American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 12, No 1 (January), 2011: pp 125-132
Available online at www.sciencedirect.com

Varying Perceived Social Threat Modulates Pain Behavior in Male Mice

Dale J. Langford,* Alexander H. Tuttle,† Ciara Briscoe,* Colin Harvey-Lewis,* Inna Baran,* Patrick Gleeson,† David B. Fischer,† Michele Buonora,† Wendy F. Sternberg,† and Jeffrey S. Mogil*

**Department of Psychology and Alan Edwards Centre for Research on Pain, McGill University, Montreal, QC, Canada.*
†Department of Psychology, Haverford College, Haverford, Pennsylvania.

Abstract: We previously demonstrated that male mice display significantly reduced pain behavior on the acetic acid abdominal constriction test when confined in close proximity to a stranger male mouse. We show here the testosterone-dependence (via castration and testosterone propionate replacement) of this phenomenon, likely a form of (social) stress-induced analgesia. However, when similar male dyads are separated by vertical metal bars, allowing only partial physical contact, we find that the mice exhibit hyperalgesia, not analgesia, in response to both acetic acid injection and noxious radiant heat, relative to testing in isolation. This finding is specific to same-sex male dyads, and no change in nociceptive sensitivity is observed when males are tested in the presence of a female conspecific. We propose that pain sensitivity varies with respect to the severity of the social threat: mild social threat produces hyperalgesia and more severe social threat produces analgesia.

***Perspective:** This work highlights the importance of social threat in modulating pain behavior in a sex-specific manner. The findings add to a growing body of evidence that social factors affect pain behavior in mice, thus allowing the study of the mechanistic underpinnings of social modulation of pain in humans.*

***Key words:** Pain, stress, threat, social, sex difference, testosterone.*

In an effort to determine the effects of the immediate social environment on pain sensitivity, we previously tested mice—using the sensitive acetic acid abdominal constriction ("writhing") test—in various dyadic (social) conditions, and compared pain behavior to that of mice tested in isolation. We observed significant modulation of pain behavior (hyperalgesia and temporal synchronization) in familiar dyads in which both mice received the acetic acid injection, and interpreted these findings as evidence for pain empathy in mice.[22] We also observed an interesting sex-specific phenomenon in stranger dyads in which only 1 mouse in the dyad was injected. Specifically, we found that a subset of male mice, when tested in the presence of a naïve stranger male, exhibited greatly reduced pain behavior relative to levels observed when testing in isolation.[22]

We speculated that this latter phenomenon was a form of social stress-induced analgesia related to threat. The impact of stress on pain sensitivity is well established; stress has been observed to inhibit or exacerbate pain perception depending on the nature and/or parameters of the stressor.[5,16] Indeed, it would be advantageous to inhibit pain behavior in a potentially dangerous situation in order to facilitate escape, whereas in other circumstances vigilance to painful stimuli might be more beneficial. We became interested in determining whether this social modulation of pain behavior could be reversed by altering the perceived threat, either by manipulating hormonal status through gonadectomy, or by manipulating the testing environment to ensure physical safety from conspecific aggression.

Castration has been shown to reduce social conflict and attack in rats and mice.[3,25] In nonhuman primates, gonadectomy during adolescence has been shown to

Received April 14, 2010; Revised May 18, 2010; Accepted June 4, 2010.
Supported by the Louise and Alan Edwards Foundation (J.S.M.) and the National Science Foundation (W.F.S.). D.J.L. was supported by a Canada Graduate Scholarship (CGS) Doctoral Award. A.H.T. was supported by the Arnold and Mabel Beckman Foundation.
Address reprint requests to Jeffrey S. Mogil, PhD, Dept. of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, QC H3A 1B1 Canada. E-mail: jeffrey.mogil@mcgill.ca
1526-5900/$36.00

doi:10.1016/j.jpain.2010.06.003

significantly impair social behavior evidenced by reduced displays of dominance and relative disinterest in unfamiliar conspecifics.[34] Conversely, testosterone administration has been shown to facilitate intermale aggression in rodents.[12] If the inhibition of pain behavior previously observed was truly due to social stress related to the possibility of intermale aggression (ie, social threat), the phenomenon should be attenuated using a gonadectomized partner and reinstated if that partner received testosterone replacement. It is also conceivable that gonadectomy of the test mouse would abolish the effect, by signaling the submissive role of the test mouse, similarly defusing the possibility of aggression.

