THE EFFECT OF CHRONIC ADOLESCENT CANNABINOID EXPOSURE ON ADULT SEXUAL BEHAVIOUR

by

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Abstract

Adolescence is a crucial sensitive period in the development of the endocannabinoid system. Perturbations to this system during adolescence using exogenous CB_1 receptor ligands produces long-lasting changes in adult behaviours, though potential effects on sexual behaviour remains to be fully explored experimentally. This was investigated in two studies: study one examined the effects of adolescent administration of cannabinoids on adult sexual behaviours in male rats, while study two did the same in female rats. In study one, adolescent male rats were administered CB_1 agonist HU-210, CB_1 antagonist AM-251, or no drug chronically during adolescence. Then they received drug abstinence until adulthood, followed by sexual behaviour testing. Study two used similar methods in females. In study one, AM-251 decreased sexual behaviour compared to control while HU-210 increased it. In study two, female sexual behaviour was not significantly affected by any drug treatment. The results for males are opposite of past findings on the effect of adult cannabinoid exposure, which show that CB_1 receptor agonism in adulthood decreases sexual functioning while antagonism increases it. This shows that the endocannabinoid system is involved in the adolescent development of normative adult male sexual behaviour. Further research is needed to fully contextualize the results for females, though it appears female sexual behaviour may be less vulnerable than male sexual behaviour to adolescent endocannabinoid system disruptions. Findings from these studies will contribute to an understanding of how the endocannabinoid system is involved in the development of sexual behaviour, how disregulation during adolescence may underlie sexual dysfunctions, and the potential risks of adolescent cannabis consumption on sexual health.

Preface

This thesis is based on a series of collaborative experiments under the supervision of Boris Gorzalka at the Department of Psychology, University of British Columbia. The original experimental designs were created by me and Tiffany Lee. Drug administration to subjects was conducted by me and Tiffany Lee. Behavioural testing was conducted by me, with assistance from Richard Rigby, Amanda Lee, Hannah Levin, Katie Cullen, and Tiffany Lee. The data analysis and composition of the thesis were my own work. All procedures were reviewed and approved by the Behavioural Research Ethics Board and Animal Care Council of the University of British Columbia (certificate numbers: A11-0369 and A13-0140).

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Introduction

Sexual behaviour is a vital aspect of life for many organisms in the animal kingdom, including humans. Copulation serves a vital function in allowing for the generation of offspring and the propagation of genes, and mating-related motivations represent major expenditures of individual resources and efforts for many animals. In people, the influence of sexuality can be seen in issues ranging from forensic systems (e.g. sexual coercion), social justice (e.g. heterosexism), public health (e.g. sexually-transmitted infections), and individual well-being (e.g. sexual dysfunctions and paraphilic disorders). For many, sex is a critical part of romantic relationships, and sexual functioning is closely associated with relationship functioning (Byers, 2005) and self-esteem (Althof, 2002). Sexual dysfunctions in particular are very common; for example, the prevalence of sexual dysfunctions has been reported to be as high as 43% for women and 31% for men (Laumann, Paik, & Rosen, 1999). These disorders are not only distressing in and of themselves, but also may potentially lead to relationship difficulties (Byers, 2005), reduced psychological health (Althof, 2002; Laumann et al., 1999), and criminal acts like sexual assault (Carvalho, Quinta-Gomes, & Nobre, 2013). Like almost all important psychological phenomena, sexuality is regulated by a complex interaction between biological and psychosocial factors. Although major strides have been made in understanding these factors and interactions, many aspects of the biopsychosocial regulation of sexual behaviours and functioning remains to be elucidated.

A particular aspect of sexuality that demands further research is the developmental etiology of functional and dysfunctional sexual behaviours. Factors that influence the development of sexual behaviours span across a lifetime. Organizational processes involving the central nervous system (CNS) and the hypothalamus-pituitary-gonadal axis are apparent during

pre-natal and early post-natal development, while activational processes are seen in during childhood and puberty. Learning from peers and changing social roles also plays a particularly crucial developmental function in shaping sexual behaviours in humans, particularly during adolescence. Puberty and adolescence represents a crucial time for the formation of sexual maturity and the reorganization of CNS and endocrine systems. It is a sensitive period where alterations and perturbations to these systems can have profound and long-lasting effects on adult sexual behaviour.

Puberty and adolescences are related and intertwined, but refer to different developmental processes. Puberty is exactly defined as the period where sexual maturation is reached (Schneider, 2008). It is initiated by increased gonadotropin-releasing hormone secretion by the hypothalamus, resulting in gonadal maturation and steroid hormone (androgens, estrogens, and progesterone) release and ultimately resulting in sexual maturation. In the common animal model, the laboratory rat, the beginning of the female pubertal period is characterized by the opening of the vaginal canal, approximately at the age of post-natal day (PND) 28 (Ojeda & Urbanski, 1994), and ends with the first estrous phase at approximately PND 40. In male rats, the beginning is characterized by balano preputial separation, at approximately PND 40 (Korenbrot, Huhtaniemi, & Weiner, 1977), and ends with the presence of mature spermatozoa in the vas deferens at approximately PND 60 (Clegg, 1960; Ojeda & Urbanski, 1994). The discrepancy between ages in each sex is consistent with findings in humans that girls reach puberty on average 1-2 years earlier than boys (Spear, 2000). Adolescence on the other hand represents the graduated behavioural change from childhood to adulthood (Schneider, 2008). These behavioural changes are reflected in the development of the CNS. Widespread neuroplasticity can be seen during this time, both at the cellular and network level. Extensive synaptic remodeling and

pruning take place, as well as alterations to neurotransmitter levels and receptor expression and functionality. This process has ill-defined boundaries in both humans and rats. For rats, it has been defined as PND 28-42, though this has been described as a conservative range that may not be exhaustive and that ages outside this range should not be considered as definitively outside of adolescence (Spear, 2000). Although puberty and adolescence are distinct processes, they are known to be intimately linked. However, the specific associations between pubertal, CNS, and behavioural development during this timeframe is currently not well understood.

The Endocannabinoid System

One neurotransmitter system that has been identified as important in during the developmental process during adolescence is the endocannabinoid system. The endocannabinoid system is a neuromodulatory system that is widespread throughout the CNS and peripheral regions in the body. It has been found to regulate a variety of physiological functions and behaviours, including cognition (Moreira & Lutz, 2008; Wotjak, 2005), emotion and mood (Hill & Gorzalka, 2009; Lutz, 2009), stress responding (Gorzalka, Hill, & Hillard, 2008; Gorzalka & Hill, 2009), motivation (Fattore, Melis, Fadda, Pistis, & Fratta, 2010), and sexual behaviour (Gorzalka & Dang, 2012; Gorzalka, Hill, & Chang, 2010).

The endocannabinoid system includes two primary G-protein coupled receptors: the cannabinoid 1 (CB_1) receptor (Herkenham, Lynn, Johnson, et al., 1991; Matsuda, Lolait, Brownstein, Young, & Bonner, 1990) and the cannabinoid 2 (CB_2) receptor (Munro, Thomas, & Abu-Shaar, 1993). CB_1 receptors are localized throughout the CNS and some peripheral tissues, but are most densely expressed in neurons of the cerebral cortex, hippocampus, amygdala, hypothalamus, basal ganglia outflow tracts, and cerebellum (Mackie, 2005). They have been found on glutamatergic, serotonergic, dopaminergic, cholinergic, GABAergic, and noradrenergic

neurons (Schlicker & Kathmann, 2001). CB_2 receptors are mostly expressed in peripheral tissues, immune cells, and glial cells. There are several endogenous ligands of these receptors, known as the endocannabinoids, of which the two most prominent are arachidonoylethanolamide (anandamide; Devane et al., 1992) and 2-arachidonoylglycerol (2-AG; Sugiura et al., 1995).

The most well-characterized endocannabinoid signaling pathway is the CB_1 receptor mediated modulation of monoamine neurotransmission in the brain. In this case, CB_1 receptors are expressed on the terminals of presynaptic neurons (Herkenham, Lynn, de Costa, & Richfield, 1991). Endocannabinoids are synthesized and released on demand (rather than being stored in vesicles) by postsynaptic neurons in response to binding of monoamines from the presynaptic cell (Piomelli, 2003; Schlicker & Kathmann, 2001). The ligands then diffuse across the synaptic clef to presynaptic CB_1 receptors. The subsequent binding of anandamide or 2-AG to the receptors on the presynaptic axon inhibits further release of neurotransmitters, allowing the postsynaptic neuron to regulate the level of incoming neurotransmission. Endocannabinoids are then removed from the synapse via cellular uptake and subject to intracellular enzymatic breakdown (Beltramo et al., 1997). Fatty acid amide hydrolase (FAAH) is primarily responsible for the metabolism of anandamide (Cravatt et al., 1996) while monoacylglycerol lipase (MAGL) is primarily responsible for the metabolism of 2-AG (Dinh, Freund, & Piomelli, 2002).

Human interaction with exogenous ligands of cannabinoid receptors is predominantly through the consumption of *Cannabis sativa*. Eighty-five cannabinoid compounds have been identified in *C. sativa*, with the most common and psychoactive natural cannabinoid being Δ^9-tetrahydrocannabinol (THC; El-Alfy et al., 2010). Cannabis and derivative products have a long history of human medicinal, recreational, and spiritual use. Estimates for the exact number of cannabis users worldwide is difficult due to the lack of recent data (Malone, Hill, & Rubino,

2010), though some surveys suggest that approximately 50% of 12th graders in the United States have used cannabis at least once in their life (Eaton et al., 2008; Rey, Martin, & Krabman, 2004). Earlier longitudinal evidence suggests that initiation of cannabis use, like most recreational drugs, occurs at an early age with a peak age of onset at 16 to 18 years old (Chen & Kandel, 1995; DeWit, Offord, & Wong, 1997). Adolescent consumption of cannabis has been investigated in the context of associations with cognitive deficits (Meier et al., 2012; Millsaps, Azrin, & Mittenberg, 1994), psychosis and schizophrenia (Malone et al., 2010), and other mental health concerns (Chadwick, Miller, & Hurd, 2013). Lifelong negative effects on sexual health and increased risks of sexual dysfunctions may be another issue among the high number of adolescents in North America that consume cannabis.

Adolescence and the Endocannabinoid System

Evidence has shown how the endocannabinoid system is linked to neurodevelopment and adolescence. The presence of CB_1 receptors, anandamide, and 2-AG indicate a functional endocannabinoid system as early as gestation days 11 to 14 in the rat CNS (Berrendero, Sepe, Ramos, Di Marzo, & Fernández-Ruiz, 1999; Rodríguez de Fonseca, Ramos, Bonnin, & Fernández-Ruiz, 1993). Similarly, functional CB_1 receptors have been discovered in human fetal tissue at gestation week 19 (Mato, Del Olmo, & Pazos, 2003). However, high levels of CB_1 receptor expression have only been detected in the amygdala and hippocampus in mid-gestation human fetal and neonatal rat brains, while expression was limited in the striatum, thalamus, and cerebral cortex (Rodríguez de Fonseca et al., 1993). In contrast, this receptor shows widespread concentrated expression the brains of adult humans and rats (Herkenham, Lynn, Johnson, et al., 1991; Jutras-Aswad, DiNieri, Harkany, & Hurd, 2009). This shows that substantial changes to this system occurs between perinatal stages and adulthood, as well as suggest that the

endocannabinoid system may also play a region-dependent role in neurodevelopment (Lee & Gorzalka, 2012). Additionally, instead of gradually increasing from neonatal to adult levels, one study showed that expression of CB_1 receptors in the rat limbic, striatal, and midbrain structures peaked during mid-adolescence (PND 30-40), before then decreasing to adult levels (Rodríguez de Fonseca et al., 1993). In contrast, another study found that levels CB_1 receptor binding increased steadily from birth to adult levels (Belue, Howlett, Westlake, & Hutchings, 1995). On a cellular level, this system is known to play multiple crucial roles in neurodevelopment. Specifically, endocannabinoid signaling appears to take part in the regulation of neural progenitor proliferation, cell lineage commitment, immature neuronal migration, axonal pathfinding, and initiation of synaptic communication in neural networks (Harkany, Keimpema, Barabás, & Mulder, 2008).

Behaviourally, there is a growing body of evidence to suggest the role of the endocannabinoid system in the adolescent development of normative adult functioning in a variety of domains. The most typical research paradigm has been the chronic administration of exogenous cannabinoids across 10 to 14 days in adolescent rats, followed by drug abstinence until behavioural testing in adulthood. Using this methodology, studies have found that adolescent perturbations to the endocannabinoid system lead to long-term alterations in adulthood of cognitive functioning, anxiety, and depression (Trezza et al., 2012). Adolescence also appears to be a crucial sensitive period of the development of appropriate hypothalamic-pituitary-adrenal axis mediated stress responding (Lee & Gorzalka, 2012). In general, specific effects in each area appears to be sex-dependent and domain specific (such as spatial memory versus inhibitory avoidance or social versus non-social anxiety), as well as varying based on methodological differences (such as specific behavioural testing paradigms and drug

administration procedures). However, the emergent pattern is that excess endocannabinoid signaling via chronic exposure to CB_1 receptor agonists causes cognitive deficits and heightened anxiety and depression in adulthood. Not only are adolescents more vulnerable to the effects of CB_1 receptor agonism, adolescent exposure can produce opposite effects as chronic exposure during adulthood.

