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Vaporized Cannabis differentially modulates sexual behavior of female rats according to the dose

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ABSTRACT

Studies exploring the effect of compounds that modulate the endocannabinoid system on sexual behavior have yielded contradictory results. However, the effect of smoked Cannabis in women has been consistently associated with an increase in sexual drive. Therefore, it can be speculated that vaporized Cannabis will augment sexually motivated components of the sexual behavior of female rats. To test this hypothesis, we compared the sexual behavior of late-proestrous female rats in a bilevel chamber after vaporizing 0, 200 or 400 mg of Cannabis flowers (containing 18% of delta-9-THC and undetectable levels of cannabidiol) during 10 min. We found that both doses of Cannabis increased the duration of the lordosis response, whereas the highest dose also reduced the lordosis quotient of females. The lowest dose of Cannabis augmented the display of hops and darts without altering the expression of sexual solicitations of females, while the highest one did not affect the expression of hops and darts but reduced sexual solicitations. These effects were not accompanied by alterations of females' ambulatory behavior. The increment of the duration of lordosis response produced by both doses of Cannabis could be associated to a general effect of this drug in sensory processing, as can be an enhancement of females' sensory reactivity to male's stimulation. However, the reduction in the display of solicitations and lordosis in response to mounting observed in females exposed to the highest dose when compared to control and 200 mg of Cannabis groups indicates a reduction of sexual receptivity and motivation. This differential effect of vaporized Cannabis according to the dose employed, suggests that it modulates sexual behavior in a complex way, impacting neural circuits that control different aspects of this social behavior.

1. Introduction

Cannabis is the most frequently used illicit recreational drug around the globe (UNODC, 2017). Recently its consumption has been legalized in different countries, both for recreational and medical uses (de la Hoz Schilling, 2015; National Conference of State Legislatures, 2017). Although the plant yields > 538 chemicals of various classes (Andre et al., 2016), its psychoactive proprieties have been mainly attributed to the phytocannabinoid Δ^9-tetrahydrocannabinol (THC) (Hložeket al., 2017). Cannabis consumption can produce a wide range of psychological and physiological effects, most of them by means of its actions on the endocannabinoid system (Trezza et al., 2008; Murillo-Rodríguez et al., 2011). This system plays a regulatory role in a variety of physiological processes and responses including sexual behavior (Di Marzo and Piscitelli, 2015; Grotenhermen, 2006; Prospéro-García et al., 2016; Zavatti et al., 2011); however, it is not completely understood how the endocannabinoid system modulates sexual behavior (Gorzalka et al., 2010).

For several decades there has been interest in the effects of Cannabis on women's sexuality, however few scientific studies have addressed this topic (Gorzalka et al., 2010; Halikas et al., 1982; Koff, 1974). There are several publications of self-reported aphrodisiac-like effect of marijuana consumption in woman (Gorzalka et al., 2010; Halikas et al., 1982; Sun and Eisenberg, 2017). Jarvik and Brecher already in 1977

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had identified several possible ways through which marijuana exerts this aphrodisiac-like effect, including the loss of inhibition, the enhancement of sensate focus, the generalized increase in enjoyment (hedonism), the slowing down of the perception of time that may cause an enjoyable activity seemingly to last longer and also a placebo effect related to its reputation as a enhancer of sexual drive (Jarvik and Brecher, 1977).

Despite the acceptance of a facilitatory effect of Cannabis on women's sexual behavior, the effect of compounds that modulate the endocannabinoid system on the sexual behavior of female rats is controversial. Thus, some authors had reported a stimulatory effect of THC on proceptive and receptive sexual responses of females (Gordon et al., 1978; Mani et al., 2001; Turley and Floody, 1981). However, two recent studies documented opposing results; the administration of a CB1 and CB2 receptors agonist decreased sexual behavior (Ferrari et al., 2000), while the administration of a cannabinoid antagonist enhanced it (López et al., 2009). These discrepancies can be, at least partially, associated to the fact that most of these studies employed hormonal-primed ovariectomized females under different hormonal treatments (Ferrari et al., 2000; Gordon et al., 1978; Mani et al., 2001; Turley and Floody, 1981). The hormonal induction of estrus is usually employed to facilitate working conditions and to homogenize the hormonal background among females. However, although it evokes an expression of sexual behavior similar to that observed in natural estrus, it is still an artificial state. As we consider important to work with natural conditions in order to be more accurate at the time of drawing conclusions, we employed late-proestrous cycling females in this study. Moreover, those studies had focused on the administration of isolated cannabinoids, while Cannabis plant is composed by a high number of active chemical substances which may have a synergic effect (Russo, 2011). In fact, Carlini et al. (1974), based on animal and human studies, determined that Cannabis extracts produced effects “two or four times greater than that expected from their THC content” (Carlini et al., 1974).

