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REVIEW

How important are sex differences in cannabinoid action?

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In humans as in animals, males and females are dissimilar in their genetic and hormonally driven behaviour; they process information differently, perceive experience and emotions in different ways, display diverse attitudes, language and social skills, and show sex-related differences in the brain anatomy and organization. Drug addiction is a widespread relapsing illness that affects both men and women. Sex-dependent differences have been frequently observed in the biological and behavioural effects of substances of abuse, including cannabis. Beside sex differences observed in the cannabinoid-induced effects related to cannabis abuse and dependence, cannabinoids have been shown to exert sex-dependent effects also in other physiological and behavioural aspects, such as food intake and energy balance (more evident in males), or anxiety and depression (more evident in females). Research has just begun to identify factors which could provide a neurobiological basis for gender-based differences in cannabinoid effects, among which, gonadal hormones seem to play a crucial role. Yet, cannabinoid pharmacodynamic and pharmacokinetic may also be important, as sex differences in cannabinoid effects might be due, at least in part, to differences in muscle mass and fat tissue distribution between males and females. Here, we will review both clinical and laboratory-based research evidence revealing important sex-related differences in cannabinoid effects, and put forward some suggestions for future studies to fill the gap in our knowledge of gender-specific bias in cannabinoid pharmacology.
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Abbreviations: CB_1, sub-type 1 cannabinoid receptor; delta9-THC, delta9-tetrahydrocannabinol

In contrast with the past, when females were excluded or underrepresented in both clinical and preclinical studies, attention and interest in possible sex differences is now developing in many fields of biomedical research. Indeed, both animal and human studies have revealed important sex-related differences in hormone and neurotransmitter functions. Over the last two decades, the endocannabinoid system has emerged as one of the neurotransmission systems most involved in both peripheral and central physiological processes. Intensive research on the possible roles of the endocannabinoid system has led to a great improvement in our knowledge of how this system modulates a number of central and peripheral functions, and has revealed important sex differences in the effects of cannabinoids (Figure 1).

In humans, gender differences have been frequently observed in the biological and behavioural effects of substances of abuse (Fattore *et al.*, 2008), including cannabis. For example, men consume marijuana in greater amounts and at higher rates than do women (Perez-Reyes *et al.*, 1981), and male high school students are more likely than female students to report problems in school and poor family relationships (Butters, 2005). Male marijuana smokers also exhibit higher circulating levels of delta9-tetrahydrocannabinol (delta9-THC) (Jones *et al.*, 2008), show larger cardiovascular and subjective effects than female smokers (Leatherdale *et al.*, 2007), display more evident withdrawal symptoms (Crowley *et al.*, 1998), are less likely to be cannabis-only users (i.e. polysubstance cannabis users) and have a higher prevalence of panic disorder and personality disorders (Hasin *et al.*,

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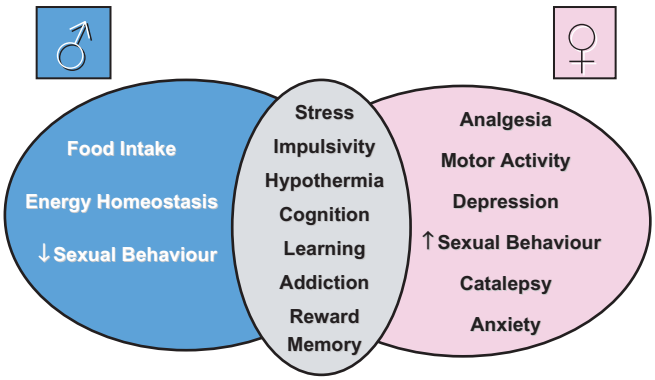

Figure 1 Sex-dependent differences in cannabinoid effects. *Blue*: cannabinoid-induced effects that are more evident in males. *Pink*: cannabinoid-induced effects more evident in females. *Grey*: fields where the effects of cannabinoids are not dependent on sex (i.e. impulsivity), are incongruent between clinical and animal studies (i.e. addiction) or data are still controversial (i.e. hypothermia).

2008). Consistent with these differences, among non-marijuana smokers, men are more sensitive to the subjective effects of delta9-THC alone than women (Haney, 2007). On the contrary, although there are no apparent gender differences in intoxication or plasma delta9-THC levels after smoking marijuana (Wall *et al.*, 1983), women report significantly more dizziness than men and are more susceptible to cannabinoid-induced haemodynamic changes and visuospatial memory impairment (Mathew *et al.*, 2003), smoke marijuana mainly when they feel anxious (Patton *et al.*, 2002) and show higher sub-type 1 cannabinoid receptor (CB_1) protein expression than men, as measured in blood samples (Onaivi *et al.*, 1999). Notably, the influence of cannabis intake on sexual behaviour and arousability appear to be dose-dependent in both men and women, although only women report facilitatory effects (Gorzalka *et al.*, 2009). No gender differences have been observed instead in the effects of delta9-THC on impulsivity (Mcdonald *et al.*, 2003).