One prominent example is the influence on depression. Important depression-like behaviours in animal models include behavioural despair, measured by a lack of escape response to negative stimuli, and anhedonia, measured by a lack of approach response towards positive stimuli. Chronic administration of CB_1 receptor agonists THC or WIN-55,212-2 during adolescence has been found of increase behavioural despair on the forced swim test (Bambico, Nguyen, Katz, & Gobbi, 2010; Realini et al., 2011; Rubino et al., 2008). Similarly, impairments on the sucrose preference test (Bambico et al., 2010; Realini et al., 2011; Rubino et al., 2008) and progressive-ratio instrumental responding (Schneider & Koch, 2003) also suggest increased anhedonia. Alterations to sleep-wake cycles were also reported by one study, perhaps similar to the sleep disturbances seen in depression in humans (Schneider & Koch, 2003). Rubino et al. also reported down-regulation of CB_1 receptor expression in associated brain regions, such as the hippocampus and amygdala, while Realini et al. showed that the adult depressive features could be reversed via up-regulation of anandamide levels using the FAAH inhibitor URB-597. Bambico et al. also demonstrated the disruption to monoamine transmission by showing the suppression of adult serotonin neurotransmission in the dorsal raphe and enhancement of norepinephrine neurotransmission in the locus coeruleus. Conversely, chronic CB_1 receptor agonism during adulthood fails to elicit depressive behaviours (Bambico et al., 2010; Realini et al., 2011; Schneider & Koch, 2003), while some studies have also suggested that

pharmacological enhancement of CB_1 receptor signaling can have antidepressive effects in adulthood (Gobbi et al., 2005; Hill & Gorzalka, 2005; McLaughlin, Hill, Morrish, & Gorzalka, 2007). These results show that adolescents are more sensitive to the effects of exogenous cannabinoids and that they show qualitatively different responses to perturbations to the endocannabinoid system. Under-development of endocannabinoid system components and/or decreased sensitivity of monoamine neurotransmission to endocannabinoid regulation represent possible mechanisms for these behavioural effects.

The Endocannabinoid System and Male Sexual Behaviour

Several lines of research have established the importance of the endocannabinoid system in regulating sexual behaviour. Results from studies in men suggest that sexual experience and sexual potency are differentially affected by cannabis consumption. Early self-report studies found that cannabis consumption was associated with better subjective sexual experience (Halikas, Weller, & Morse, 1982; Kolodny, Masters, & Johnson, 1979; Tart, 1970). Tart (1970) found that men reported increased sexual arousal, number of sexual thoughts, and duration of intercourse following cannabis consumption. It was also found that a majority of men reported improved sexual pleasure and quality of orgasm following cannabis use, while a proportion reported increased length of sexual performance. Some have proposed that cannabis slows the user's attention and perception of time, making sex seem to last longer while allowing the user to focus more on the present (Melges, Tinklenberg, Hollister, & Gillespie, 1971). Others have raised that cannabis may enhance sensate focus and alter how sexual pleasure is experienced in the body (Gawin, 1978).

There appears to be a dose-dependent effect, with lower amounts of cannabis (such as one cannabis joint) being more likely to be reported as causing increased sexual pleasure than

larger amounts (such as two or more joints) (Abel, 1981; Koff, 1974). Larger doses were also associated with a deleterious effect on sexual performance, which was attributed to a broad suppression of all behaviors by heavy exposure to the drug (Abel, 1981; Chopra & Jandu, 1976). There have also been some reports that chronic cannabis use is associated with erectile dysfunction (Kolodny, Masters, Kolodner, & Toro, 1974; Scher, 1970). Cohen (1982) reported that daily cannabis users displayed twice the rate of erectile dysfunctions compared to non-users, from 8% to 19%. Earlier studies have been hampered by a lack of reliable measures for erectile dysfunction, relying primarily on self-report (Shamloul & Bella, 2011). In a more current study, Aversa et al. (2008) found an association between cannabis use in men and erectile dysfunction as measured by venocclusive plethysmography; early epithelial damage may be a mechanism for the link between cannabis and erectile dysfunction. Recent epidemiological studies are inconsistent in finding a relationship between cannabis use and sexual dysfunction, with one study finding no relationship between cannabis use and sexual dysfunctions (Johnson, Phelps, & Cottler, 2004), while another found that regular cannabis use among men was associated with orgasmic difficulties (Smith et al., 2010).

In male rodent models, endocannabinoid activation has generally been associated with decreases in sexual behavior, although some conflicting findings also exist in this area. Administration of THC to male rats increased the latency before mounting, latency before ejaculation, and latency before sexual behavior after an ejaculation when the male was presented with a sexually receptive female (Merari, Barak, & Plaves, 1973). Similar findings have been reported in mice (Cutler & Mackintosh, 1984). Chronic administration of THC also produces similar negative effects on male sexual behavior (S. L. Dalterio, 1980; Dhawan & Sharma, 2003). Unlike many effects of cannabinoids such as a global locomotor deficit, male rats do not

seem to develop tolerance to the sexual effects of THC over longer durations of exposure (Frischknecht, Sieber, & Waser, 1982). This also suggests that the deleterious effects of THC on sexual behavior, at least following chronic exposure, are not purely secondary to its effects on locomotor activity. As well, the negative effects of THC on sexual function occurred even in mice that were castrated and then administered testosterone (Shrenker & Bartke, 1985). This shows that THC does not exert its effects of sexual function purely by influencing androgen levels. Ferrari et al. (2000) found that exposure to potent CB_1 receptor agonist HU-210 caused a dose-dependent reduction in male sexual behavior. Chronic administration of HU-210 was also effective in suppressing sexual functioning in male rats, even at lower doses that did not produce an acute inhibitory effect. Furthermore, acute administrations of CB_1 receptor antagonist AM-251 decreased the time and number of intromissions required before male rats reached ejaculation (Gorzalka, Morrish, & Hill, 2008). At the same time, anandamide reuptake inhibitor/FAAH inhibitor AM-404 only increased latency prior to intromission at very high doses, while FAAH inhibitor URB-597 failed to affect sexual functioning at all. This suggests that endocannabinoid regulation of sexual behaviour may be more dependent on the effects of 2-AG or other potential endogenous CB_1 receptor ligands than anandamide.

However, Martinez-Gonzalez et al. (2004) found that, although administration of high doses of endocannabinoid anandamide decreased male sexual behavior, low doses of anandamide increased ejaculation frequency. Anandamide administration also appeared to transform some, but not all, naturally non-copulating rats into sexually active animals (Canseco-Alba & Rodríguez-Manzo, 2013). Riebe, Lee, Hill, & Gorzalka (2010) chronically administered male rats with HU-210, which were were either taken off the drug (spontaneous withdrawal), given AM-251 (precipitated withdrawal), or continued to be given HU-210 (drug maintenance)

during the day of sex testing. The reduction in sexual behavior caused by HU-210 was attenuated in, and only in, the precipitated withdrawal condition, suggesting the chronic effects of cannabinoids on sexual behavior is directly mediated by the CB_1 receptor and not due to the direct effects of drug withdrawal. Riebe et al. (2010) interpreted the effects of chronic cannabinoid exposure as likely being caused by neuroadaptive changes, though other interpretations are possible (such as HU-210 not being completely metabolized in the timeframe of the study). These results show that the role of the endocannabinoid system in modulating male sexual behavior may be more complex, with endocannabinoids and exogenous cannabinoids having potentially opposite effects.

The Endocannabinoid System and Female Sexual Behaviour

For the most part, the evidence suggests that the use of cannabinoids increases subjective appraisals of sexual experience. Multiple early studies have found that some women reported increases in sexual desire following use of cannabis (Koff, 1974; Kolodny et al., 1979; National Commission on Marihuana and Drug Abuse, 1972). Women generally reported that cannabis consumption caused higher sexual responsiveness that was associated with greater sensitivity to touch and relaxation. More physiologically oriented functions such as vaginal lubrication, orgasm frequency, or orgasm intensity were not reported to have increased. Koff also found that women reported increased sexual desire and pleasure following cannabis consumption, and attributed this increase to psychosocial factors such as decreased inhibition. As well, approximately 70% of women who consumed one cannabis joint reported increased sexual desire, while less 50% of women who consumed two or more cannabis joints reported increased sexual desire, suggesting a dose-dependent effect similar to the pattern seen in men. Other more

recent studies have also found a facilitative effect of cannabis use on sexual experience, again emphasizing the enhanced subjective experience (Halikas et al., 1982; Weller & Halikas, 1984).

However, in a large epidemiological study (Johnson et al., 2004) found, after controlling for demographics, general health, and psychiatric disorders, cannabis use was associated with inhibited orgasm and sexual pain. Women reporting chronic cannabis use displayed lower physiological sexual arousal, measured by vaginal pulse amplitude plethysmography, in response to erotic stimuli compared to women who did not use cannabis (Klein, 2011). At the same time, increased physiological arousal in response to erotic stimuli, in non-cannabis using women, was associated with concurrent decreases in concentrations of circulating anandamide and 2-AG (Klein, Hill, Chang, Hillard, & Gorzalka, 2012). This suggests a potential inhibitory role of endocannabinoid system activation on female sexual arousal. These diverging results may be explained by cannabis consumption and endocannabinoid activation enhancing the experience of sex but reducing physiological sexual function. This would be consistent with older findings that cannabis use is associated with increased more subjective elements such as sexual enjoyment and desire but not more objective aspects like vaginal lubrication.

Findings from animal models of female sexual behaviour, done primarily in rats, are also somewhat contradictory. Gordon, Bromley, Gorski, & Zimmerman (1978) found that, in female rats, administration of low doses of THC increased the receptive behavior of lordosis, while higher doses reduced receptivity. Acute administrations of THC have also been found not to only increase receptive behavior, but also mate soliciting proceptive behavior in rodents (Turley & Floody, 1981). Unlike in humans, the sexual response of female rodents depends on estrogen and progesterone rather than adrenal androgens (Gorzalka et al., 2010). THC administration into the intracerebral ventricles of estradiol-primed female rats produced similar rates of lordosis as rats

primed with both progesterone and estradiol (Mani, Mitchell, & O'Malley, 2001). Furthermore, the stimulatory effects of THC were blocked by suppression of progesterone signaling through the administration of either a progesterone antagonist or an antisense progesterone receptor oligonucleotide. Administration of a dopamine $D_{1/5}$ receptor antagonist also blocked the effect of THC on sexual behavior, while a CB_1 receptor antagonist attenuated the sexually facilitative effect of either progesterone or dopamine. These results suggest that THC, and by extension the endocannabinoid system, may regulate sexual behavior by facilitating the central interaction between progesterone and dopamine.

Conversely, administration of the potent CB_1 receptor agonist HU-210 has been shown to reduce receptive and proceptive behavior in female rats (Ferrari et al., 2000). As well, CB_1 receptor antagonist/inverse agonist AM-251 was reported to increase female sexual motivation, as shown by increasing the time that females spent with males in a novel runway task (López, Webb, & Nash, 2009). Conversely, another study showed that CB_1 receptor antagonist SR 141716 reduced female sexual receptivity but did not affect female sexual proceptivity or motivation (Zavatti, Carnevale, Benelli, & Zanoli, 2011). Some of these divergent results may be due to methodological differences between studies. For example, some groups used a runway apparatus where the female was measured on how quickly she approached a male and how long she spent with the male to test sexual motivation (López et al., 2009), while others used a partner selection task where the female was measured on how often she spent time with a male rat or another female rat (Zavatti et al., 2011). Sexual testing can also be done in a manner that allows either the male or the female to regulate the pace of the sexual encounter. As well, different drugs, despite being in the same class, may show different biochemical properties. For example, HU-210 is a potent full agonist of CB_1 receptors while THC is only a partial agonist that also

displays antagonist properties (Paronis, Nikas, Shukla, & Makriyannis, 2012), and antagonists AM-251 and SR 141716 show different properties in blocking the ionotropic responses of CB_1 receptors to anandamide binding (Ford, Honan, White, & Hiley, 2002). Despite these methodological differences, it is clear that further research is needed to fully understand the role of the endocannabinoid system in female sexual behavior.

Potential Mechanisms of Action

Although it has been established that the endocannabinoid system plays a role in regulating sexual behavior, the mechanisms of action remain unclear. One potential mechanism for the influence of the endocannabinoid system might be via interactions with the gonadal hormones. In humans, studies on the acute and chronic effects of cannabis consumptions on gonadal hormone levels are inconsistent, with some finding that the drug caused a decrease in circulating levels of testosterone (Cone, Johnson, Moore, & Roache, 1986), while another finding no effect (Mendelson, Kuehnle, Ellingboe, & Babor, 1974). It is possible that inconsistency in average amount of cannabis consumed, and different methodologies in measuring levels of circulating hormones.

However, non-human models have found more consistent results. Features of the endocannabinoid system, such CB_1 receptors, endocannabinoids, and endocannabinoid synthesis and breakdown enzymes, have been found throughout the hypothalamic-pituitary-gonadal axis (Gorzalka & Dang, 2012). In general, increased CB_1 receptor activity, either through endogenous endocannabinoid signaling or exposure to exogenous cannabinoids, suppresses the release of gonadotropin-release hormone (GnRH) from the hypothalamus and luteinizing hormone (LH) release from the pituitary gland, which subsequently causes a secondary reduction in estradiol, progesterone, or testosterone levels (Dalterio, 1980; Kumar & Chen, 1983; Murphy, Steger,

Smith, & Bartke, 1990). Chronic heightened CB_1 receptor stimulation may also cause reduced function at the gonads directly, including degeneration of testosterone-producing Sertoli cells of the testes in males (Dixit, Arya, & Lohiya, 1975) and decreases in LH receptor expression of the corpus luteum in females (Tsutahara et al., 2011). Both *in vivo* and *in vitro* studies in animal models produce similar findings (Dalterio, Bartke, & Burstein, 1977; Jakubovic, McGeer, & McGeer, 1979).