If smoking Cannabis exerts an increase in sexual drive in women (Halikas et al., 1982; Koff, 1974) it could be expected that the inhalation of Cannabis will also augment sexually motivated components of the sexual behavior of female rats while interacting with a male. An alternative inhalation-based route to smoking of Cannabis is its administration through vaporization, as it provides delivery characteristics similar to smoking, without the toxicants that are present due to combustion (Shiplo et al., 2016). On these bases, the aim of the present study was to characterize the acute effects of Cannabis flowers vaporization on the sexual behavior of late-proestrous female rats assessed in the bilevel chamber. The bilevel chamber model was selected because it has been designed to improve the discernment of the motivational components of sexual behavior repertoire of females (Mendelson and Gorzalka, 1987; Mendelson and Pfaus, 1989; Pfaus et al., 1999) and it has been shown that virgin sexually naive females display the full sexual behavior repertoire in this model (Afonso et al., 2007).

2. Methods

2.1. Animals

Female and male adult rats (*Rattus norvegicus*, Charles Rivers-derived Wistar strain) from the *Laboratorio de Experimentación Animal* of the Facultad de Ciencias, Universidad de la República, were used in this study. Females employed in this study were between 120 and 140 days old, and their weights did not differ between the experimental groups (0.0 mg: 274.0 ± 3.3, 200 mg: 267.3 ± 7.6 and 400 mg: 271.3 ± 7.5, $F_{(2, 19)} = 0.277$, $p = 0.8$, one-way ANOVA). All animals were housed in a temperature and humidity-controlled environment (21 ± 1 °C and 50–70%, respectively) under a 12 h light-dark cycle (lights on at 05:00 h). Rats of the same sex were housed in groups of 4 with food and water ad libitum. Males and females shared the same animal room; however, after weaning they have never been housed together in the same cage. Animal care and experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the NIH and the Uruguayan law (number 18611) for the care and use of laboratory animals. The experimental protocol employed was approved by the Ethical Committee on Animal Use (CEUA) of the Facultad de Ciencias, Universidad de la República (protocol number: 240011-000903-18).

The estrous cycle of virgin, sexually naive females, was followed through the inspection of daily vaginal smears performed during the light phase of the cycle (Marcondes et al., 2002), and they were tested on late-proestrous stage of their estrous cycle (when sexual behavior is expressed), after showing at least two regular cycles.

2.2. Cannabis flowers composition and administration

Fresh flowers of *Cannabis sativa* L. were obtained from the Institute of Regulation and Control of Cannabis (“*Instituto de Regulación y Control de Cannabis, IRCCA*”), grounded, homogenized and preserved in a protection plastic bag at −18 °C until its use. Cannabinoids (THC and CBD) content was determined by gas chromatography (GC) (AHP, 2014). The quantification of cannabinoids was performed with an external calibration, using the average values of three sets of standards containing target compounds at concentrations ranging from 1 to 250 μg/mL in MeOH. The limit of quantification for both compounds was 1.5 μg/mL, and the correlation coefficients were ≥0.995. The cannabinoid content in the grounded material was: THC = 18.0% and CBD < 0.05%.

Cannabis flowers (0, 200 or 400 mg) were vaporized in a plastic chamber of 4.6 L of capacity at 180 °C by means of thermostated plates for 10 min. The vaporization was performed inside of a laminar flow hood. The selection of the amounts of Cannabis flowers employed, as well as the vaporizing temperature and time was based on Mondino et al. (2019), which is, to our knowledge, the first study to report the behavioral effects of vaporized Cannabis flowers in rats. Thus, the lowest dose of Cannabis (200 mg) was chosen because Mondino et al. (2019) had already demonstrated that its vaporization produces changes in sleeping behavior. However, as this effect was moderate, we decided to also investigate the effect of a higher dose (400 mg).

2.3. Sexual behavior test

Sexual behavior was assessed in a transparent acrylic bilevel chamber (60 cm long × 51 cm high × 15 cm wide) during the dark phase of the light cycle (between 19:00 and 21:00 h) under a dim red light. Females were habituated to the bilevel chamber for 10 min each day, the three days before been tested. On the testing day, each female was placed into the bilevel chamber for 5 min and the total number of level changes was recorded (this measure includes intermediate levels). Afterwards, a sexually active male (trained for performing one ejaculation in < 15 min) was introduced and the following components of female's sexual behavior were recorded during 20 min: the number of a. hops and darts (short distance hopping and running near by the male, usually with a “rigid” body posture), b. sexual solicitations (approach toward or orientation of the head to the male, and a rapid run away from it to another level; usually this behavior ends in a hop and dart or presenting posture; in this case the whole action was considered as a sexual solicitation and the ending hop and dart was not counted as a separate component) and c. lordosis postures in response to male's mounting, as well as the latency to the first proceptive and receptive displays (Pfaus et al., 2000). Additionally, we assessed the number of level changes performed by the females. The measurement of male's sexual behavior included the assessment of the number of mounts (including those with and without intromission), ejaculations and their respective latencies (Fernández-Guasti et al., 1990). Female's sexual receptivity was assessed by the lordosis quotient (LQ), calculated as the

number of lordosis postures that females acquired in response to male's mounting (number of lordosis/number of mounts including intromissions and ejaculations) (Fernández-Guasti et al., 1990, 1986; Pfaus et al., 1999). In addition, the duration of lordosis postures (in seconds) was recorded and the mean duration of the lordosis posture in response to a mount per female was calculated.

