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Chronic aerobic exercise: Lack of effect on brain CB1 receptor levels in adult rats

Sabrina Swenson[a], John Hamilton[a,b], Lisa Robison[c], Panayotis K. Thanos[a,b,*]

[a] Behavioral Neuropharmacology and Neuroimaging Laboratory on Addictions (BNNLA), Research Institute on Addictions, Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14203, United States of America
[b] Department of Psychology, University at Buffalo, Buffalo, NY, 1403, United States of America
[c] Department of Neuroscience and Experimental Therapeutics, Albany Medical College, Albany, NY 12208, United States of America

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ABSTRACT

Introduction: Exercise programs have been shown to be effective for both reducing risk for, and intervention following, substance abuse behaviors in both clinical and preclinical studies. Less is known, however, regarding the underlying neurobiological substrates involved in these changes in drug seeking behavior. In this study, we assessed cannabinoid receptor (CB1) levels throughout the brain which are key in endocannabinoid signaling following chronic aerobic exercise.
Methods: Male and female Lewis young adult rats were grouped into exercise and sedentary groups at 8 weeks of age. Exercise rats ran on a treadmill at 10 m/min, 5 days/week, for 6 weeks, whereas sedentary rats remained in their home cage. Rats were euthanized after 6 weeks, and *in vitro* receptor autoradiography was performed using [^{3}H] SR141716A to quantify CB1 receptors throughout the brain.
Results: Exercise rats did not show significantly different [^{3}H] SR141716A binding levels as compared to sedentary rats; however, an overall sex effect was found, where males had 29% higher [^{3}H] SR141716A binding within the pyramidal layer of the hippocampus when compared to females. The chronic aerobic exercise regimen did not produce any changes in CB1 receptor levels.
Conclusions: The present study found that chronic exercise during young adulthood did not alter cannabinoid CB1 receptor levels in the brain. Therefore, previously reported decreased cocaine preference in parallel treated cohorts did not involve exercise induced changes in CB1 levels which is key for endocannabinoid signaling.

1. Introduction

Aerobic exercise can mitigate the risk of several diseases and negative health outcomes including cancer and obesity-related disorders (*e.g.* type II diabetes mellitus, stroke, osteoarthritis) [1]. Cognitive benefits have also been noted; including enhanced executive function, learning and memory, and protection against cognitive decline during aging and Alzheimer's disease [2]. Psychologically, exercise is linked to several mental health benefits, including enhanced mood, and reduced stress, anxiety, and depression [3]. A meta-analysis of 22 randomized control trials assessed the efficacy of chronic exercise as an intervention for substance abuse in adults, and found that exercise increased abstinence rates across follow-up periods, drug class, and exercise type and intensity, while mitigating withdrawal symptoms, anxiety, and depression [4]. The mechanisms underlying these changes in behavior and mood are still unknown.

A possible explanation for these results could be alterations in endocannabinoid (eCB) signaling as a result of exercise [5]. Exercise has been shown to activate the eCB system [6], and it has been hypothesized that the mechanism behind the sensation of a “runners high” is through the eCB system rather than the opiate system (for review see [7]). The cognitive benefits of emotional regulation, including alleviating effects of depressive and anxious behaviors, enhanced memory, along with reduced perception of pain have shown to possibly be mediated by the eCB system [8]. Exercise has also been shown to affect the central reward pathway and related behaviors [9,10]. The eCB system has been shown to interact with this dopaminergic system [11]. Both dopamine and eCB neurotransmission in emotional brain circuits contribute to regulating motivational and emotional neural processing [12]. There has also been evidence showing that CB1 and D2 receptors form heterodimers coupled to stimulatory G-proteins that enhance cyclic nucleotides in heterologous expression systems and in striatal

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[*] Corresponding author at: 1021 Main Street, Buffalo, NY 14203-1016, United States of America.
E-mail address: pthanos@ria.buffalo.edu (P.K. Thanos).

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neurons [13,14]. This highlights the direct cross-talk between the two signaling systems. Recently we found that exercise alters dopaminergic transmission, presenting a possible biological pathway that reflects the attenuation of drug-seeking and taking behavior in that dopamine type 2-like receptor increased and dopamine type 1-like receptor decreased [9].

