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Clitoral stimulation modulates appetitive sexual behavior and facilitates reproduction in rats☆

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ABSTRACT

In rats, sexual reward, appetitive sexual behaviors and reproduction are modulated by the amount and rate of vaginocervical stimulation. Here the effect of clitoral stimulation (CLS) on proceptivity was assessed. In Exp 1, ovariectomized, hormone-primed Wistar females formed three groups: G1 (1 CLS every second), G2 (1 CLS every 5 s) and G3 (no CLS). Precopulatory CLS consisted of 5 cycles of 1 min of stimulation with the tip of a cotton swab connected to a vibrator device, followed by 1–2 min of rest. CLS increased proceptive behavior in G1 compared to G2, but not compared to G3. In Exp 2, gonadally-intact rats in late proestrous received CLS prior to copulation. No differences in sexual behavior were detected between the groups, but CLS enhanced reproduction in females that received >9 intromissions. 28, 66 and 10% of females became pregnant in G1, G2, and G3, respectively. These data indicate that precopulatory CLS affects proceptive behaviors depending on the pattern and rhythm of stimulation in hormone-primed females. In virgin rats that have received sufficient vagino cervical stimulation CLS also increases fertility.

1. Introduction

Despite a wealth of human data, the role of clitoral stimulation (CLS) in sexual behavior and reproduction in other animals has not received extensive attention. A few decades ago, experiments on cows showed that 10 s of manual CLS following artificial insemination (AI) hastened ovulation by 4.3 h as compared to control females that did not receive CLS [1], and a higher pregnancy rate was shown in stimulated females (75%) compared to non-stimulated females (67.45%) [2]. However, another study on more than 4100 cows failed to demonstrate any benefit from 30-second CLS one or more times during estrus [3], suggesting that the pattern of CLS affected the reproductive outcome.

In laboratory rats, sexual motivation and reproduction has been assessed with the paradigm of paced sexual stimulation. The ability of female rats to regulate or "pace" the pattern of sexual stimulation they receive from males induces a reward state sufficient to induce both conditioned place preference (CPP) [4,5] and a preference for odors or strain cues of partners associated with this reward state [6,7]. Place and partner preferences conditioned by paced copulation are blocked by treatment with the opioid receptor antagonist naloxone [8,9] whereas the dopamine antagonist flupenthixol blocks the development of conditioned partner preference for an odor, but not for strain cues [10] or CPP in female rats [11]. Naloxone infusions to the medial preoptic area (mPOA), ventromedial hypothalamus (VMH), or medial amygdala (MEA), but not nucleus accumbens (NAcc), block the development of pacing-induced CPP [12]. Thus, opioid activation within those structures plays an important role in pacing-induced sexual reward.

In addition to the facilitation of reward, paced copulation exerts a bimodal effect on reproduction, facilitating or disrupting it, depending on the number of intromissions received from the males. Coopersmith and Erskine [13] reported that approximately 75% of female rats became pregnant when they received a large number of paced intromissions before ejaculation (≥9), whereas only 67% became pregnant when they received an equal number of nonpaced intromissions. In contrast, if females received a low number of intromissions (≤8) during paced copulation only 40% became pregnant compared to 71% of females that received low nonpaced intromissions. Necropsies 1 week after copulation showed that the low pregnancy rate of paced, low intromission females was a consequence of a failure of activation of the corpora lutea, indicating that those females did not receive the minimum required stimulation to trigger appropriate neuroendocrine responses, such as nightly prolactine surges [14–16] and/or progesterone release [17]. Interestingly, closer observation of females that

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became pregnant with paced, but low intromissions revealed that they had significantly more pups per litter than females with an equal number of nonpaced intromissions (14.3 vs. 11 pups, respectively). This was attributed to a compensatory effect of the temporal patterning of intromissive stimulation that pacing accrues to fertility [13].

