then examined in both genotypes anxiety-related behaviors using the elevated plus-maze (see above), and cued fear memory expression and extinction during fear recall tests. In the elevated plus-maze, wheel running was found to exert hypolocomotor influences, as assessed by the number of visits to the closed (i.e., protected) arms of the maze (Figure 3.3A). On the other hand, wheel running counteracted the decrease in the percent time spent in the open (i.e., unprotected) arms of the maze that was displayed by the mutant mice (Figure 3.3B). Taken with the data mentioned above, this set of observations thus suggests that wheel running in mice does not bear intrinsic anxiolytic effects but may be able to reverse innate anxiety. Whether this counteracting effect of wheel running extends to environmental situations that further raise anxiety levels is an issue that surely deserves future investigation. When Glu-CB1$^{-/-}$ and male Glu-CB1$^{+/+}$ mice were cued fear conditioned by a tone-shock pairing, fear expression responses to single tone exposure 1 to 3 days later revealed that wheel running had an influence in mutant, but not in wild-type mice (Figures 3.3C and 3.3D). Thus, wheel running

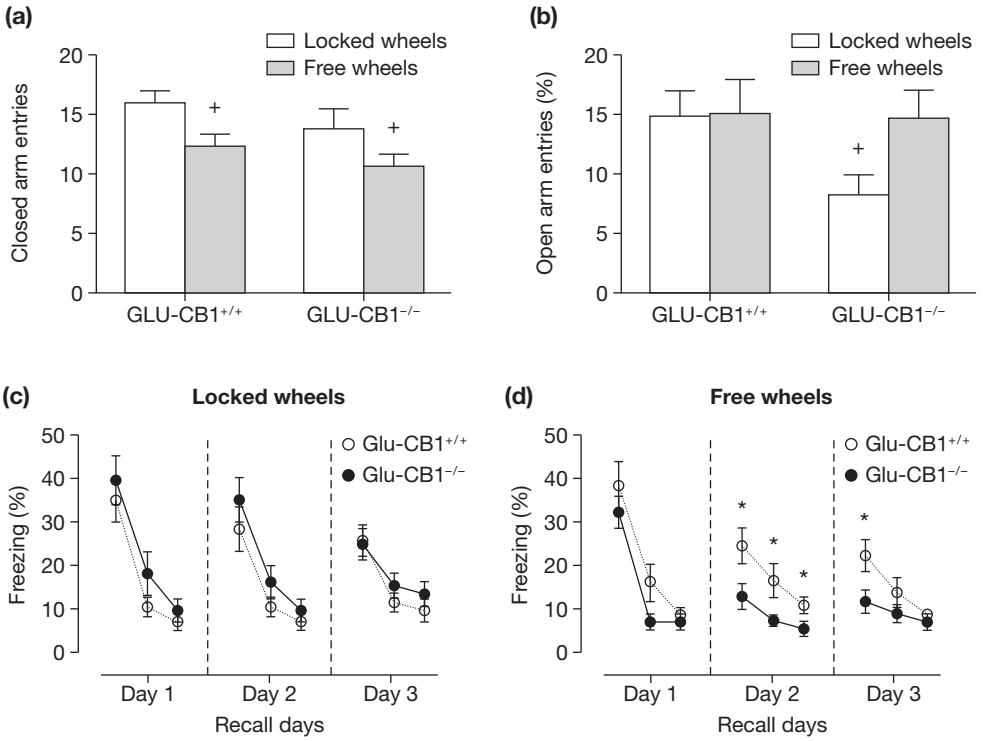

Figure 3.3 CB1 receptors and behavioral consequences of wheel running. (a) Activity and (b) anxiety-related behavior in the elevated plus-maze of Glu-CB1$^{+/+}$ and Glu-CB1$^{-/-}$ mice housed for 3 weeks with either locked or free wheels. Cued fear memory during recall sessions in Glu-CB1$^{+/+}$ and Glu-CB1$^{-/-}$ mice housed for 3 weeks with locked wheels (c) or with free wheels (d). Values are the mean ± S.E.M. of 13–15 mice/group. $^{+}$ $P < 0.05$ for the main influence of the housing condition in the two genotypes. ⋆ at least $P < 0.05$ for the difference between sedentary and running subjects.

