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REVIEW

Sex-Dependent Effects of Cannabis and Cannabinoids: A Translational Perspective

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Recent policy changes have led to significant increases in the use of cannabis for both medical and recreational purposes. Although men are more likely to endorse past month cannabis use and are more frequently diagnosed with Cannabis Use Disorder relative to women, a growing proportion of medical cannabis users are reported to be women. The increased popularity of cannabis for medical purposes and the narrowing gap in prevalence of use between men and women raises questions regarding sex-dependent effects related to therapeutic efficacy and negative health effects of cannabis and cannabinoids. The objective of this review is to provide a translational perspective on the sex-dependent effects of cannabis and cannabinoids by synthesizing findings from preclinical and clinical studies focused on sex comparisons of their therapeutic potential and abuse liability, two specific areas that are of significant public health relevance. Hormonal and pharmacological mechanisms that may underlie sex differences in the effects of cannabis and cannabinoids are highlighted.
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INTRODUCTION

Over the last two decades, the policy landscape of cannabis legalization for medical and recreational use has undergone significant changes in the U.S. California was the first state to legalize the use of cannabis for medical purposes in 1996. Since then, medical cannabis has been legalized in 28 states and the District of Columbia for a multitude of indications that vary from state to state (MPP (Marijuana Policy Project), 2016). These indications include chronic pain and chemotherapy-induced nausea, for which there is substantial evidence supporting the clinical utility of cannabis and cannabinoids (chemical constituents of the cannabis plant and synthetic compounds), to other indications that lack strong scientific support from randomized clinical trials, including psychopathologies (anxiety and post-traumatic stress disorder (PTSD)), neurological disorders (Tourette syndrome, Parkinson's and Huntington's Disease), and other health conditions (eg, irritable bowel syndrome) (National Academies of Sciences, 2017). As of 2016, 8 states and the District of Columbia have also legalized cannabis for recreational use (NCSL (National Conference of State Legislatures), 2017). With the growing acceptance of cannabis use for medical and recreational purposes have come widespread changes in cannabis availability, decreases in perceived risk, and expanding motivations for use (Hasin *et al*, 2015; Compton *et al*, 2017). Rates of cannabis use and problematic cannabis use (ie, cannabis use disorder; CUD) have also increased (Hasin *et al*, 2015); for example, past-month cannabis use increased from 6.2% to 8.3% between 2002 and 2015 (Center for Behavioral Health Statistics and Quality, 2016). Although epidemiological reports consistently demonstrate that men use cannabis more frequently than women (Substance Abuse and Mental Health Services Administration, 2014a), men seek treatment for cannabis use more often than women (Substance Abuse and Mental Health Services Administration, 2014b), and men are at higher risk for developing CUD (Stinson *et al*, 2006), a growing number of women report cannabis use for medical purposes (McConnell *et al*, 2014; Finseth *et al*, 2015; Ryan-Ibarra *et al*, 2015). This increase in medical cannabis use by women raises the question of whether its therapeutic efficacy differs as a function of sex. It also raises concerns about the increased susceptibility to developing CUD in women, who show an accelerated progression from initiation of cannabis use to problematic use relative to men, called the 'telescoping effect' (Hernandez-Avila *et al*, 2004; Ehlers *et al*, 2010; Schepis *et al*, 2011). Given the widespread use of cannabis for both medical and recreational purposes, and the emerging

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TABLE 1 Studies of Sex Differences in Acute Analgesic Effects of Cannabinoids

Species, strain, age	Drug	Route	Pain test [a]	Sex difference [b]	Reference
Rat, Sprague-Dawley PND 75–105	THC 11-OH-THC CP55,940	i.p.	Tail immersion, paw pressure	F > M (both tests) F > M (TI only) F > M (TI only)	Tseng and Craft (2001)
Rat, Wistar PND 40	CP55,940	s.c.	Tail Immersion	F > M	Romero *et al* (2002)
Rat, Sprague-Dawley PND 90–150	THC	i.c.v.	Tail immersion, paw pressure	Late proestrous F > M	Wakley and Craft (2011)
Rat, Sprague-Dawley PND 60–85	THC CP55,940	i.p.	Tail immersion, paw pressure	F > M F > M (PP only)	Craft *et al* (2012)
Rat, Sprague-Dawley PND 60–100	THC	i.p.	Tail immersion, paw pressure	F > M	Wakley *et al* (2014)
Rat, Sprague-Dawley PND 56	ACPA	i.m.	CFA masseter muscle: mechanical allodynia	M > F	Niu *et al* (2012)
Rat, Sprague-Dawley PND 60–90	THC	i.p. intraplantar	CFA hindpaw: mechanical allodynia, heat hyperalgesia	F > M (thermal hyperalgesia only) F > M (both tests)	Craft *et al* (2013)
Human, non-cannabis users; 18–30 years old	Nabilone (0.5, 1.0 mg)	p.o.	Heat hyperalgesia (temporal summation after tonic heat applied to forearm)	F > M (1.0 mg)	Redmond *et al* (2008)
Human, daily cannabis users; 21–50 years old	Cannabis (3.56–5.60% THC)	Smoked	Cold pressor test	M > F	Cooper and Haney (2016)

[a]Complete Freund's adjuvant (a model of persistent inflammatory pain). [b]F > M: drug was significantly more potent, or more effective (at one or more doses) in females compared to males; M > F: drug was significantly more potent, or more effective (at one or more doses) in males compared to females.

trend of more women using cannabis and cannabis-derived products, identifying potential sex differences in the therapeutic effects and risks associated with cannabis use is a public health imperative.

This review synthesizes findings from preclinical (animal) and clinical (human) sex difference studies guided by epidemiological findings that underscore two significant public health issues arising from changing legislation regarding cannabis for medical and recreational use: therapeutic potential and abuse liability. End points that correspond to cannabis's and cannabinoids' potential therapeutic utility focus primarily on neurobiological and behavioral/psychological effects rather than on other physiological End points such as cancer biology and immunology. Literature addressing the effects of adolescent cannabinoid exposure on adult behaviors and cannabinoid sensitivity is not included in this review, nor is the clinical literature on sex differences in cannabis use and brain structure, as these topics have been recently reviewed elsewhere (eg, Viveros *et al*, 2011, 2012; Ketcherside *et al*, 2016; Dow-Edwards and Silva, 2017). However, a few sex difference studies examining acute cannabinoid effects in adolescent rats (commonly defined as post-natal day (PND) 28–42, with males maturing slightly later than females (Spear, 2000)) are included, as these results suggest that sexual differentiation of cannabinoid sensitivity may occur well before adulthood for some cannabinoid effects. Pharmacological (eg, cannabinoid metabolism and cannabinoid receptor density) and hormonal mechanisms that may underlie sex differences in cannabinoid effects are briefly reviewed.

POTENTIAL THERAPEUTIC EFFECTS OF CANNABIS AND CANNABINOIDS

Preclinical Evidence for Sex-Dependent Effects

Analgesia. Given the demonstrated pain-relieving effects of cannabinoids in clinical trials (Lynch and Ware, 2015) and the commonly reported use of cannabis to reduce chronic pain (Bonn-Miller *et al*, 2014; Cuttler *et al*, 2016), most preclinical sex difference studies published to date have focused on determining whether cannabinoids are equipotent and -efficacious analgesics in females *vs* males. Details of animal and human studies are shown in Table 1. Cannabinoids act at two types of cannabinoid receptors 1 (CB1) and 2 (CB2), with many addiction-related and therapeutic effects known to be due to actions at the CB1 receptor (Pertwee, 2008). In animals, several cannabinoid receptor agonists that act at the CB1 receptor, including THC (the primary psychoactive constituent of cannabis), its primary active metabolite 11-OH-THC, and the synthetic cannabinoid CP55,940 have been shown to be more potent in female compared to male rats on tests of acute thermal and mechanical pain (Tseng and Craft, 2001; Romero *et al*, 2002; Craft *et al*, 2012). Few cannabinoids have been compared in females *vs* males using models of persistent pain, which are likely to be more predictive of cannabinoid analgesic potential in humans. In the complete Freund's adjuvant (CFA) model of paw inflammatory pain, systemically administered THC produced greater anti-allodynic or anti-hyperalgesic effects in female than in male rats, when tested 1, 3, or 7 days after the induction of pain (Craft *et al*, 2013). Local (intraplantar) THC administered to CFA-

treated female and male rats also produced greater anti-allodynic and anti-hyperalgesic effects in females, suggesting that sex differences in peripheral cannabinoid function contribute to sex differences in cannabinoid antinociception. Given the consistently greater antinociceptive effect of THC in female rats, surprisingly, THC's anti-edema effect in the CFA model was significantly greater in *males* (Craft *et al*, 2013). One other study has reported a sex difference in peripheral antinociceptive effects of a cannabinoid: using a model of orofacial pain in which CFA is injected into the masseter muscle, a CB1-selective agonist was a substantially more potent and efficacious anti-allodynic agent in male rats compared to females (Niu *et al*, 2012). At this point it is unclear whether the discrepant sex difference between the two studies of peripheral antinociception is related to the selectivity of the cannabinoid, the locus of the pain, or some other methodological difference. Additionally, we do not yet know whether sex differences in cannabinoid antinociception occur at other levels of the neuraxis, for various types of pain. One study demonstrated greater intracerebroventricular (i.c.v.) THC-induced antinociception against acute thermal pain in late proestrous female rats compared to males (Wakley and Craft, 2011), suggesting that a second locus of sex differences is supraspinal. Although intrathecal cannabinoids can be effective analgesics in male (Gu *et al*, 2011; Brownjohn and Ashton, 2012) and female (Cui *et al*, 2011) rats or mice, we are not aware of any studies comparing spinal cannabinoid antinociception between males and females of any species.

