Behavioral/Cognitive

Pain Reduces Sexual Motivation in Female But Not Male Mice

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Chronic pain is often associated with sexual dysfunction, suggesting that pain can reduce libido. We find that inflammatory pain reduces sexual motivation, measured via mounting behavior and/or proximity in a paced mating paradigm, in female but not male laboratory mice. Pain was produced by injection of inflammogens zymosan A (0.5 mg/ml) or λ-carrageenan (2%) into genital or nongenital (hind paw, tail, cheek) regions. Sexual behavior was significantly reduced in female mice experiencing pain (in all combinations); male mice similarly treated displayed unimpeded sexual motivation. Pain-induced reductions in female sexual behavior were observed in the absence of sex differences in pain-related behavior, and could be rescued by the analgesic, pregabalin, and the libido-enhancing drugs, apomorphine and melanotan-II. These findings suggest that the well known context sensitivity of the human female libido can be explained by evolutionary rather than sociocultural factors, as female mice can be similarly affected.

Key words: **motivation; paced mating; pain; sex difference; sexual behavior**

Introduction

Sex differences in sexual motivation and reward can be demonstrated in laboratory rodents. Male rodents develop conditioned place preferences (CPP) for compartments paired with copulation and postejaculatory satiety, whereas female rodents only develop CPP in specific contexts, when they are allowed to selectively pace (i.e., control the timing and rate of) sexual activity (Pfaus et al., 2003). Whether there are sex differences in the disruption of sexual motivation has never been assessed. Even genetically "fit" animals invariably face circumstances that result in injury or illness, which can compromise reproductive success. Injury can hamper the physical act of copulation, as well as the motivational states that drive animals to seek out and mate with desirable partners. Pain, which often accompanies physical injury and disease, modulates diverse motivational states (Leknes and Tracey, 2008), and thus pain may diminish the rewarding properties of sexual activity in a sex-specific manner consistent with sexual selection strategies (Trivers, 1972). Across numerous species, for instance, physical and environmental stress can diminish female mate preference (Cotton et al., 2006), suggesting that perceived mate value and sexual behavior is context-dependent. In contrast, environmental novelty, sensory disruption, and even castration do not affect the sexual behavior of sexually experienced male rats (Pfaus et al., 2003). In classical animal studies, intense electrical shock inhibits male sexual performance (Beach et al., 1956); it is currently unclear whether shock-induced fear or pain is responsible for this effect.

The link between pain and sexual motivation is evident in human sexual relations. The widespread aphorism, "Not tonight, dear, I have a headache" refers to a lack of sexual motivation due to pain. No clinical data exist on the direct impact of pain on sexual motivation, yet high prevalence of reduced sexual desire in chronic pain populations (Basson et al., 2010; Fine, 2011) suggest that pain may adversely influence sexual motivation. The current study was designed to evaluate the effect of pain on sexual behavior in male and female mice.

Materials and Methods

Animals. Mice were sexually experienced outbred CD-1 (ICR:Crl, Charles River) males (ages 10–25 weeks) and ovariectomized (by the supplier, at 5 weeks of age) females (ages 7–15 weeks). For the female paced mating paradigm (see below) it was critical for the females to be significantly smaller in size (<25 g) than the males (>45 g). Mice were maintained on a 12 h light/dark cycle (lights off at 19:00 h) in a vivarium maintained at ≈21°C, with access to food (Harlan Teklad 8604) and tap water *ad libitum*. All mating testing was conducted during the dark phase, between 20:00–24:00 h. All procedures were approved by the McGill University Animal Care Committee and are fully consistent with Canadian Council on Animal Care guidelines.

Apparatus. For the male open field (M-OF) paradigm, mating occurred in clear Plexiglas chambers (8 × 8.5 × 14 inches), in which the

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male had unrestricted access to a sexually receptive female. The female paced mating (F-PM) paradigm used identical chambers except for a clear Plexiglas partition bisecting the chamber into two halves, a "male" side (8 × 8.5 × 7 inches) and an "escape" side (8 × 8.5 × 7 inches). Four semicircular openings ~1" in diameter (with 0.75 inch space separating each opening) were made in the bottom of the partition that were large enough for females to freely traverse between the male and escape sides, but were too small to permit the larger male mouse to cross. The partition thus allowed the female to enter and leave the male side of the chamber, allowing her to control the timing or "pace" of mating, as the male was confined to one side. Red lights installed above each mating chamber enabled clear video capture of sexual behavior and female position, with cameras mounted 12 inches from the male side, allowing visualization of the male side during F-PM and entire cage during M-OF testing. The video feeds were recorded live using Virtual Dub v1.6.15 software, and Lab Spy v1.5.2 software was used by observers blinded to drug condition to later code the behaviors.

Sexual vigor training. Sexual receptivity was induced in ovariectomized females with subcutaneous (s.c.) injections of estradiol benzoate (5 μg/0.1 ml in sesame oil; Sigma-Aldrich) given 48 h pretest, and progesterone (500 μg/0.1 ml in sesame oil) 5.5 h pretest. These parameters are known to induce a state of optimal sexual receptivity, comparable to the estrus phase (Jones et al., 2013). Hormonally primed ovariectomized females were paired with novel sexually experienced males for "sexual vigor" training sessions (1–2 times/week) in the same mating chambers (in the M-OF configuration) used for testing. Each vigor session lasted 30 min, and only male–female pairs that exhibited ≥10 intromissions (representing <30% of tested pairs) were used (together) for experimental testing, and randomly assigned to be either the test subject or mating partner in either the conventional (M-OF) or paced mating (F-PM) paradigms. This procedure ensured comparable baselines of sexual behavior across groups and ensured that differences in mate preference would not confound results. Vigor testing also served as habituation to the testing environment.