Previous studies have identified vaginocervical stimulation (VCS) as a critical stimulus for sexual reward and reproduction [18,19]. Artificial VCS can be induced by insertion of a glass rod or plunger with either a level of force or stimulation frequency that mimics intromissions [20–22] or parturition [21], and the CPP induced by artificial VCS in rats can be blocked by lesions to the mPOA [23]. An outstanding question remains as to whether VCS induces these effects by a common pathway or by activating distinct sets of neural systems. Because VCS activates the internal part of the clitoris contained in the vaginal wall along with the cervix, and in turn hypogastric, pelvic, pudendal, and vagus nerves [22,24], it is unclear which pathway(s) may carry the reward and reproductive-related signals.

Recently, artificial clitoral stimulation (CLS) was reported to induce CPP in the rat [25]. This stimulation was applied by brushing the clitoris with a fine paint brush either in a distributed manner (1 stimulation every 5 s) or nondistributed manner (1 stimulation every second) to mimic the effect male pelvic thrusting that makes direct contact with the external clitoris during copulation [26]. During conditioning trials both types of CLS were applied during 1 min, followed by 1 min of rest, for a total of 5 cycles, associating CLS with one distinctive side of the CPP box. In the final test, only rats that received distributed CLS showed a clear CPP. This indicates that CLS, like VCS, induces a reward state that is sensitive to the rate and distribution of the stimulation.

The present study examined whether paced (distributed) or unpaced (nondistributed) CLS would facilitate appetitive sexual behaviors displayed by ovariectomized (OVX), hormone-primed females during copulation with male rats (Exp. 1), and whether such stimulation could enhance the fertility in gonadally-intact rats (Exp. 2).

2. Experiment 1

2.1. Method

2.1.1. Animals and surgery

Twelve males and 15 female Wistar rats were used, weighting between 250 and 300 g. They were housed in groups of three to five per cage (50×30×20 cm) containing wood chip bedding (Rismart México), and kept in a room maintained at 22±4 °C and under a 12:12-h light–dark schedule (lights on at 22:00 h). Rodent chow 18% protein (Harlan, México) and drinking water were available *ad libitum*. The experimental protocols in these studies were approved by a Review Committee of the graduate program in Neuroethology, Universidad Veracruzana, Mexico.

Females were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected intraperitoneally (as in [27]) in a volume of 1 ml/kg of body weight. Anesthetized females were then OVX bilaterally via lumbar incisions. Females were given post-surgical treatment with daily subcutaneous injections of Flunixin meglumine (2.5 mg/kg) for analgesia, and Enrofloxacin (5 mg/kg) during 3 days to prevent post-surgical bacterial infections. All females were given a week of post-surgical recovery prior to CLS experience.

Gonadally-intact Wistar male rats that served as partner stimuli during the copulatory test had at least 4 tests of sexual behavior prior to the start of the experiments. These males were sexually vigorous and initiated copulatory activity with females within 15 s of being placed into the chambers. For all behavioral tests, sexual receptivity was induced in all females by subcutaneous injections of estradiol benzoate (10 μg) 48 h and progesterone (500 μg) 4 h before each test.

2.1.2. Experience with clitoral stimulation

Sexually naïve females were divided in three groups ($n=5$/group) depending on the rate of stimulation to receive. Animals of Group 1 received nondistributed (non paced) CLS (1 stimulation every 1 s); Group 2 received distributed (paced) CLS (1 stimulation every 5 s); and Group 3 received no stimulation (control). During CLS females were slightly lifted by the tail so that their rear limbs were hanging, but the front limbs were firmly on the cage floor. One cycle of CLS consisted of 1 min of stimulation and 1 min of rest (5 cycles in total = 10 min). Stimulation was provided with a soft touch using the tip of a cotton swab (3 cm long) connected to a commercial vibrating bullet (operated with two AA batteries) that provided between 4.5 and 5 oscillations per millisecond as measured with a polygraph (stimulus isolation unit TP511, amplifier 7PI, Grass Instruments; Polyview software). During the period of rest females were placed in their home cage. In the control condition, rats were held in place, with the vibrator turned on, but did not receive CLS. All females received CLS every 4 days for a total of 6 trials before being given their first sexual experience with a sexually active male. Multiple trials of CLS in Experiment 1 served to acclimate females to the stimulation.