The frequency of the sexual responses of females and males are reported before the first ejaculation of the male and along the whole test, in order to detect putative variations in the expression of sexual behavior related to the progression of the sexual interaction. One female of the control group was excluded from the whole test analysis due to technical problems with the digital recording.

2.4. Ambulatory activity test

Locomotor activity was assessed during 5 min in an open field consisting of a rectangular arena (36 cm wide × 53 cm long × 25 cm high) with transparent plastic walls. The number of crosses (crossing gridlines with the four paws), rearings (standing on both hind paws in a vertical upright position) and groomings (rubbing, licking or nibbling the head and body) were registered.

2.5. Experimental procedure

In order to study the effect of Cannabis on the sexual behavior of female rats, cycling females in late proestrus were placed in the vaporization chamber where 0 mg (C_0, n = 9), 200 mg (C_{200}, n = 9) or 400 mg (C_{400}, n = 7) of Cannabis flowers were vaporized for 10 min. Immediately after removing the rats from the chamber, they were submitted to the ambulatory activity test during 5 min, in order to assess the putative influence of locomotor effects of Cannabis on females' sexual performance. Afterwards, females were placed during 5 min in the bilevel chamber, and subsequently, the sexual behavioral test was conducted.

Due to problems with the availability of the stock of Cannabis flowers used in this study, the C_{400} group was composed of 7 animals.

2.6. Determination of THC levels in plasma after vaporizing 200 mg of Cannabis flowers

In order to estimate the range of levels of THC reached in plasma with the vaporization methodology employed, we determined the concentration of THC in plasma of 8 females in late proestrus (between 19:30 and 20:30 h) 10 min after vaporizing the lower dose of Cannabis. With this aim, 200 mg of Cannabis flowers were vaporized employing the same methodology used in the main experiment. Afterwards, the rats were maintained in a quiet cage for 10 min and then euthanized by decapitation. Blood was collected in ice-chilled K2-EDTA coated collection tubes. Immediately after the collection, blood was centrifuged 15 min at 1200*g* (Giuffrida et al., 2000; Takahashi et al., 2014). Plasma was collected in cryotubes and stored at −80 °C for later analysis. Plasma THC levels were determined by ELISA with high sensitivity for Δ9-THC and 11-OH-THC (Neogen Corporation, Lansing, USA). Δ9-THC used was T-005-1ML of (−)-Δ9-THC Certified Reference Material at 1.0 mg/mL in Methanol from Cerilliant. Each plasma sample was evaluated following the manufacturer's instructions. Briefly, a 25 µL aliquot of each plasma sample was transferred to individual wells of the ELISA plates together with 25 µL of optimization buffer and incubated for 60 min in the dark with gently shaking. Once this incubation stage was finished, 50 µL of drug-enzyme conjugate was added to each well and incubated in the dark at room temperature for 30 min, gently shaking the plate. Following the incubation, the liquid was dumped from the wells. After washing with buffer, 100 µL K-Blue® (TMB) substrate was added to each well and incubated for 30 min in the dark. The reaction was stopped with 100 µL H_2SO_4 (1 N) and the plate was read at 450 nm. The intensity of the color development was inversely proportional to the concentration of drug in the sample. A calibration curve was done for 1 ng/mL, 2 ng/mL and 5 ng/mL in blank plasma using Δ9-THC reference material. Sensitivity was determined by running a drug-free plasma sample and analyzing spiked at 0.5 ng/mL.

A negative control was also prepared and analyzed. A control plasma sample with 2 ng/mL was included; and, at the beginning and at the end of each batch, a blank sample was run.

Due to unavailability of the amount of Cannabis flowers of the strain employed in the behavioral experiment (with its specific chemical composition), the plasma determinations of THC levels were only assessed after vaporizing the lowest dose of Cannabis flower and not the highest one.

2.7. Statistical analysis

Behavioral data were expressed as medians and semi-interquartile ranges (SIQRs) and were analyzed using non-parametric tests. Comparisons among groups were made by the Kruskal Wallis ANOVA followed by Mann Whitney *U* test (Siegel and Castellan, 1988). Plasma levels of THC were expressed as means ± standard error (SE).