Inflammatory pain. Different combinations of inflammatory mediators and injection sites (both genital and nongenital) were used, to establish generalizability and address various possibilities for confounds, for example the possibility that tenderness in the hind paw might physically impede sexual behavior by affecting weight-bearing ability. Individual male and female mice received, pseudorandomly: (1) a subcutaneous injection of zymosan A (0.5 mg/ml in 10 μl physiological saline, Sigma-Aldrich) into the genitalia (center-posterior vulva or center-dorsal penile shaft) or dorsal right hind paw, (2) a subcutaneous injection of 2% λ-carrageenan (Sigma-Aldrich) dissolved in 10 μl saline into the ventral tail (halfway from base to tip) or right cheek (mid-masseter), (3) a subcutaneous injection of saline only into one of these same locations, or (4) no injection. The mating partner was not injected in any experiment. Both zymosan (a glucan derivative of *Saccharomyces cerevisiae* cell walls) and carrageenan (a linear sulfated polysaccharide extracted from seaweed) are inflammogens used widely to produce tonic inflammatory pain in preclinical studies.

In separate groups of male and female mice not used for mating (n = 6/sex), mechanical sensitivity was quantified immediately before and 4 h postzymosan injection using von Frey monofilaments using the up/down psychophysical method of Dixon (Chaplan et al., 1994).

In separate groups of male and female mice not used for mating (n = 4/sex), the presence of spontaneous pain was assessed immediately before and 4–4.5 h postzymosan injection via facial expressions using the Mouse Grimace Scale (Langford et al., 2010).

Sexual behavior testing (F-PM and M-OF). F-PM and M-OF testing lasted 60 min, based on pilot experiments showing that this duration captured the vast majority of mouse mating behavior, with most mice initiating sexual contact 10–20 min into testing. The following groups were tested in males and females (n = 6–10 per group, except for no injection groups; n = 8–13): (1) vulva (F-PM) or penis (M-OF) zymosan, (2) vulva (F-PM) or penis (M-OF) saline control, (3) hind paw zymosan, (4) hind paw saline control, (5) tail carrageenan, (6) tail saline control, (7) cheek carrageenan, (8) cheek saline control, or (9) no injection control. Injections were given 3 or 4 h pretest (for carrageenan and zymosan, respectively, based on pilot data establishing the peak mechanical allodynia of the hind paw from these inflammatory agents) to mice briefly anesthetized with isoflurane/oxygen.

The male (for M-OF) or female (for F-PM) test mouse was placed into the mating chamber 15 min before the introduction of the opposite-sex, uninjected mating partner. For F-PM testing, males were placed on the side of the partition nearest to the cameras (male side). Immediately following testing, mice in all pain groups were euthanized.

Behavioral measures. Extensive pilot studies revealed differences between mouse sexual behavior and published descriptions of rat sexual behavior, both in the open field and paced mating paradigms (Johansen et al., 2008). Notably, very little hopping and darting was observed by female mice, and sexually receptive females showed variable intensities of lordosis posture during mating. The following behaviors were observed and analyzed: (1) frequency of mounts (defined as direct genital contact by the male onto flanks of the female without achieving penetration, often accompanied by female lordosis), (2) frequency of intromissions (defined as two or more distinct thrusts with penetration), (3) ejaculation frequency, (4) latency to first mount (with or without intromission), (5) interintromission interval, (6) average intromission duration, and (7) ratio of mounts to intromissions. For paced mating, additional variables included: (8) number of exits/entrances to male side, (9) total time spent on male side (overall and following first intromission), and (10) average return latency following each intromission.

Sexual receptivity testing. To test whether zymosan would alter sexual reflexes in female mice, a separate group of virgin female mice (n = 15) were ovariectomized and given the same hormonal priming as described above. Mice were given two tests of receptivity, one before and another 4 h after zymosan injection to the hind paw. The lubricated suction end of a malleable polyethylene transfer pipette (Fisher catalog #13-711-9AM; outer diameter, 3 mm) was inserted into the vagina and pressed against the cervix for 5 s, then withdrawn for another 10 s while the experimenter held the mouse at the base of the tail. A receptive posture was noted when the mouse became immobile, hunched the lower axial muscles, and splayed the hind legs outward, reminiscent of the posture taken during intromission. Each incidence of an artificially generated receptive posture was summed, of a total of 10 applications per test, to generate a receptivity quotient.

Drug administration. To test whether pain inhibition would restore sexual behavior, some mice were pretreated with an analgesic drug, the $\alpha_2\delta$ ligand, pregabalin, at a dose (200 mg/kg; 10 ml/kg, i.p.) we have previously demonstrated to be effective in the amelioration of both spontaneous pain and thermal pain hypersensitivity in mice (Chesler et al., 2003). Pregabalin or saline vehicle were injected 30 min before the start of sexual behavior testing, which occurred in mice injected with carrageenan or vehicle into the hind paw (n = 4–8/group). Pregabalin was kindly provided by Pfizer Canada as part of the Pfizer Pain Research Award program.