Using similar logic, we also predicted that we might block the phenomenon by limiting the opportunity for physical aggression, presumably thereby reducing the inherent social threat. In our original paradigm,[22] mice were tested in a Plexiglas cylinder (15-cm diameter; 22.5-cm high), with no barriers between the mice. In the present study, we placed a barrier (vertical metal bars) between the 2 mice, eliminating the possibility of effective attack (because mice can easily retreat beyond the reach of the aggressing mouse), but still allowing social interaction. We also predicted that female-female dyads as well as male-female dyads tested in this paradigm would not evince any threat-related pain modulation, since female mice pose no threat to larger males (≈20 g versus ≈35 g, respectively). Females show no aggression against males unless they are attacking their pups or trying to mount them when they are not in estrus, and female-female aggression rarely occurs in group-housed laboratory mice.[28]

Methods

These studies were conducted at Haverford College and McGill University. All procedures were approved by local Institutional Animal Care and Use Committees, subject to national (U.S. and Canadian) guidelines.

Subjects

Mice used in this study were of the outbred CD-1 (ICR) strain (Harlan, Indianapolis, IN, or Charles River, Boucherville, QC). Animals were housed in a light- (12:12 hour light:dark cycle, lights on at 0730), and temperature (20°C)-controlled facility, housed in standard shoebox cages in same-sex groups of 3 to 6 mice, with food (Harlan Teklad 8604) and tap water available ad lib. Mice were habituated to the vivarium for at least 1 week before testing. All experiments were conducted during the animal's light cycle.

Nociceptive Assays

Acetic Acid Abdominal Constriction ("Writhing") Test

In this assay, .9% acetic acid is injected intraperitoneally (10 mL/kg). This test of tonic inflammatory nociception involves a clear quantifiable pain behavior (stretching of the abdominal musculature), and is relatively mild in intensity, allowing the detection of subtle modulatory factors.[17] After a 30-minute habituation period, mice were injected with acetic acid and immediately returned to the testing apparatus (see below). Animals were digitally videotaped for 30 minutes postinjection, and pain behavior was quantified by an observer blinded in as much as possible to experimental condition, using an accurate and reliable time-sampling method in which the presence/absence of writhing was scored for the first 5 seconds of every 20-second interval.[22]

Radiant Heat Paw-Withdrawal Test

In this assay,[15] mice are confined atop a ¼ -inch-thick glass floor located 6 cm above a projector lamp bulb (Model 336 Plantar Analgesia Meter; IITC Life Science Inc, Woodland Hills, CA). A 2- to 3-hour-long habituation time is necessary to reduce activity levels sufficiently to allow testing.[6] After habituation, a noxious radiant heat stimulus (20% maximal intensity; ≈45 W) was applied to the plantar surface of the hind paw, and the latency to purposeful paw withdrawal was recorded to the nearest .1 second. Five measurements per hind paw (separated by at least 20 seconds) were recorded and averaged for each subject.

Testing Apparatus

In some experiments, mice were habituated and tested in transparent Plexiglas observation cylinders (15-cm diameter; 22.5-cm high), allowing completely unimpeded physical contact between them. Mice were either tested in isolation, or in a "One Writhing" condition as previously described,[22] in which 1 mouse of a dyad was injected with acetic acid (as above) and the other was not. Overt physical aggression was rare, but did in fact occur in ≈4% of intact male dyads. No physical aggression whatsoever was seen in dyads containing a castrated male or female mouse. In other experiments, mice were habituated and tested in adjacent Plexiglas observation cubicles (9 × 5 × 5-cm high), separated by thin (2-mm wide) vertical metal bars. Social interactions could and did occur between the 2 mice, but physical aggression was not possible, since the attacked mouse could simply withdraw beyond the biting range of the attacker. In the "No Neighbor" version of this paradigm, the vertical bars were left in place. We have determined in pilot studies (data not shown) that the different dimensions of the testing apparatuses do not affect writhing behavior.