2.1.3. Sexual behavior test

The day of the sexual behavior test, hormone-treated females received CLS according to their group and immediately after were placed in a cylindrical Plexiglas chamber (50 cm high×60 cm in diameter) with a thin layer of wood chip bedding and a sexually vigorous male for a 30-min period. This test was video recorded and scored using the computerized Behavioral Observation Program (BOP) [28]. Sexual behavior was assessed by measuring the latency and frequency for female solicitation (defined as a head-wise orientation to the male followed by a runaway, forcing the male to chase her), hops and darts, female–male mounting, lordosis magnitude (on a scale from 1 to 3, with 1 representing low magnitude, 2 representing moderate magnitude, and 3 representing high magnitude, as in [29]). Olfactory investigation was considered as a single event from the moment a female started sniffing the male's body until she moved her head away. Male mounts, intromissions and ejaculations were also recorded.

2.1.4. Statistical analysis

One-way Analysis of Variance (ANOVA) was used to determine differences in the frequencies and latencies of sexual behaviors between the three groups. For all significant main effects, Fisher LSD posthoc tests were conducted to assess differences between individual means. The level of significance for all comparisons was $p \leq .05$.

2.2. Results

Table 1 shows the summary of descriptive results for female and male behavior. The ANOVA detected a significant effect of CLS on solicitation frequency $F(2, 14) = 3.6$ $p = 0.05$. Posthoc tests indicated that the number of solicitations displayed by Group 1 ($M = 54.6$) was significantly higher than those of Group 2 ($M = 31.8$), but not Group 3 ($M = 44$) (Fig. 1). No other sexual behaviors were affected significantly by CLS (Table 1).

3. Experiment 2

This experiment was designed to assess changes in sexual behavior and reproductive performance of virgin rats that received CLS prior to copulation with sexually experienced male rats.

3.1. Method

3.1.1. Animals and procedure

Wistar rats (12 males, 54 females) were housed and maintained in the same conditions as in Experiment 1. Gonadally-intact males that served

Table 1
Sexual behavior of rats in Experiment 1. Ovariectomized, hormone-primed and sexually naïve females received different patterns of clitoral stimulation during a 10 min period prior to copulation with sexually experienced males. Values are expressed in mean ± SEM.

Variable	Group 1 1 stimulation every 1 s	Group 2 1 stimulation every 5 s	Group 3 Control no stimulation	*F*	*p*
Female behavior					
Genital investigation	8 ± 2.1	5.6 ± 2.5	14.4 ± 6.3	1.2	.33
Solicitations	54.6 ± 4.5	31.8 ± 6.7	44 ± 6.2	3.6	.05*
1st solicitation latency (seconds)	49.32 ± 13.3	126 ± 42	57.6 ± 21	2.2	.15
Hops and darts	201 ± 52	100.8 ± 14	119 ± 25	2.3	.13
Lordosis magnitude 1	1.8 ± 1.5	3.2 ± 0.8	3.8 ± 2.2	.39	.68
Lordosis magnitude 2	30.4 ± 4.5	22 ± 5.9	20.2 ± 9.2	.62	.55
Lordosis magnitude 3	15.6 ± 12.1	22.4 ± 13	11.2 ± 6.7	.26	.77
Male behavior					
Mounts	28.2 ± 6.2	23.6 ± 5.7	20 ± 7.7	.38	.68
Intromissions	18.6 ± 6.8	22.6 ± 4.9	14.2 ± 5.2	.52	.60
Ejaculations	3 ± 0.3	2.4 ± 0.8	2 ± 0.5	.71	.50

* Significant differences between groups were considered when $p \leq 0.05$.

as stimulus during the copulatory test had at least 10 tests of sexual behavior and had produced progeny prior to the start of the experiments, whereas females were left gonadally intact and were sexually naive.

Reproductive status was assessed via vaginal smears. The tip of a cotton swab was dampened with water to facilitate a gentle smear of the first third of the caudal portion of the vagina. Females in vaginal proestrous were selected to be tested for sexual behavior on that afternoon, during the second third of the dark circadian period. Females not in proestrous on that day were identified and smeared again when proestrous was expected.