Other mice received one of two drugs known to affect libido in rats (Pfaus, 2009). Some received an injection of the nonselective dopamine D1/D2 receptor agonist, apomorphine (100 mg/kg in 50% polyethylene glycol, 10 ml/kg, i.p., Sigma-Aldrich), or vehicle, 45 min before the start of mating testing. Others received injections of melanotan-II (3 mg/kg, 10 ml/kg, i.p., Alpha Diagnostic International), or vehicle, once per day for 5 d before the start of mating testing (including the last injection 5 min before testing). In both cases, mating testing occurred in mice injected with carrageenan or vehicle into the hind paw (n = 4–8/group). Melanotan-II is a prodrug that is converted to the melanocortin-3/melanocortin-4 receptor agonist, bremelanotide, which produces penile erections in rats and primates (Molinoff et al., 2003).

In separate groups of mice not used for mating (n = 6–8/drug), spontaneous pain was quantified immediately before, 3.5–4 h postcarrageenan injection, and 30–60 min postdrug injection via facial expressions using the Mouse Grimace Scale (Langford et al., 2010). The comparison of the latter two time points allowed the quantification of the influence of pregabalin, apomorphine, and melanotan-II on spontaneous pain.

Statistical analysis. Data were analyzed by ANOVA and/or Student's t test as appropriate, using Systat v13 (SPSS). Where appropriate, *posthoc*