Anxiolysis. Surveys suggest that over 50% of medical cannabis users are using cannabis to reduce anxiety/stress (Web and Web, 2014; Cuttler *et al*, 2016). In animals, demonstrating anxiolytic effects of cannabinoids has been challenging, perhaps because cannabinoid effects on anxiety-like behavior are dose-dependent, with lower doses tending to be anxiolytic and higher doses tending to be anxiogenic (eg, Rubino *et al*, 2007, 2008). Furthermore, animal studies typically involve un-signaled, non-contingent cannabinoid administration by the experimenter, which is presumably stressful compared to self-administration, the mode of cannabinoid intake by humans seeking to reduce anxiety/stress. We found only one published study that included a comparison of cannabinoid effects on anxiety-like behavior in adult (PND60 or older) male *vs* female rats: on the elevated plus-maze, a moderate dose of THC (2 mg/kg) increased closed arm time and decreased stretch-attend postures (peering out to open arms from closed arms) in female but not male rats, suggesting increased anxiety-like behavior in females only (Macúchová *et al*, 2016). Several studies have examined acute cannabinoid effects on anxiety-like behavior in adolescent female *vs* male rats, with variable results: for example, the synthetic cannabinoid CP55,940 was more potent in PND44 females than males in increasing anxiety-like behavior on the elevated plus-maze (Marco *et al*, 2006), whereas 2 mg/kg THC was anxiolytic in both sexes tested on PND35 (Harte-Hargrove and Dow-Edwards, 2012). Silva *et al* (2016) recently demonstrated that pre-pubertal male and female rats were more sensitive to the anxiolytic effects of 3 mg/kg THC than post-pubertal rats, and they note that the two sexes do not reach puberty at the same time so that testing both sexes on the same PND may contribute to the inconsistency in sex differences and drug results across studies. Cannabinoid effects on fear- and anxiety-like behaviors can also be test-dependent (Simone *et al*, 2015). Thus, future research is needed to determine whether sex differences observed to date in the effects of cannabinoids on anxiety are consistent across a broader range of ages, drug doses, and behavioral tests in animals, and of course, whether there are sex differences in the effects of cannabis and cannabinoids on anxiety in humans.

Other potential therapeutic effects for which there are not yet any analyses by sex. While clinical literature suggests that cannabinoids have demonstrated therapeutic effects for the treatment of auto-immune disorders (Ware *et al*, 2010; Notcutt, 2015; Katchan *et al*, 2016; Katz *et al*, 2016), there are no sex difference studies of cannabinoid effects in animal models of auto-immune disease, despite single-sex studies demonstrating promising results (eg, Malfait *et al*, 2000; Gui *et al*, 2015; Moreno-Martet *et al*, 2015). Given that auto-immune disorders are more prevalent in women than men (Klein and Flanagan, 2016), determining whether the greater acute cannabinoid effects observed in female rodents compared to males on some relevant End points (eg, inflammatory pain) extend to models of auto-immune disease is warranted.

Adverse effects. In a review of adverse effects of medical cannabinoid use, Wang *et al*, 2008 note that nearly 97% of adverse events reported across 31 studies (including 23 randomized controlled trials) were not serious. The most common non-serious adverse effect was dizziness, followed by gastrointestinal complaints such as nausea/vomiting. Dizziness has not been assessed in animal studies. However, motoric effects are frequently assessed in animal studies of cannabinoid effect, and it is likely that dizziness contributes to altered movement. Cannabinoids such as THC, 11-OH-THC, and CP55,940 have been reported to be more potent in decreasing spontaneous locomotor activity and lever-pressing, and increasing catalepsy in adult female rats compared to males (Tseng and Craft, 2001; Craft *et al*, 2012; Weed *et al*, 2016; Wiley *et al*, 2017). Sex differences in motoric effects of the synthetic cannabinoid agonists WIN55,212-2, CP47,497, and JWH018 were smaller and not statistically significant (Wiley *et al*, 2017). Greater decreases in locomotor activity have also been reported in adolescent female compared to male rats given CP55,940 (Llorente-Berzal *et al*, 2011) and THC (Harte and Dow-Edwards, 2010); however, the sex difference with THC is not always observed and may depend on the age at which young rats are tested, as well as procedural factors (Harte and Dow-Edwards, 2010; Harte-Hargrove and Dow-Edwards, 2012; Wiley *et al*, 2007). Only one sex difference study in rats has reported a significant

THC-induced *increase* in locomotor activity, and this was observed in females only, at the lowest THC dose tested (Wiley and Burston, 2014). In mice, which are more likely than rats to show cannabinoid-induced increases in locomotor activity, females but not males showed THC-induced increases in locomotion (Wiley, 2003). Overall, rodent studies indicate that females may be more sensitive than males to the effects of cannabinoids on movement.

In regard to cannabinoid-elicited nausea, Hempel *et al* (2017) showed that THC was more potent and efficacious in female rats compared to males in decreasing saccharin consumption in a conditioned taste aversion procedure, suggesting that females are more sensitive than males to the aversive effects of THC, or females were better than males at learning the drug-taste association (which the authors concluded may be the case). Interestingly, considerable animal research suggests that cannabinoid treatment could be useful for alleviating chemotherapy-induced nausea and vomiting (Rock and Parker, 2016). Given that women may be more susceptible than men to chemotherapy-induced nausea (Hilarius *et al*, 2012), sex comparisons of cannabinoids' anti-nausea and anti-emetic effects in animal studies (and analysis of results by sex in human studies) are warranted.

Clinical Evidence of Sex-Dependent Effects

Survey/self-report data obtained from medical cannabis dispensaries. Although rates of cannabis use are consistently higher among men, data from surveys of medical cannabis users demonstrate that the gender gap is narrowing. In 2007–2008, close to 40% of chronic pain patients who sought legal qualification for medical cannabis to help manage their pain in Washington State were women (Aggarwal *et al*, 2009). In a recent survey of 1000 patients diagnosed with a rheumatic condition, nearly half of respondents who reported of medical cannabis to treat symptoms were women (Ste-Marie *et al*, 2016). These demographics are similar for other patient populations who use medical cannabis to alleviate symptoms other than pain. For instance, over 50% of Israeli cancer patients who received a permit for medical cannabis to manage appetite, weakness, nausea, and pain were women (Waissengrin *et al*, 2015). Men and women appear to use medical cannabis for different reasons. A survey of cannabis users found that of those using cannabis for medical purposes, women were more likely than men to report that they used it to help alleviate anxiety, nausea, anorexia, irritable bowel syndrome, and headache (Cuttler *et al*, 2016). Significant differences in methods of use also emerged, with more women than men reporting using edible cannabis preparations, whereas men more frequently reported using joints, blunts, vaporizers, and concentrates (Cuttler *et al*, 2016). These survey findings demonstrate that a significant proportion of the population using medical cannabis to treat specific symptoms are women, however, sex comparisons of self-reported efficacy of cannabis and cannabis-derived products are limited. One recent survey on perceived efficacy of cannabis to treat a variety of medical conditions found that men reported more relief from headache relative to women (Cuttler *et al*, 2016). Data regarding self-reported efficacy of medical cannabis for other symptoms are not typically analyzed by sex.