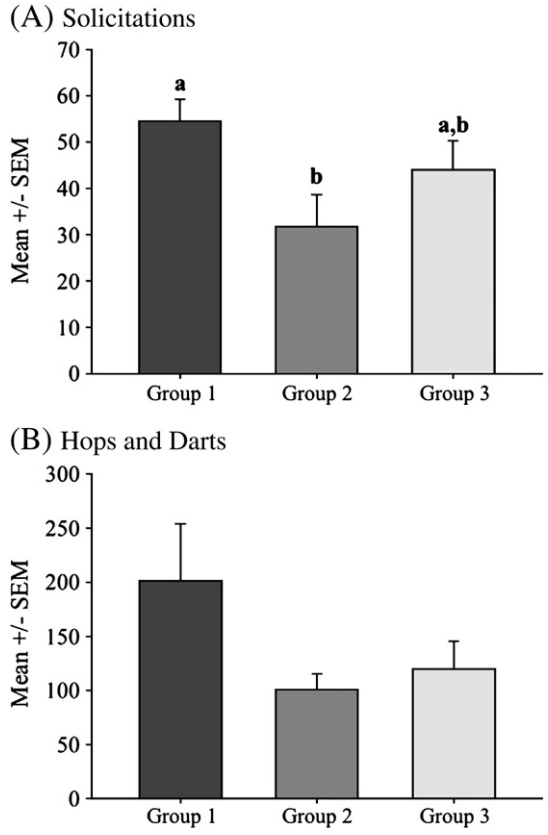

Fig. 1. (A) Mean ± SEM of solicitations and (B) of hops and darts of ovariectomized, hormoned-primed female rats that received precopulatory clitoral stimulation. Group 1 (1 stimulation every second), Group 2 (1 stimulation every 5 s), Group 3 (control, no stimulations). Different letters indicate significant differences.

3.1.2. Grouping by clitoral stimulation and ejaculations

Groups were formed as in Experiment 1 to receive CLS: Group 1 received 1 stimulation every 1 s; Group 2 received 1 stimulation every 5 s; Group 3 was a control and received no stimulation. Each Group was further divided to receive either 1 or 2 ejaculations (see Table 2). Females in Experiment 2 received only one experience of CLS during late proestrous, on the day of copulation, which served to assess the unconditioned effects of CLS on reproduction.

3.1.3. Sexual behavior and reproductive performance

Immediately after receiving CLS, females were placed into a cylindrical chamber with a sexually experienced male chosen randomly from those that had ≥4 days since their last copulation. Sexual behavior was recorded on video until females had received the required number of ejaculations from the males (1 or 2) according to their group, after which the couple was gently separated. Females were housed in groups of 4–5 for approximately 2 weeks after which they were housed in single Plexiglas cages until parturition (days 21–22), or until day 25 post-copulation in, cases where delivery did not occur.

3.1.4. Statistical analyses

Variables of sexual behavior were measured as in Experiment 1. One-way Analysis of Variance (ANOVA) was used to determine differences in the frequencies and latencies of sexual behaviors between the three groups. For the reproductive performance, we first identified females that received high (≥9) or low (≤8) intromission frequency and compared the proportion of pregnant/non-pregnant females per groups with the Fisher's exact test. Then litter size was analyzed by using a Student *t* test between stimulated females (groups 1 and 2 together) vs. control (Group 3). The level of significance for all tests was $p \leq 0.05$.

3.2. Results

Table 2 shows the summary of descriptive results for female and male behavior, as well as the reproductive outcome of the females.

3.2.1. CLS + 1 ejaculation

The statistical analysis did not reveal any significant effect of clitoral stimulation on the sexual behaviors of males and females: mounts $F(2, 21) = 0.3$ $p = 0.74$; intromissions $F(2, 21) = 0.18$ $p = 0.83$; ejaculation latency $F(2, 21) = 1.2$ $p = 0.31$; lordosis magnitude 1 $F(2, 21) = 1.3$ $p = 0.29$; lordosis magnitude 2 $F(2, 21) = 0.22$ $p = 0.80$; lordosis magnitude 3 $F(2, 21) = 0.59$ $p = 0.56$; genital investigation frequency $F(2, 21) = 0.84$ $p = 0.44$; solicitation frequency $F(2, 23) = 0.47$ $p = 0.63$; first solicitation latency $F(2, 21) = 1.3$ $p = 0.28$; hops and darts $F(2, 21) = 0.11$ $p = 0.89$.