Similar sex differences in responses to cannabinoids have been found in preclinical studies. For example, males are more sensitive to the hyperphagic and hypophagic effects of the CB_1 receptor agonists and antagonists, respectively (Diaz *et al.*, 2009), as well as to their hypothermic and hyperthermic effects (Farhang *et al.*, 2009). Conversely, cannabinoids elicit comparatively greater catalepsy, antinociception and locomotor effects in females than in males (Tseng and Craft, 2004), and decrease both exploratory behaviour and emotionality/anxiety levels in female, but not male, rodents (Biscaia *et al.*, 2003). More consistent are animal studies showing that the endocannabinoid system may be differentially sensitive in its modulation of appetitive behaviour in females versus males. That is, intravenous self-administration of the CB_1 receptor agonist WIN 55,212-2 in female Long Evans and Lister Hooded rats is more rapidly acquired, more robustly maintained and more slowly extinguished than in their male counterparts (Fattore *et al.*, 2007). Moreover, after both drug and cue priming, intact female rats reinstate responding for the cannabinoid at higher level than males and ovariectomized females (Fattore *et al.*, 2010). Importantly, perinatal exposure to delta9-THC decreases proenkephalin gene expression in the caudate-putamen of female but not male rats (Corchero *et al.*, 1998), while female but not male rats that have been perinatally exposed to delta9-THC self-administer more morphine once they are adults (Vela *et al.*, 1998).

Sex-dependent differences in mammalian behaviour, and in drug effects on behaviour, generally have been found to rely on activational effects of gonadal steroid hormones. For example, sex differences in cannabinoid-induced behavioural effects in rats have been attributed to activational effects of testosterone in males and/or oestradiol in females, as cycling females result more sensible to delta9-THC-induced effects when tested in oestrous (i.e. in a high-oestradiol state) than in dioestrous (i.e. in a low-oestradiol state) (Craft and Leitl, 2008). Consistent with this, gonadal hormones such as oestradiol are known to regulate cannabinoid receptor density (Busch *et al.*, 2006), transcription (González *et al.*, 2000) and signal transduction (Mize and Alper, 2000) in some areas of the adult rodent brain, suggesting that CB_1 receptor function may be sexually dimorphic. That is, in some regions of the brain, the endocannabinoid levels (Bradshaw *et al.*, 2006) and the CB_1 receptor density and affinity (Rodríguez de Fonseca *et al.*, 1994) fluctuate as a function of sex and hormonal cycle, supporting the hypothesis of possible sex hormone-dependent differences in the sensitivity of certain neuronal processes triggered by cannabinoid treatment. Oestrogen exerts profound effects on mood, mental state and memory by acting on both monoamine and neuropeptide transmitter mechanisms in the brain. Low levels of oestrogen in women are, in fact, associated with the premenstrual syndrome, postnatal depression and post-menopausal depression (Fink *et al.*, 1996). However, one cannot exclude the possibility that sex differences in the behavioural effects of cannabinoids are related to sex differences in drug disposition and body fat distribution. Cannabinoids are lipophilic, and a high concentration is sequestered in fat tissue. Women have a higher percentage of body fat than men, suggesting that women experience weaker effects because more delta9-THC is retained by fat cells. Moreover, cannabinoids may be differentially metabolized to active and inactive metabolites in men and women. In contrast to human males, male rodents have a higher percentage of body fat, which could account, at least partly, for the different results reported from human and animal studies. For example, female rodents express greater amounts of hepatic cytochrome P-450 isozymes and aldehyde oxygenase activity that may facilitate conversion of delta9-THC to potent bioactive metabolites such as 11-hydroxy-THC (Narimatsu *et al.*, 1991). In line with this, levels of delta9-THC metabolites in brain tissue, including 11-hydroxy-delta9-THC, are higher in females than in males, likely contributing to the greater behavioural effects of delta9-THC in female compared to male rats (Tseng *et al.*, 2004).