Clinical studies. The potential clinical efficacy of cannabis and cannabinoids for various therapeutic indications is supported by findings from randomized, double-blind, and placebo-controlled studies. These studies assessed a range of therapeutic End points as a function of treatment with various CB1 agonists, including oral THC (dronabinol), nabilone, or cannabis containing THC, and various routes of administration including smoked, vaporized, and oral. However, few of these studies speak to the possible sex-dependent nature of cannabis and cannabinoid effects, with most testing only one sex or not including sex as a factor in data analysis. This is most evident for studies examining the analgesic effects of cannabinoids in patient populations for various types of pain, including cancer pain (Noyes *et al*, 1975), chronic non-cancer pain (Issa *et al*, 2014; Ware *et al*, 2015), chronic neuropathic pain (Karst *et al*, 2003), multiple sclerosis-associated pain (Svendsen *et al*, 2004; Rog *et al*, 2007), HIV-associated neuropathy (Abrams *et al*, 2007; Ellis *et al*, 2009), postoperative pain (Buggy *et al*, 2003), chronic headache (Pini *et al*, 2012), post-traumatic or postsurgical neuropathic pain (Ware *et al*, 2010), neuropathic pain from spinal cord injury (Wilsey *et al*, 2016), and diabetic neuropathy (Wallace *et al*, 2015). This is also the case for most laboratory investigations of cannabinoid-induced analgesia in healthy populations (Greenwald and Stitzer, 2000; Naef *et al*, 2003; Wallace *et al*, 2007; Kraft *et al*, 2008; Lee *et al*, 2013b). Therapeutic effects of cannabis or cannabinoids have also been demonstrated in multiple sclerosis patients (Notcutt, 2015), and cannabinoids may be useful for the treatment of other auto-immune disorders such as rheumatoid arthritis, fibromyalgia, and Crohn's disease (Ware *et al*, 2010; Katchan *et al*, 2016; Katz *et al*, 2016). Although it is difficult to recruit a balanced sample of each sex for human trials of auto-immune disease, one wonders whether the beneficial effects that have been reported thus far are due to the prevalence of women participants in these studies. Other therapeutic indications that have been explored but do not include sex-dependent analyses include cannabinoid treatment of PTSD (Jetly *et al*, 2015), chemotherapy-induced nausea and vomiting (ie, Ungerleider *et al*, 1982), and AIDS- and HIV-induced cachexia (ie, Beal *et al*, 1997). Although sex-dependent therapeutic effects have not been assessed *a priori*, the following sections describe investigations that included exploratory assessments of drug effects as a function of sex, or that assessed sex differences in retrospective analyses of data pooled from several studies.

Analgesia. Although sex differences in cannabinoid-induced analgesia have not been assessed in a patient population, these differences have been explored in one study of healthy

participants. The analgesic and antihyperalgesic effects of nabilone (0.5 and 1.0 mg) were compared to placebo using a model of noxious, long-lasting heat stimulation in non-cannabis-using, healthy participants. In this study, nabilone did not reduce pain intensity relative to placebo. However, the high dose decreased hyperalgesia in women but not men (Table 1; Redmond *et al*, 2008). Based on these findings, the authors suggest that cannabinoids may be helpful for women suffering from chronic pain.

A recent analysis sought to determine if cannabis-using men and women differed in cannabis-induced analgesia. In this double-blind, placebo-controlled study, healthy, and daily cannabis smokers were recruited, and the effects of smoked cannabis with THC (active cannabis) *vs* placebo cannabis (cannabis with no THC) were determined using the Cold Pressor Test, a laboratory pain assay that has predictive validity for clinically effective analgesics used for chronic pain. Men and women did not differ in subjective ratings of analgesia such as bothersomeness of the painful stimulus. However, for objective measures of pain, cannabis elicited greater analgesia compared to placebo-cannabis in men relative to women: men exhibited a robust analgesic response as measured by latency to feel pain, whereas women did not. Active cannabis also increased participants' ability to tolerate the painful stimulus to a greater extent in men than in women. Despite the differences in cannabis-induced analgesia, men and women showed comparable subjective effects related to abuse liability (Table 1; Cooper and Haney, 2016). These findings demonstrated that among daily cannabis smokers, women do not appear to be sensitive to the analgesic effects of cannabis, while adverse effects associated with abuse liability are retained. These results were limited by the inclusion of a single strength of active cannabis (3.56–5.6% THC), the smoked route of administration, which is not the preferred route for the therapeutic use of cannabinoids, and the study population consisting of normal healthy controls rather than a patient population.

Chemotherapy-induced nausea. A significant proportion of randomized, placebo-controlled studies of the therapeutic utility of cannabinoids has investigated their effectiveness for chemotherapy-induced nausea and vomiting, and HIV- and cancer-related cachexia (Whiting *et al*, 2015), yet few studies have analyzed sex-dependent differences in these clinical End points. One study explored possible sex differences in the effectiveness of cannabinoids to stimulate appetite in patients with cancer-related anorexia-cachexia syndrome. Patients were randomized to receive oral THC (2.5 mg), a cannabis extract that contained cannabidiol (1 mg) and THC (2.5 mg), or placebo twice a day for 6 weeks. Self-reported measures of appetite, mood, nausea, and quality of life were compared between men and women as a function of medication condition. Women exhibited greater improvements in appetite under the medication conditions (oral THC alone and the cannabis extract that included THC and cannabidiol), yet no sex differences were observed for other End points, including ratings of nausea or quality of life measures (Strasser *et al*, 2006).

Adverse effects. As mentioned previously, adverse events associated with cannabis use in clinical trials are rarely serious, and are predominately reports of dizziness followed by gastrointestinal complaints including nausea and vomiting (Wang *et al*, 2008). There is little evidence that the type and frequency of adverse events in response to cannabinoid administration differs between men and women; however, a single laboratory study suggests that women may be more sensitive than men to cannabinoid-induced dizziness (Mathew *et al*, 2003). There have also been recent reports of intense bouts of vomiting and gastrointestinal distress in chronic cannabis smokers, called cannabinoid hyperemesis syndrome (CHS); whether these cases are recreational or medical cannabis users is not clear. A recent analysis synthesizing findings from case reports found that men were overwhelmingly more likely to be diagnosed with CHS relative to women (72.9 *vs* 27.1%; Sorenson *et al*, 2016). However, this sex discrepancy may reflect heavier cannabis use reported among men relative to women (ie, Cuttler *et al*, 2016) rather than a sex-specific sensitivity to this adverse effect of cannabis.

In summary, survey data suggest that women and men utilize medical cannabis at similar rates, although there is evidence that the symptoms for which they use medical cannabis differ, as do their methods of use (Cuttler *et al*, 2016). Recent efforts have sought to elucidate differences in efficacy of FDA-approved therapeutics between men and women to better help guide treatment decisions. However, few studies have explored whether sex-dependent therapeutic effects of cannabinoids exist. Data from surveys, laboratory-based studies, and one study in a clinical population suggest that men and women may differ in their responses to cannabinoids, yet the direction of this sex difference may depend on the End points used and the population studied (patients, non-cannabis-using healthy controls, or cannabis-using healthy controls). Important pharmacological variables that are likely to influence sex-dependent effects are the drug tested (ie, nabilone, dronabinol, cannabis, or cannabidiol), route of administration (ie, smoked, oral, or vaporized), dose administered, and duration of treatment. More comprehensive sex comparisons of the therapeutic effects of cannabis and cannabinoids in human populations are needed.

CANNABINOID ABUSE LIABILITY AND DEPENDENCE

Preclinical Evidence for Sex-Dependent Effects

Preclinical models/markers of drug abuse. Several animal studies suggest that females may be more sensitive than males to the reinforcing and discriminative effects of cannabinoids. First, female rats of two strains acquired stable drug self-administration faster, self-administered more of the synthetic cannabinoid agonist WIN55,212-2 during

the maintenance phase, took longer to extinguish responding when vehicle was substituted for WIN55,212-2, and showed greater drug- and cue-primed reinstatement than males (Fattore *et al*, 2007, 2010). Female rats also learned to discriminate THC from vehicle at a lower dose than males (1 *vs* 3 mg/kg), and acquired the discrimination significantly faster (Wiley *et al*, 2017). THC discrimination was also acquired in ~ 30% fewer sessions in female compared to male mice (Wiley *et al*, 2011). The only study comparing cannabinoid place preference/aversion between the sexes reported no significant THC effect in either sex (Hempel *et al*, 2017), although the highest dose tested, 6 mg/kg, reduced time spent on the THC-paired side by ~ 30% in male but not female rats. Thus, the limited number of animal studies published to date suggests that female rodents are more sensitive than males to the reinforcing and discriminative stimulus effects of cannabinoids.