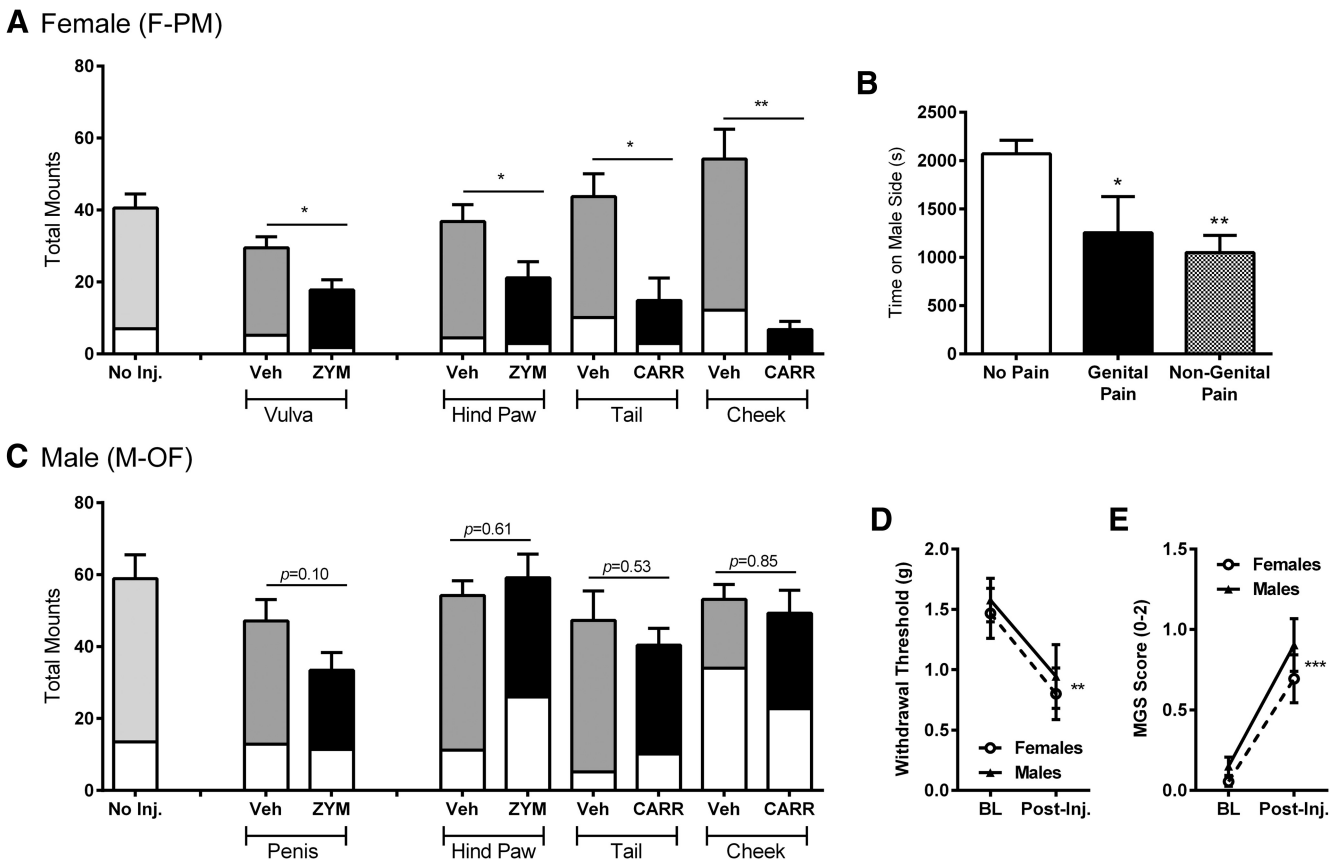

Figure 1. Reduction of sexual behavior in female but not male mice by inflammatory pain. ***A***, Decreased mounting behavior in a paced mating (F-PM) paradigm when female mice receive zymosan (ZYM) or carrageenan (CARR) injections to the vulva, hind paw, tail, or cheek, compared with uninjected female mice (No Inj.). Bars represent mean ± SEM mounts with (shaded) or without (open) intromissions; $n = 7–12$ mice/condition. ***B***, Female mice with genital (vulvar; $n = 6$) or nongenital (combined hind paw, tail, and cheek; $n = 23$) pain spend less time on the side containing the male in a paced-mating chamber compared with mice receiving uninjected or vehicle injected mice (No Pain; $n = 43$). Bars represent mean ± SEM time on male side (s). ***C***, No decreases in mounting behavior in an open field (M-OF) when male mice are treated similarly to female mice. Bars as in *A*; $n = 6–10$ mice/condition. ***D***, No sex difference in mechanical allodynia produced by hind paw zymosan. Symbols represent mean ± SEM threshold (g) to withdraw from von Frey fibers before (BL) and after (Post-Inj.) bilateral zymosan injection; $n = 6$ mice/sex. ***E***, No sex difference in facial grimacing produced by hind paw zymosan. Symbols represent mean ± SEM Mouse Grimace Scale (MGS) score before (BL) and after (Post-Inj.) bilateral zymosan injection; $n = 4$ mice/sex; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with vehicle (***A***, ***C***), No Pain (***B***), or BL (***D***, ***E***).

testing was performed using Dunnett's case-comparison test or Student's *t* test. A criterion $\alpha = 0.05$ was adopted in all cases. In four cases, statistical outliers (studentized residual >3) were omitted from the analyses.

Results

Reduced sexual activity and solicitation behavior in female mice with inflammatory pain

In the F-PM paradigm (Fig. 1*A*), vehicle injection showed no significant effect on total mount frequency compared with no injection ($F_{(4,39)} = 2.3$, n.s.). A two-way ANOVA performed on all groups except uninjected mice revealed a highly significant main effect of pain (zymosan/carrageenan vs vehicle: $F_{(1,54)} = 34.9, p < 0.001$) and a significant pain × site interaction ($F_{(3,54)} = 3.2$, $p < 0.05$). Because each inflammatory stimulus/site was tested concurrently with its own vehicle controls, we then compared each pain group to its vehicle group by Student's *t* test. Inflammatory pain in females produced a reduction in total mounting frequency across stimuli and body sites (Fig. 1*A*), including the vulva ($t_{(14)} = 2.4, p < 0.05$), hind paw ($t_{(15)} = 2.2, p < 0.05$), tail ($t_{(12)} = 2.7$, $p < 0.05$), and cheek ($t_{(13)} = 4.0$, $p < 0.005$). Highly similar results were obtained if mounts with intromissions or mounts without intromissions were analyzed separately (main effects of pain: $F_{(1,54)} = 25.4$, $p < 0.001$, $F_{(1,54)} = 34.2, p < 0.001$, respectively; pain × site interaction: $F_{(3,54)} = 3.4$, $p < 0.05$, $F_{(3,54)} = 3.0, p < 0.05$, respectively). Thus the presence of female pain, genital or nongenital, resulted in an overall reduction in sexual behavior in the paced mating paradigm. Virgin females given vaginocervical stimulation to induce a sexually receptive posture showed a small but nonsignificant reduction in intensity after zymosan injection into the hind paw (baseline receptivity quotient: 9.3 ± 0.4; postzymosan receptivity quotient: 7.8 ± 1.0; repeated-measures ANOVA: $F_{(1,11)} = 2.4$, $p = 0.15$).

Given that mounting frequency is a behavioral measure that involves male participation, we sought to determine whether these robust differences were indeed the result of reduced female sexual motivation. Females in genital or nongenital pain spent significantly less time on the male side ($F_{(2,69)} = 8.7, p < 0.001$; Fig. 1*B*), suggesting that the overall reduction in sexual behavior was indeed due to reduced female sexual motivation. Furthermore, this lowered solicitation behavior was not due to pain-related impairments in motor functioning, given that the total number of barrier crossings did not differ between mice with and without pain ($F_{(2,70)} = 0.4$, n.s.; Table 1). Sexual motivation of the male mating partner seemed unaffected by the pain status of the female mouse, as there were no group differences in latency to mount the female once the female appeared in the male side

Table 1. Sexual behaviors affected by inflammatory pain in female mice in the paced mating (F-PM) paradigm