Studies using genetic knockout models have also found an inhibitory role for the endocannabinoid system. For example, Wenger, Ledent, Csernus, and Gerendai (2001) reported that male wild type mice displayed decreased LH levels in their bloodstream following anandamide administration, but such a response was not seen in CB_1 receptor knockout mice. However, the specific mechanism by which these interactions affect sexual behavior in males or females is still unclear. The acute effects of CB_1 receptor signaling on gonadal hormones may be less likely to directly influence sexual behavior, as Shrenker and Bartke (1985) found that the acute effects of THC in males are independent of testosterone levels. However, the endocannabinoid system may still influence gonadal hormone signaling in other ways, such as reducing binding to androgen receptors (Purohit, Ahluwahlia, & Vigersky, 1980), that then alter sexual functioning. As well, Mani et al. (2001) reported findings that suggest a crucial role for central progesterone activity in mediating the effect of THC on female sexual function.

Monoamine neurotransmitters represent another potential mediator for the effects of the endocannabinoid system on sexual function. In addition to progesterone, Mani et al. (2001) showed role of the dopaminergic activity in the endocannabinoid regulation of sexual behavior. Dopamine, opioid, and oxytocin signaling has been well established as being important to sexual motivation and behavior in both males and females (Graham & Pfaus, 2012; Pfaus & Phillips,

1991; Pfaus et al., 2012). Noradrenergic signaling may also be important, as Murphy, Gher, Steger, & Bartke (1994) showed that acute THC administration blocked the noradrenergic and dopaminergic surge in the medial basal hypothalamus that typically occurs when a male rat is exposed to a sexually receptive female rat. As well, serotonin and the hypothalamic-pituitary-adrenal stress response may also play a role. Both higher levels of chronic stress and serotonergic activity have been found to be associated with decreased sexual behavior in male rats but increased sexual behavior in female rats (Gorzalka, Hanson, & Brotto, 1998). At the same time, the endocannabinoid system has been reported to partially regulate physiological and behavioral responses to stress (Gorzalka, Hill, et al., 2008), as well as sertonergic activity in some receptor subtypes (Hill, Sun, Tse, & Gorzalka, 2006). The differential effects of serotonin activity and stress on males and females may be related to the differential effects that cannabinoids have on male and female sexual behavior. However, Shrenker and Bartke (1985) showed that the acute effects of THC on male sexual behaviour were dependent on changes to neither serotonin nor dopamine levels. Overall, there are interesting associations between monoamines, endocannabinoids, and sexual behavior, though the development of a clear model of the interaction between the three areas remains to be fully elucidated.

The endocannabinoid system also plays a role in modulating penile erectile function. CB_1 receptors have been shown to be expressed on neurons in the paraventricular nucleus of the hypothalamus (Herkenham, Lynn, de Costa, et al., 1991), the brain region that directly regulates erectile function. Cannabinoid receptors have also been found in the cavernous smooth muscle in the penis (Ghasemi et al., 2006); the relaxation of this muscle allows erections to occur and be maintained. Melis, Succu, Mascia, and Argiolas (2004) reported that CB_1 receptor antagonists infused directly into the paraventricular nucleus can induce erections in rats. CB_1 receptor

agonists attenuated the effect of the antagonist, but did not have an effect when administered alone. Conversely, administration of anandamide induced greater relaxation in the cavernous smooth muscle, therefore facilitating erectile function in rats (Ghasemi et al., 2006). However, anandamide had the opposite effect in rhesus monkeys. These findings relating to erectile function highlight the potential differential effects of the endocannabinoid system in different regions of the body, as well as in different species. Only one published study to date (Klein et al., 2012) has investigated role of endocannabinoids on physiological aspects of female sexual function, and this topic requires further investigation.

Current Studies

The current series of studies seeks to further elucidate the role of the endocannabinoid system in the development of adult sexual behaviours. The first study will investigate male sexual behaviour, and make use of the paradigm that has been utilized previously for investigating the effects of adolescent cannabinoid exposure on cognition and emotionality. Adolescent rats will be exposed to chronic administrations of CB_1 receptor agonist or antagonists, and their sexual functioning will be assessed in adulthood. No other studies to date have targeted the adolescence or pubertal sensitive period in investigating the role of endocannabinoid signaling on male sexual behaviour. Knowledge from this investigation may represent the first step in fully understanding how the endocannabinoid system can contribute to the development of sexual dysfunctions and other sexual psychopathologies, as well as shed light on potential risks for sexual functioning in adolescents who consume cannabis. For males, it is hypothesized that excess CB_1 receptor activity during adolescence will cause a compensatory down-regulation of endogenous endocannabinoid signaling, which will subsequently manifest in a hypoactive state in adulthood. Given the inhibitory effects of CB_1 receptor signaling on male

sexual behaviour in adulthood, it is predicted that rats exposed to CB_1 receptor agonists will exhibit increased sexual behaviour in adulthood. Similarly, given the facilitatory effects of CB_1 receptor antagonism on adult sexual behaviour, it is predicted an analogous but opposite process, namely inhibited adult sexual behaviour, following adolescent exposure to CB_1 receptor antagonists.

The second study will use a similar methodology to investigate the influence of chronic adolescent cannabinoid exposure on adult sexual behaviour in female rats. Only one study has previously investigated the role of the endocannabinoid system in the adolescent development of female sexual behaviour. Chadwick et al. (2013) previously used a similar protocol, and found that daily administration of CP-55,940 during PND 35 to 45 attenuated adult female sexual motivation. Sexual receptivity, estrous cyclicity, and CB_1 receptor expression in the ventral-medial nucleus of the hypothalamus and the nucleus accumbens were not affected. Although Chadwick et al.'s study was illuminating, the current study seeks to expand on some of its limitations. Specifically, its injection timeframe is targeted towards late adolescence and after the onset of puberty. The current study will target early adolescence, before the onset of puberty, which may cause more profound effects. As well, the measures of sexual behaviour used were the runway apparatus for motivation and a single instance of lordosis for sexual receptivity. The current study will utilize a more comprehensive and standard measure of sexual receptivity. In addition, the current study will also investigate the effects of antagonist exposure. As with study one, the results of the current study will further our understanding of the role of the endocannabinoid system in the development of sexual pathologies, and in characterizing the risks of early cannabis exposure. Given the finds of Chadwick et al., it is expected that exposure

to CB_1 receptor agonists in adolescence will caused decreased sexual receptivity in adulthood, and that exposure to CB_1 receptor antagonists will cause increased sexual receptivity.

Methods

Study One

Subjects

Fifty-one male Sprague-Dawley rats, from Charles River (Montreal, Canada) were used as subjects for this study. The animals were 20 days old upon arrival, and received two weeks of habituation to their housing conditions and colony room before the start of any experimental procedures. All subjects were pair housed in clear plastic bins (48 x 27 x 20 cm) with aspen chip bedding and provided with free access to tap water and Purina rat chow. Opaque PVC tubes and paper towels were provided for enrichment. Fresh bins, bedding, and enrichment materials were provided twice weekly. The colony room was ventilated, kept at 21±1ºC, and on a reversed 12 hour light-12 hour dark (lights on at 9:00 am) cycle. Eighteen adult ovariectomized female Sprague-Dawley rats were used as stimuli animals for sex testing, and were housed in identical conditions. Male and female animals were kept in separate colony rooms with only other animals of the same sex. All procedures involving animals were reviewed and approved by the Animal Care Council of the University of British Columbia, with adherence to guidelines from the Canadian Council for Animal Care.

Drugs Conditions

The subjects were divided into five experimental groups. Each group received one of the following treatment conditions: 25 ug/kg HU-210 (n = 10), 75 ug/kg HU-210 (n = 10), 5 mg/kg AM-251 (n = 10), vehicle only control (n = 10), and no injection control (n = 11). The HU-210

doses were based on previous findings that found a significant effect on sexual behaviour following chronic administration at the high dose, but not the low dose (Ferrari et al., 2000). The AM-251 dose was based on previous findings that found a significant effect on sexual behaviour following acute administration (Gorzalka, Morrish, et al., 2008). Drugs were administered via intraperitoneal injections at 1 ml/kg, and were dissolved in a 1:1:8 vehicle mixture of dimethyl sulphoxide, polysorbate 80, and 0.9% saline. Estradiol benzoate and progesterone were used to ensure sexual receptivity of the stimuli females. These hormones were administered via subcutaneous injections at 10 mg/animal and 500 mg/animal respectively and were dissolved in peanut oil. HU-210 was ordered from Tocris Bioscience (UK), AM-251 from Cedarlane (Ontario, Canada), and estradiol benzoate and progesterone from Sigma-Aldrich (Ontario, Canada).

Procedures

Subjects received daily injections with their assignment treatment condition from the ages of PND 35 to 45. Injections occurred once per day during the first one-third of the dark phase of their light cycle, between 10:00 am and 12:00 pm. Following the last injection, the animals were then placed on drug abstinence until PND 100. Then, they received four practice test sessions of sexual behaviour, followed by one sexual behaviour test session. Each session was spaced between 3 and 7 days apart from the preceding and following sessions. Sexual testing procedures were based on the methodologies of previous studies in this area (e.g. Gorzalka, Morrish, et al., 2008).

Before each testing session, artificial estrous was induced in the female stimuli animals via injections of estradiol benzoate 48 hours and progesterone 3 to 5 hours prior to testing. Testing sessions occurred during the second one-third of the dark phase of the male rats' light

cycle, between 1:00 pm and 6:00 pm. Testing sessions took place in clear rectangular Plexiglas chambers (30 x 30 x 45 cm) filled with approximately 3 cm of Aspen chip bedding. During each testing session, a male subject would be placed into the chamber and given 5 minutes to explore and habituate to the testing environment. Subsequently, a stimuli female was placed into the chamber and the animals were allowed to interact. Every 10 minutes, the stimuli female was replaced to prevent sexual satiation in the male rat. Testing ended after the male had interacted with three females, for a total of 30 minutes. After testing, all animals were returned to their home cages and colony room. Testing took place in a quiet room under low-light conditions and without the presence of research personnel (except briefly during the transfer of animals). Testing sessions were recorded on videotape and scored at a later date by trained observers blind to the experimental conditions.

The following sexual behaviours were evaluated: mounts, intromissions, and ejaculations. Mounts occur when the male approaches the female from the rear and climbs on top of the female's back, and is generally followed by some pelvic thrusting. Intromissions are defined by the entry of the male's penis into the female's vaginal canal during a mount; it can be observed from the presence of vigorous thrusting and a stereotyped dismount characterized by a flailing extension of the forepaws. Ejaculations are defined by the production of semen from the male's penis, and can be noted via a series of convulsions from the male during an intromission followed by a more gradual dismount. Following ejaculation, the male will enter into a refractory period where it is no longer interested in the female. The post-ejaculation interval (PEI) is defined as the time between the ejaculation and the next time the male mounts a female. For each behaviour, a latency and a frequency is recorded. Latency represents the duration between the start of testing and the male engaging in a behaviour. Frequency is the number of times the male

engages in a behaviour; for mounts and intromissions, frequency is defined as the number of times the behaviour occurs before the first ejaculation, while for ejaculation, frequency is defined as the number of times the behaviour occurred over the entire session. Therefore, the variables measured are mount latency, mount frequency, intromission latency, intromission frequency, ejaculation latency, ejaculation frequency, and PEI. For intromissions, subjects that did not display the associated behaviour were assigned a value of 1800 s. Subjects that did not mount following ejaculation or did not ejaculate were excluded from the PEI analysis.

Data Analysis

All behaviours were analyzed for significant mean differences using one-way ANOVA, followed by *post hoc* analyses using Tukey's HSD. The alpha level was set at $\alpha = .05$. Previous pilot studies using similar methods suggested that an estimated effect size of $d = .70$ is reasonable, which, in consideration of the current sample size of $n = 10$ per group, would allow an estimated power of $\beta = .35$. Although this value is low, the sample size of this study is consistent with past research in this field and was deemed acceptable.

Study Two

Subjects

Fifty-one intact female Sprague-Dawley rats, from Charles River (Montreal, Canada) were used as subjects for this study. Animals were 15 days old upon arrival. Eighteen sexually proficient adult male Sprague-Dawley rats were used as stimuli animals for sex testing. Animals were housed in the same conditions as in the first study.

Drugs Conditions

The subjects were divided into five experimental groups. Each treatment condition received one of the following treatment conditions: 75 ug/kg HU-210 (*n* = 10), 5 mg/kg AM-251 (*n* = 10), 75 ug/kg HU-210 + 5 mg/kg AM-251 (*n* = 10), vehicle only control (*n* = 10), and no injection control (*n* = 11). In study one, no significant differences were found between the high and low doses of HU-210 (see below). Therefore, only the high dose was retained while the low dose was replaced with a combined agonist and antagonist treatment. The combined dose allowed for further investigation into whether the effects of adolescent HU-210 exposure on adult female sexual behaviour are mediated by CB_1 receptor signaling during adolescence. Otherwise, the drugs were obtained, handled, and administered using the same procedures as study one.

Procedures

As in study one, subjects received daily injections with their assignment treatment. Since females enter puberty sooner than males, drug administration was done at PND 25-35. Injections occurred once per day during the first one-third of the dark phase of their light cycle, between 10:00 am and 12:00 pm. Following the last injection, the animals were then placed on drug abstinence until PND 100. Then, they received one session of sexual behaviour testing.