However, with regard to reproduction, clitoral stimulation had some effects. The analysis indicated that only the groups that received CLS had pregnant females: Group 1, (2 out of 8, 25%) and in Group 2 (2 out of 8, 25%); whereas in Group 3 none of the females became pregnant (0 out of 8). A supplementary analysis of pregnant females with high or low intromission frequency revealed that in Group 1 only high intromission females became pregnant, but none with low intromission frequency. In Group 2, however, the opposite occurred. None of the females given high intromission frequency, and 2 females with low intromission frequency were pregnant, respectively. In Group 3, none of the females became pregnant. Of the pregnant females in Groups 1 and 2, we ran a student's *t* test to compare litter size, but this did not reveal any significant difference between Group 1 (10.5 ± .7) and Group 2 (8.5 ± 7.7), $t(2) = -0.36$, $p = 0.75$.

3.2.2. CLS + 2 ejaculations

In Experiment 2, each female received 2 ejaculatory series preceded by CLS according to the groups. The statistical analysis

Table 2
Sexual behavior of rats in Experiment 2. Gonadally-intact, sexually naïve females received different patterns of clitoral stimulation prior to copulation with sexually experienced males. Values of sexual behavior and litter size are expressed in mean ± SEM. Significant differences between groups were considered when $p \leq 0.05$.

Variable	Group 1 1 stimulation every 1 s		Group 2 1 stimulation every 5 s		Group 3 Control No stimulation	
Ejaculatory series plus CLS[a]	1 ($n=8$)	2 ($n=10$)	1 ($n=8$)	2 ($n=10$)	1 ($n=8$)	2 ($n=10$)
Female behavior						
Genital	8.25 ± 2.2	2 ± 0.8	8.7 ± 2.6	4.6 ± 2.3	5.1 ± 1.3	2.7 ± 1.7
Investigation						
Solicitations	6.3 ± 2	7 ± 2.2	8.6 ± 2.6	5.8 ± 1.7	5.8 ± 1.5	5.4 ± 1.1
1st solicitation latency (seconds)	124 ± 34	30 ± 9.1	99 ± 44	31.3 ± 9.5	49.5 ± 11.6	69 ± 36.6
Hops and darts	51 ± 16	124 ± 18	46.6 ± 7.7	121 ± 22	43.7 ± 5.7	103 ± 10
Lordosis 1	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.4	1.7 ± 0.7	1.2 ± 0.4	1.4 ± 0.6
Lordosis 2	15.7 ± 6.7	22 ± 2.6	12.8 ± 2.6	19.5 ± 1.9	11.8 ± 1.4	19.8 ± 2.2
Lordosis 3	0.3 ± 0.2	1 ± 0.4	0.8 ± 0.3	0.4 ± 0.2	0.8 ± 0.4	0.3 ± 0.2
Male behavior						
Mounts	9.6 ± 5	9.8 ± 1.8	6.1 ± 1.9	8.4 ± 1.7	7.2 ± 1.5	7.4 ± 1.7
Intromissions	7.1 ± 2.11	11.8 ± 1.8	8.2 ± 1.1	12.3 ± 1.7	7.1 ± 0.9	13.7 ± 1.5
1st ejaculation latency	558 ± 126	337 ± 67	452 ± 84	367 ± 88	356 ± 41	343 ± 88
2nd ejaculation latency	–	591 ± 143	–	574 ± 132	–	514 ± 105
Pregnant/total (%)	2/8 (25%)	2/10 (20%)	2/8 (25%)	4/10 (40%)	0/8 (0%)	1/10 (10%)
Pregnant with ≥9 intromissions	2/2 (100%)	2/7 (28.5%)	0/2 (0%)	4/6 (66.6%) *	0/3 (0%)	1/9 (11.1%)
Pregnant with ≤8 intromissions	0/6 (0%)	0/0 (0%)	2/6 (33.3%)	0/0 (0%)	0/6 (0%)	0/0 (0%)
Litter size (mean ± SEM)	10.5 ± 0.5	7 ± 2	8.5 ± 5.5	8.2 ± 1.1	0	6 ± 0