Condition	*n*	Mount - I (no.)	Mount + I (no.)	Ejac. (no.)	1st Mount (s)	III (s)	ID (s)	M/I (no.)	Crossings (s)	Male side (s)	Return (s)
No treatment	13	7.0 (2.1)	33.5 (3.9)	0.4 (0.1)	609 (178)	70 (10)	12 (2)	0.8 (0.04)	43.4 (13.6)	1962 (222)	159 (61)
Saline-vulva	8	5.1 (1.4)	24.4 (3.1)	0.5 (0.2)	1020 (238)	79 (7)	13 (1)	0.8 (0.03)	38.6 (8.4)	1611 (317)	210 (119)
Saline-hind paw	9	4.4 (1.3)	32.3 (4.7)	0.7 (0.2)	293 (120)	75 (12)	12 (1)	0.9 (0.04)	41.0 (15.2)	2090 (333)	60 (19)
Saline-tail	7	10.1 (3.4)	33.6 (6.3)	0.3 (0.2)	428 (175)	60 (6)	15 (3)	0.8 (0.06)	27.3 (11.2)	1905 (302)	90 (29)
Saline-cheek	7	12.1 (2.5)	42.0 (8.3)	0.3 (0.2)	1245 (218)	34 (7)	9 (1)	0.7 (0.11)	14.0 (7.2)	2721 (426)	31 (20)
No pain	**44**	**7.5 (1.0)**	**32.8 (2.3)**	**0.4 (0.1)**	**691 (96)**	**65 (5)**	**12 (1)**	**0.8 (0.02)**	**34.8 (5.7)**	**2036 (141)**	**115 (31)**
ZYM-vulva (*Genital pain*)	**8**	**1.8 (0.4)****	**16.0 (2.8)****	**0.2 (0.2)**	**765 (450)**	**112 (26)**	**22 (4)****	**0.9 (0.02)**	**41.0 (15.1)**	**1254 (375)***	**211 (114)**
ZYM-hind paw	8	2.9 (0.8)	18.2 (4.5)	0.1 (0.1)	1076 (315)	133 (36)	12 (1)	0.9 (0.05)	30.1 (11.4)	1430 (397)	392 (117)
CARR-tail	7	2.9 (1.1)	12.0 (6.2)	0.6 (0.2)	582 (147)	137 (58)	17 (5)	0.6 (0.14)	40.6 (15.5)	1025 (272)	109 (39)
CARR-cheek	8	0.9 (0.6)	5.8 (2.3)	0 (0)	602 (400)	78 (34)	7 (3)	0.4 (0.19)	15.4 (4.8)	686 (191)	377 (144)
Nongenital pain	**23**	**2.2 (0.5)****	**12.0 (2.7)****	**0.2 (0.1)**	**761 (181)**	**116 (25)***	**12 (2)**	**0.6 (0.08)***	**28.4 (6.5)**	**1048 (179)****	**290 (68)***

Values represent mean (SEM). Mount - I, Frequency of mounts (defined as direct genital contact by the male onto flanks of the female without achieving penetration, often accompanied by female lordosis) without intromission; Mount + I, frequency mounts with intromission (defined as two or more distinct thrusts with penetration); Ejac., ejaculation frequency; 1st Mount, latency to first mount (with or without intromission); III, average inter-intromission interval; ID, average intromission duration; M/I, ratio of mounts to intromissions; Crossings, number of entries/exits into the male side; Male side, total time spent on male side; Return, average latency to return to the male side following intromission; CARR, carrageenan; ZYM, zymosan.

*$p < 0.05$ compared with No Pain; **$p < 0.01$ compared with No Pain. ANOVAs were followed by Dunnett's case-comparison posthoc test with No Pain as the comparator group.

Table 2. Sexual behaviors not affected by inflammatory pain in male mice in the open field mating (M-OF) paradigm

Condition	*n*	Mount - I (no.)	Mount + I (no.)	Ejac. (no.)	1st Mount (s)	III (s)	ID (s)	M/I (s)
No treatment	8	13.5 (5.1)	45.4 (6.6)	0 (0)	432 (177)	63 (10)	11 (1)	0.8 (0.05)
Saline-penis	7	12.9 (7.1)	34.3 (5.9)	0.4 (0.2)	451 (331)	82 (17)	13 (1)	0.8 (0.11)
Saline-hind paw	6	11.2 (4.7)	43.0 (4.1)	0.5 (0.2)	195 (69)	52 (6)	11 (1)	0.8 (0.06)
Saline-tail	7	5.1 (1.6)	42.1 (8.1)	0.3 (0.2)	220 (75)	78 (28)	13 (2)	0.7 (0.12)
Saline-cheek	8	34.0 (18.8)	19.1 (4.1)	0.8 (0.2)	220 (67)	85 (18)	15 (1)	0.5 (0.10)
No pain	**36**	**16.0 (4.7)**	**36.4 (3.0)**	**0.4 (0.1)**	**314 (81)**	**73 (8)**	**13 (1)**	**0.7 (0.04)**
ZYM-penis (*Genital pain*)	**10**	**11.4 (3.5)**	**22.0 (5.0)**	**0.4 (0.2)**	**540 (219)**	**83 (23)**	**12 (1)**	**0.6 (0.10)**
ZYM-hind paw	7	26.0 (9.5)	33.1 (6.5)	0.4 (0.2)	168 (94)	78 (14)	11 (1)	0.6 (0.13)
CARR-tail	8	10.1 (2.3)	30.2 (4.7)	0.6 (0.2)	376 (76)	103 (22)	10 (1)	0.8 (0.05)
CARR-cheek	7	22.7 (5.1)	26.6 (6.4)	0.8 (0.1)	269 (43)	103 (30)	14 (1)	0.5 (0.07)
Nongenital pain	**22**	**11.4 (3.5)**	**30.0 (3.3)**	**0.6 (0.1)**	**282 (44)**	**96 (13)**	**11 (1)**	**0.6 (0.05)**

Values represent mean (SEM). Mount - I, Frequency of mounts (defined as direct genital contact by the male onto flanks of the female without achieving penetration, often accompanied by female lordosis) without intromission; Mount + I, frequency mounts with intromission (defined as two or more distinct thrusts with penetration); Ejac., ejaculation frequency; 1st Mount, latency to first mount (with or without intromission); III, average inter-intromission interval; ID, average intromission duration; M/I, ratio of mounts to intromissions; CARR, carrageenan; ZYM, zymosan.