Gonadectomy and Hormone Replacement

Gonadectomy

Castration surgery was performed under isoflurane/oxygen anesthesia. Bilateral incisions were made in the scrotum, and testes were isolated and exposed. A hemostat was used to clamp the vas deferens, and the testis and testicular fat was removed from each side. Sutures were placed using 3-0 silk as necessary to close the incision. Sham gonadectomy was performed under similar conditions, except that no testicular tissue was removed.

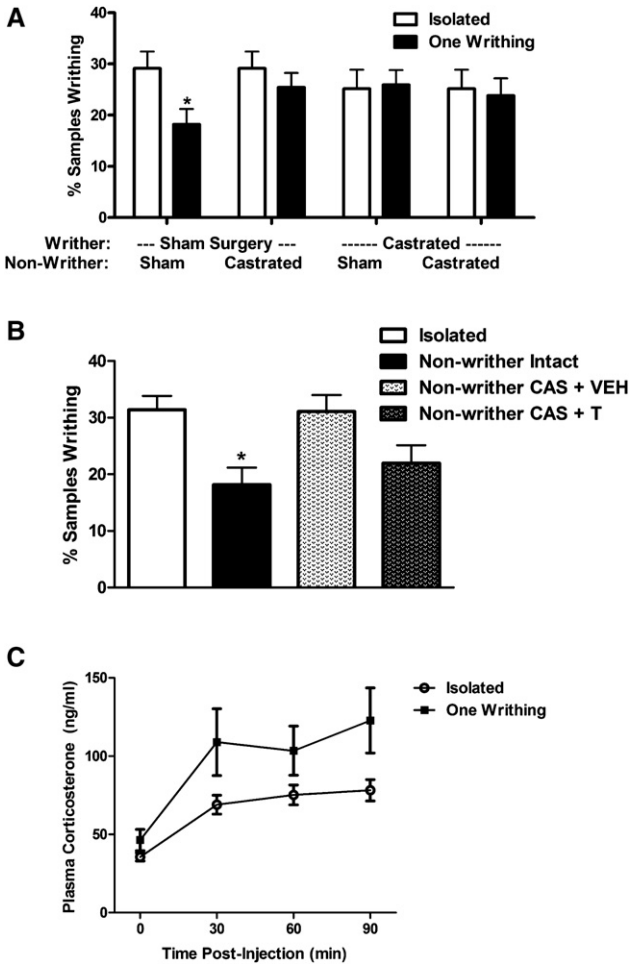

Figure 1. Reduction of pain behavior in stranger male dyads in which the nonwrither is hormonally intact or testosterone replaced and full contact is permitted. Bars represent mean ± S.E.M. percentage of samples featuring writhing behavior over 30 minutes (n = 13-18/condition). **(A)** Male mice injected with acetic acid ("Writher") tested in the presence ("One Writhing" condition) of a hormonally intact, unaffected, stranger male mouse ("Non-writher") significantly inhibit their writhing behavior compared to mice tested in isolation (Isolated). **(B)** Castration (CAS) of the non-writher abolishes the effect, and testosterone propionate (T) replacement reinstates the inhibition. **(C)** Increased plasma corticosterone levels in "writher" mice in the One Writhing condition compared to those tested in isolation. Symbols represent mean ± S.E.M. plasma corticosterone (ng/mL) (n = 18-24/condition). VEH = vehicle treatment (sesame oil). *P < .05 compared to Isolated condition.

Behavioral testing commenced no less than 2 weeks following surgery.

Hormone Replacement

Approximately 1 week following gonadectomy, Silastic tubing (.062" id) was cut to a length of 15 mm, and packed with crystalline testosterone propionate to a length of 10 mm (≈10 mg). The ends of the capsule were sealed with Silastic adhesive. Pellets were cured overnight in PBS and rinsed with 70% ethanol followed by sterile saline just prior to implantation. These procedures are adapted from Lindzey et al,[24] who found capsules of these dimensions to restore 80% of seminal vesicle weight and reverse the plasma testosterone reduction resulting from castration in male mice. Empty pellets were used as a control. Pellets were implanted subcutaneously at the shoulder, under isoflurane/oxygen anesthesia. The incision was closed with 3-0 silk. Behavioral testing commenced no less than 2 weeks following pellet implantation.