[a] Each ejaculatory series was preceded by CLS according to each group.

failed to reveal any effect of CLS on the sexual behaviors of males or females: mounts $F(2, 27) = 0.73$ $p = 0.48$; intromissions $F(2, 27) = 0.21$ $p = 0.80$; first ejaculation latency $F(2, 27) = 0.03$ $p = 0.96$; second ejaculation latency $F(2, 27) = 0.09$ $p = 0.90$; lordosis magnitude 1 $F(2, 27) = 1.6$ $p = 0.21$; lordosis magnitude 2 $F(2, 27) = 0.63$ $p = 0.53$; lordosis magnitude 3 $F(2, 27) = 1.7$ $p = 0.19$; genital investigation frequency $F(2, 27) = 0.55$ $p = 0.58$; solicitation frequency $F(2, 27) = 0.39$ $p = 0.67$; first solicitation latency $F(2, 27) = 0.96$ $p = 0.39$; hops and darts $F(2, 27) = 0.33$ $p = 0.72$.

The analysis of the reproductive performance, however, showed that in Group 1: 2 out of 10 females (20%) became pregnant; in Group 2: 4 out of 10, (40%), and in Group 3: 1 out of 10 (10%), although no significant differences were observed. A supplementary analysis of females that received high or low intromission frequency revealed that only those with high intromission frequency (≥9) became pregnant: Group 1: 2 out of 7 females (28.5%) became pregnant; in Group 2: 4 out of 6, (66.6%), and in Group 3: 1 out of 9 (11.1%), whereas none with low intromission (≤8) was pregnant in either group. The Fisher's exact test showed significant differences in the proportion of pregnancy between Group 2 (paced) vs. Group 3 (control) χ^2 (df 1) =, $p = 0.04$; but not in any other pair comparison: Group 1 (nonpaced) vs. Group 2 (paced) χ^2 (df 1) = 1.89, $p = 0.20$; or Group 1 vs. Group 3, χ^2 (df 1) = .79, $p = 0.40$ (Fig. 2). With regard to litter size the student t test failed to reveal significant differences between Group 1 (7 ± 2) and Group 2 (8.2 ± 1.1), $t(4) = -0.6$, $p = 0.57$.