After PND 100, each animal was tested individually when they entered estrous. Flank stimulation was used to check for behavioural estrous (Pfaff, Montgomery, & Lewis, 1977). During flank stimulation, the distal surfaces of a female's thighs were gently, briskly, and repeatedly brushed with the researcher's thumb and index finger. Estrous is indicated by a behavioural response of hopping, ear wiggling, and lordosis (see below). Animals were screened with this procedure three times a day, at 9:00 am, 12:00 pm, and 3:00 pm. Animals that

displayed estrous behaviours were immediately entered into sexual behaviour testing and excluded from future flank stimulation. Animals that did not display behavioural estrous were placed in this procedure again the following day for up to two weeks. Animals that failed to respond within this time were excluded from the study.

Sexual behaviour testing consequentially took place in the first and second one-third of the subjects' light cycle. Prior to sexual behaviour testing, all stimuli males were trained for sexual proficiency using the sexual behaviour testing procedures from study one. Stimuli females from study one were used again as stimuli females for these training sessions. Sexual proficiency in the stimuli males were defined as at least one ejaculation within 30 minutes. Test sessions took place in bi-level testing chambers first described by (refer to figure 1; Mendelson & Gorzalka, 1987). During each testing session, a stimuli male would be placed into the chamber and given 5 minutes to explore and habituate to the testing environment. Subsequently, a female subject was placed into the chamber and the animals were allowed to interact. Every 10 minutes, the female subject was moved to a new test chamber with another male, again in order to account for the Coolidge effect. Testing ended after the female had interacted with three males, for a total of 30 minutes. Other aspects of testing proceeded in the same fashion as study one.

Receptive lordosis behaviour was measured in the female subjects. Lordosis is the inversion of the curvature in a sexually receptive female rat's back, from convex to concave, upon mounted by a male. This is typically accompanied by the tilting back of the head, and raising of the posterior can also occur. Lordosis scoring procedures were based on the system described by previous research on this behaviour (Hardy & DeBold, 1972). Each time the female was mounted by the male, its lordosis behaviour was given a rating on the following scale: 0 (none), 1 (minimal), 2 (normal), or 3 (exaggerated). A rating of 0 was given when the female

was mounted but does not display a concave back arrangement. Minimal lordosis occurs when the back assumes a horizontal position with a head tilt that is below 30º from the horizontal. Normal lordosis occurs when the back assumes a concave position, the head is tilted between 30º and 45º from the horizontal, and there is minor raising of the posterior. Exaggerated lordosis occurs when the back assumes an extreme concave position, the head is tilted more than 45º from the horizontal, and there is prominent raising of the posterior. For each subject, a lordosis quotient is calculated, which the ratio of the number of lordosis responses to the number of mounts received over the course of the testing session. An average lordosis rating is also calculated for each subject, which is the mean of all lordosis ratings made over the course of the testing session. Finally, in order to allow for comparisons to Chadwick et al. (2013), the lordosis rating for the first mount the female receives was also recorded.

Data Analysis

Given that it is possible for the flank stimulation procedures to not detect all instances of behavioural estrous, and that some animals may not have regular estrous cycles either due to chance or the experimental manipulations, chi-square test was used to test whether non-estrous animals were evenly distributed across treatment conditions. Otherwise, data analysis was done in the same fashion as in study one.

Results

Study One

One-way ANOVA showed that adolescent exposure to exogenous CB_1 receptor ligands had a significant effect on mount latency, $F(4, 42) = 4.02$, $p = .008$, $\eta^2 = .28$, and ejaculation frequency, $F(4, 42) = 3.64$, $p = .012$, $\eta^2 = .26$. A trending effect was also seen in intromission

latency, $F(4, 42) = 2.42$, $p = .063$, $\eta^2 = .19$. No significant effects were seen for mount frequency, $F(4, 42) = .90$, $p = .473$, $\eta^2 = .08$; intromission frequency, $F(4, 42) = .59$, $p = .673$, $\eta^2 = .05$; ejaculation latency, $F(4, 42) = 2.03$, $p = .11$, $\eta^2 = .16$, and PEI, $F(4, 30) = .93$, $p = .462$, $\eta^2 = .11$. Tukey's HSD for mount latency indicated that the AM-251 group displayed significantly increased mean mount latency compared to the 25 ug/kg HU-210 group, $p = .009$, $d = 1.12$, 95% CI [.12, 2.12], and the 75 ug/kg HU-210 group, $p = .010$, $d = 1.12$, 95% CI [.12, 2.12], as well as a marginally significantly increased compared to the no injection group, $p = .049$, $d = .88$, 95% CI [-.07, 1.84], and the vehicle group, $p = .057$, $d = .84$, 95% CI [-.18, 1.87] (refer to figure 2). The AM-251 group also displayed significantly decreased mean ejaculation frequency compared to the 25 ug/kg HU-210 group, $p = .018$, $d = -1.86$, 95% CI [-2.97, -.75], and the 75 ug/kg HU-210 group, $p = .018$, $d = -1.51$, 95% CI [-2.56, -.46] (refer to figure 3). Non-significant trends were also seen for intromission latency (refer to figure 4) and ejaculation latency (refer to figure 5), where the AM-251 group had higher mean latencies compared to other groups.

As no significant mean differences were seen between the vehicle and no injection control groups, nor between the 25 ug/kg HU-210 and 75 ug/kg HU-210 groups, they were collapsed into an aggregated control group and an aggregated HU-210 group. An independent samples *t*-test was then used to compare the mean difference between the HU-210 group and the control group. Compared to the control group, HU-210 significantly decreased mount latency $t(37) = 2.16$, $p = .038$, $d = .69$, 95% CI [.04, 1.34], and intromission latency, $t(37) = 2.40$, $p = .022$, $d = .77$, 95% CI [.12, 1.42] (refer to figures 6 and 7 respectively). A marginally significant increase was seen in ejaculation frequency, $t(37) = -2.02$, $p = .050$, $d = -.65$, 95% CI [-1.29, -.003], while a non-significant decreasing trend was seen in ejaculation latency, $t(37) = 1.90$, $p = .066$, $d = .61$, 95% CI [-.03, 1.25] (refer to figures 8 and 9 respectively). No significant effects

were seen for mount frequency, $t(37) = .68$, $p = .503$, $d = .22$, 95% CI [-.41, .84]; intromission frequency, $t(37) = -1.51$, $p = .141$, $d = .48$, 95% CI [-.15, 1.12]; and PEI, $t(29) = 1.51$, $p = .141$, $d = .48$, 95% CI [-.15, 1.12].

Study Two

Behavioural estrous was detected in 39 of the 50 subjects. Chi-square test showed there was no significant difference in the frequency of non-estrous animals between any group, $\chi^2(4, N = 50) = 1.63$, $p = .80$, $\varphi = .18$. One-way ANOVA showed that adolescent exposure to exogenous CB_1 receptor ligands did not have a significant effect on lordosis quotient, $F(4, 34) = .74$, $p = .571$, $\eta^2 = .08$ (refer to figure 10); average lordosis rating, $F(4, 34) = .90$, $p = .48$, $\eta^2 = .10$ (refer to figure 11); and first lordosis rating $F(4, 34) = 1.63$, $p = .189$, $\eta^2 = .16$ (refer to figure 12).

Discussion

Study One

In male rats, adolescent chronic exposure to AM-251 decreased adult sexual behaviour, with significant and large effects in mount latency and ejaculation frequency and non-significant but moderate sized effects in intromission latency and ejaculation latency. At the same time, adolescent chronic exposure to HU-210 increased adult sexual behaviour, with significant and moderate to large effects in mount latency, intromission latency, and ejaculation frequency and non-significant but moderate sized effects in ejaculation latency. However, these changes were only apparent after an increase in statistical power via aggregation the HU-210 treatment groups and the control groups. AM-251 versus control groups overall had much larger effect sizes than HU-210 versus control groups. The same trends were seen for both manipulations compared to controls (and each other), even in sexual behaviour domains that did not show significant effects.

Overall, this suggests that chronic exogenous agonism of CB_1 receptor signaling during adolescence facilitates adult sexual behaviour in male rats, while antagonism attenuates the same behaviour. These findings are in contrast to previously established results, which show that acute or chronic administration of HU-210 during adulthood decreased sexual behaviour (Ferrari et al., 2000), while acute exposure to AM-251 caused an increase (Gorzalka, Morrish, et al., 2008). Adolescent cannabinoid treatment had a qualitatively different and opposing effect as administration of the same cannabinoids during adulthood.

Overall, these results are in support of the hypothesized model. For adolescent HU-210 exposure, excess CB_1 receptor signaling caused by the presence of an exogenous agonist triggers a compensatory down-regulation of endocannabinoid system. Subsequently, as adulthood is reached, the brain becomes less plastic and the endocannabinoid system manifests in a permanent hypoactive state. As CB_1 receptor signaling inhibits male sexual behaviour in adulthood, under-activation of this system then leads to increased sexual behaviour. The reciprocal process likely occurs for adolescent AM-251 exposure, where the inhibition of CB_1 receptor signaling causes the endocannabinoid system to crystallize in an over-active state in adulthood, leading to increased suppression of male sexual behaviour. This is similar to the pattern seen in the effect of chronic adolescent exposure versus adult exposure to CB_1 receptor agonists on depression-like behaviours. Similar to the current findings, chronic adult exposure to cannabinoid agonists has either no effect or an antidepressant effect, while chronic adolescent exposure has been found to increase depression-like behaviours in adulthood.

However, further research is needed to fully characterize the neurobiological mechanisms of the observed behavioural effects. One competing explanation is that instead of direct alterations to adult CB_1 receptor signaling, adolescent cannabinoid exposure instead acutely

disrupts endocannabinoid regulation of the development of other neurotransmitter and hormone systems. Therefore, long-term physiological changes might be seen not in the endocannabinoid system, but other pathways important to sexual functioning, such as dopamine, serotonin, or testosterone. In addition, if the endocannabinoid system is the direct site of alteration following adolescent treatment, it also remains to be seen which downstream systems play major roles in modulating observable behavioural responses. It is also unknown which features of the endocannabinoid system, such as CB_1 receptor expression, endocannabinoid synthesis enzymes, or basal anandamide tone, contributes to the behavioural effects of this manipulation. Past studies show that alterations to CB_1 receptor expression, anandamide production, and regulation of serotonin and norepinephrine may occur following this type of manipulation (Bambico et al., 2010; Realini et al., 2011; Rubino et al., 2008), and multiple sites of perturbation may exist. Adolescent exposure to exogenous cannabinoids may disrupt maturation of both the endocannabinoid system itself and other monoamine systems whose development is regulated by the endocannabinoid system, while these disruptions may have further indirect effects in adulthood.

Past research in the area of stress responding have suggested that alterations to basal anandamide tone may be particularly important to long-term changes to endocannabinoid-regulated behaviours (Gorzalka, Hill, et al., 2008). Compensatory up or down regulation of anandamide tone may also represent a potential mechanism for the effects seen in the current study. Increased levels of anandamide in response to adolescent exposure to AM-251 may explain over-regulation of sexual behaviour, while exposure to HU-210 leads to decreased levels of anandamide and greater sexual behaviour. However, this is in contrast to previous findings that show inhibition of FAAH activity, and therefore increases in anandamide content, in

adulthood does not prominently alter copulatory behaviours in male rats (Gorzalka, Morrish, et al., 2008). One possibility is that basal anandamide tone is less important for adult sexual behaviour, but is involved in adolescent development of functions that will ultimately affect sexual behaviour. Alternatively, other endocannabinoids, such as 2-AG, may be more important in regulating sexual behaviour, and the synthesis and breakdown of this ligand may be particularly important in understanding the current findings. Future studies could potentially focus on investigating 2-AG signaling specifically, potentially via MAGL inhibitors. Investigating alterations to basal anandamide levels following adolescent endocannabinoid perturbations may also prove fruitful.

The current study measured male sexual behaviour by observing the subjects copulating with female conspecifics. This method has the advantages of being more ecologically valid, representing a broader range of male sexual behaviours, and being methodologically consistent with the largest proportion of past studies. However, it is limited in that it cannot easily disentangle specific facets of sexual behaviour, namely sexual motivation versus sexual potency. Mount and intromission latencies may be seen as more reflective of motivation, as they measure the approach towards engaging with a preferred stimulus. Conversely, ejaculation latency and frequency may measure physiological competence as they directly assess how quickly and often ejaculation and insemination can occur. The current results suggest that both facets of sexual behaviour are affected by adolescent cannabinoid exposure. The current testing paradigm nevertheless allows motivation and potency to interact, and therefore measures the two in a confounded fashion that is difficult to interpret at a more specific level. The runway apparatus described previously (Chadwick et al., 2013; López et al., 2009) represents one possible paradigm that measures motivation. Additional research using alternative or novel

methodologies may be useful for exploring whether effects observed are due to alterations in motivation, physiological competence, or both.

The results from this experiment also show that adolescent AM-251 had a much stronger effect than HU-210, as indicated by the substantially larger effect sizes. However, this should be interpreted with caution. The dosages of AM-251 and HU-210 are not directly comparable, and the pharmacokinetics of the both drugs have not yet been fully characterized, particularly in adolescent rats. It is possible that the dosage of AM-251 used represented a much more severe physiological challenge than the dosages of HU-210. As well, given that the control groups were not sexually impaired, there is potentially a limit on how much any treatment can increase sexual behaviour. Therefore, the improved sexual functioning caused by HU-210 may have been attenuated by a ceiling effect. However, the large deficits seen via chronic AM-251 exposure suggest that antagonism of CB_1 receptor signaling during adolescences may cause profound changes in other domains as well. Further investigations on the effects of inhibited endocannabinoid signaling during adolescence, which so far has received limited attention compared to enhanced signaling, may also uncover crucial associations with other dysfunctions.