3. Results

3.1. Sexual behavior test

3.1.1. Females' sexual receptivity

The administration of Cannabis affected the lordosis quotient of females (Fig. 1), an effect that was more pronounced when the whole test was analyzed (Fig. 1B; $H_{(2)} = 8.6$, $p < 0.05$, Kruskal Wallis ANOVA). Thus, the highest dose of Cannabis employed reduced the LQ of females in the whole test when compared to C_0 and C_{200} groups (Fig. 1B, C_0 vs. C_{400}: $U_{(8,7)} = 6.0$, $p < 0.01$; C_{200} vs. C_{400}: $U_{(9,7)} = 11.5$, $p < 0.05$, Mann Whitney U test).

Both doses of Cannabis significantly increased the mean duration of the lordosis responses before the first ejaculation (Fig. 1A, $H_{(2)} = 7.4$, $p < 0.05$; C_0 vs. C_{200}: $U_{(9,9)} = 12.0$, $p < 0.05$, and C_0 vs. C_{400}: $U_{(9,7)} = 12.0$, $p < 0.05$). This effect did not reach statistical significance when the whole test was considered (Fig. 1B, C_0 vs. C_{200}: $U_{(8,9)} = 17.0$, $p = 0.08$; C_0 vs. C_{400}: $U_{(8,7)} = 12.0$, $p = 0.07$).

The latency to display lordosis did not differ among groups; however, it tended to be shorter in the C_{400} group (Table 1, $H_{(2)} = 5.6$, $p = 0.06$).

3.1.2. Females' proceptive behaviors

Cannabis inhalation differentially modified the expression of hops and darts ($H_{(2)} = 6.1$, $p < 0.05$) and sexual solicitations performed by the females ($H_{(2)} = 7.2$ $p < 0.05$) before the first ejaculation (Fig. 2A). Thus, 200 mg of Cannabis significantly increased the number of hops and darts (C_0 vs. C_{200}: $U_{(9,9)} = 12.0$, $p < 0.05$), without affecting the number of sexual solicitations (C_0 vs. C_{200}: $U_{(9,9)} = 22.5$, p = NS) when compared to the control group. On the other hand, as shown in Fig. 2A, the number of hops and darts of females treated with 400 mg of Cannabis did not differ from C_0 ($U_{(9,7)} = 20.5$, p = NS) or C_{200} ($U_{(9,7)} = 22.5$, p = NS) rats. However, these females, exposed to the highest dose of the drug, exhibited a significant reduction of the number of sexual solicitations when compared to control (Fig. 2A, C_0 vs. C_{400}: $U_{(9,7)} = 10.5$, $p < 0.05$).

As shown in Fig. 2B, the number of hops and darts exhibited by C_{200} females throughout the whole test remained higher than the one displayed by control females ($U_{(8,9)} = 11.5$, $p < 0.05$), and tended to be greater than the one displayed by C_{400} females ($U_{(9,7)} = 14.0$ $p = 0.07$). Moreover, when the whole test was considered, sexual solicitation of C_{400} females were significantly reduced when compared to both C_0 and C_{200} rats (Fig. 2B, C_0 vs. C_{400}: $U_{(8,7)} = 7.0$, $p < 0.05$ and C_{200} vs. C_{400}: $U_{(9,7)} = 10.5$, $p < 0.05$).