Discrepancies have been reported on the effects of exercise in the role of addiction and between sexes (for review see [15]). Overall, exercise has been shown to be effective in the initiation/acquisition, maintenance, escalation, extinction, and relapse of drug-seeking and preference across several drug classes (alcohol, nicotine, stimulants, and opiates) [16,17]. Generally it has been found that females are more susceptible to drugs during all phases of the addiction process [18]. A better understanding of the relationship between exercise, sex and the eCB system has potential to aid in addiction treatments.

2. Materials and methods

2.1. Animals

Male (n = 16) and female (n = 16) Lewis rats (Taconic, Hudson, NY) at 8 weeks of age were individually housed under standard laboratory conditions at 22.0 °C ± 2 °C with a 12 h reverse light/dark cycle (lights off: 08:00–20:00 h). Food and water were available *ad-libitum* for the duration of the study. All subjects were handled daily. The experiment was conducted in accordance with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals (1996) and University at Buffalo Institutional Animal Care and Use Committee.

2.2. Treadmill

A custom-made motorized treadmill divided into eight lanes by Plexiglas walls and by a sheet of metal at its end to keep the rats enclosed on the treadmill was used as in previous studies [16]. The dimensions of the running lanes were 56 cm long x 9 cm wide x 31 cm high.

2.3. Exercise regimen

Following one week of habituation, male and female rats were randomly assigned to be in either the sedentary or exercise group (n = 8 per group). Rats in the exercise group were placed on the treadmill (10 m/min) five days a week for six weeks. The first day of exercise lasted 10 min and was increased by 10 min daily until the final duration of 60 min was reached. A ten-minute break followed the first half hour of exercise. During the course of the 6 weeks rats ran a total of approximately 16.5 km, rats in the sedentary group remained in their cages and received no exercise other than normal cage ambulation, as in previous studies [9,10,19].

2.4. In-vitro receptor autoradiography

[^{3}H] SR141716A binding: All mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), and the brains were rapidly extracted, flash frozen in isopentane and stored at −80 °C. Sagittal sections, 12 μm thick, were cut with a Leica cryostat and stored at −80 °C until receptor binding was performed. *In vitro* CB1R autoradiography was performed as previously reported [20]. Briefly, sections were pre-incubated for 10 min in assay buffer solution (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl, 1 mM MgCl, pH 7.4) at room temperature and then incubated for 90 min in assay buffer in the presence of 0.4 nM [^{3}H] SR 141716A (Amersham) at room temperature. Non-specific binding was determined in the presence of 100 μM HU-210 (Tocris Bioscience). Incubation was followed by 3 × 30 min washes in ice-cold assay buffer and a rapid rinse in ice-cold distilled H_2O. The data were expressed in counts per minute per millimeter squared and converted to μCi/g tissue using a brain paste standard of known radioactivity value and mass [20].

2.5. Region of interest analysis

Regions of interest analyzed included the following brain regions as per the rat stereotaxic brain atlas [21]: Cingulate (Cg), Insular (Ins), Sensory cortex (S- Barrel field (bf), Hind limb (hl), Jaw (j), Shoulder (sh), Trunk (tr), Upper lip (up)), Motor (Primary (M1) and Secondary (M2)), Visual (Primary (V1) and Secondary (V2)), Piriform (Piri), Prelimbic (Prl), and Infralimbic (In) cortices, Striatum (Dorsal and Ventral), Globus Pallidus (GP), Nucleus Accumbens (NAc), Substantia Nigra (SN), Superior and Inferior colliculi (S colli, I colli), and Periaquaductal Grey (PAG).

2.6. Statistics

A two-way ANOVA was conducted within each region of interest to determine the effects of sex and exercise treatment on CB1 receptor binding. Significance level was set at $\alpha = 0.05$ and all statistical analyses were performed with Sigmaplot 11.0 (Systat Software, Inc., Chicago, Illinois).