($F_{(2,70)} = 0.8$, n.s.; Table 1). Thus, the decreased mounting observed appeared to be purely due to the male-avoidance behavior of the female mouse.

No change in sexual activity in male mice with inflammatory pain

In the M-OF paradigm (Fig. 1*C*), vehicle treatment showed no effect on total mount frequency compared with no injection ($F_{(2,65)} = 0.9$, n.s.). A two-way ANOVA performed on all groups except uninjected mice revealed no significant main effects or interactions (all $p > 0.05$). Inflammatory pain in male mice was found to have no effect on total mount frequency in any body site (Fig. 1*C*, Table 2), including the penis ($t_{(15)} = 1.6$, n.s.), hind paw ($t_{(11)} = 0.5$, n.s.), tail ($t_{(13)} = 0.7$, n.s.), and cheek ($t_{(13)} = 0.2$, n.s.).

Females and males show equivalent sensitivity to inflammatory pain

Although highly dependent on strain and assay (Mogil et al., 2000), female mice are sometimes found to be more sensitive to pain than males (Mogil, 2012), and this might explain our current observations if pain levels in females but not males were above some threshold level. Zymosan was administered into the hind paws of a separate group of mice using identical parameters as used in the mating studies, and mechanical hypersensitivity (allodynia) and spontaneous pain were measured using von Frey fibers and the Mouse Grimace Scale, respectively. Zymosan produced marked allodynia and spontaneous pain, as expected (repeated measures: $F_{(1,10)} = 14.7$, $p < 0.005$, $F_{(1,6)} = 38.5$, $p = 0.001$, respectively), but no significant main effects or interactions with sex were observed (Fig. 1*D*,*E*).

Pregabalin reverses reductions in sexual behavior due to female inflammatory hind paw pain

The administration of pregabalin, an analgesic drug acting at the $\alpha_2\delta$ calcium channel site, while producing no significant changes in mounting behavior *per se* ($t_9 = 0.4$, n.s.), was able to fully reverse the reduction in mounting behavior produced by hind paw carrageenan in female mice ($t_{(8)} = 2.3$, $p < 0.05$; Fig. 2*A*). The analgesic efficacy of pregabalin at the 200 mg/kg dose was confirmed using the Mouse Grimace Scale (drug × repeated measures: $F_{(2,20)} = 6.7$, $p < 0.005$; Fig. 2*B*).

Prosexual drugs reverse reductions in sexual behavior due to female inflammatory hind paw pain

The administration of apomorphine, a dopamine agonist, although producing no significant changes in mounting behavior *per se* ($t_{(14)} = 0.6$, n.s.), was able to fully reverse the reduction in mounting behavior produced by hind paw carrageenan in female mice ($t_{(14)} = 2.8$, $p = 0.01$; Fig. 2*C*). Apomorphine also increased time spent on the male side in the presence of pain ($t_{(13)} = 2.5$, $p < 0.05$), but not in the absence of pain ($t_{(14)} = 0.8$, n.s.; data not shown). As expected, apomorphine had no effect on carrageenan-induced pain (drug × repeated measures: $F_{(2,28)} = 0.5$, n.s.; Fig. 2*D*).