Sex-dependent tolerance and dependence. Several studies have examined sex differences in the development of tolerance to THC in adult female rats compared to males. In two studies comparing tolerance development to the 'tetrad' of cannabinoid effects (antinociception, locomotor suppression, catalepsy, and hypothermia) in male *vs* female, adolescent *vs* adult rats (Wiley *et al*, 2007; Wiley and Burston, 2014) or in adolescents bred in-house *vs* shipped (Wiley and Evans, 2009), robust tolerance developed to all four 'tetrad' effects and no sex differences in tolerance development were observed. However, the high-dose (10 mg/kg), 10-day chronic THC regimen led to nearly complete flattening of most dose-effect curves in all groups, so the magnitude of tolerance development was difficult to distinguish among groups. Tolerance to THC's cataleptic effect did appear to be greater in adolescent females than males in one of these studies, although group sample sizes were relatively small and the sex difference was not statistically significant (Wiley and Burston, 2014). In a subsequent study using doses of THC lower than 10 mg/kg to induce tolerance, female rats developed significantly more antinociceptive tolerance than males, measured as a greater decrease in THC potency in females than males (Wakley *et al*, 2014). Similar sex differences were reported in a later tolerance study from the same lab, using the same acute pain tests (Wakley *et al*, 2015). Notably, in the latter two studies the tolerance-inducing dose of THC was sex-specific (~30–40% lower in females than males) to adjust for greater THC potency in females before tolerance induction. Thus, even when treated with lower doses than males, females developed greater tolerance than males. In contrast, another study in adult rats showed that when 10 mg/kg THC was administered daily, more rapid tolerance occurred in *males* compared to females responding on a repeated acquisition and performance operant task (Weed *et al*, 2016). Finally, another study in which a low dose of THC (2 mg/kg) was given chronically to juvenile (PND22–40) or late adolescent rats (PND41–60), no tolerance development was observed in either sex (Harte and Dow-Edwards, 2010). In the only study examining the effects of chronic THC in male *vs* female mice, several days of THC treatment resulted in significantly altered locomotor activity only in females: tolerance developed to the stimulant effects of a low THC dose (1 mg/kg) and sensitization developed to the stimulant effects of a high dose (30 mg/kg; Wiley, 2003). In summary, the few studies that have examined sex differences in the development of tolerance to cannabinoids suggest that females develop tolerance more readily than males to the antinociceptive effects of THC, but there are very few studies of tolerance development to cannabinoid effects other than antinociception. Additionally, cannabinoid tolerance is known to be dose-dependent and to develop at different rates for different effects (Gonzalez *et al*, 2005), so in the future it will be important to conduct sex comparisons of cannabinoid tolerance to a variety of effects using a variety of dose ranges and dosing frequencies.

In regard to cannabinoid dependence, several studies report sex differences in animals. In one study, a high dose of THC (30 mg/kg) was administered twice-daily for 6.5 days to adult female and male rats, and then withdrawal was precipitated with the CB1 antagonist rimonabant (10 mg/kg). The only significant sex difference among the many withdrawal symptoms assessed was that females showed more retropulsion (walking backwards) than males did (Marusich *et al*, 2014). A second study conducted in adult rats also used rimonabant (1.0 mg/kg)-precipitated withdrawal: after 40 days of 10 mg/kg/day THC treatment, females showed more disrupted performance (increased errors, decreased response rate) than males in the performance component of a repeated acquisition, 3-response sequence task (Weed *et al*, 2016). A third study examined spontaneous withdrawal symptoms after termination of chronic THC treatment of adolescent rats. Rats were given either 2, 7.5, or 15 mg/kg daily from PND35–41, and then tested on the elevated plus-maze on PND 42, 44, and 56. Early in withdrawal, males that had been treated with the higher doses of THC showed evidence of decreased anxiety whereas females showed evidence of increased anxiety (Harte-Hargrove and Dow-Edwards, 2012). Overall, limited evidence suggests that female rats may develop greater tolerance and greater cannabinoid dependence than males, although results may be end point-specific, and may also depend on the dose and duration of the chronic cannabinoid treatment.

Clinical Evidence for Sex-Dependent Effects

Epidemiology studies have consistently demonstrated that men have a higher rate of problematic cannabis use marked by increased chronicity of use (Preston, 2006), longer episodes of CUD, and greater cannabis use as measured by number of cannabis cigarettes smoked per day (Khan *et al*, 2013). Additionally, prevalence rates of CUD are higher among males relative to females during adolescence (Hayatbakhsh *et al*, 2009, and see Farmer *et al*, 2015: lifetime prevalence rates of DSM-IV cannabis abuse or dependence from 16 to 30 years of age was 22.5% of the male

population *vs* 16.4% of the female population), and in adulthood (Haberstick *et al*, 2014: lifetime prevalence of DSM-IV cannabis dependence diagnosis in men was 10.9 *vs* 6.1% in women). The increase in risk observed among men relative to women may not be due to biological underpinnings, but rather due to social factors that limit exposure to cannabis and decrease the likelihood of cannabis initiation and use in females, including greater perception of risk, decreased cannabis use among peers, and greater childcare responsibilities. However, epidemiological studies are showing that sex differences in cannabis use are shrinking, suggesting that societal constraints on cannabis use among females are abating. An analysis of data from the National Youth Risk Behavior Survey, a large, biennial, school-based survey, demonstrated that while boys consistently show higher prevalence rates than girls for lifetime and current cannabis use, the gap decreased over a 14-year period between 1999 and 2013, with a difference of 7.6 percentage points between boys and girls in 1999 for prevalence of lifetime cannabis use *vs* a difference of 2.9 percentage points in 2013 (Johnson *et al*, 2015); this shrinking gender gap has also been observed at the national level among adolescent and adult respondents (Substance Abuse and Mental Health Services Administration, 2014a,b,). Furthermore, findings from the annual National Survey on Drug Use and Health (NSDUH) from 2002 through 2014 show that past-month cannabis use increased among pregnant women from 2.37% to 3.85%. Among non-pregnant women, past month use increased by close to 4% in women 18–25 years of age, and close to 3% in women overall (18–44 years of age; Brown *et al*, 2017). Given the narrowing gap in cannabis use among adolescent and adult males *vs* females, identifying sex-dependent effects of cannabis associated with the development of CUD and probing the biological basis for these differences is a public health imperative.

Abuse-related subjective effects. Sex differences in response to the acute effects associated with abuse liability of cannabis and cannabinoids have been examined using double-blind, placebo-controlled laboratory procedures. End points that have been assessed include subjective drug ratings related to positive drug effects and cue-induced craving. Similar to studies investigating sex differences in the therapeutic effects of cannabinoids, sex differences related to abuse liability were mostly analyzed as an exploratory aim or by pooling data from several studies in order to obtain sufficient statistical power to detect a significant difference between men and women. A recent laboratory study compared the discriminative stimulus and subjective effects of oral THC in male and female non-treatment-seeking cannabis users. While men and women did not appear to be differentially sensitive to the discriminative stimulus effects of oral THC, a dose-dependent sex difference emerged with respect to THC's subjective effects that are indicative of abuse liability. Women exhibited greater sensitivity to the lowest THC dose tested (5 mg) and reported higher ratings related to abuse liability, including ratings of drug liking, desire to take the dose again, and good drug effect. In contrast, men reported higher subjective ratings of drug liking than women for the higher THC dose (15 mg). The sample used for this study consisted of men and women who did not differ in cannabis use, with average use reported to be 4.5 days per week and about 3 cannabis cigarettes/day (Fogel *et al*, 2017). An earlier study investigating the effects of higher oral THC doses (20 and 40 mg) in male and female cannabis smokers failed to find sex-dependent effects with regard to subjective ratings. However, differences emerged when assessing these effects in non-cannabis users: men were more sensitive than women to the intoxicating effects (ie, ratings of 'High') of oral THC (2.5–10 mg) (Haney, 2007).

Studies with smoked cannabis reveal mixed effects. One study that investigated the subjective ratings of smoked cannabis included participants whose smoking frequency ranged from once per month to three times per week. Women were reported to have a slower latency to detect cannabis's psychoactive effects and a shorter duration of its effects compared to men, suggesting that men were more sensitive to cannabis's subjective effects (Penetar *et al*, 2005). This study did not specify if men and women differed in current cannabis use. Given the wide range of cannabis use frequency among participants in this study (one time per month to three times a week), differences in current cannabis use between men and women may have influenced the findings. A second study assessing sex-dependent subjective effects of smoked cannabis matched men and women according to current cannabis use (Cooper and Haney, 2014). Among daily cannabis smokers, females were more sensitive than males to subjective effects related to abuse liability, including ratings of drug liking and willingness to take the drug again. However, males and females did not differ in ratings of intoxication ('High'). These findings indicate that under double-blind, placebo-controlled procedures, female daily cannabis smokers are more sensitive than their male counterparts to the subjective experience that is associated with cannabis abuse liability. Although subjective effects provide a measure of abuse potential, drug self-administration is the best measure of abuse liability because it best reflects drug-taking in the natural ecology. To date, no studies have assessed possible differences in cannabis or cannabinoid self-administration between men and women.

Together, these findings suggest that sex differences in THC-induced subjective effects depend on dose, route of administration (oral *vs* smoked), and population (cannabis users *vs* non-users). These findings are directly relevant to the population using cannabis for medical indications, which varies in previous and current exposure to cannabis and cannabis-derived products. In addition, these findings highlight the differences across studies that can emerge as a function of route of administration, a variable that should be addressed in future studies, particularly given the increasing popularity of edibles (Cuttler *et al*, 2016).