3. Results

A two-way ANOVA was conducted with the factors of Sex [Male, Female] and Treatment [Exercise, Sedentary] within each region of interest for CB1 binding. No effect of exercise was found for any region. However, an overall sex effect was found within the pyramidal layer in the hippocampus [$F(1,24) = 6.018$; $p < .05$; See Fig. 1], where males displayed 29% greater CB1 receptor binding compared to females.

4. Discussion

In the present study, we examined the effect of chronic forced aerobic (treadmill running) exercise during on CB1 receptor expression in young adult Lewis rats. Exercise began in early adulthood, as there are critical neurodevelopmental periods during adolescence noted where the eCB system is more sensitive to environmental stimuli [22] including endocannabinoids [23] and exercise [24]. Both male and female rats were used, as the eCB system has been shown to play an important neurodevelopmental role that differs between sex [25]. Lastly, treadmill exercise, rather than voluntary wheel running exercise was chosen so that each rat's exercise in terms of speed, frequency, duration, and intensity would be the same. Additionally, it has been hypothesized that forced running mimics the behavior of the average person, while voluntary running mimics the behavior of high endurance athletes, as rodents will voluntarily engage in large volumes of exercise when given access to a running wheel [26]. With this in mind, forced daily exercise is an excellent model for studying the effects of exercise as an addiction intervention.

While prior research has provided several lines of evidence that exercise has an effect on endocannabinoid (eCB) transmission (for review see [7]), the results of the present study show that this specific 6 week aerobic exercise regimen (treadmill running at 10 m/min, 60 min/day, 5 days/week) produced no significant changes in cannabinoid CB1 receptor levels in any brain region measured. This study was in agreement, however, with others showing differences between male and female endocannabinoid signaling in the hippocampus, specifically in the pyramidal layer [27].

Pyramidal cells play a role in hippocampal plasticity [28], which also varies by sex [27]. The eCB system regulates aspects of synaptic plasticity, including long-term depression [29] pyramidal cell specification, long range axon patterning [30] and interneuron migration [31]. Preclinical studies have shown plasticity, neurogenesis and