Corticosterone Assay

A separate group of gonadally intact male mice was used for this assay. Tail blood was sampled from the mouse exposed to the noxious stimulus by removing the distal end of the tail with sharp surgical scissors, and collecting beads of blood into a sample tube containing 6-μl EDTA. Samples were collected at 4 time points: just prior to injection with acetic acid, upon removal from the observation cylinder (30 minutes postinjection), and at 30-minute intervals thereafter (60 and 90 minutes postinjection). Approximately 50 μl was collected during each draw. A styptic pencil was used to curb bleeding in between draws. Samples were stored on ice until the last draw, then all samples were centrifuged at 4°C for 15 minutes at 5,000 rpm. Plasma was pipetted from each sample and stored at –70°C until processing.

Plasma corticosterone was assayed through the use of an enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI; #500655). Samples were processed at a 1:10 dilution in EIA buffer in duplicate, and the assay proceeded according to the kit protocol. Single absorbance readings of the assay plate at 450 nm were obtained (Tecan plate reader; Tecan US Inc, Durham, NC) and used for the calculation of plasma corticosterone levels (ng/mL) based on a linear regression of the standard curve values.

Fecal Boli

Fecal boli deposits were determined by counting visible boli immediately preinjection, and subtracting this amount from the number of boli counted at the end of the postinjection testing period. Initial boli counts did not differ between groups (data not shown).

Statistical Analyses

All data were analyzed using SYSTAT v.10 (SPSS, Inc, Chicago, IL) and graphically displayed using Prism 5.0 (GraphPad Software; La Jolla, CA). A criterion α = .05 was set for all statistical tests.

Results

Pain Inhibition and Increased Corticosteroid Release Produced by Unhindered Social Interaction

As shown in Fig 1A, only test mice from stranger "one writhing" dyads in which both mice received sham operations (ie, were androgenically intact) displayed significantly reduced pain behavior (t_{25} = 2.4, P < .05) relative to the appropriate isolated condition.

In the testosterone replacement experiment, a 1-way ANOVA performed on writhing behavior indicated