Additional translational issues should also be considered before these findings can inform clinical and public health practice. The results from this study potentially suggest that cannabis consumption by adolescent can increase sexual behaviour in adulthood. However, it is important to consider that the cannabis plant contains a diverse range of different cannabinoid compounds with varying CNS effects. THC itself is a partial agonist of CB_1 and CB_2 receptors that can also exhibit antagonist properties, as opposed to the highly potent and selective CB_1 receptor agonist properties of HU-210 (Paronis et al., 2012). Important species differences also exist between humans and rats. Although the most prominent neurological features that regulate most

behaviours are conserved across humans and rodents, quantitative differences such as rates of drug metabolism do exist. Nevertheless, given the consistent convergence between human and animal models in past research on cannabinoids and male sexual behaviours, it may be reasonable to expect patterns seen in the current study to generalize to physiological aspects of human sexual behaviour.

More prominently however, important psychosocial factors in human sexuality that are not present in animal models may interact with the endocannabinoid regulation of biological aspects of sexual behaviour. For instance, although more rapid and frequent copulations and ejaculations may be reproductively advantageous for male rodents, sexual pleasure for oneself and for one's partner may be the predominant sexual functioning concerns in men. Under-controlled hypersexuality is also often problematic when it manifests in people. Finally, adolescent development in human boys also represents a time of learning about one's own relation to sexuality, which is a process that could interact in other ways with both the direct psychoactive effects of cannabis as well as the social context of its use as a controversial recreational or medicinal drug. Despite these caveats, the current findings are consistent with other research that have characterized the potential long-term disruptions to normal functioning as a result of early cannabis exposure, and adds evidence in another domain that should be considered when discussing the potential consequences of adolescent cannabis use.

Study Two

In female rats, no measure of sexual behaviour was statistically significant, while only first lordosis displayed a moderate effect size. However, first lordosis is also the least reliable of the three receptivity measures (as it relies on a single observation of behaviour). Overall, this suggests that adolescent exposure to HU-210 or AM-251 did not have an effect on adult female

sexual behaviour. Similarly, though behavioural estrous was not detected in all female subjects, the association between whether estrous was detected and drug treatment was neither strong nor significant. This suggests that adolescent cannabinoid treatment did not have a significant effect on estrous cyclicity; animals that did not demonstrate behavioural estrous may have either entered into estrous during hours outside the times that they were tested or did not cycle due to biological variability.

These results, although in contrast to the effects of adolescent cannabinoid exposure in males, are consistent with the findings of Chadwick et al. (2013) despite methodological differences, such as different age of cannabinoid administration, between the current study and theirs. In that study, significant results were only seen in the runway paradigm, and not in either estrous cyclicity or first lordosis. These results are also consistent in part with the findings of Mani et al., (2001), which showed that the effect of adult exposure to THC on lordosis is dependent on progesterone signaling. Lordosis itself has been shown to be a reflex-like response largely regulated by ovarian hormones, with estrogens being crucial while progesterone playing a further facilitatory role (Pfaff & Sakuma, 1979). Given that estrous cyclicity is not affected by adolescent cannabinoid treatment in our study or Chadwick et al., it is perhaps unsurprising that lordosis is likewise not significantly altered by adolescent exogenous CB_1 receptor perturbations. Overall, it is possible that the endocannabinoid system does not play a large role in the development of ovarian hormone mediated behaviours during adolescence, despite being implicated in the onset of adolescence and interacting with estrogens and progesterone in adulthood (Gorzalka & Dang, 2012). It is possible that development of the endocannabinoid system itself is regulated by gonadal hormones during the course of puberty.

Conversely, Chadwick et al. (2013) did report an attenuation of female sexual motivation following adolescent THC exposure. Female sexual motivation appears to be dependent on factors other than ovarian hormones, such as opioid or oxytocin receptor activity and activation of hypothalamic and mesolimbic dopamine systems (Pfaus et al., 2012). These systems may be more vulnerable to adolescent endocannabinoid system disruptions than ovarian hormones, leading to subtle changes in more complex motivated behaviours than gross changes in reflexive sexual receptivity. Although Chadwick et al. did not report changes to CB_1 receptor expression in the nucleus accumbens and globus pallidus, alterations in anandamide tone, CB_1 receptor expression in other parts of brain, or network-level reconfiguration in other monoamine pathways may still occur. Future studies should focus on how the endocannabinoid system and other relevant neurotransmitter systems are altered by adolescent cannabinoid exposure in order to understand how this system regulates the development of female sexual behaviours. Further replications of existing findings using motivation paradigms, as well as proceptive behaviours, which may also reflect aspects of female sexual motivation, will also be helpful.

The results of the current study may suggest that adolescent use of cannabis in human women does not lead to adult deficits in sexual functioning. However, as is the case in males, more research is required before the current findings can be generalized to humans. The factors discussed in the previous study holds here as well. Furthermore, since female sexual responding in humans is largely regulated by adrenal androgens, it is possible that the consequences to adolescent cannabinoid exposure in women may be more similar to that of the effects in males. This is possibly reflected in past findings showing that a decrease in circulating levels of endocannabinoids is correlated with increases in female sexual arousal (Klein et al., 2012). It is also currently not fully understood how men and women may differ (or not differ) in their

neurophysiological regulation sexual behaviour. As well, for both men and women, fertility represents another crucial aspect of sexual health that is not captured by the current study. Given the influence of the endocannabinoid system on fertility (Bari, Battista, Pirazzi, & Maccarrone, 2011), this is another aspect that needs to be explored to fully understand the impact of cannabinoids on the development of sexuality. Overall, more research is needed before the role of the endocannabinoid system on human female sexual functioning can be used to inform clinical practice.

General Discussion

Overall, this pair of studies represents novel evidence for the role of the endocannabinoid system in the development of sexual behaviours during adolescence. In males, adolescent exposure to CB_1 receptor agonists and antagonists has the inverse effect compared to exposure to the same ligands in adulthood. These effects can be potentially explained by the hypothesized model where exogenous facilitation of CB_1 receptor signaling in adolescence causes compensatory down-regulation of endocannabinoid development, eventually leading the system to manifest in a hypoactive fashion in adulthood. A similar but opposite occurs when CB_1 receptor signaling is suppressed by antagonists in adolescence. In females, no significant effects were seen, suggesting that the development of the sexual behaviours measured is not directly regulated by the endocannabinoid system. Specifically, sexual receptivity is modulated by ovarian hormones, which may not be affected by alterations to the endocannabinoid system during puberty. More subtle changes in central neurotransmission and sexual motivation remain as other potential sites for the influence of cannabinoids.

These results can contribute to our understanding of sexual dysfunctions and non-disordered variations in sexuality in people. External factors such as chronic stress may disrupt

the development of the endocannabinoid system (Gorzalka et al., 1998; Lee & Gorzalka, 2012), which in term causes this system to manifest in an abnormal way in adulthood and lead to sexual dysfunctions. This would be consistent with similar patterns that are being observed in research surrounding cognition and emotionality (Trezza et al., 2012). Various life stressors can strain the growth of the endocannabinoid system during adolescence, which could then crystallize in an abnormal state in adulthood. It is possible that cognitive aspects of dysfunctional sexuality, such as negative beliefs and emotions surrounding oneself and sex, may also be represented at a neurophysiological level by disturbances in the endocannabinoid system and other processes. At the same time, these results also serve as further caution towards the use of cannabis amongst youth, especially boys. Puberty and adolescence appears to be a sensitive period where chronic perturbations to the endocannabinoid can have long-term consequences for normative adult sexual functioning.

Research into how the endocannabinoid system influences the development of adult sexuality is still in its infancy, and a large amount of additional research is required to support, improve, or refute the interpretations presented. Possible areas of further investigation include disentangling the effects on sexual motivation versus sexual potency, elucidating the more specific neurological mechanisms of the observed effects, and how these physiological phenomena interact with psychosocial factors in humans. Eventually, the endocannabinoid system may represent a target for novel pharmacological agents in treating sexual dysfunctions. The current results fill in one piece of a cohesive understanding of sexual development from a biopsychosocial perspective. Ultimately, this knowledge will hopefully allow for educational and clinical programs to prevent sexual dysfunctions and other problematic aspects of sexual

experience, and allow for more effective combined psychological and pharmacological interventions that build healthy and adaptive sexualities.

Figures

Chamber walls are made of clear Plexiglas while platform levels are made of wire mesh.

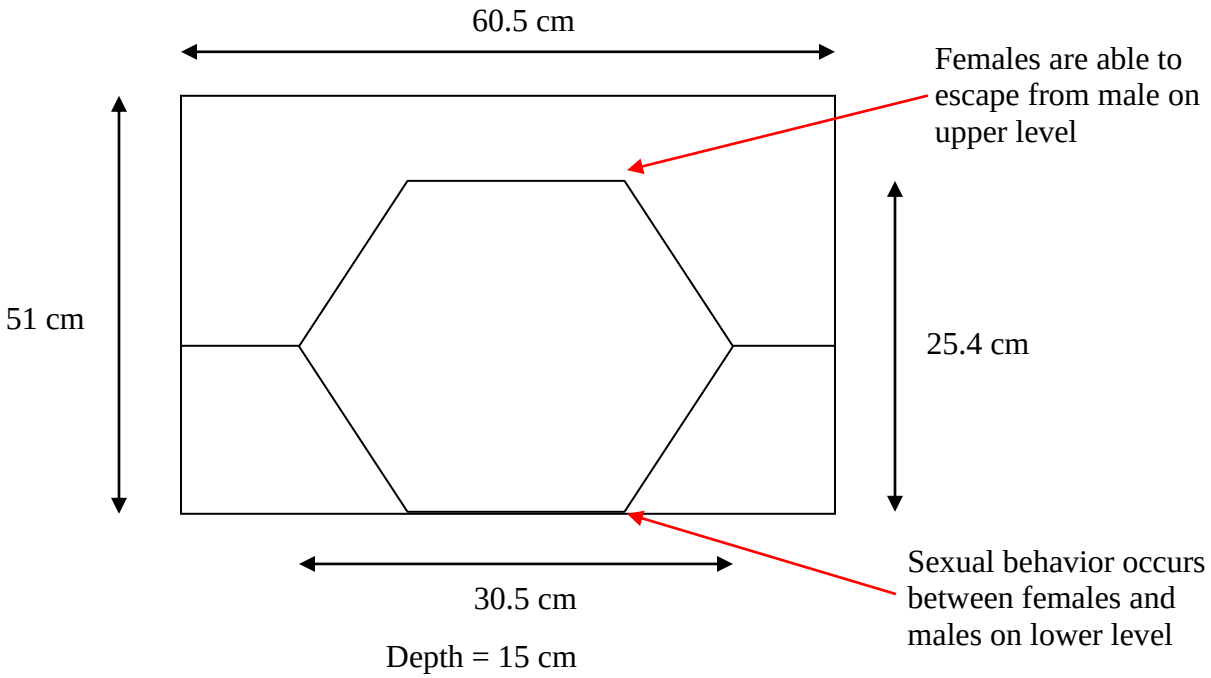

Figure 1. Diagram of the bi-leveled chamber for testing female sexual behaviour.

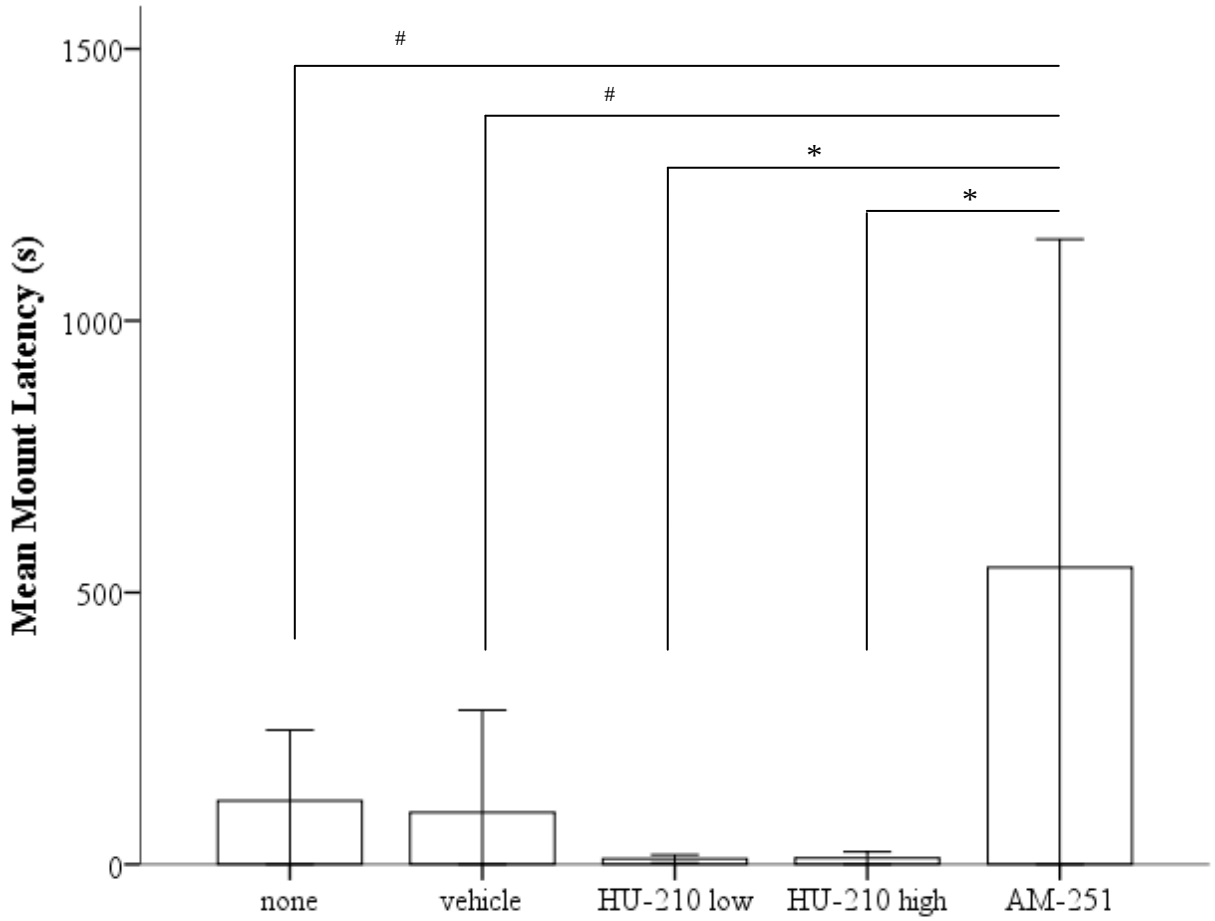

Figure 2. Mean mount latencies of male rats following adolescent exposure to no injections (n = 11), vehicle (n = 8), 25 ug/kg HU-210 (n = 10), 75 ug/kg HU-210 (n = 10), or 5 mg/kg AM-251 (n = 8). * indicates significant comparisons ($p < .05$). # indicates marginally significant comparisons ($p \approx .05$). Bars represent $M \pm$ 95% CI.