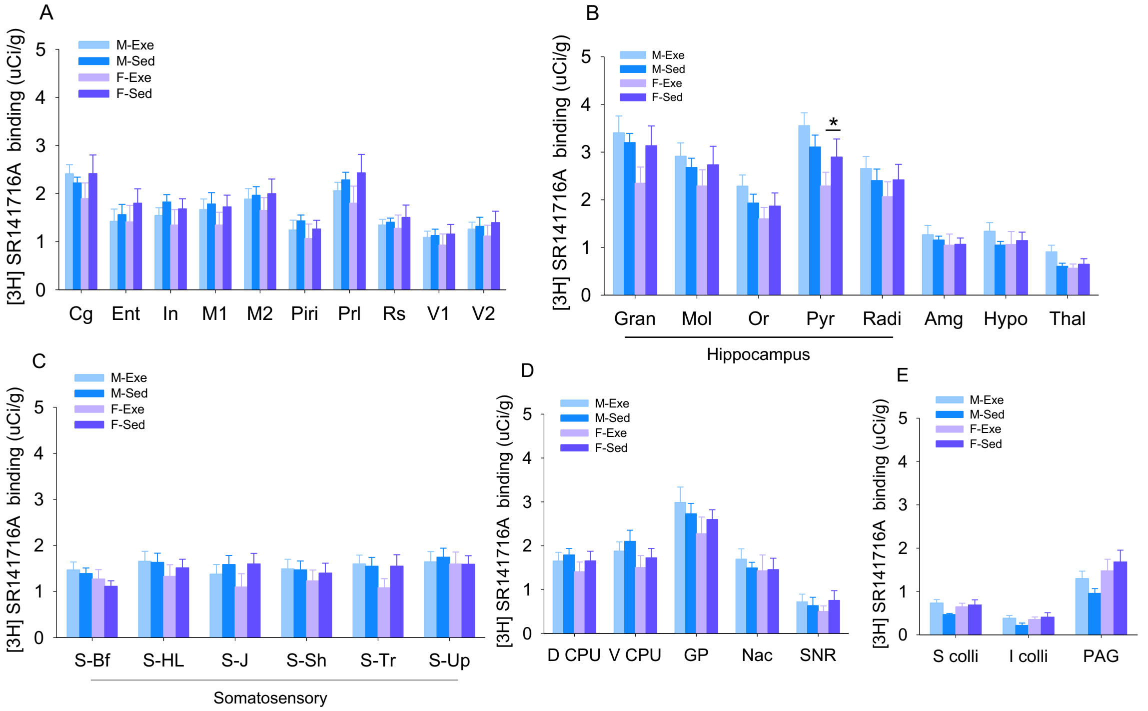

Fig. 1. Mean (+SEM) [3H] SR141716A binding for CB1 receptors in male and female sedentary *versus* exercised rats. There was no exercise effect found on CB1 receptor binding in any region examined. Overall sex effect, where males showed higher [3H] SR141716A binding in the pyramidal layer of the hippocampus [$p < .05$].

neurotransmitter systems were affected by sex hormones, which could be an underlying reason for differences between sexes in drug addiction [32,33].

The eCB system has been shown to play a crucial role in establishing normal sex differences in the brain [34]. During adolescence, females have been found to have slightly lower Cb1 receptor density expression compared to males [35]. This slight difference becomes clear in adult rats. Males have been found to have greater CB1 receptor expression in the hippocampus [36] while females have been found to have higher CB1 receptor-mediated G protein function in the hippocampal formation [37]. Testicular hormones are responsible for the increase in the density of dendritic spines in hippocampal neurons during puberty, which then decrease after [38] There has been evidence showing that levels of CB1 fluctuate during the estrus cycle in females [39]. Estradiol treatment also has been shown to regulate hundreds of hippocampal genes [40]. Frester et al. also found that estradiol synthesis by hippocampal neurons maintain long-term potentiation (LTP) and synapses in females but not males [32]. Cognition in women has been found to be impaired when estradiol was not within optimal levels [41]. The effects of estradiol do vary between age and duration of estrogen decline in ovariectomized rats [42]. Estradiol has also been found to be a large contributor to female vulnerability to addictive behaviors [43].

Ovarian hormones have been shown to enhance drug addiction related behaviors (acquisition, maintenance, and escalation) [44,45]. Estradiol has also been shown to facilitate positive subjective effects of amphetamine and enhances the acquisition for cocaine [46,47]. On the contrary there also have been studies that have found no difference between males and females in either phase (luteal or follicular) of the estrus cycle in regards to the effects of cocaine [48]. Testosterone has been shown to attenuate the response to cocaine in male subjects [49]. Interestingly, Cb1 has a role in the production of sex hormones, one of which is to repress testosterone production [50].

Previous studies suggest a relationship between exercise and the endocannabinoid system. Clinically, acute voluntary aerobic exercise on a stationary bike has been shown to increase AEA levels [51]; preclinical wheel running increased sensitization of CB1 receptors in the striatum [52]. It was also found that voluntary wheel running was dependent on the presence of Cb1 receptors [53]. CB1 receptors also have a strong implication in voluntary wheel running behavior and neurogenesis in the hippocampus. CB1 deficient mice exhibited decreased wheel running behavior as well as a decrease in immature neurons in the dentate gyrus [54]. Increases in CB1 binding site density due to wheel running in the hippocampus has been found to be required for the exercise-induced increase in cell proliferation [55]. While these effects are present in voluntary wheel running behavior, there is evidence of more robust results in regards to neurogenesis and plasticity with forced exercise [26,56].

There have been limited studies, however, directly looking at the effects of forced exercise on CB1 receptor density. There has been a study with similar findings of forced treadmill running showing no change in gene expression in the liver [57]. The lack of effect of exercise on CB1 receptors found here is in contrast to another study in rats that found that chronic treadmill running decreased CB1 binding in the striatum and hippocampus [58]. It is possible that the exercise "dose" or prescribed exercise regimen was not within an intensity range to show changes in Cb1 binding. The previous study had rats run at a speed of 18 m/min, nearly double that used in the current study. This is a similar finding to another study which one month old rats performed a forced swim test for three times a week for six months and also found decreased Cb1 expression in adipose tissue [59] This possibility is supported by findings that, along with subjective reports of "runners high" conditions, there have been studies showing eCB signaling is modulated by intensity of exercise [6,51], although these were clinical studies and looking at AEA and or 2-AG, which are peripheral indicators of Cb1 signaling. Although it is clear that both short-term, voluntary exercises and long-term forced exercises increase eCb ligand concentrations, only long-term-forced exercise shows sustained increases of eCB's and predictable CB1 downregulation [60–62].