The latency to display hops and darts was reduced in the C_{400} group

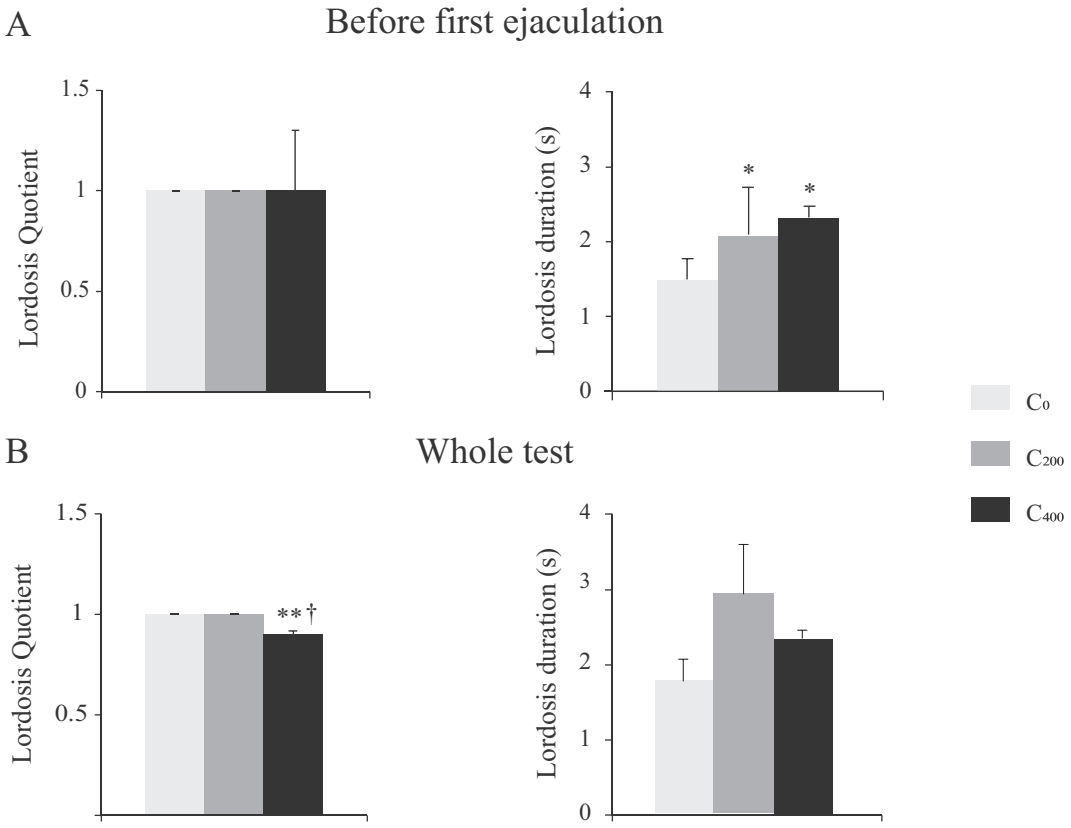

Fig. 1. Lordosis quotient (number of lordosis/total number of mounts and ejaculations) and mean duration of the lordosis response (s) of females, until the first ejaculation of the male (panel A), after vaporizing 0 (C_0 light gray bars, n = 9), 200 (C_{200}, darker gray bars, n = 9) or 400 (C_{400}, black bars, n = 7) mg of Cannabis flower. Panel B shows lordosis quotient and duration during the whole test (C_0 n = 8, C_{200} n = 9 and C_{400} n = 7). Data are expressed as median (SIQRs), *p < 0.05 vs. C_0, **p < 0.01 vs. C_0 and †p < 0.05 vs. C_{200}, Mann Whitney *U* test.

Table 1
Latencies to the display of different sexual components of females.

Latencies of female sexual responses (s)			
	C0	C200	C400
Proceptive	28.0 (9.5)	27.0 (10.5)	12.5 (3.3)*,†
Lordosis	30.0 (17.0)	42.0 (7.5)	17.0 (7.8)

Data are expressed as medians (SIQRs).
* p < 0.05 vs. C_0.
† p < 0.05 vs. C_{200}, Mann Whitney *U* test.

when compared to C_0 and C_{200} groups (Table 1, C_0 vs. C_{400}: $U_{(9,7)} = 7.0$, $p < 0.05$; C_{200} vs. C_{400}: $U_{(9,7)} = 8.5$, $p < 0.05$).

3.1.3. Ambulation in the bilevel chamber

The number of level changes performed by the females during the 5 min before the test was not affected by drug treatment (C_0: 32.0 (10.4), C_{200}: 47.0 (11.5) and C_{400}: 27.0 (6.3); $H_{(2)} = 0.56$, p = NS). In line with this result, the number of level changes performed by females during the whole test (C_0: 78.5 (27.0), C_{200}: 69.5 (32.0), C_{400}: 74.0 (18.3)) did not differ among groups ($H_{(2)} = 1.2$, p = NS).

3.1.4. Males' sexual responses

The sexual behavior of the males did not differ among groups, either during the time period until the first ejaculation or during the whole test (Table 2). Furthermore, the latency to display the first mount and the first ejaculation did not vary between groups, although it tended to be shorter in males interacting with C_{400} females (Table 2, $H_{(2)} = 5.8$, p = 0.06).

3.2. Ambulatory test

As shown in Fig. 3, the number of crosses and rearings performed by the three groups of females in the ambulatory test did not vary among groups (crosses: $H_{(2)} = 4.6$, p = NS and rearings: $H_{(2)} = 0.8$, p = NS). Besides, the administration of Cannabis flowers did not affect grooming behavior (C_0: 6.0 (2.1), C_{200}: 2.0 (1.4) and C_{400}: 5.0 (0.3)), although it tended to be reduced in C_{200} females ($H_{(2)} = 5.0$, p = 0.09).

3.3. THC plasma levels after vaporization of 200 mg of Cannabis flowers

THC plasma concentration 10 min after ending the vaporization of 200 mg of Cannabis flowers was 22.0 ng/mL/kg ± 1.2 (mean obtained from plasma of 8 females weighting 260.1 ± 7.0 g).