Cue-induced craving. Cannabis craving as a measure of addiction severity has been studied as a function of participant sex. Men and women who smoked similar amounts of

cannabis were presented with tactile, visual, and olfactory cannabis-related cues and were asked to rate their craving for, urge, and desire to smoke cannabis. No sex differences were detected in baseline craving. While craving for, urge, and desire to smoke cannabis increased in response to the cues, differences between men and women were not detected (Lundahl and Johanson, 2011). A second study probed the potential utility of oral THC (10 and 20 mg) for treating CUD in daily cannabis smoking men and women by determining its effects on cannabis cue-elicited craving (Lundahl and Greenwald, 2015). In this study, cannabis cues increased ratings associated with compulsivity, or inability to control cannabis use, anxiety, and urge to smoke cannabis relative to a neutral cue. Oral THC decreased cue-elicited craving and anxiety, an effect that did not differ between men and women. However, sex-dependent effects were observed for ratings of compulsivity, with women exhibiting significant decreases on this measure after receiving either dose of oral THC, whereas oral THC failed to attenuate this rating in men (Lundahl and Greenwald, 2015). These data suggest that THC differentially attenuates aspects of subjective responses related to continued cannabis use between men and women, and have implications for the use of THC as a potential pharmacotherapy for CUD.

Withdrawal. Although women and men report similar patterns of cannabis use, women seeking treatment for problematic use score higher on a measure of severity of drug dependence (Copeland *et al*, 2001). One indication of severity of drug dependence is the presence and severity of withdrawal symptoms after cessation of cannabis use. Earlier studies examining withdrawal symptoms among non-treatment-seeking male and female cannabis users found that men and women reported different symptoms: women reported nausea, whereas men endorsed goose-bumps and cannabis craving (Agrawal *et al*, 2008; Copersino *et al*, 2010). A later survey similarly assessed sex differences in self-reported withdrawal symptoms and also found that more women reported nausea and anxiety, whereas men more frequently reported sleep disruptions (Cuttler *et al*, 2016). A recent study sought to study incidence and severity of withdrawal symptoms as a function of sex during a cannabis quit attempt in a population of treatment-seeking cannabis users (Herrmann *et al*, 2015). This study carefully controlled for cannabis dependence inclusion criteria, excluded other drug dependencies (eg, alcohol) and characterized participants based on amount of cannabis use in the previous 90 days, ensuring that men and women were matched for current cannabis use. Women reported more withdrawal symptoms relative to men and had significantly higher scores on symptoms related to mood (including increased restlessness, irritability) and gastrointestinal symptoms (nausea and stomach pain). Women also reported greater symptom severity related to mood symptoms. A later study of treatment-seeking cannabis users similarly found that women endorsed more withdrawal symptoms and negative impact of withdrawal symptoms relative to men who were matched for past-month cannabis use; sex differences in the severity of specific symptoms were found for hot flashes, headache, mood swings, irritability, gastrointestinal symptoms, and sleep disturbances (Sherman *et al*, 2017). While men and women had similar patterns of cannabis use, women were more likely to have psychiatric diagnoses and reported lower quality of life compared to men, which may have contributed to differences in self-reported incidence and severity of withdrawal symptoms.

Clinical trials for CUD. Clinical trials investigating potential pharmacotherapies to treat CUD do not typically assess outcomes according to sex (recent studies include Allsop *et al*, 2014; Levin *et al*, 2016). However, recent studies investigated the potential for sex-dependent treatment effects with the selective serotonin receptor inhibitor and partial 5-HT1A agonists, vilazodone and buspirone, for cannabis dependence (McRae-Clark *et al*, 2015, 2016). While these pharmacotherapies were not effective in reducing cannabis use relative to placebo for either sex, the authors found that women had worse outcomes relative to men in relation to cannabis craving and in difficulty achieving abstinence. These findings agree with earlier reports demonstrating that women report greater withdrawal symptom severity relative to men.

Given that severity of withdrawal is hypothesized to contribute to ongoing use and relapse to use in a treatment setting (Budney *et al*, 2008; Haney *et al*, 2013), these studies suggest that women may experience greater difficulty than men achieving and maintaining abstinence from cannabis use. Understanding how men and women differ in regard to incidence and severity of withdrawal symptoms during abstinence can help to guide research into sex-specific pharmacotherapies for cannabis dependence, increasing treatment success for both men and women. A limitation of these studies is that assessments were based on retrospective self-report measures, requiring participants to recall from memory the incidence and severity of their withdrawal symptoms. In addition, it is not clear whether cannabis exposure immediately prior to the quit attempt was comparable among men and women. Future studies should examine withdrawal symptoms according to both self-report and clinician-based assessments in real time, optimally after participants have been exposed to a known quantity and strength of cannabis. Accurate assessment of pharmacotherapies to target sex-specific withdrawal symptoms can be best examined under these circumstances.

Other End points related to cannabis addiction severity. Differences among men and women in neural correlates associated with severity of cannabis addiction were recently explored in a positron emission tomography (PET) study that measured brain glucose metabolism utilizing the radiotracer [^{18}F]deoxyglucose. Differences in brain glucose metabolism were compared in healthy, non-cannabis-using controls relative to daily cannabis users who met DSM-IV criteria for cannabis abuse or dependence in response to placebo administration and a methylphenidate challenge

(Wiers *et al*, 2016). Under placebo conditions, cannabis users exhibited lower glucose metabolism relative to healthy controls in the frontal cortex and anterior cingulate. In response to the methylphenidate challenge, cannabis users exhibited blunted regional metabolism compared to healthy controls; moreover, regional increases in glucose metabolism in response to methylphenidate were negatively correlated with addiction severity. In regard to sex differences, the differences between healthy controls and cannabis users in both placebo and methylphenidate-induced changes in metabolism were driven by the female cannabis users. That is, female cannabis users exhibited lower glucose metabolism after placebo administration relative to female healthy controls, an effect that was not observed when comparing cannabis-using men to non-cannabis-using men. Furthermore, increases in methylphenidate-induced glucose metabolism were apparent in the female healthy controls but not in the female cannabis users. The authors conclude that women who use cannabis may be more susceptible than men to the adverse neurobiological effects of cannabis, including negative emotionality and severity of addiction (Wiers *et al*, 2016).

The above findings demonstrate that men and women differ in End points associated with cannabis's abuse liability, withdrawal from cannabis, and neurobiological End points associated with cannabis addiction severity. These effects were assessed in response to smoked cannabis and oral THC in populations of non-cannabis users, light users, and heavy users. Multiple studies report that cannabis-dependent women experience more difficulty than cannabis-dependent men with withdrawal symptoms, a finding that is consistent with results showing enhanced negative effects of cannabis in women who are daily smokers, including increases in subjective effects that reflect abuse liability, and deficits in neurobiological markers associated with addiction severity. Given the differences observed across studies as a function of cannabis exposure, these findings highlight the need to match women and men according to current cannabis use in order to control for the potential effects of tolerance that may alter adverse effects of cannabis, withdrawal, cue-induced craving, and investigation of neural markers of addiction severity. A significant gap in our knowledge regarding differences between men and women in relation to CUD is self-administration during non-abstinent and abstinent conditions, and a systematic assessment of withdrawal symptoms as a function of sex while controlling for cannabis exposure preceding cessation of use, as well as withdrawal assessments that include clinician-based ratings and objective measures.

MECHANISMS UNDERLYING SEX-DEPENDENT EFFECTS OF CANNABINOIDS

Gonadal Hormones

Sexual differentiation of cannabinoid sensitivity could arise from the direct influence of sex chromosomes (eg, genes on the X *vs* Y chromosomes that control cannabinoid receptor expression), or indirectly from organizational (early developmental) or activational (pubertal to adult) effects of gonadal hormones on the endocannabinoid system (Becker *et al*, 2005). Direct sex chromosome influence has not yet been investigated for any cannabinoid effect. Sex differences in behavioral effects of cannabinoids have been observed on some End points in adolescent rats (PND40: Romero *et al*, 2002; PND41: Harte and Dow-Edwards, 2010) and even in pre-pubertal rats (PND28–29: Llorente-Berzal *et al*, 2011; but see Borcel *et al*, 2004 and Wiley *et al*, 2007), suggesting that sex differences begin to develop early in life. No studies have been conducted to determine whether neonatal gonadectomy, a method typically undertaken to examine organizational effects of gonadal hormones, abolishes sex differences in behavioral effects of cannabinoids. Studies of activational hormone effects (eg, gonadectomy with or without hormone replacement in adulthood) suggest that testosterone can dampen gonadectomized male and female rats' sensitivity to the motor-impairing effects of systemic THC, while enhancing females' but not males' sensitivity to THC's antinociceptive effect against acute pain; conversely, estradiol can enhance female and male rats' sensitivity to THC's antinociceptive effect without significantly altering their sensitivity to THC's motor-impairing effects, although the estradiol effect is not consistent across all studies (Craft and Leitl, 2008; Wakley *et al*, 2014, 2015; Craft *et al*, 2017). Progesterone alone has been reported to decrease systemic THC's antinociceptive potency (Wakley *et al*, 2015), but it did not significantly affect i.c.v. THC-induced antinociception (Wakley *et al*, 2014) in female rats ovariectomized as adults. In gonadally intact female rats, acute antinociceptive sensitivity to THC also has been shown to fluctuate across the estrous cycle, with peak sensitivity to THC at the time of ovulation or shortly thereafter (Craft and Leitl, 2008; Wakley *et al*, 2011). Endocannabinoids such as anandamide and 2-arachidonyl glycerol (2-AG) have also been shown to fluctuate across the rat estrous cycle in some brain areas, with the greatest changes around the time of ovulation or shortly thereafter (Bradshaw *et al*, 2006). In contrast to results obtained with systemically administered THC and tests of acute pain, using a model of masseter muscle inflammatory pain, Niu *et al* (2012) showed that adult male rats' antinociceptive sensitivity to a peripherally (i.m.) administered CB1 receptor-selective agonist was decreased by castration, and this appeared to be due to a testosterone-dependent decrease in trigeminal ganglia CB1 receptor density. In contrast to studies of acute cannabinoid antinociception, gonadectomy and hormone replacement did not significantly alter the development of tolerance to THC-induced antinociception (Wakley *et al*, 2015), although flattening of the post-chronic THC dose-effect curves in this study limited the precision of tolerance quantification. In a study of cannabinoid dependence (rimonabant-precipitated withdrawal) in gonadectomized and hormone-replaced adult male and female rats, it was concluded that estradiol and progesterone tended to promote dependence in females