Another alternative explanation lies in the sensitivity of the CB1 system due to the age of our subjects. Our animals began exercise at young adulthood (ages 2 through 3.5 months), while the aforementioned studies utilized exercise intervention starting in adolescence (age 3 weeks, and one month old respectively). Adolescence is marked by high neural plasticity, which decreases with age [63], and sensitivity to environmental stimuli [22], including exposure to endocannabinoids [23] and exercise [24].

There has been evidence showing that eCB signaling through CB1 receptors can modulate substance abuse-related behavior; therefore, the aim of the current study was to determine whether the exercise-induced reductions in drug-seeking behavior previously noted could be mediated in part by changes in CB1 receptor binding. Additionally, the same exercise regimen used in the current study was previously shown to attenuate conditioned place preference for cocaine in a different cohort of male and female rats of the same age and strain [16]. Pretreatment with CB1 antagonists decreases relapse to cocaine [64], while mice lacking the CB1 receptor showed decreased preference for and intake of alcohol [65]. CB1 antagonists also reduce nicotine and Δ^9-tetrahydrocannabinol (THC) self-administration, decrease morphine-induced conditioned place preference and block reinstatement of methamphetamine-seeking behavior in rats [66–68]. On the contrary cannabinoid administration produces cross- sensitization to the locomotor effects of amphetamine [69].

While our studies and others have demonstrated that exercise is effective in reducing susceptibility to drug addiction [15,16,19,70], the present findings suggest that these effects were not be mediated by changes in CB1 receptor levels (at least during young adulthood). There have been reported sex differences in many phases of addiction [16,44,71]. Possibly due to the role of sex- hormones, there have also been inconsistent results between studies the effects of sex specific exercise interventions [72–74].

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References

[1] Physical activity guidelines for Americans, Available from, 2008. https://health.gov/paguidelines/pdf/paguide.pdf.

[2] A.F. Kramer, K.I. Erickson, S.J. Colcombe, Exercise, cognition, and the aging brain, J Appl Physiol (1985) 101 (4) (2006) 1237–1242.

[3] J. Vina, et al., Exercise acts as a drug; the pharmacological benefits of exercise, Br. J. Pharmacol. 167 (1) (2012) 1–12.

[4] Wang, D., et al., Impact of physical exercise on substance use disorders: a meta-analysis. PLoS One, 2014. 9(10): p. e110728.

[5] A.G. Brellenthin, et al., Endocannabinoid and mood responses to exercise in adults with varying activity levels, Med. Sci. Sports Exerc. 49 (8) (2017) 1688–1696.

[6] D.A. Raichlen, et al., Exercise-induced endocannabinoid signaling is modulated by intensity, Eur. J. Appl. Physiol. 113 (4) (2013) 869–875.

[7] A. Dietrich, W.F. McDaniel, Endocannabinoids and exercise, Br. J. Sports Med. 38 (5) (2004) 536–541.

[8] R.G. Pertwee, Cannabinoid receptors and pain, Prog. Neurobiol. 63 (5) (2001) 569–611.

[9] L.S. Robison, et al., Exercise reduces dopamine D1R and increases D2R in rats: implications for addiction, Med. Sci. Sports Exerc. 50 (8) (2018) 1596–1602.

[10] L.S. Robison, L. Alessi, P.K. Thanos, Chronic forced exercise inhibits stress-induced reinstatement of cocaine conditioned place preference, Behav. Brain Res. 353 (2018) 176–184.