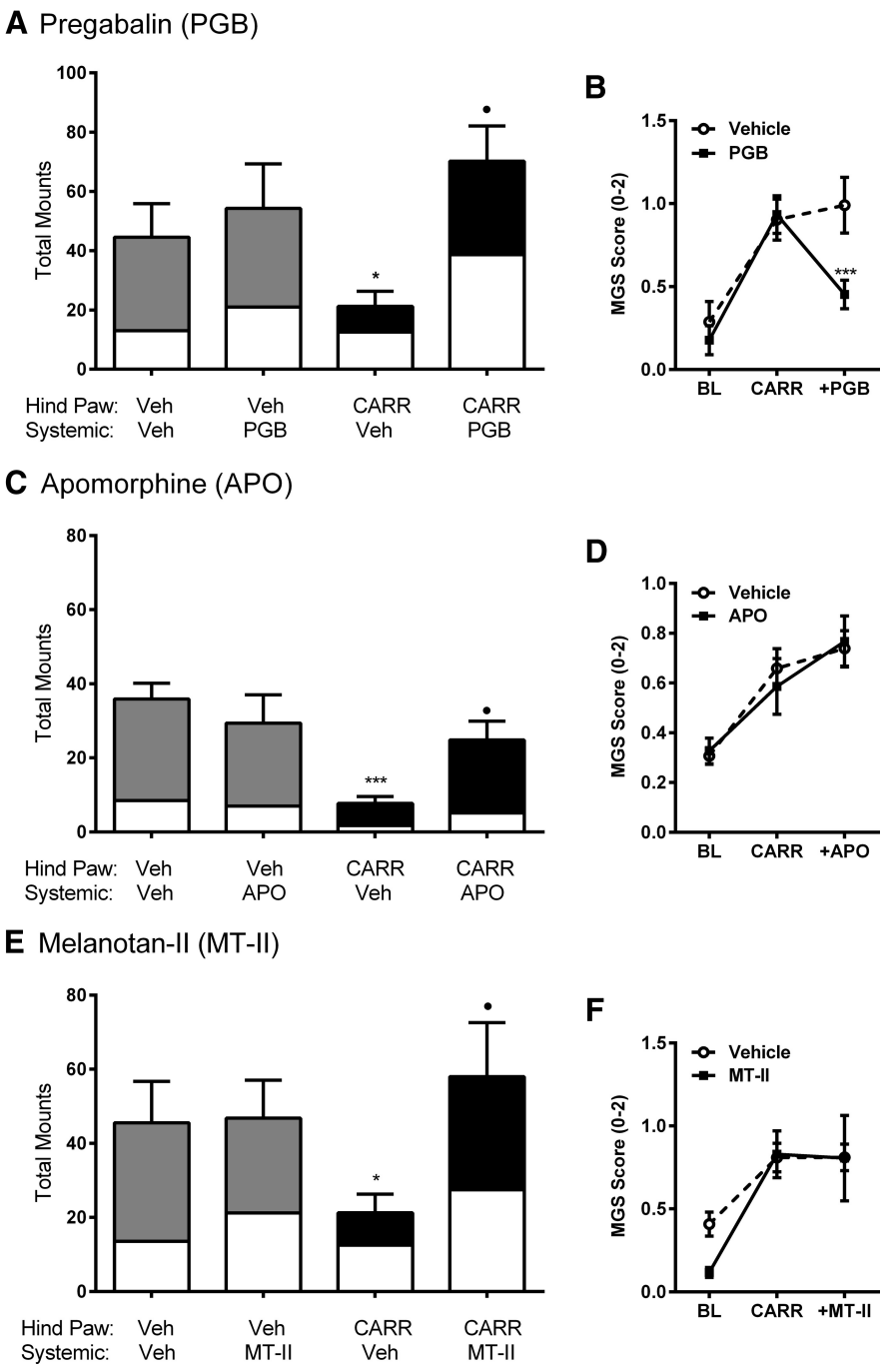

Figure 2. Reversal of hind paw carrageenan (CARR)-induced reduction in female sexual behavior (all F-PM) by the analgesic, pregabalin (PGB; ***A***), and the libido-enhancing drugs apomorphine (APO; ***C***) and melanotan-II (MT-II; ***E***). The analgesic efficacy of PGB against was confirmed (***B***), as was the lack of effect on pain by APO (***D***) and MT-II (***F***). Bars in ***A***, ***C***, ***E*** represent mean ± SEM mounts with (shaded) or without (open) intromissions; $n = 4–8$ mice/condition. Symbols in ***B***, ***D***, ***F*** represent mean ± SEM Mouse Grimace Scale (MGS) score before carrageenan (BL), after carrageenan but before drug (CARR), and after drug (+PGB/+APO/+MT-II); $n = 6–8$ mice/drug; $^{*}p < 0.05$, $^{***}p < 0.001$ compared with Veh/Veh group (***A***, ***C***, ***E***) or vehicle (***B***, ***D***, ***F***); $^{\bullet}p < 0.05$ compared with CARR/Veh group.

Similarly, the administration of melanotan-II, an α-melanocyte-stimulating hormone analog, although producing no significant changes in mounting behavior *per se* ($t_9 = 0.1$, n.s.), was able to fully reverse the reduction in mounting behavior produced by hind paw carrageenan in female mice ($t_{(7)} = 2.4, p < 0.05$; Fig. 2*E*). As expected, melanotan-II had no effect on carrageenan-induced pain (drug × repeated measures: $F_{(2,24)} = 1.9$, n.s.; Fig. 2*F*).

Discussion

We have observed sex differences in the effect of pain on sexual behavior in rodents. The sex difference observed here appears regardless of the site of pain and the inflammatory compound used. Female mice in pain reduce the amount of time voluntarily spent in proximity to previously preferred male mice, leading to reduced copulatory behavior in a paced mating paradigm. This reduction occurred despite the fact that pain did not significantly affect the ability of the female to assume a sexually receptive posture during artificial vaginocervical stimulation, suggesting that pain did not compromise the physiological "ability" of the female mouse to mate. The reduced sexual motivation displayed by female mice can be rescued either by reducing the pain itself, with the analgesic pregabalin, or by pretreatment with libido-enhancing drugs apomorphine or melanotan-II. In contrast, male sexual behavior is unaffected by pain. We observed a trend toward reduced mounting behavior in the presence of penile pain ($p = 0.10$), but in contrast to female mice, absolutely no reductions were observed in male mice with nongenital pain. We are aware of only one previous study examining the effect of pain on sexual behavior, in which male rats with nerve injury (and neuropathic mechanical and cold allodynia) were found to have unaltered mounting behavior (Keay et al., 2004). Another group observed no obvious change in sexual behaviors in female rats with surgically induced endometriosis (Clark et al., 2011), but in that study the presence of pain was never assessed.