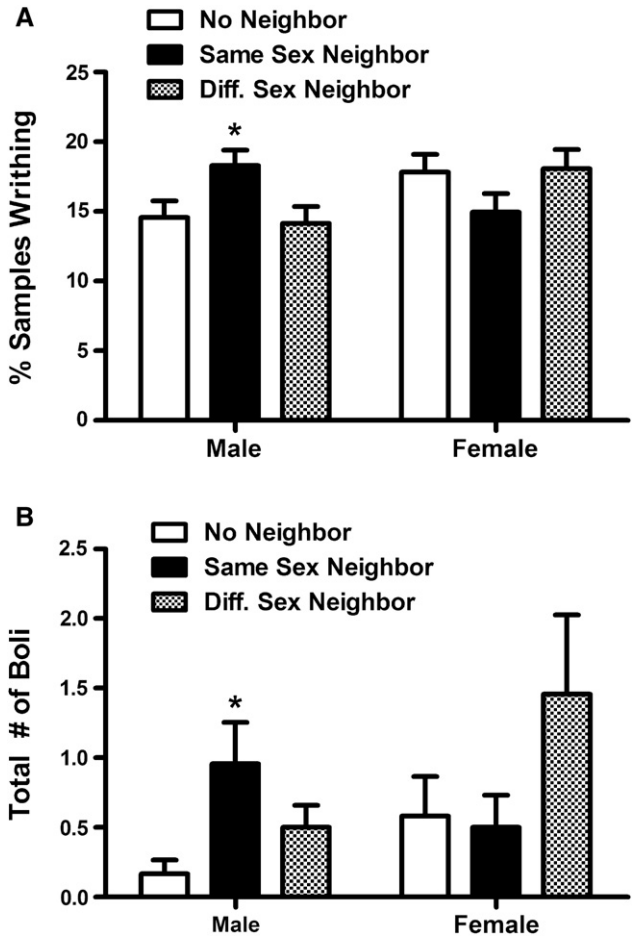

Figure 2. Hyperalgesia in same-sex male dyads in which only limited contact was permitted. **(A)** Male mice display significantly increased writhing behavior in the presence of an unfamiliar neighboring male non-writher ("Same Sex Neighbor") separated by vertical metal bars, but not an unfamiliar neighboring female non-writher ("Diff. Sex Neighbor"). Bars represent mean ± S.E.M. percentage of samples featuring writhing behavior (n = 24/sex/condition). *P < .05 compared to relevant isolated ("No Neighbor") condition. Female mice display a strong but nonsignificant trend (P = .12) in the opposite direction when the neighbor mouse is female. **(B)** Male mice deposit significantly more fecal boli when tested in the presence of an unfamiliar male non-writher. Females and mice tested in the presence of the opposite sex deposit similar numbers of fecal boli compared to those tested in isolation. Bars represent mean ± S.E.M. number of fecal boli deposited during the writhing test itself (ie, after habituation). *P < .05 compared to relevant No Neighbor condition.

a significant main effect of condition ($F_{3,57}$ = 4.7, P < .01). Post hoc testing revealed that mice tested in the presence of an (uninjected) intact or castrated but hormonally replaced male mouse exhibited significantly less writhing behavior than those tested either in isolation or in the presence of an (uninjected) castrated male mouse (Fig 1B).

As shown in Fig 1C, a 2-way (condition × time) repeated measures ANOVA of corticosterone levels at 30, 60, and 90 minutes postinjection revealed a significant main effect of condition ($F_{1,40}$ = 4.6, P < .05), time ($F_{2,120}$ = 41.8, P < .001), and a significant condition × time interaction ($F_{3,120}$ = 3.1, P = .05). Subsequent t-tests indicated a significant effect of condition at 30 minutes (t_{40} = 2.0, P < .05) and 90 minutes (t_{40} = 2.3, P < .05), but not 60 minutes (t_{40} = 1.8, P = .08) postinjection. When corrected for the 3 relevant comparisons (30, 60, and 90 minutes) using the Bonferroni method, however, none of these differences between condition remained significant (.11 < P < .29, respectively), and should thus be regarded as strong trends.

Pain Hypersensitivity Produced by Limited Social Interaction With Unaffected Stranger Male Mice

In the limited social interaction (ie, barrier) experiments, a 2-way (sex × testing condition) ANOVA performed on writhing behavior revealed a significant interaction ($F_{2,138}$ = 5.2, P < .01). Significant main effects of testing condition were observed in male mice only ($F_{2,69}$ = 3.9, P < .05); subsequent Dunnett post hoc tests comparing to the isolated (No Neighbor) condition revealed significant differences in writhing of 2 males tested beside each other (separated by jail bars), who each displayed significantly more writhes (P < .05; Fig 2A). A trend towards decreased writhing in female-female dyads was also observed (P = .12). In contrast, no significant changes were observed in mixed-sex dyads under similar conditions. Male mice in same-sex dyads deposited significantly more fecal boli than isolated males (interaction $F_{2,134}$ = 3.1, P < .05) (Fig 2B). Statistically significant neighbor effects were not observed in same-sex female dyads or in different-sex dyads.

A 2-tailed Student's t-test performed on paw-withdrawal latencies also indicated a significant effect of condition, such that male mice, tested in the presence of a same-sex unfamiliar, unaffected, physically separated conspecific, displayed significantly reduced paw-withdrawal latencies relative to isolated testing (t_{46} = 2.03, P < .05; Fig 3A). No difference relative to the isolated condition was observed among different-sex dyads (t_{42} = .64, ns; Fig 3B).

Laboratory Effects

Baseline (ie, isolated condition) writhing levels differed considerably between the experiments reported here (compare Isolated condition in Fig 2 versus Isolated conditions in Figs 3A and B). This is likely due to the different testing environments, as the cylinder experiments were conducted at Haverford College, and the cubicle experiments conducted at McGill University. This is not particularly surprising considering the substantial effect of varying laboratory environments on behavior.[8-10] The other possibility is that the variability is related to the different suppliers of CD-1 mice (Harlan versus Charles River).