decreased fear expression and accelerated fear extinction on recall sessions 2 and 3 in Glu-CB1$^{-/-}$, but not in Glu-CB1$^{+/+}$, mice. Such a differential effect of wheel running in the two genotypes closely resembles that measured in constitutive CB1 mutants, as opposed to their wild-type littermates (see above). This indicates that (1) wheel running controls in a tonic manner cued fear memory expression/extinction and (2) that such a control involves CB1 receptors located on cortical glutamatergic neurons. In turn, the former finding opens the promising hypothesis that physical exercise in humans may set back to normal levels abnormally high fear expression and/or delayed extinction (as observed, e.g., in patients suffering post-traumatic stress disorder). Our finding reinforces former evidence for a tight control of fear memory by the ECS (Marsicano et al., 2002); however, how such a control is exerted in exercising subjects remains to be explored.

The endocannabinoid system and the neurogenic impact of wheel running

Adult neurogenesis, i.e., the formation of new neurons, occurs in the hippocampus where it is highly sensitive to wheel running (Ernst, Olson, Pinel, Lam, & Christie, 2006; van Praag, 2009). Thus, housing with running wheels increases neurogenesis in the dentate gyrus, an effect already observed after 1 week of wheel running. However, such a stimulatory impact of exercise is not specific to running as environmental enrichments also share that capacity (Kempermann, Kuhn, & Gage, 1997). As already underlined above with regard to the emotional effects of wheel running, the neurogenic impact of an enrichment in the environment raises the question of the intrinsic consequence of the presence of the wheel. Indeed, a recent study wherein mice were housed in standard cages or in cages complemented with either locked wheels or free wheels revealed the following. Housing with wheels, whether locked or unlocked, stimulated the proliferative phase of the neurogenic process, compared with standard housing (Bednarczyk et al., 2010). However, when focusing on later stages of the neurogenic process (i.e., survival, differentiation, maturation), mice housed with free wheels displayed increased neurogenesis, as compared to the two other mouse groups. This indicates that exercise has an intrinsic stimulatory influence on the last phases of the neurogenic process only.

Several studies have analyzed whether the ECS contributes to the stimulatory effects of wheel running on neurogenesis. In the aforementioned study in which a CB1 receptor antagonist was administered daily for 8 days to male rats, it was observed that such a treatment prevented the stimulatory influence of wheel running on hippocampal cell proliferation (Hill et al., 2010). Supporting this, one study using female mice reported that wheel running elicited cell proliferation in the dentate gyrus, as compared to standard housing, a change that was absent in CB1 mutant mice (Wolf et al., 2010). On the other hand, another study using male CB1 mutant and wild-type mice reported that although CB1 receptor mutation decreased neurogenesis, in line with past results (Aguado et al., 2006; Jin et al., 2004) and running performance (see above), the percent stimulatory influence of wheel running on neurogenesis was independent from the mouse genotype (Dubreucq et al., 2010). Taken together, these results indicate the need for future experiments to delineate the role of CB1 receptors on exercise-induced neurogenesis. In keeping with the contradictory data that have emerged on that subject, these experiments will need to detail the respective roles of CB1 receptors at each stage of the neurogenic process and compare the effects of wheel running to standard housing and housing with locked wheels.

Conclusions

The ECS plays a major role in the regulation of central functions, including well-being. This chapter has tried to provide arguments in favor of a role for that key modulatory system during

exercise. The data reviewed above highlight the likelihood that the ECS is involved in the control of exercise performance, but much remains to be done before identifying the mechanisms underlying such a control. Besides, there is experimental support for a role of the ECS in the modulation of fear memory and neurogenic processes in exercising subjects, but again much work is needed to detail such a relationship. We have seen that the main experimental evidence for a role of the ECS during exercise stems from animal studies. In turn, this indicates the need for a real appreciation of the limits of animal models of exercise. This chapter has tried to raise several of them, including the need to compare exercising animals to appropriate controls. However, besides these issues, other ones that were not covered in the present chapter need to be taken into account before translating animal studies to the clinics. These include the difficulty of building the most appropriate animal models of human psychopathologies such as depression, and the awareness that laboratory animals live in impoverished environments. Accordingly, wheel running activity, even if compared with that related to the simple presence of a wheel, may bear a valence that largely exceeds that usually measured in human beings offered a vast array of occupational activities.

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