[11] L. Dazzi, et al., Involvement of the cannabinoid CB1 receptor in modulation of dopamine output in the prefrontal cortex associated with food restriction in rats, PLoS One 9 (3) (2014) e92224.

[12] S.R. Laviolette, A.A. Grace, The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction, Cell. Mol. Life Sci. 63 (14) (2006) 1597–1613.

[13] C.S. Kearn, et al., Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? Mol. Pharmacol. 67 (5) (2005) 1697.

[14] A. Jarrahian, V.J. Watts, E.L. Barker, D2 dopamine receptors modulate Galpha-subunit coupling of the CB1 cannabinoid receptor, J. Pharmacol. Exp. Ther. 308 (3) (2004) 880–886.

[15] W.J. Lynch, et al., Exercise as a novel treatment for drug addiction: a neurobiological and stage-dependent hypothesis, Neurosci. Biobehav. Rev. 37 (8) (2013) 1622–1644.

[16] P.K. Thanos, et al., Chronic forced exercise during adolescence decreases cocaine conditioned place preference in Lewis rats, Behav. Brain Res. 215 (1) (2010) 77–82.

[17] T. Archer, R.D. Badgaiyan, K. Blum, Physical exercise interventions for drug addictive disorders, J Reward Defic Syndr Addict Sci 3 (1) (2017) 17–20.

[18] J.B. Becker, M. Hu, Sex differences in drug abuse, Front. Neuroendocrinol. 29 (1) (2008) 36–47.

[19] P.K. Thanos, et al., Daily treadmill exercise attenuates cocaine cue-induced reinstatement and cocaine induced locomotor response but increases cocaine-primed reinstatement, Behav. Brain Res. 239 (2013) 8–14.

[20] P.K. Thanos, et al., Leptin receptor deficiency is associated with upregulation of cannabinoid 1 receptors in limbic brain regions, Synapse 62 (9) (2008) 637–642.

[21] G. Paxinos, C. Watson, *The Rat Brain in Stereotaxic Coordinates*, in *123Library*, Academic Press, 2007.

[22] S.L. Andersen, Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci. Biobehav. Rev. 27 (1) (2003) 3–18.

[23] M. Schneider, E. Schomig, F.M. Leweke, Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats, Addict. Biol. 13 (3–4) (2008) 345–357.

[24] S. Gomes da Silva, et al., Physical exercise during the adolescent period of life increases hippocampal parvalbumin expression, Brain and Development 32 (2) (2010) 137–142.

[25] M.P. Viveros, et al., The endocannabinoid system in critical neurodevelopmental periods: sex differences and neuropsychiatric implications, J. Psychopharmacol. 26 (1) (2012) 164–176.

[26] J.L. Leasure, M. Jones, Forced and voluntary exercise differentially affect brain and behavior, Neuroscience 156 (3) (2008) 456–465.

[27] W.A. Koss, K.M. Frick, Sex differences in hippocampal function, J. Neurosci. Res. 95 (1–2) (2017) 539–562.

[28] . Dale, E., et al., Vortioxetine disinhibits pyramidal cell function and enhances synaptic plasticity in the rat hippocampus. Journal of Psychopharmacology (Oxford, England), 2014. 28(10): p. 891–902.

[29] P.J. Sjostrom, G.G. Turrigiano, S.B. Nelson, Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors, Neuron 39 (4) (2003) 641–654.

[30] J. Mulder, et al., Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning, Proc. Natl. Acad. Sci. U. S. A. 105 (25) (2008) 8760–8765.

[31] P. Berghuis, et al., Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor, Proc. Natl. Acad. Sci. U. S. A. 102 (52) (2005) 19115–19120.

[32] L. Fester, G.M. Rune, Sexual neurosteroids and synaptic plasticity in the hippocampus, Brain Res. 1621 (2015) 162–169.

[33] C.L. Wetherington, Sex differences and gonadal hormone influences in drug addiction and sexual behavior: progress and possibilities, Horm. Behav. 58 (1) (2010) 2–7.

[34] M.-P. Viveros, et al., Framework for sex differences in adolescent neurobiology: a focus on cannabinoids, Neurosci. Biobehav. Rev. 35 (8) (2011) 1740–1751.