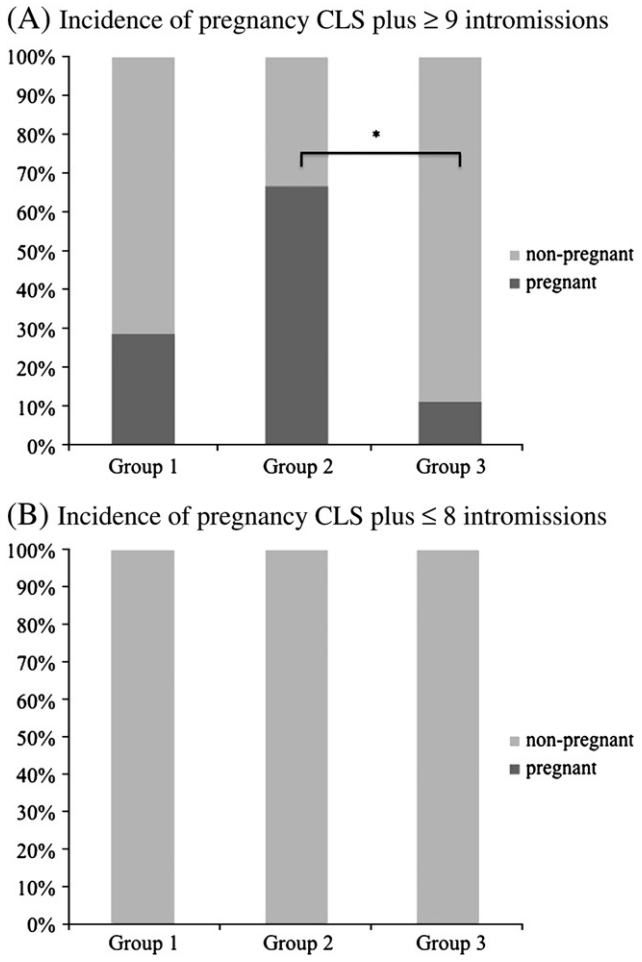

Fig. 2. (A) Percentage of pregnant females that received precopulatory clitoral stimulation plus high frequency of intromissions during copulation (≥9). Group 1 (1 stimulation every second), Group 2 (1 stimulation every 5 s), Group 3 (control, no stimulations). * = $p<0.05$. (B) when females received low intromission frequency (≤8).

4. Discussion

The present study shows that experience with CLS prior to a female rat's first copulatory experience with a male rat modulates the frequency of solicitations. According to our results, females that received distributed CLS solicited less than females that received nondistributed CLS. In addition, this study indicates that distributed CLS may facilitate reproduction once females have received sufficient vaginocervical stimulation (<9 intromissions).

4.1. Effects of CLS on sexual motivation

In Experiment 1, solicitations were either increased or decreased by CLS, depending on the pattern. Ovariectomized, hormone-primed rats that received nondistributed CLS (1 stimulation/s) prior to

copulation with a stud male, were more proceptive (as observed with more solicitations and a trend toward more hops and darts) than females that received distributed CLS (1 stimulation/5 s). However, neither Group 1 nor 2 were different in proceptivity with regard to Group 3 (control) although the mean values showed opposite directions (Group 1 being higher, and Group 2 being lower than Group 3, respectively). This finding is interesting in light of evidence that distributed stimulation (as in Group 2) induced CPP whereas nondistributed stimulation did not [25], although we cannot rule out the possibility that the discrepancy is due to the strain of rat used (albino Wistar rats in the present study, and pigmented Long–Evans rats in the previous study). Solicitations in female rats are directly related to the reward value of copulation, and are used as a preclinical index of sexual desire in female rats [30,31]. We have shown previously that solicitations are directed toward males bearing cues that females have come to associate with paced, relative to nonpaced copulation [7], and that solicitations are selectively reduced in situations of sexual nonreward, e.g., if opioid receptors are blocked by naloxone during early copulatory experience [8]. In addition, it is possible that distributed CLS may induce more "sexual satiety" (if induces CPP may be also more rewarding). Thus, satiety would reduce their number of solicitations relative to the nondistributed group. Regardless of the mechanism, it is clear that experience with distributed or nondistributed CLS affects the ability of OVX, hormone-primed females to solicit during their first copulatory experience.

4.2. Differences between OVX, hormone-primed and gonadally-intact females

The present study also highlights differences in sexual responding between OVX, hormone-primed rats relative to gonadally-intact rats in Proestrus. Whereas distributed CLS enhanced solicitations in the former group, it did not do so significantly in the latter group. Although at first glance this appears as a discrepancy, there are several factors that make those two groups of females very different. The first involves circulating hormone levels. Females in the first group likely had more circulating progesterone than the females in Proestrus. The intact group was assessed for the stage of the ovulatory cycle in the morning and tested approximately 4 h later. This would correspond to an afternoon phase of Proestrus, when estradiol levels have peaked and progesterone levels are beginning to rise. Solicitations are critically dependent on progesterone effects [32–34], thus the increased number of solicitations displayed by the control animals in Experiment 1 relative to the control animals in Experiment 2 likely reflects a difference in circulating progesterone levels. Second, estradiol and progesterone increase sensory responsiveness to tactile stimulation of the perineal skin [35,36]. Although those studies were designed to examine regions of tactile stimulation that induce lordosis, it is equally important to consider sensory input in terms of perineal stimulation induced by the male's pelvic thrusts that would induce natural CLS in addition to the sensory inputs provided by VCS during intromissions (some of which estradiol and progesterone blunt, rather than enhance [37]). Thus, estradiol and progesterone may enhance the ability of CLS to activate excitatory sexual systems in the brain, just as they blunt certain inhibitory sexual systems activated by VCS.