4. Discussion

Present results show that Cannabis flowers vaporization significantly modified the sexual behavior of female rats and this effect was not due to a locomotor affectation. Moreover, the fact that both doses of Cannabis similarly affected the duration of the lordosis response, while only the highest one reduced the expression of sexual solicitations and receptivity, suggests an impact of Cannabis on different elements of the circuitry controlling sexual behavior.

Since the display of the lordosis posture in response to male's mounting is a reflexive behavior, strongly dependent on sensory inputs provided by the male (Lisk and Baron, 1982; Pfaus et al., 2000), the increase in lordosis duration suggests that the vaporization of Cannabis flowers could have augmented females' response to sensory stimulation. Supporting this idea, several studies have demonstrated that Cannabis

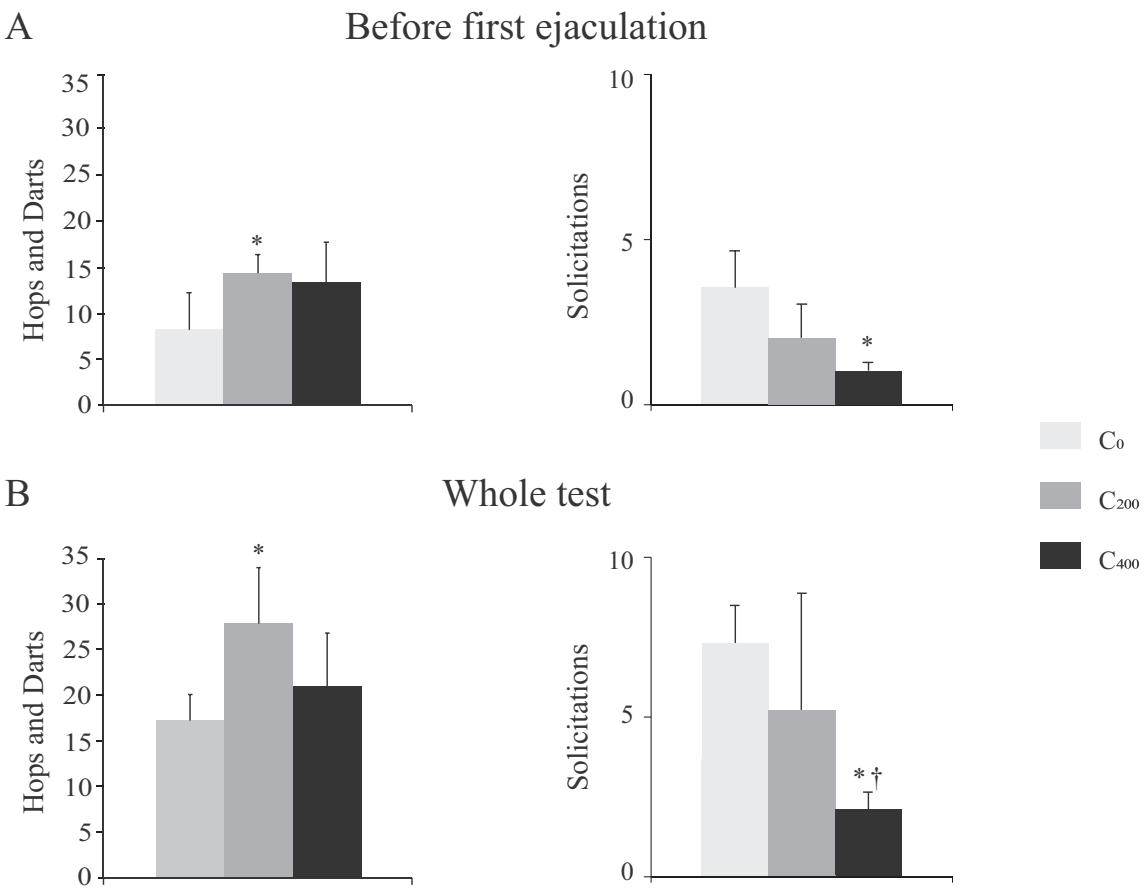

Fig. 2. Number of hops and darts and sexual solicitations displayed by females, until the first ejaculation of the male (panel A), and during the whole test (panel B) after vaporizing 0 (C_0 light gray bars), 200 (C_{200}, darker gray bars) or 400 (C_{400}, black bars) mg of Cannabis flower. Before first ejaculation: C_0 n = 9, C_{200}, n = 9 and C_{400} n = 7 and during the whole test: C_0 n = 8, C_{200}, n = 9 and C_{400} n = 7. Data are expressed as median (SIQRs), *p < 0.05 vs. C_0 and †p < 0.05 vs. C_{200}, Mann Whitney U test.

Table 2
Latencies to the display of different sexual components and number of sexual responses of males.