while testosterone tended to blunt dependence in males, although hormone effects tended to be subtle and inconsistent across End points (Marusich *et al*, 2015).

Several other behavioral effects of cannabinoids have been shown to be modulated by gonadal hormones in adulthood. For example, adult ovariectomy decreased female rats' self-administration of WIN55,212-2 (Fattore *et al*, 2007), and self-administration was restored to that of gonadally intact females by estradiol replacement (Fattore *et al*, 2010). In several other studies female rats were ovariectomized peripubertally (PND30) and then studied as adults; in these cases, early ovariectomy decreased females' later sensitivity to THC's disruptive effects on a repeated acquisition operant task (Winsauer *et al*, 2011; Winsauer and Sutton, 2014), and decreased the slope of a THC discrimination curve (ie, THC's efficacy appeared to be lower in ovariectomized females compared to gonadally intact females: Winsauer *et al*, 2012). It is not yet known whether modulation of THC's cognitive effects by ovariectomy is due to estradiol, progesterone, or both. In addition to these presumably genomic (slower) effects of gonadal hormones, rapid membrane estrogen receptor (ER)-mediated effects on the cannabinoid system have been documented. For example, WIN55,212-2-induced hyperphagia in ovariectomized guinea pigs is attenuated by estradiol acting at both nuclear ER-alpha and membrane ER in hypothalamic neurons (Washburn *et al*, 2013). In summary, there is growing evidence that sexual differentiation of cannabinoid drug sensitivity is due at least in part to activational effects of gonadal steroid hormones. Given that peri-pubertal ovariectomy of female rats can also modulate cannabinoid sensitivity (Winsauer *et al*, 2011; Winsauer *et al*, 2012; Winsauer and Sutton, 2014), and administration of the same hormone to adult male and female gonadectomized rats does not change cannabinoid sensitivity in the same way (Craft *et al*, 2017), it is likely that organizational gonadal hormone milieu and possibly sex chromosomes also contribute to sexual differentiation of cannabinoid sensitivity.

In humans, there is also evidence that gonadal hormones influence the cannabinoid system. For example, endocannabinoid tone has been shown to vary across the menstrual cycle. Peak plasma anandamide levels are observed during ovulation and are positively associated with estradiol, luteinizing hormone, and follicle stimulating hormone suggesting that these hormones may play a role in regulating anandamide levels (El-Talatini *et al*, 2010). Interestingly, low levels of anandamide and high levels of fatty acid amide hydrolase, the enzyme that degrades anandamide, are detected after ovulation during the luteal phase in the peripheral lymphocytes of normally cycling, non-cannabis smoking females (Lassarin *et al*, 2004). Given the evidence in the preclinical literature that changes in endocannabinoid tone across the female's cycle are also mediated by changes in cannabinoid receptor density, affinity and function (eg, Rodriguez de Fonseca *et al*, 1994), menstrual phase-related changes in endocannabinoid tone should influence the behavioral effects of cannabis and cannabinoids. However, very few studies address this issue in the human literature assessing therapeutic or abuse-related effects of cannabinoids. Most studies examining effects of cannabinoids in women either did not control for menstrual cycle phase or only assessed effects during the mid-follicular phase when circulating reproductive hormones (progesterone, estradiol, FSH, and LH) are low (Normandin *et al*, 2015; Weirs *et al*, 2016). An early study investigated the effect of menstrual cycle phase on self-reported measures of mood, behavioral changes, and cannabis smoking in daily female cannabis smokers using daily diary entries; phase was estimated based on daily diary reports that tracked the onset of menses. No changes in cannabis use were detected as a function of menstrual cycle phase (follicular, periovulatory, luteal, premenstrual, and menstrual phases) over three cycles (Griffin *et al*, 1986).

Pharmacokinetics

In rats, sex differences in the metabolism of THC contribute to sex differences observed in behavioral effects of THC. After acute THC injection, females produce more of the major active metabolite 11-OH-THC than males do (Tseng *et al*, 2004; Wiley and Burston, 2014; Britch *et al*, 2017). This sex difference in 11-OH-THC production is modulated by gonadal hormones (Craft *et al*, 2017), observed in adolescent as well as adult rats, and increases in magnitude with repeated THC treatment (Wiley and Burston, 2014). Greater production of 11-OH-THC by females appears to be due to different cytochrome P450 enzymes produced by the female *vs* male rat liver, such that females metabolize THC primarily to 11-OH-THC and the major inactive metabolite THC-COOH, whereas males produce less 11-OH-THC but a wider array of (mostly inactive) metabolites (Narimatsu *et al*, 1991, 1992). Blocking the hydroxylation of THC to 11-OH-THC by pre-treating rats with a non-selective cytochrome P450 inhibitor decreases THC-induced antinociception and catalepsy in female but not male rats, essentially eliminating the sex difference in these THC effects (Tseng and Craft, 2004). Sex differences in liver enzymes involved in THC metabolism have also been documented in mice (Watanabe *et al*, 1992). In humans, an early study reported no significant sex differences in THC metabolism after oral or intravenous (i.v.) THC administration (Wall *et al*, 1983). However, a later study with oral THC (that had double the sample size of the earlier study) demonstrated significantly greater C_{max}, shorter t_{max}, and greater AUC in women than in men, particularly for 11-OH-THC (Nadulski *et al*, 2005). Although THC dosing was not adjusted by body weight in this study, correction for sex differences in body weight did not eliminate the sex difference in metabolic indices, and blood levels of cannabinoids were also not correlated with BMI (Nadulski *et al*, 2005). No studies to date have investigated differences between men and women in THC pharmacokinetics after smoked cannabis administration. Thus, it would appear that sex differences in THC metabolism occur across species, although the differences may be

larger in rats than in humans. It is not known to what extent sex differences in metabolism underlie sex differences in behavioral effects of cannabinoids besides THC. In addition, sex differences in THC's effects have been observed when THC is administered i.c.v. (Wakley *et al*, 2011) and locally into the rat hindpaw (Craft *et al*, 2013), routes that preclude hepatic THC metabolism, suggesting that sex differences in cannabinoid pharmacodynamics must also be important contributors to sex differences in behavioral effects of cannabinoids.

Pharmacodynamics

Preclinical studies demonstrate that the antinociceptive effects of THC and CP55,940 are CB1 and/or CB2 receptor-mediated in rats of both sexes (Craft *et al*, 2012, 2013). Systemically administered rimonabant, a CB1 receptor-selective antagonist, was significantly more potent in females than males in blocking the acute antinociceptive effects of systemic THC and CP55,940 (Craft *et al*, 2012), suggesting that rimonabant has greater affinity for the CB1 receptor in females than males. However, when given supraspinally or spinally, rimonabant was more similar in its potency in male and female rats to block the acute antinociceptive effects of systemic THC (Tseng and Craft, 2004), suggesting that the greater potency of systemically administered rimonabant in females compared to males may reflect a pharmacokinetic sex difference. THC's peripheral antinociceptive effect (intraplantar injection of antagonist plus THC) was similarly antagonized in both sexes by a low dose of rimonabant; however, THC's local antinociceptive effect was also blocked by a low dose of the CB2 receptor-selective antagonist SR144528 in females but not males (Craft *et al*, 2013). Two other studies report limited antagonism of THC's behavioral effects by SR144528 in females but not males (Craft *et al*, 2012; Wiley *et al*, 2017), suggesting that females may have more CB2 receptors than males do, or that THC has greater affinity for or efficacy at CB2 receptors in females than males.