4.3. Effects of CLS on reproduction

CLS alone activates Fos within hypothalamic and limbic regions, including the NAcc, piriform cortex, anterior mPOA, dorsomedial aspect of the ventromedial hypothalamus, and posteroventral aspect of the medial amygdala [25]. The anterior portion of the mPOA contains neurons that stain for GnRH (gonadotrophin releasing hormone), and previous studies have shown that VCS activates Fos within GnRH neurons in this region [38]. It remains to be determined whether CLS may also activate GnRH neurons. Previous studies have also shown that lidocaine infusions to the medial amygdala block the ability of copulatory stimulation to induce pseudopregnancy in gonadally-intact rats [39]. Thus it is possible that CLS could potentiate reproduction by activating systems that underlie ovulation and pseudopregnancy.

Accordingly, the clitoris is innervated by the sensory branch of the pudendal nerve [40], which converges in the L6-S1 trunk of the spinal cord, and neurons of this segment are sensitive to paced or nonpaced sexual stimulation [41]. A direct pathway was also demonstrated from spinal cord to the diencephalon and telencephalon [42] in which axons from different levels of the spinal cord (including lumbosacral) project to the lateral hypothalamus, the posterior hypothalamic area, the dorsal hypothalamic area, suprachiasmatic nucleus, paraventricular nucleus, and to the lateral POA and mPOA. The telencephalic areas with projections from the spinal cord include the ventral pallidum, globus pallidus, substantia innominata, central amygdala, medial and lateral septum, bed nucleus of the stria terminalis, NAcc, infralimbic cortex, and medial orbital cortex, among others [42]. The different patterns of CLS may potentially reach brain areas such as the posterodorsal medial amygdala [43] involved in the processing of unconditioned stimuli that become sexually rewarding depending on the pattern, and areas that participate in both unconditioned and conditioned processing of sexual reward such as the mPOA [30,38,44,45]. Thus, CLS appears to activate both reward- and reproductive-related processes at the same time.

The positive effect on reproduction might also depend on autonomic reflexes associated with sperm transport. Different reflexes that induce uterine contractions following vaginocervical stimulation have been described in women [46] and rats [47], but also mechanostimulation of the clitoris evokes reflexive contractions of the ilio- and pubococcygeus muscles [48]. In cows, manual CLS induces a clitoris–cervix reflex, observed with enlargement of the cervix lumen [49] and uterine contractility [3]; Shafik et al. [46] reported a so-called "clitorouterine reflex" in women which has not yet been reported in rats. Such a reflex could evoke the "suction-pumping" action that aids in sperm transport from the vagina to uterus. Accordingly, precopulatory CLS would be expected activate this reflex more efficiently so that sperm could be transported to the uterus with more efficacy, increasing the likelihood of pregnancy once females have received sufficient intromissions (<9).

In a recent study, the same patterns of clitoral stimulation were studied on sows [50,51]. The authors showed that CLS prior to artificial insemination increased the incidence of pregnancy. In that case, females that received nondistributed CLS (as in Group 1 of the present study) had a higher incidence of pregnancy (up to 95%), followed by females that received distributed CLS (up to 82%), and finally control females (75%). Taken together, the data of rats and sows indicate that CLS may affect reproduction in a positive manner, depending on the pattern of stimulation.

In conclusion, the data of our two experiments indicate that precopulatory clitoral stimulation may increase or decrease solicitations depending on the pattern and rhythm of stimulation in OVX, hormone-primed rats and enhance fertility in gonadally-intact rats that have received sufficient vagino cervical stimulation. Although the actual mechanisms by which CLS modulates appetitive sexual behavior and reproduction remain to be determined, it is likely given previous findings that they involve an activation of reward- and reproductive-related neural, neuroendocrine, and autonomic processes.

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