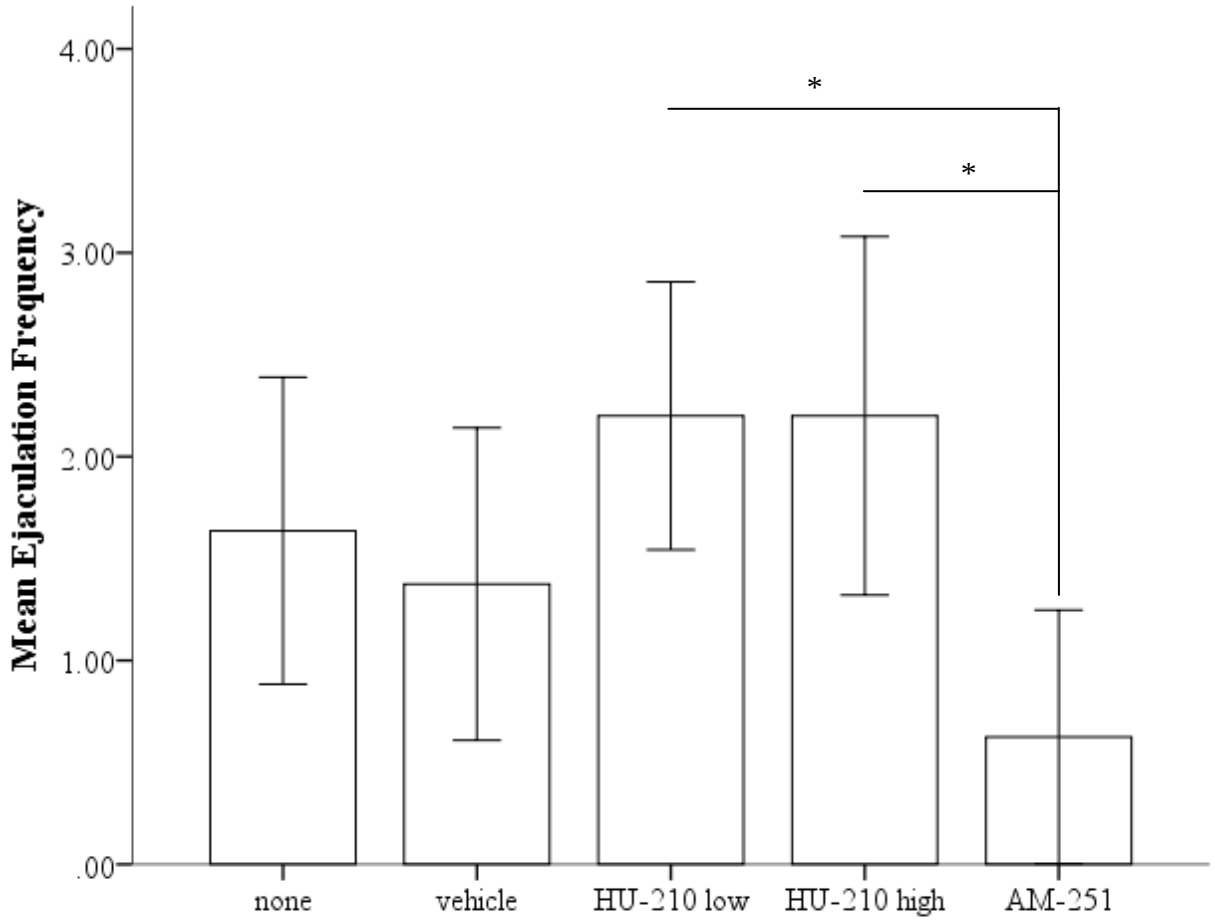

Figure 3. Mean ejaculation frequencies of male rats following adolescent exposure to no injections (n = 11), vehicle (n = 8), 25 ug/kg HU-210 (n = 10), 75 ug/kg HU-210 (n = 10), or 5 mg/kg AM-251 (n = 8). * indicates significant differences ($p < .05$). Bars represent $M \pm$ 95% CI.

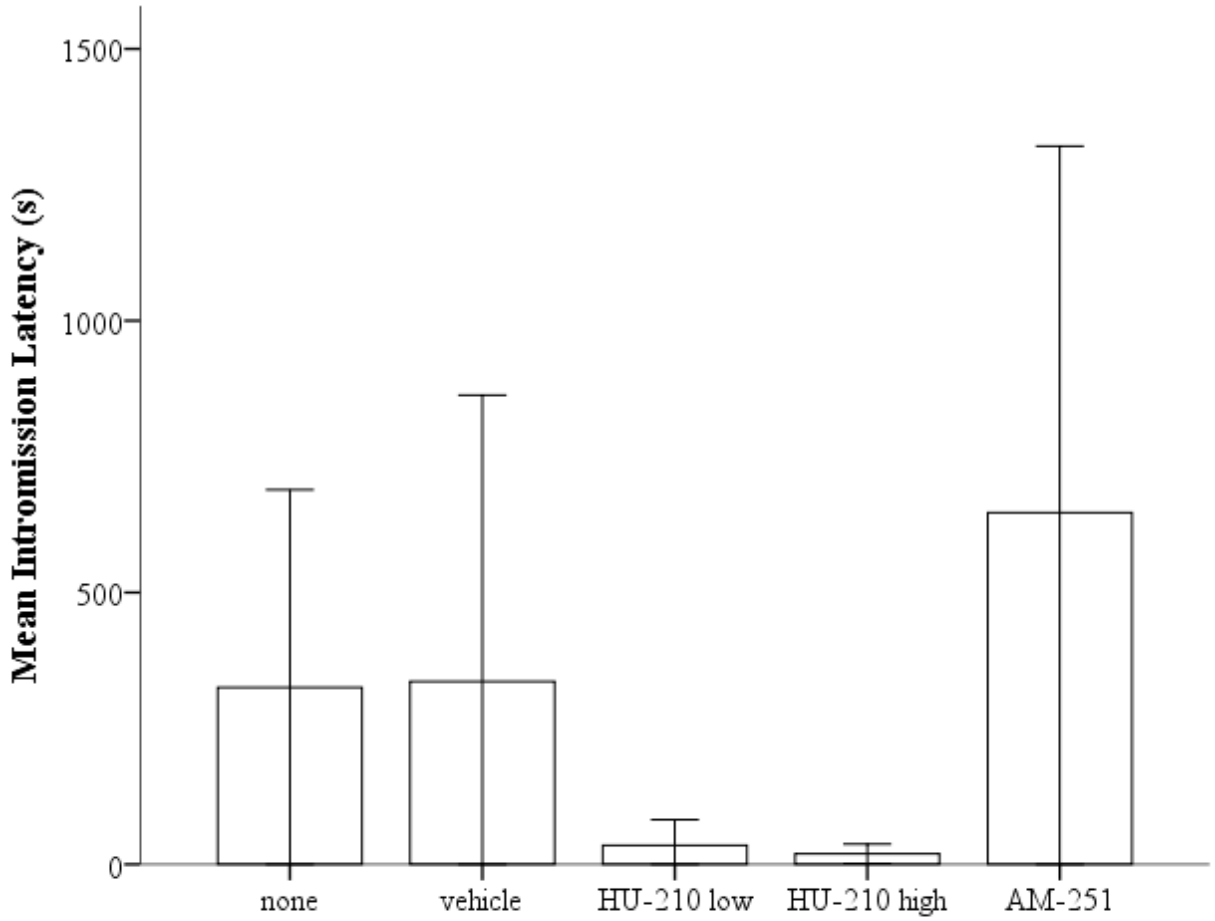

Figure 4. Mean intromission latencies of male rats following adolescent exposure to no injections (*n* = 11), vehicle (*n* = 8), 25 ug/kg HU-210 (*n* = 10), 75 ug/kg HU-210 (*n* = 10), or 5 mg/kg AM-251 (*n* = 8). Bars represent *M* ± 95% CI.

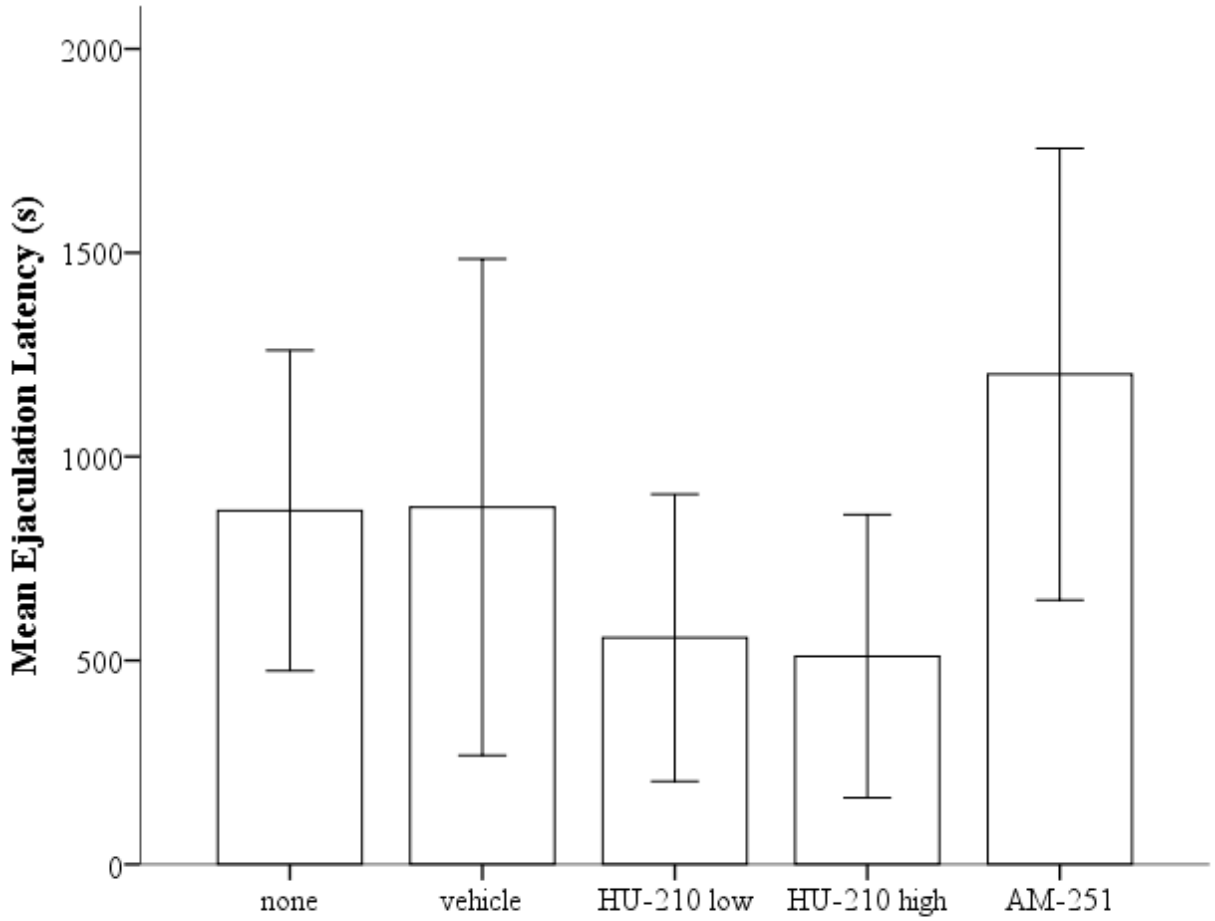

Figure 5. Mean ejaculation latencies of male rats following adolescent exposure to no injections (*n* = 11), vehicle (*n* = 8), 25 ug/kg HU-210 (*n* = 10), 75 ug/kg HU-210 (*n* = 10), or 5 mg/kg AM-251 (*n* = 8). Bars represent *M* ± 95% CI.