[35] E.M. Marco, et al., Subchronic nicotine exposure in adolescence induces long-term effects on hippocampal and striatal cannabinoid-CB1 and mu-opioid receptors in rats, Eur. J. Pharmacol. 557 (1) (2007) 37–43.

[36] C.G. Reich, M.E. Taylor, M.M. McCarthy, Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats, Behav. Brain Res. 203 (2) (2009) 264–269.

[37] B. Mateos, et al., Adolescent exposure to nicotine and/or the cannabinoid agonist CP 55,940 induces gender-dependent long-lasting memory impairments and changes in brain nicotinic and CB(1) cannabinoid receptors, J. Psychopharmacol. 25 (12) (2011) 1676–1690.

[38] G. Meyer, R. Ferres-Torres, M. Mas, The effects of puberty and castration on hippocampal dendritic spines of mice. A Golgi study, Brain Res. 155 (1) (1978) 108–112.

[39] S. Gonzalez, et al., Sex steroid influence on cannabinoid CB(1) receptor mRNA and endocannabinoid levels in the anterior pituitary gland, Biochem. Biophys. Res. Commun. 270 (1) (2000) 260–266.

[40] K.K. Aenlle, T.C. Foster, Aging alters the expression of genes for neuroprotection and synaptic function following acute estradiol treatment, Hippocampus 20 (9) (2010) 1047–1060.

[41] T.C. Foster, Role of estrogen receptor alpha and beta expression and signaling on cognitive function during aging, Hippocampus 22 (4) (2012) 656–669.

[42] A.L. Markowska, A.V. Savonenko, Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats, J. Neurosci. 22 (24) (2002) 10985–10995.

[43] V.L. Hedges, N.A. Staffend, R.L. Meisel, Neural mechanisms of reproduction in females as a predisposing factor for drug addiction, Front. Neuroendocrinol. 31 (2) (2010) 217–231.

[44] J.J. Anker, M.E. Carroll, Females are more vulnerable to drug abuse than males: evidence from preclinical studies and the role of ovarian hormones, Curr. Top. Behav. Neurosci. 8 (2011) 73–96.

[45] A.C. Segarra, et al., Estrogen receptors mediate estradiol's effect on sensitization and CPP to cocaine in female rats: role of contextual cues, Horm. Behav. 65 (2) (2014) 77–87.

[46] A.J. Justice, H. de Wit, Acute effects of estradiol pretreatment on the response to d-amphetamine in women, Neuroendocrinology 71 (1) (2000) 51–59.

[47] W. Zhao, J.B. Becker, Sensitization enhances acquisition of cocaine self-

administration in female rats: estradiol further enhances cocaine intake after acquisition, Horm. Behav. 58 (1) (2010) 8–12.

[48] S.L. Collins, et al., Intranasal cocaine in humans: effects of sex and menstrual cycle, Pharmacol. Biochem. Behav. 86 (1) (2007) 117–124.

[49] R. Menéndez-Delmestre, A.C. Segarra, Testosterone is essential for cocaine sensitization in male rats, Physiol. Behav. 102 (1) (2011) 96–104.

[50] T. Wenger, et al., The central cannabinoid receptor inactivation suppresses endocrine reproductive functions, Biochem. Biophys. Res. Commun. 284 (2) (2001) 363–368.

[51] P.B. Sparling, et al., Exercise activates the endocannabinoid system, Neuroreport 14 (17) (2003) 2209–2211.

[52] V. De Chiara, et al., Voluntary exercise and sucrose consumption enhance cannabinoid CB1 receptor sensitivity in the striatum, Neuropsychopharmacology 35 (2) (2010) 374–387.

[53] Wolf, S.A., et al., Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. Cell communication and signaling : CCS, 2010. 8: p. 12–12.

[54] S. Dubreucq, et al., CB1 receptor deficiency decreases wheel-running activity: consequences on emotional behaviours and hippocampal neurogenesis, Exp. Neurol. 224 (1) (2010) 106–113.

[55] M.N. Hill, et al., Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus, Hippocampus 20 (4) (2010) 513–523.