Latency of male's sexual responses (s)			
	C0	C200	C400
Mount	30.0 (11.0)	42.0 (7.5)	17.0 (7.8)
Ejaculation	278.0 (94.0)	280.0 (73.0)	284.0 (69.2)

Number of male's sexual responses			
	C0	C200	C400
Mounts before 1st ejaculation[a]	9.0 (2.5)	13.0 (1.5)	13.0 (2.5)
Mounts in the whole test[a]	20.0 (1.5)	22.0 (5.5)	21.0 (5.8)
Ejaculations	2.0 (0.5)	2.0 (0.5)	2.0 (0.5)

Data are expressed as medians (SIQRs).
[a] Mounts performed with and without intromissions.

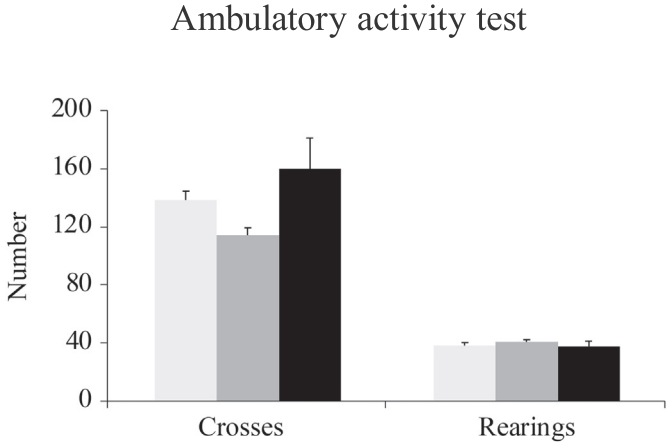

Fig. 3. Total number of crosses and rearings performed by females in the ambulatory test after vaporizing 0 (C_0 light gray bars, n = 9), 200 (C_{200}, darker gray bars, n = 9) or 400 (C_{400}, black bars, n = 7) mg of Cannabis flower. Data are expressed as median (SIQRs), p = NS Kruskal Wallis ANOVA.

consumption can enhance sensory perception (Green et al., 2003; Rodin et al., 1970; Schwin et al., 1974; Vale, 2007). Moreover, in women, one of the most commonly self-reported effects of Cannabis on sexual behavior is the increase of sensations associated to the sexual activity; being tactile and gustatory senses the most often stated (Halikas et al., 1982). Additionally, the hedonic effects (pleasure) of Cannabis consumption are more often reported than motivational or aphrodisiac ones (Androvicova et al., 2017; Halikas et al., 1982; Weller and Halikas, 1984). Regarding a biological consequence of this increment of lordosis duration evoked by the drug, Schulze and Gorzalka (1991) proposed that the lordosis duration could be related to lordosis quality, however this hypothesis has not been proved yet.

Interestingly, the expression of hops and darts was increased after 200 mg of Cannabis flowers vaporization. Although, these behaviors are thought to reflect the motivational state of females (Erskine, 1989; Pfaus et al., 1999), this increment in their expression was not

accompanied by changes in the display of sexual solicitations, suggesting that this dose of Cannabis did not affect sexual motivation. In this line of thought, even if hops and darts are used as a measure of sexual motivation (Erskine, 1989; Pfaus et al., 1999), the display of these paracopulatory behaviors have some characteristics typical of more reflexive behaviors, as they are quite stereotype, strongly dependent on stimulation by a male (Bergheim et al., 2015; Blaustein et al., 2009), and can be exhibited in a non-reproductive context: for example, they can be triggered by manually stroking the hind flanks (Cummings and Becker, 2012). Therefore, this increment in the number of hops and darts could also be indicative of an enhancement of the sensory perception produced by the vaporization of Cannabis flowers.

Sexual solicitations, on the other hand, appear to be mainly dependent of the motivational state of females since the expression of this behavior is more susceptible to procedures that increase or decrease female's sexual motivation (Kim et al., 2013; Pfaus, 2009; Pfaus et al., 1999). While 200 mg of Cannabis flowers did not affect the number of sexual solicitations, 400 mg reduced their expression, an effect that was accompanied by a decrease in sexual receptivity. This result indicating a reduction of sexual motivation by this dose of Cannabis, agrees with previous reports showing that the administration of THC or CB1 agonists to fully hormonal-primed ovariectomized rats (submitted to an estradiol plus progesterone treatment) reduces (Ferrari et al., 2000; López et al., 2010), while treatment with CB1 antagonist increases (López et al., 2009) their sexual performance. Although other studies, have found a promoting effect of THC or CB1 agonist on females' sexual motivation and receptivity, these reports employed ovariectomized females primed only with estradiol (Gordon et al., 1978; Mani et al., 2001; Turley and Floody, 1981; Zavatti et al., 2011), a hormonal treatment that evokes a suboptimal display of sexual behavior (Pfaus et al., 1999). Besides the clear differences in the hormonal background of females between studies, that could have influenced the effect of the drugs, it is hard to compare the results of previous studies to ours since they route of administration was different (parenteral or intracerebroventricular vs. pulmonary) and it has been shown that the THC plasma levels achieved by inhalatory or parenteral routes are very different (Nguyen et al., 2016). Our findings showing a detrimental effect of the vaporization of 400 mg of Cannabis flowers on the sexual behavior of late-proestrous females, during their sexual heat, support the idea that THC could inhibit the sexual behavior of highly sexually active females.