In the human population, a gender difference in cannabis use has been identified in several clinical studies and in anecdotal observations, although the nature of the differences have not been well explored. The most consistent finding arising from the surveys conducted so far on this topic is that boys are more likely to be 'heavy users' than girls (Kohn *et al.*, 2004). For example, according to the Ontario Student Drug Use Survey conducted between 1999 and 2003, daily marijuana smoking appears to be more common among boys

(6.2%) rather than girls (2.2%) (Adlaf and Paglia, 2003). Population-based surveys of adolescents in Belgium have also found a gender difference in the prevalence rate of marijuana smoking, with males being higher frequency users than females (Kohn *et al.*, 2004). Yet, the factors associated with marijuana smoking by boys and girls use are not well described, and it is not known whether the risk factors differ by gender. It is possible that cannabis smoking is associated with different emotional or mental states in males versus females, some anxiety disorders being more frequent in women than in men. Moreover, some possible bias in the results coming from epidemiological studies may occur because women appear to receive more health care information than men, possibly due to women's superior general communication skills. A recent survey has also revealed interesting gender differences in correlates of both frequent and heavy cannabis use. In particular, girls appear to be less influenced by the cannabis use of their peers or the social milieu established in school. Second, girls reporting relatively poor mental health are more at risk than boys for frequent and heavy cannabis use (Tu *et al.*, 2008). Thus, mental health status is correlated with girls' cannabis use but not boys' cannabis use, suggesting that the use of cannabis may be very different for the two sexes. In addition, it should be considered that for girls, the drug is typically obtained through their social relationships with boys, supporting the conclusion that girls smoke marijuana to impress boys, whereas boys 'get high' for the sake of the experience.

The studies outlined above describe differences in a variety of cannabinoid effects between sexes; yet, most data come