[56] M. Ploughman, et al., Exercise intensity influences the temporal profile of growth factors involved in neuronal plasticity following focal ischemia, Brain Res. 1150 (2007) 207–216.

[57] S. Yasari, et al., Effects of exercise training on molecular markers of lipogenesis and lipid partitioning in fructose-induced liver fat accumulation, Journal of nutrition and metabolism 2012 (2012) 181687.

[58] S. Gomes da Silva, et al., Physical exercise in adolescence changes CB1 cannabinoid receptor expression in the rat brain, Neurochem. Int. 57 (5) (2010) 492–496.

[59] Z.C. Yan, et al., Exercise reduces adipose tissue via cannabinoid receptor type 1 which is regulated by peroxisome proliferator-activated receptor-δ, Biochem. Biophys. Res. Commun. 354 (2) (2007) 427–433.

[60] E.M. Marco, et al., Long-term consequences of URB597 administration during adolescence on cannabinoid CB1 receptor binding in brain areas, Brain Res. 1257 (2009) 25–31.

[61] C.S. Breivogel, et al., Chronic ∆9-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain, J. Neurochem. 73 (6) (1999) 2447–2459.

[62] L.J. Sim-Selley, B.R. Martin, Effect of chronic administration of < em > R < / em > -(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3- < em > de < /em >]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone Mesylate (WIN55,212-2) or Δ < sup > 9 < /sup > -tetrahydrocannabinol on cannabinoid receptor adaptation in mice, J. Pharmacol. Exp. Ther. 303 (1) (2002) 36.

[63] G. Lynch, C.S. Rex, C.M. Gall, Synaptic plasticity in early aging, Ageing Res. Rev. 5 (3) (2006) 255–280.

[64] . Filip, M., et al., Involvement of cannabinoid CB1 receptors in drug addiction: effects of rimonabant on behavioral responses induced by cocaine. Pharmacol. Rep., 2006. 58(6): p. 806–19.

[65] P.K. Thanos, et al., Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors, Behav. Brain Res. 164 (2) (2005) 206–213.

[66] C. Cohen, E. Kodas, G. Griebel, CB1 receptor antagonists for the treatment of nicotine addiction, Pharmacol. Biochem. Behav. 81 (2) (2005) 387–395.

[67] M. Mas-Nieto, et al., Reduction of opioid dependence by the CB(1) antagonist SR141716A in mice: evaluation of the interest in pharmacotherapy of opioid addiction, Br. J. Pharmacol. 132 (8) (2001) 1809–1816.

[68] K. Anggadiredja, et al., Endocannabinoid system modulates relapse to methamphetamine seeking: possible mediation by the arachidonic acid cascade, Neuropsychopharmacology 29 (8) (2004) 1470–1478.

[69] J.W. Muschamp, S.M. Siviy, Behavioral sensitization to amphetamine follows chronic administration of the CB1 agonist WIN 55,212-2 in Lewis rats, Pharmacol. Biochem. Behav. 73 (4) (2002) 835–842.

[70] M.A. Smith, W.J. Lynch, Exercise as a potential treatment for drug abuse: evidence from preclinical studies, Frontiers in Psychiatry 2 (2011) 82.

[71] W.J. Lynch, et al., Exercise as a novel treatment for drug addiction: a neurobiological and stage-dependent hypothesis, Neurosci. Biobehav. Rev. 37 (8) (2013) 1622–1644.

[72] K.P. Cosgrove, R.G. Hunter, M.E. Carroll, Wheel-running attenuates intravenous cocaine self-administration in rats: sex differences, Pharmacol. Biochem. Behav. 73 (3) (2002) 663–671.

[73] A.B. Peterson, D.P. Hivick, W.J. Lynch, Dose-dependent effectiveness of wheel running to attenuate cocaine-seeking: impact of sex and estrous cycle in rats, Psychopharmacology 231 (13) (2014) 2661–2670.

[74] Y. Zhou, C. Zhou, R. Li, Sex differences in exercise and drug addiction: a mini review of animal studies, J. Sport Health Sci. 3 (3) (2014) 163–169.