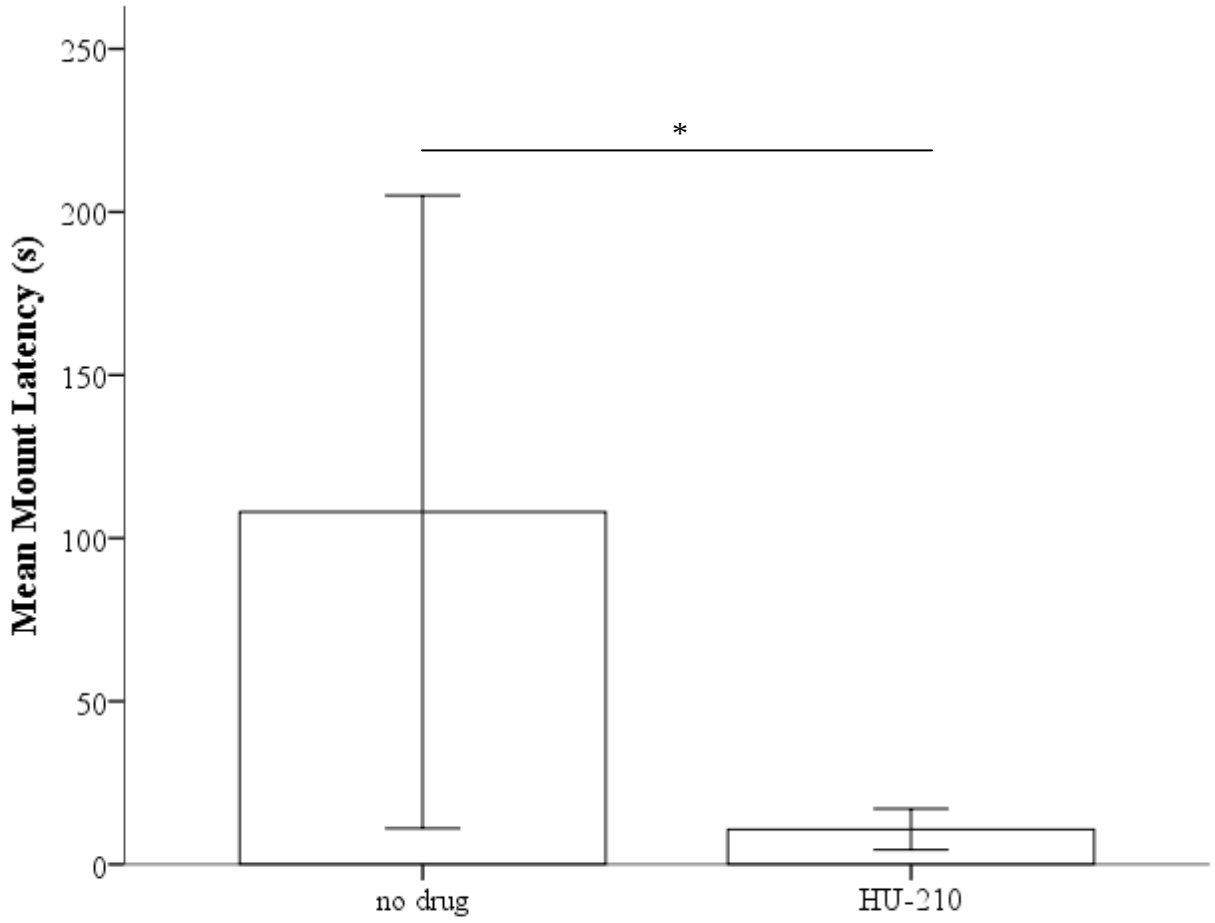

Figure 6. Mean mount latencies of male rats of the aggregated control (n = 19) and aggregated HU-210 (n = 20) groups. * indicates significant differences ($p < .05$). Bars represent $M \pm 95\%$ CI.

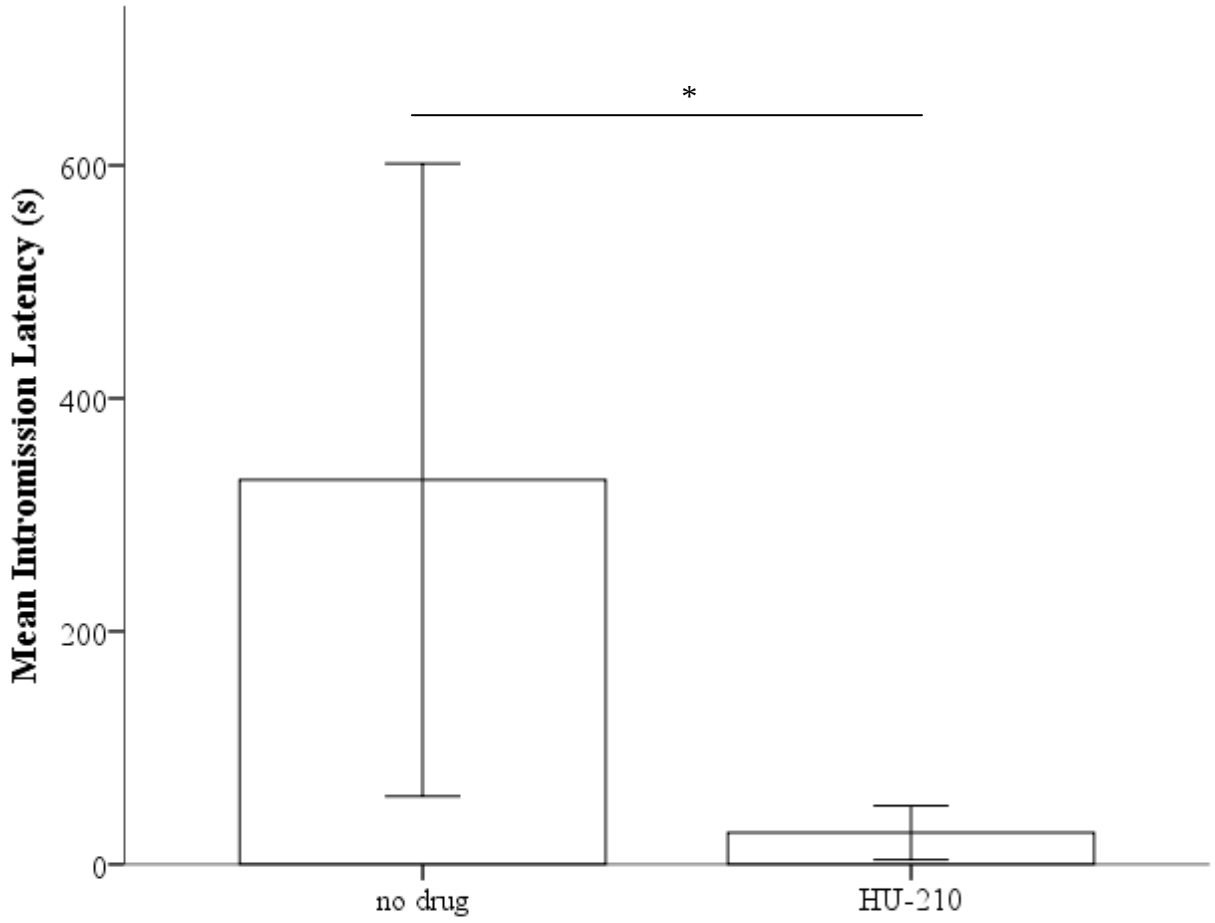

Figure 7. Mean intromission latencies of male rats of the aggregated control (n = 19) and aggregated HU-210 (n = 20) groups. * indicates significant differences ($p < .05$). Bars represent $M \pm$ 95% CI.

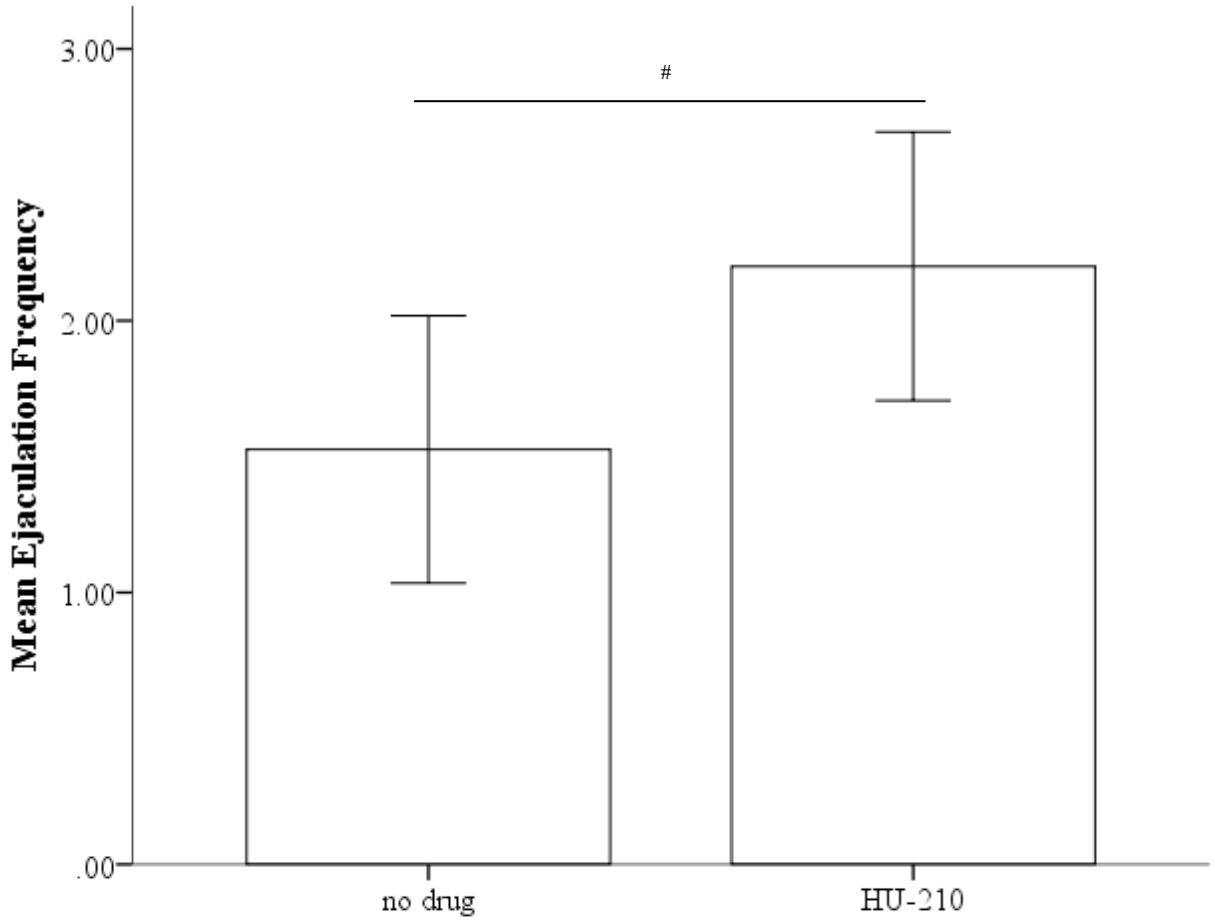

Figure 8. Mean ejaculation frequencies of male rats of the aggregated control (n = 19) and aggregated HU-210 (n = 20) groups. # indicates marginally significant differences ($p \approx .05$). Bars represent $M \pm$ 95% CI.

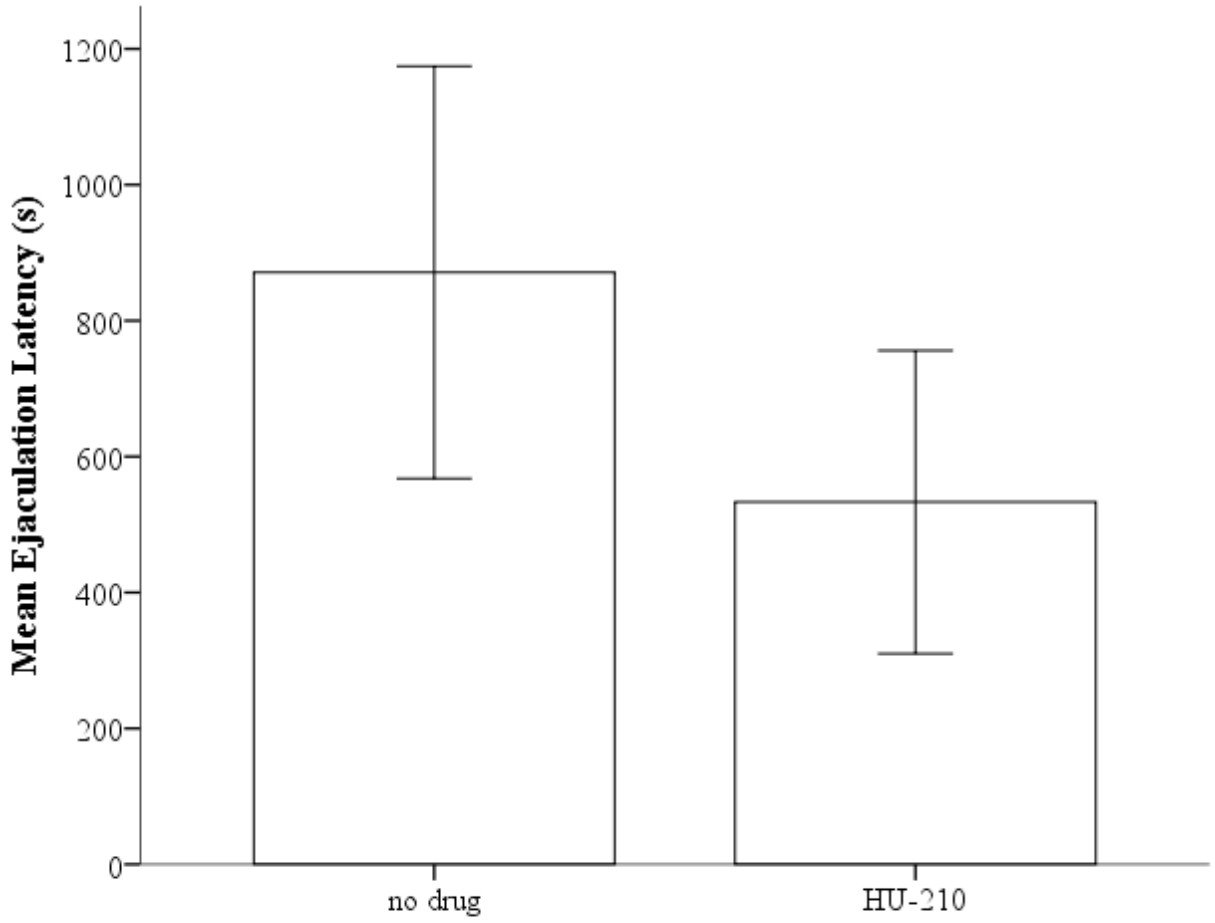

Figure 9. Mean ejaculation latencies of male rats of the aggregated control (*n* = 19) and aggregated HU-210 (*n* = 20) groups. Bars represent *M* ± 95% CI.