Interestingly, our study suggests that a different threshold of Cannabis exposure is necessary to affect the lordosis duration and the expression of sexual motivation, pointing toward an impact of Cannabis on different aspects of the expression of sexual behavior. If Cannabis at the lowest dose vaporized increases sensory reactivity without affecting sexual motivation, it would represent an excellent model for exploring differences in the hedonic response evoked by genitosensory stimulation (Pfaus et al., 2016), and the unconditioned incentive value of the male that promotes searching responses (Eliasson and Meyerson, 1975; Nofrey et al., 2008). This idea guarantees future experiments. On the other hand, although the decrease in the display of sexual solicitations indicates a reduction in female's sexual motivation (Jones et al., 2017; Kim et al., 2013; Pfaus, 2009), in the present study the behavior of the females was evaluated during sexual interaction, without using specific models designed for assessing sexual motivation (Ventura-Aquino and Paredes, 2017). Therefore, motivational tests, like a sexual preference test (Agrati et al., 2019; Ellingsen and Agmo, 2004), should be employed in order to clarify how Cannabis impacts the expression of female's sexual motivation.

It is important to stress that the effect of Cannabis flowers vaporization on females' sexual behavior, do not seem to depend on motor actions of the drug, as none of the doses administered to the rats did affect the locomotor activity, either in the ambulatory test or in the bilevel chamber. We considered essential to measure this effect since it has been reported a biphasic (Katsidoni et al., 2013) and even triphasic (Sañudo-Peña et al., 2000) effect of Cannabis on locomotor activity (Katsidoni et al., 2013), with high doses decreasing locomotor and exploratory activity (Bruijnzeel et al., 2016) and producing catalepsy (Prescott et al., 1992; Sañudo-Peña et al., 2000).

The THC plasma levels obtained after the vaporization of the lowest dose of Cannabis, which affected the sexual behavior without detectable effects in ambulation, were around 22 ng/mL/kg (approximately 6 ng/mL). This concentration is considered low since, higher levels of THC in blood are needed to trigger some physiological changes, for example, to induce hypomotility (Bruijnzeel et al., 2016; Javadi-Paydar et al., 2018). However, none of these previous studies employed vaporization of fresh Cannabis flowers as we did. Mondino et al. (2019) were the first in describing the effects of this administration route of Cannabis flowers in sleep; finding moderate changes in sleep with similar THC plasma levels as the ones found in the present study (Mondino et al., 2019). Additionally, these THC plasma levels are in the same range of that needed to show both objective and subjective effects in humans (Brenneisen et al., 1996; Ohlsson et al., 1980). Although we were not able to measure the THC plasma levels related to the vaporization of the highest dose of Cannabis flowers employed (due to limitations in obtaining this particular stock), the levels obtained in response to the lower dose (200 mg) demonstrate a low range of plasma THC levels associated with this method of administration. We consider that for medical purposes it is essential to study low doses of THC trying to discover beneficial properties while avoiding the potential side effects. In this line of thought, this is the first study to show that Cannabis vaporization associated to low plasma ranges of THC, significantly affects the expression of sexual behavior in the female rat. Further studies are needed in order to determine the THC plasma levels necessary to evoke the different effects.

As we administrated a Cannabis variety with high TCH and undetectable concentrations of CBD, we hypothesized that the effect of Cannabis vaporization on sexual behavior was primary due to it THC content. However, the plant is composed by > 530 chemicals (Andre et al., 2016) and it has been already determined that some of those compounds may have a synergic effect (Carlini et al., 1974; Mechoulam and Ben-Shabat, 1999). Although we did not find any research on the effect of terpenes on sexual behavior, it must be acknowledged that these substances could be playing a role in the effects of vaporized Cannabis on female's sexual behavior.

In summary, this study shows that Cannabis vaporization can differentially impact components of female's sexual behavior repertoire according to the dose. The reduction of sexual receptivity and motivation of females after 400 mg of vaporized Cannabis flower indicates that this drug directly affects areas that control sexual behavior, while the increment in the duration of the lordosis response by both doses suggests an affectation of sensory processing that might compromise more general systems. Therefore, it is possible that the disparity of results obtained regarding the effect of this drug on sexual behavior, both in rat and human studies, are due to the impact of Cannabis at circuits that control different aspects of sexual behavior. This idea points toward a complex modulation of sexual behavior by this drug.

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