In rats, sex differences in CB1 receptor mRNA or receptor protein density or binding affinity have been reported in prefrontal cortex (male > female: Castelli *et al*, 2014), amygdala (male > female: Castelli *et al*, 2014; female > male: Riebe *et al*, 2010), cerebellum (female > male: Xing *et al*, 2011), hippocampus (male > female: Reich *et al*, 2009; Weed *et al*, 2016), hypothalamus (male > female: Reibe *et al*, 2010), brainstem (female > male: Xing *et al*, 2011), mesencephalon (male > female: Rodriguez de Fonseca *et al*, 1994), and anterior pituitary (male > female: Gonzalez *et al*, 2000). However, sex differences are not entirely consistent across studies for the same brain area, and some studies report no sex differences in some of these same brain areas (eg, Rodriguez de Fonseca *et al*, 1994; Riebe *et al*, 2010; Casteels *et al*, 2014; Castelli *et al*, 2014), suggesting that methodological details such as how rats are handled (ie, stress), what estrous stage females are in, and various biochemical parameters of the mRNA or protein assay may be crucial. Only one study in CB1 receptor knockout animals has been conducted. Male but not female CB1 receptor knockout mice showed greater anxiety-like behavior than wild-type controls, despite the fact that rimonabant increased anxiety-like behavior in knockouts of both sexes (Bowers and Ressler, 2016), further suggesting that CB1 receptors may function differently in the two sexes. Finally, a comprehensive study of sex differences in brain endocannabinoid content in the rat revealed sex differences in multiple brain areas. For example, females had greater 2-AG levels than males in hypothalamus and pituitary, and lower 2-AG levels in cerebellum, and estrous cycle-dependent sex differences were additionally found in hippocampus (anandamide) and midbrain (2-AG and anandamide; Bradshaw *et al*, 2006).

In addition to studies demonstrating sex differences in CB1 receptor number or affinity, some studies report sex differences in CB1 receptor-mediated signal transduction (typically assessed via GTPγS assay) in addition to, or in the absence of sex differences in CB1 receptor density or affinity (eg, Castelli *et al*, 2014), and it has been suggested that sex differences in cannabinoid receptor density *vs* affinity *vs* signal transduction may functionally compensate for each other (for review, see Rubino and Parolaro, 2011).

Beyond sex differences in cannabinoid receptor pharmacology, there is growing preclinical evidence that gonadal hormones, particularly estradiol, may influence cannabinoid receptor density or function. Estrous cycle and/or ovariectomy plus hormone replacement have been reported to modulate CB1 receptor density or affinity (Rodriguez de Fonseca *et al*, 1994; Riebe *et al*, 2010; Castelli *et al*, 2014; Wakley *et al*, 2014) as well as endocannabinoid content (Bradshaw *et al*, 2006) in various brain areas. Furthermore, estradiol-induced decreases in CB1 receptor signaling observed in the cortex and hippocampus of ovariectomized female rats were observed when estradiol was given acutely but not when it was given chronically (Mize and Alper, 2000), a potentially important finding given that many behavioral studies of estradiol effect involve chronic estradiol treatment. Testosterone has also been shown to modulate CB1 receptor density in brain (Rodriguez de Fonseca *et al*, 1994) and in trigeminal ganglia (Niu *et al*, 2012; Lee *et al*, 2013a) of male rats.

Various studies have also documented sex differences in, or gonadal hormone modulation of, the effects of stress or chronic THC exposure (in adolescence or adulthood) on brain cannabinoid receptor pharmacology. For example, chronic THC exposure during adolescence or adulthood decreased CB1 receptor density similarly in all groups of rats, but CB1 receptor desensitization was greater in adolescent females than males in multiple brain areas (Burston *et al*, 2010). Chronic adolescent THC exposure also increased CB1 receptor mRNA and decreased GTPγS signaling in the amygdala of female but not male rats (Silva *et al*, 2016; but see Mateos *et al*, 2011), increased hippocampal CB1 receptor density in adult female rats but decreased it in males (Weed *et al*, 2016), and decreased

CREB phosphorylation in the prefrontal cortex and hippocampus (and increased CREB phosphorylation in nucleus accumbens) of adult female but not male rats (Rubino *et al*, 2008). Chronic stress has also been reported to decrease hippocampal CB1 receptor density in male rats while increasing it in females (Reich *et al*, 2009). In summary, although there are some inconsistencies across studies, sex differences in cannabinoid receptor pharmacology have been demonstrated under conditions of no cannabinoid exposure, and acute and chronic cannabinoid exposure, suggesting that sex differences in behavioral effects of cannabinoids are due at least in part to sex differences in cannabinoid receptor pharmacology.

Several clinical studies have compared cannabinoid receptor pharmacology between men and women. Onaivi *et al* (1999) reported greater cannabinoid receptor density in healthy, non-cannabis using women compared to men, using blood samples of leukocytes (cells known to be involved in inflammatory pain responses), an intriguing finding that remains to be confirmed. PET studies with the radiotracer [^{11}C]OMAR developed for imaging of the CB1 receptor recently reported findings suggesting that among healthy, non-cannabis using participants who were 18–65 years of age, women ($N=5$) tested during their follicular phase had higher CB1 availability relative to men ($N=5$; Normandin *et al*, 2015). This effect was also observed in a study investigating [^{11}C]OMAR volume of distribution in both non-cannabis using healthy controls and participants diagnosed with PTSD. Female participants exhibited greater CB1 receptor availability relative to men in both the healthy control and PTSD groups (Neumeister *et al*, 2013). These findings are in contrast to studies using a different CB1 receptor radiotracer, [^{18}F]MK-9470 in healthy non-cannabis using participants, which suggest greater density in men than in women, for example in the cortico-striato-thalamic circuit (Van Laere *et al*, 2008). Interestingly, in this study, density increased with age among women specifically in the limbic system, basal ganglia, and lateral temporal cortex, an effect that was not observed in men. While recent PET studies with [^{11}C]OMAR suggest that cannabis use is associated with CB1 receptor downregulation in men (D'Souza *et al*, 2016), to date, no studies have assessed CB1 receptor radiotracer binding in cannabis users as a function of sex. The greater CB1 receptor availability in women observed in some of these studies suggests that relative to men, women may be more sensitive to both the therapeutic and adverse effects of CB1 receptor agonists, like THC in cannabis.

SYNTHESIS OF FINDINGS FROM PRECLINICAL AND CLINICAL STUDIES

Although cannabis is the most frequently used illicit drug (Center for Behavioral Health Statistics and Quality, 2016), scientific investigations exploring how men and women may differ in response to its effects are just beginning. Few clinical studies have investigated sex differences in the acute and long-term effects of cannabis and cannabinoids in regard to therapeutic and abuse-related effects. Data that do exist are largely from observational and retrospective approaches rather than *a priori* investigation, which can have a significant impact on the quality of the findings, as it is difficult to control for variables including drug history, current drug use, and demographics that may influence sex-dependent effects in these types of study designs. For studies on the therapeutic effects of cannabis and cannabinoids, differences in severity of disease state and concomitant medications between men and women are factors that should be taken into account. Furthermore, studies investigating these effects (1) across a range of routes of administration (ie, smoked, vaporized, and oral), and (2) in non-cannabis using populations are important in guiding our understanding and in generalizability of the therapeutic utility of cannabis and cannabinoids. Ensuring that men and women are matched according to severity of dependence and frequency of current cannabis use is critical for cannabis abuse liability and dependence studies. Despite the paucity of data from clinical studies, growing evidence from preclinical *a priori* investigations into sex-dependent effects of cannabinoids have provided a foundation from which predictions can be made regarding sex-dependent effects in humans. Findings from preclinical studies provide insight into variables that significantly influence sex differences, such as age of first exposure, end point of interest, hormonal status (ie, estrous cycle phase), and degree of exposure (dose, frequency and duration of exposure).

While some of the evidence from clinical studies regarding differences between men and women does not directly correspond to preclinical findings, commonalities arise that strongly support the value of translational investigations of these effects. In agreement with preclinical studies demonstrating that females are more sensitive than males to the antinociceptive effects of acute cannabinoid administration, healthy, non-cannabis using women, but not men, were shown to be sensitive to the anti-hyperalgesic effect of nabilone (Redmond *et al*, 2008). In contrast, a laboratory-based study with healthy, daily cannabis users found that men were more sensitive to the cannabis-elicited analgesia relative to women (Cooper and Haney, 2016), findings that may be considered to align with preclinical data demonstrating greater development of tolerance to cannabinoid-induced antinociception in females than males (Wakley *et al*, 2014, 2015). That is, after repeated exposure to THC, THC may be a more potent antinociceptive agent in male compared to female rats. Results from clinical studies examining sex differences of the negative effects of cannabis related to its abuse liability are also mixed, although the aggregate data suggest that withdrawal severity is greater in women relative to men (Agrawal *et al*, 2008; Copersino *et al*, 2010; Herrmann *et al*, 2015; Sherman *et al*, 2017). These observations agree with preclinical findings that female rats show greater withdrawal effects under spontaneous and precipitated withdrawal, such as more disruption in

performance on cognitive tasks (Weed *et al*, 2016), more anxiety-like behavior (Harte-Hargrove and Dow-Edwards, 2012), and more retropulsion (Marusich *et al*, 2014) relative to males. These findings have significant implications for taking sex into consideration when addressing treatment for CUD.