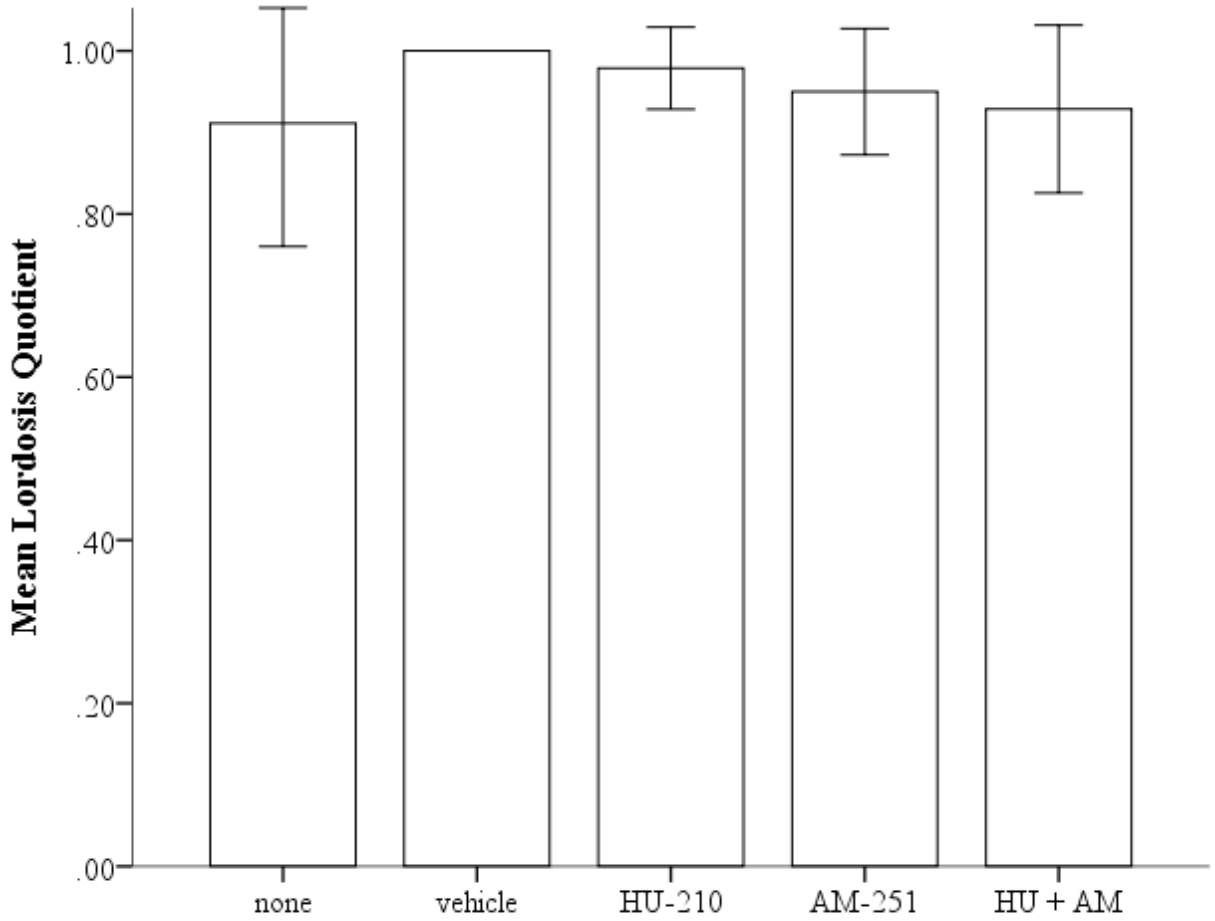

Figure 10. Mean lordosis quotients of female rats following adolescent exposure to no injections (n = 9), vehicle (n = 7), 75 ug/kg HU-210 (n = 8), 5 mg/kg AM-251 (n = 8), or 75 ug/kg HU-210 + 5 mg/kg AM-251 (n = 7). No significant differences between drug conditions were detected, $p > .05$. Bars represent $M \pm 95\%$ CI.

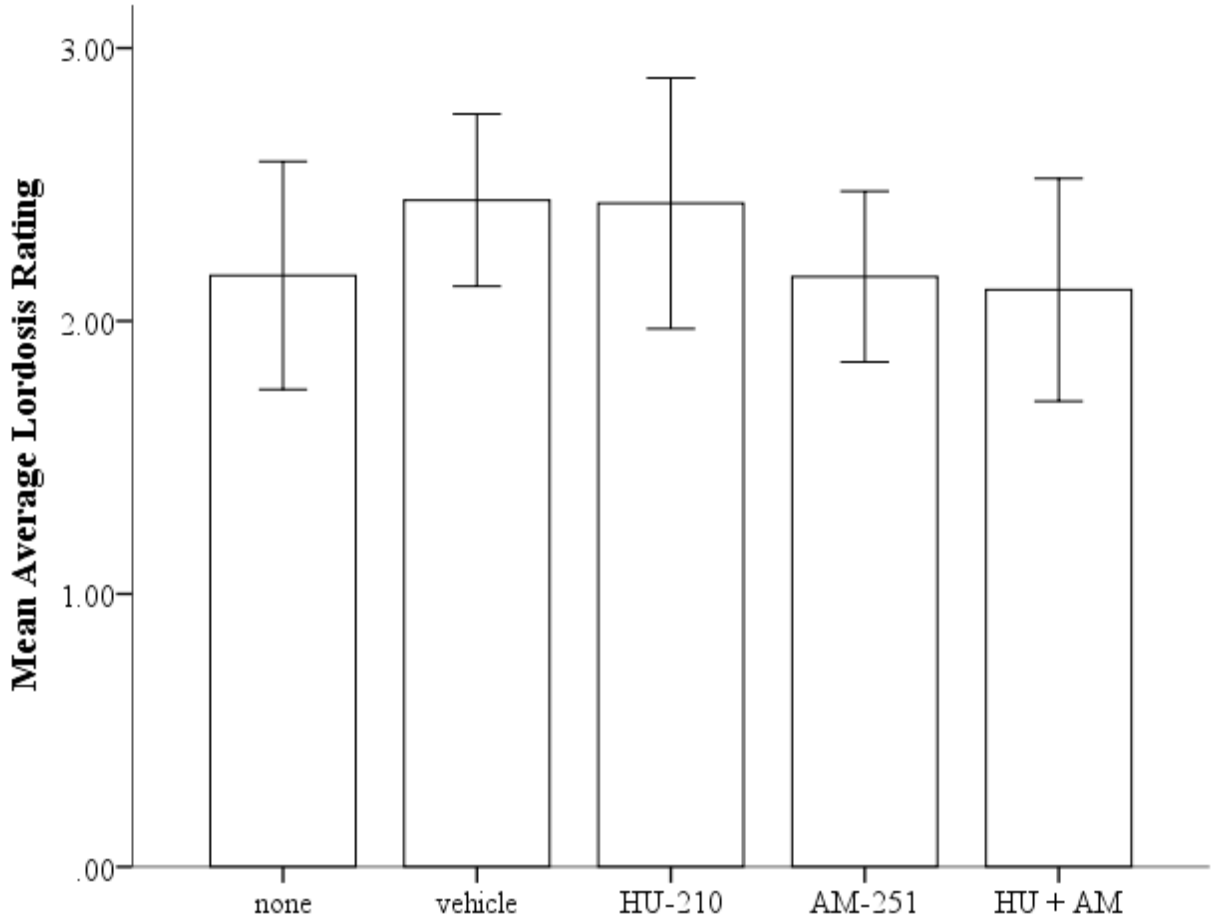

Figure 11. Mean average lordosis ratings of female rats following adolescent exposure to no injections (n = 9), vehicle (n = 7), 75 ug/kg HU-210 (n = 8), 5 mg/kg AM-251 (n = 8), or 75 ug/kg HU-210 + 5 mg/kg AM-251 (n = 7). No significant differences between drug conditions were detected, $p > .05$. Bars represent $M \pm 95\%$ CI.

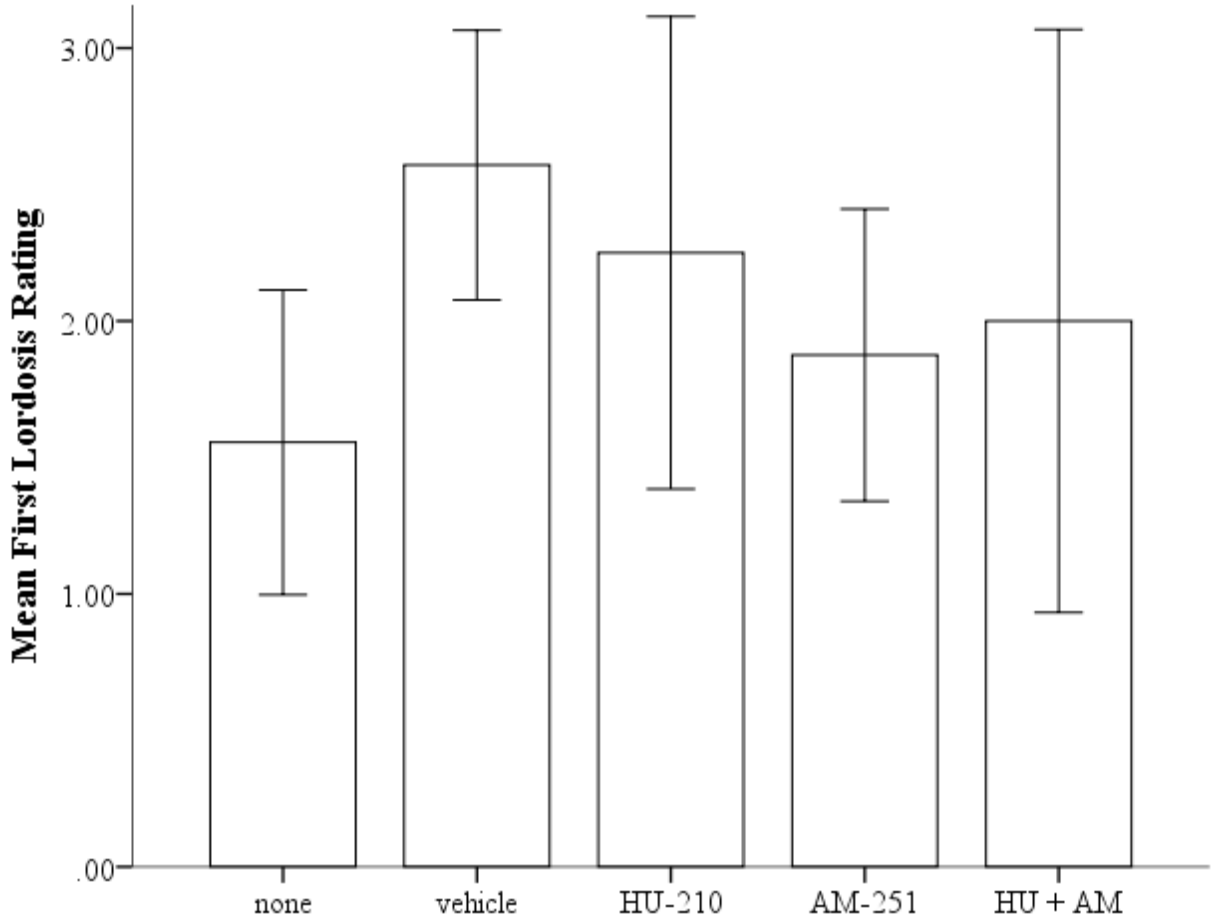

Figure 12. Mean first lordosis ratings of female rats following adolescent exposure to no injections (n = 9), vehicle (n = 7), 75 ug/kg HU-210 (n = 8), 5 mg/kg AM-251 (n = 8), or 75 ug/kg HU-210 + 5 mg/kg AM-251 (n = 7). No significant differences between drug conditions were detected, $p > .05$. Bars represent $M \pm$ 95% CI.

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