In the paced mating paradigm, motivation is inferred from the amount of time spent on the male side, given that the female regulates the timing and frequency of sexual activity. When pain-free females are allowed to pace sexual activity, the preferred frequency of mounting is reduced compared with nonpaced situations wherein males have unfettered access to receptive females (Fig. 1*A*,*C*; compare uninjected groups), as has been observed in rats (Erskine, 1985; Martínez and Paredes, 2001). We find here that the presence of pain dramatically reduces the time a female spends with a previously preferred sexually vigorous male, and results in decreased frequency of copulation. This effect cannot be explained by sex differences in pain sensitivity, which depend on stimulus modality and genotype (Mogil et al., 2000) and were not observed here. Nor can the reduction in mounting behavior in females be attributed to discomfort due to weight bearing, with the affected hind paw (or perhaps also the

tail) being aggravated by the physical act of being mounted, given that cheek pain produced equivalent reductions in sexual activity. Although reduced cutaneous sensitivity on the rump or genitals may adversely impact lordosis and copulation rate (Pfaff et al., 1977), nongenital pain was equally effective as genital pain at impairing behavioral indices of female sexual motivation. Analgesia associated with mating has been reported for male as well as female rodents (i.e., vaginocervical stimulation-evoked analgesia), but has only been shown to relieve pain for extremely brief periods of time (Gómora et al., 1994; Gonzalez-Mariscal et al., 1994), and would be expected to produce effects in the opposite direction to those observed. Therefore it is highly unlikely that purely sensory mechanisms can account for the present findings.

Dopamine and melanocortin modulation with apomorphine and melanotan-II, respectively, largely restored the pain-induced sexual behavior deficit in female mice, suggesting either that these drugs enhance sexual motivation (restoring the impairment of sexual motivation caused by pain), or that they have analgesic properties like pregabalin. However, the doses we administered produced no alterations in facial grimacing due to zymosan, and thus it is unlikely that they produced analgesia. Prosexual effects in rodents and humans have been reported with dopamine and melanocortin-3/-4 receptor agonists, with both enhancing female solicitations (Pfaus, 2009). In our hands, and at the doses used, these drugs did not affect paced mating behavior *per se*. Thus, the reversal of pain-induced reductions in female-paced sexual behavior likely reflects an enhanced incentive value of the paced mating context, indicating that motivational mechanisms can overcome the effects of pain. We suggest that restoration of pain-induced loss of libido may provide a more sensitive test of prosexual drugs than current paradigms.

Female rodents show a CPP to female-paced sexual encounters (Martínez and Paredes, 2001), indicating that pacing is in itself rewarding. Furthermore, we controlled for baseline differences in female mate preference by only testing couples that had previously demonstrated a high rate of copulation, and thus the incentive value of their mates were roughly equivalent. Thus, the female susceptibility to the effects of pain indicates that a previously rewarding context (paced mating of a preferred male) has lost its incentive value. This shift in incentive value is not inherent to pain in general, given that males continue to seek sexually rewarding contact with females despite equivalent discomfort. Rather, the motivational shift is unique to the females' experience of sexual reward. It is therefore feasible that the reduction in time spent with the male reflects a reduced intensity of mate preference. Comparative evidence supports this hypothesis, as a number of stressors including dietary deprivation, extreme temperatures, and predation can reduce a female preference for a high-value male partner (Cotton et al., 2006). Alternatively, the female's reduction in sexual interest may reflect a decision regarding her current physiological state. The female's heightened sensitivity to pain states is consistent with the behavior of an organism with high parental investment (Trivers, 1972). A female in pain may lack the motivation and energy required to sustain pregnancy and rear pups, or may lose sexual interest in reaction to the chances of successful pregnancy and pup rearing being low. Male mice, on the other hand, are under intense evolutionary pressure to copulate regardless of their or their mate's current circumstances.

The possibility remains that the differences between paced and nonpaced sexual activity could have, in part, reflected reduced motivation of the male. Pursuit of a female is reinforcing for male rats, such that the pharmacological inhibition of female pacing and solicitation behavior impairs the male's initiation of sexual behavior (Everitt, 1990). Given that female mice in pain showed reduced pacing compared with pain-free females, it might be argued that their behavior may have been less reinforcing to males than higher-pacing females. Similarly, male mice may find the scent of a female in pain less appealing and reduce their approach behavior. However, in rats even aversive scents, such as cadaverine on a receptive female, fail to inhibit male sexual behavior (Pfaus et al., 2012), suggesting that aversive scent does not impact male sexual motivation.

Although sexuality relies on biological mechanisms to facilitate reproduction, it is widely believed that the expression of sexuality in our species is dictated by culturally constructed expectations of sexual motivation and behavior (Eagly and Wood, 1999). Cross-sectional self-report data indicate that, compared with men, women's sexual desire and arousal are regulated more by inhibitory factors that restrict sexual motivation and behavior (e.g., negative mood, distraction, fear of pregnancy or disease; Carpenter et al., 2008). Our results suggest that such inhibitory factors reduce sexual motivation in mice as well as humans. The interaction between pain and sexual motivation conforms to the patterns of sexual behavior predicted by parental investment theory, and suggests that the context-dependence of female sexuality (Meana, 2010) across species has a biological rather than sociocultural basis.

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