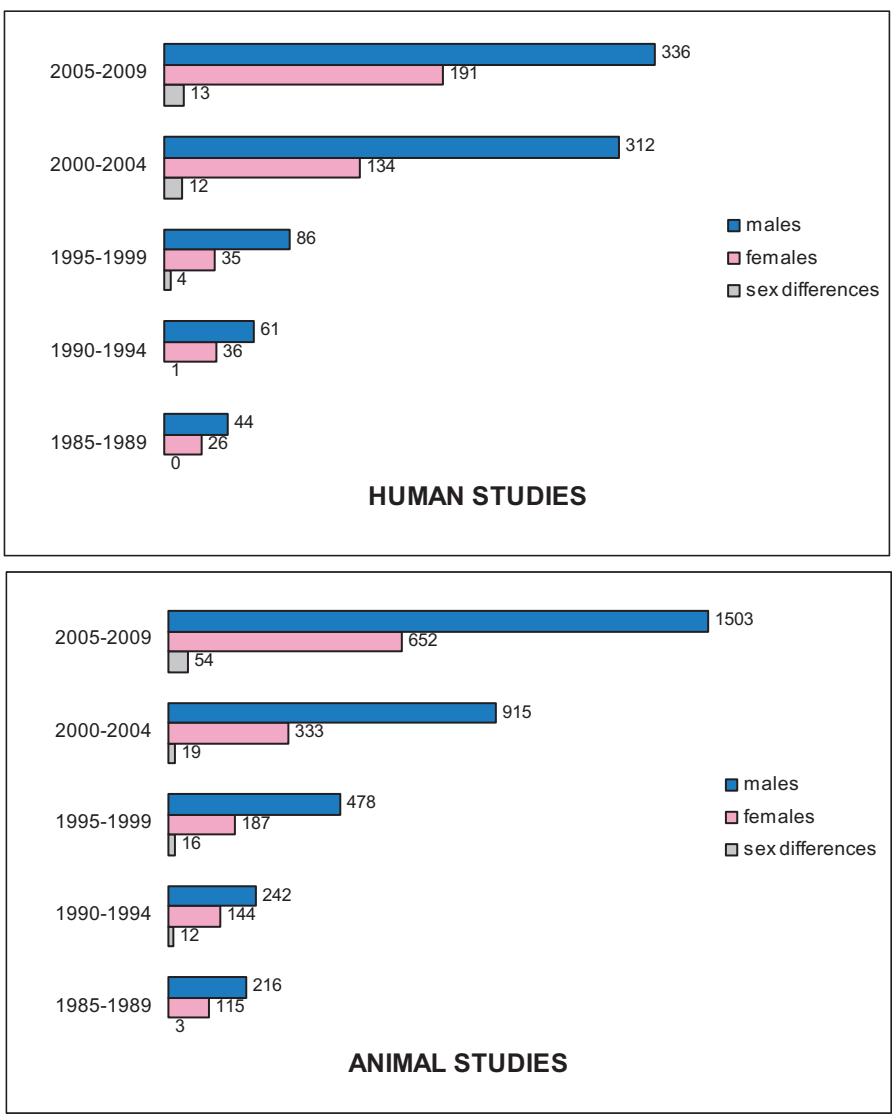

Figure 2 Number of clinical (*top*) and preclinical (*bottom*) studies using either male (*blue*) or female (*pink*) subjects, or specifically focused on the study of sex differences in cannabinoid effects (*grey*) published during 5 year time intervals. Source: Pub-Medical. Searching for human studies was conducted using 'cannabinoid + male + men' (blue), 'cannabinoid + female + women' (pink), and 'cannabinoid + sex differences' (*grey*) as keywords, and limiting the search to the following types of studies: *Clinical Trial, Randomized Controlled Trial, Case Reports, Clinical Conference, Clinical Trial, Phase I, Clinical Trial, Phase II, Clinical Trial, Phase III, Clinical Trial, Phase IV, Comparative Study, Controlled Clinical Trial.* Searching for animal studies was conducted using 'cannabinoid + male' (blue) or 'cannabinoid + female' (pink) or 'cannabinoid + sex differences' as keywords, and limiting the search to the following types of studies: *Classical Article, Journal Article.*

from studies with rodents and systematic comparisons of cannabinoid effects in humans are needed.

The knowledge that cannabinoid action is regulated differently between the sexes and that the majority of these differences are dependent on changes across the oestrous cycle sets the stage for future experiments aimed at elucidating the relationships between endocannabinoids and hormonal milieu. Brain imaging represents another methodology that holds promise for facilitating mechanistic and translational advancements. Unfortunately, although neuroimaging studies have frequently examined brain activations elicited by emotional stimuli in either men or women, they have seldom directly compared men and women within the same study, often because the sample sizes for each sex were insufficient.

Although research regarding gender and the endocannabinoid system has continued to expand and generate novel findings, to date, there has been limited clinical impact of this new knowledge. In this era of translational research, an important goal for scientists is to gain information that may improve clinical treatment of patients of both sexes. For example, as the endocannabinoid signalling pathways affect the multifaceted process of reproduction and may impact both male and female fertility, researchers are working with the hope of designing and developing endocannabinoid-oriented next-generation therapeutics for the treatment of infertility (Wang *et al.*, 2006). An opportunity that could help translation comes from animal models with well-established face and predictive validities, which could serve as a translational link between laboratory findings from animals and human patients. Genetic research may also be useful for this translational continuum, by determining, for example, whether sex-related associations between genetic variants and common diseases described in animal studies may help us to understand why men and women often differ in their susceptibility to the development of diseases and in their response to medicines (pharmacogenetics).

Since the motivations for smoking cannabis are different between sexes especially in adolescents, a higher consideration of the gender differences in smoking marijuana may be important in the design and implementation of prevention or treatment programs for young users. Empirical attention to these issues will further advance knowledge regarding sex and behaviour, and could lead to sex-specific enhancements in clinical management in the not too distant future.

On the other hand, given the remarkable therapeutic potential of cannabinoid agonists or antagonists for the treatment of neuropathic pain, glaucoma, multiple sclerosis, migraine, movement disorders and eating/appetite disorders, some of which occur disproportionately in women, further examination of possible gender differences in cannabinoid pharmacological effects is warranted. Gathering in-depth information on how endocannabinoid signalling is differentially regulated in men and women is essential for developing endocannabinoid system-oriented drugs for selectively targeting central or peripheral tissues thus avoiding adverse effects in unrelated tissue types.

In conclusion, although few important insights into the neurobiological bases of individual differences in cannabinoid effects have been achieved, investigation of such differences is still at an early stage (Figure 2). We would like to emphasize important issues that we feel are worth noting and demand future consideration and investigation. First, mainly in the last decade, clinical research has paid more attention to female patients although, similarly to preclinical research, females are still underrepresented: there is no longer a justification for limiting research to only one sex. Besides cannabis use, a considerable part of the clinical disorders focused on by preclinical research are female predominant (think of anxiety-related disorders or eating disorders), so that preclinical researches that exclude females appear necessarily incomplete and biased. Second, studies specifically aimed at investigating sex-dependent differences in cannabinoid action are now emerging: if both preclinical and human studies routinely included subjects of both sexes, greater progress in the field would be reached in a shorter time. Clinical studies should also report all findings, whether positive or negative, in order to quantitatively define the issues related to the gender differences in cannabis consumption.

Conflict of interest

Authors declare to have no conflict of interest.

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