Divergent findings between laboratory animal and human studies may be due to multiple methodological differences. For instance, the route of cannabis and cannabinoid administration in human studies is restricted to smoking, vapor inhalation, or oral, whereas animal studies primarily employ local, i.c.v., i.v., or i.p. cannabinoid administration. Another significant discrepancy between rodent and human studies is the degree of control that animal studies afford regarding history and current cannabinoid exposure. Although matching men and women according to current and history of cannabis use can be achieved with regard to smoking frequency and amount smoked per day, the amount of THC and other cannabinoids in cannabis can vary considerably between participants. As such, total THC exposure can differ regardless of matching for frequency of use. Animal studies regarding sex-dependent tolerance to CB1 receptor agonists suggest that adjusting chronic THC exposure as a function of initial THC sensitivity between males and females may be important to 'equalize exposure' between the sexes (Wakley *et al*, 2014, 2015), but this cannot be done in human studies of established cannabis users. One way to circumvent this issue when assessing cannabis- and cannabinoid-induced effects is to test the end point in question only after exposing the study population to known quantities of cannabis of a particular strength (ie, THC concentration) for some period of time. This strategy would limit differences in current cannabis exposure that may impact sex-dependent effects and is a strategy that has been implemented to assess cannabis withdrawal in the laboratory (ie, Haney *et al*, 2013). Another important difference between animal and human studies is the ability to control for the effects of fluctuating reproductive hormones. There is significant evidence from animal studies demonstrating that reproductive hormones can modify the effects of cannabinoids (ie, Craft and Leitl, 2008; Fattore *et al*, 2010; Wakley and Craft, 2011; Castelli *et al*, 2014; Marusich *et al*, 2015; Wakley *et al*, 2015). While sex-dependent effects have emerged in several human studies that have not controlled for menstrual cycle phase, evaluating effects according to phase would help to elucidate how reproductive hormones contribute to differences between men and women. However, it should be noted that the largest changes in the brain endocannabinoid system and in sensitivity to exogenous cannabinoids in rats appear to occur around the time of ovulation. Given that the ovulatory period in women is quite short relative to other cycle phases (Reed and Carr, 2015), the impact of menstrual cycle over a long period of cannabis or cannabinoid use may not be particularly important. Studying cannabinoids in animal models that incorporate variables that impact cannabinoid effects in the clinic, such as route of administration and duration of exposure, would likely improve translation from the bench to the clinic.

IMPLICATIONS OF FINDINGS

Sex differences in any phenomenon can be categorized as quantitative – phenomena that are essentially the same but differ in magnitude between the sexes, or qualitative – phenomena that occur only in one sex, or that differ in underlying mechanism (Mogil and Bailey, 2010). At this point primarily quantitative sex differences in cannabinoid effects have been demonstrated in animal and, to a lesser degree, in human studies. That is, cannabinoid drugs have been found to be more or less potent, and in a few cases more or less efficacious, in one sex compared to the other, on a variety of End points. Small sex differences in therapeutic drug potency are unlikely to be important clinically, because drug dose can be readily adjusted up or down to compensate for any individual difference in drug effect. In contrast, large sex differences in therapeutic drug potency, or sex differences in therapeutic drug efficacy are more likely to be clinically significant, since escalating drug dose to compensate for relative lack of effect results in no gain in effect (when efficacy is low), and/or overwhelming adverse effects. Increasing dose to compensate for a lack of potency or efficacy can also lead to greater development of tolerance and dependence. These consequences of increasing drug dose have significant implications when considering the potential utility of cannabis and cannabinoids as a viable therapeutic option. Considering that cannabis is widely used recreationally, sex differences in initial potency and efficacy may also affect the likelihood of developing CUD, and achieving and maintaining abstinence in people who seek treatment for this disorder. Findings from preclinical studies demonstrate that females are particularly sensitive to the reinforcing effects of a CB1 receptor agonist (Fattore *et al*, 2007, 2010). These findings coupled with epidemiological and smaller observational studies reporting that women accelerate to problematic cannabis use at a faster rate than males and exhibit more severe withdrawal symptoms when abstinent underscore the importance of addressing sex-dependent effects of cannabis and cannabinoids for both therapeutic and abuse-liability perspectives.

LIMITATIONS AND FUTURE RESEARCH DIRECTIONS

In addition to the suggestions noted above regarding clinical studies, we note several caveats to the published preclinical and clinical research on sex differences in cannabinoid effects, as well as some potential future directions. First, developmental factors in preclinical studies such as prepubertal or adolescent cannabinoid or other drug exposure, and environmental factors such as diet, and whether rats are bred in-house *vs* shipped have been shown to modulate cannabinoid effects in adult rats, in some cases in a sex-

specific manner (Marco *et al*, 2006; Wiley and Evans, 2009; Wiley *et al*, 2011; Silva *et al*, 2016). Given the important role of the endocannabinoid system in brain development and response to stress (Dow-Edwards and Silva, 2017), this is not surprising, and it likely contributes to discrepancies in results across studies. Researchers are encouraged to be aware of methodological variables that have been shown to have sex-specific impact on outcomes of interest, and to choose methods accordingly. Second, some published sex difference studies may be under-powered. For example, very similar effect sizes using larger *vs* smaller samples can be found in studies that report statistically significant *vs* non-significant sex differences, respectively, in THC-induced antinociception (eg, Craft *et al*, 2012 *vs* Wiley *et al*, 2007), and discrimination (Wiley *et al*, 2017 *vs* Wiley *et al*, 2011). Thus, researchers are encouraged to power studies appropriately to test for sex differences, rather than add more confusion to the literature by concluding that there are no sex differences based on inadequately sized samples. For clinical studies, investigating sex-dependent effects should be prioritized when designing a study, to increase the quality of findings and to control for variables that limit interpretations of observational and retrospective studies. Third, more animal research needs to be done using models of cannabinoid self-administration to examine male *vs* female propensity to 'self-medicate' under conditions of stress/anxiety, chronic pain, etc. Control over (or prediction of) drug administration has been shown to produce different effects for a variety of drugs (see Donny *et al*, 2006), and addressing discrepancies between human and animal studies in this regard may help to optimize translation of results from animals to humans. Fourth, animal studies of sex differences in cannabinoid effects have focused on adolescents to young adults, and even human studies tend not to include older participants (ie, above 55 years of age), but there may be age-related differences in cannabinoid effects. The growing demographic of older cannabis users (Hasin *et al*, 2015; Han *et al*, 2017) highlights the importance of assessing age in future sex differences research. Fifth, although there are animal studies examining sex differences in behavior after chronic cannabinoid administration, with the exception of studies by the Winsauer lab (2011; 2012; 2014; 2016), the longest periods of administration tend to be 10 days; in contrast, many human studies of cannabinoid effect are conducted in chronic cannabis users who have been using for years. To the extent that there may be sex differences in the development of tolerance to and dependence on cannabinoids, continuing to examine only acute effects of cannabinoids in animals will provide only limited information relevant to human use. For clinical studies, it is also important to assess the safety, tolerability, and efficacy of cannabis and cannabinoids in non-cannabis users as a function of sex, as patients who turn to medical cannabis and cannabinoids are not necessarily current cannabis users. Sixth, the major non-psychoactive component of cannabis, cannabidiol (CBD), is emerging as a cannabis constituent with potentially diverse therapeutic effects, but there are almost no sex comparisons of its effects, even in animals. Given the emerging evidence that TRPV1 channels—a major CBD target—are estradiol-modulated (Yamagata *et al*, 2016), examining sex differences in a variety of CBD effects is warranted.

Finally, it should be noted that to date, only a few laboratories have examined sex differences in either therapeutic or abuse-related effects of cannabinoids in either animals or humans, so it remains to be seen whether sex differences observed in the early studies reviewed herein will be replicated by other laboratories using more strains and species as well as a wider spectrum of End points. In addition, very few animal (or human) sex difference studies have obtained complete dose- and time-effect curves for the cannabinoid effects of interest. These parameters are crucial for determining whether observed sex differences reflect sex differences in drug potency, drug efficacy, onset and/or duration of action. It can be argued that the most clinically important sex differences in drug effect would be sex differences in drug efficacy, since a lesser maximal effect in one sex compared to the other has many implications for treatment as well as abuse liability (see implications). Thus, it will be important in future studies to comprehensively characterize sex differences in all pharmacological parameters of cannabinoid effect.

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