Regardless of the reason for the variability, in both experiments, pain behavior in the isolated condition is at intermediate levels, allowing for the observation of hyperalgesia or analgesia (ie, no floor or ceiling effects). Note also that the cylinder experiments (Fig 2) conducted at Haverford College include a direct replication of a phenomenon also demonstrated previously at McGill University,[22] suggesting that despite differences in absolute pain levels, it is appropriate to directly

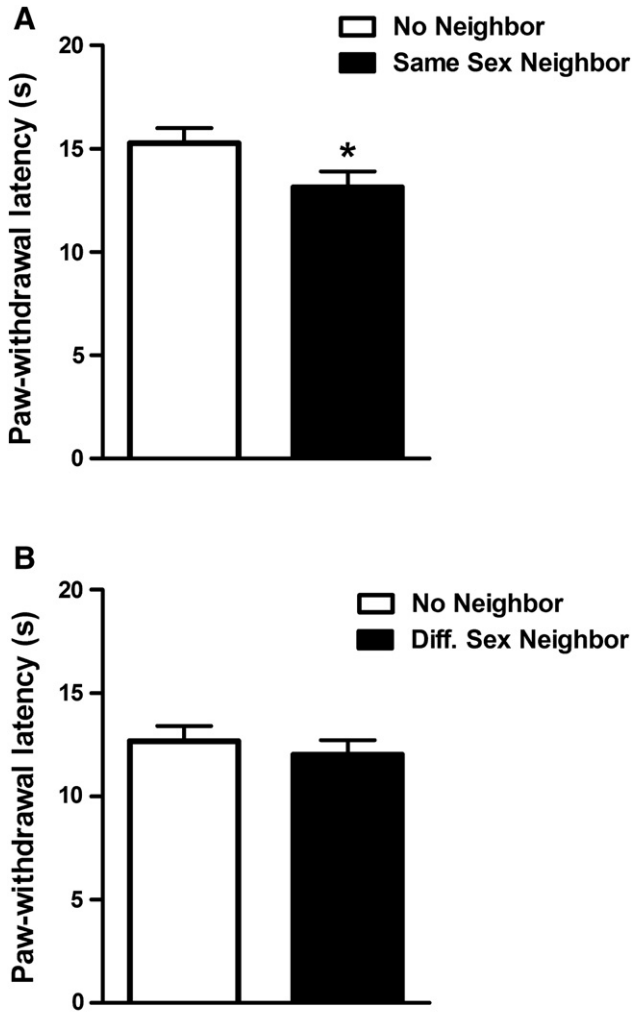

Figure 3. Thermal hypersensitivity among same-sex male dyads in which limited contact is permitted. Bars in **A** and **B** (2 experiments run separately) represent mean ± S.E.M. latency to withdraw from a noxious thermal stimulus applied to the hind paws (average of 10 stimulations) (n = 22-24 condition). **(A)** Male mice display significantly reduced paw-withdrawal latencies (ie, hyperalgesia) when tested in the presence of an unfamiliar male (Same Sex Neighbor) mouse. **(B)** Male mice in different-sex (Diff. Sex Neighbor; ie, with a female mouse) dyads show no alterations in noxious thermal sensitivity. *P < .05 compared to relevant No Neighbor condition.

compare relative data (eg, group differences) from the 2 sites. We note that this was also the conclusion of Crabbe et al[10] with respect to differential mouse strain performance on behavioral assays.

Discussion

These results replicate, in a different laboratory, our previous finding of decreased pain behavior of male mice tested in the presence of an unaffected stranger male.[22] We find that this inhibition is dependent on gonadal hormone status, such that when either member of the dyad has undergone castration, the effect is abolished and can be reinstated by testosterone replacement. This phenomenon is wholly specific to males, in that females do not display reduced pain behavior in the presence of an unfamiliar female,[22] and the effect cannot be induced by administration of testosterone to females in adulthood (unpublished observations).

Furthermore, we find that by limiting physical contact between 2 stranger male mice, not only is the analgesia in the test mouse abolished, but pain is actually modulated in the entirely opposite direction (ie, hyperalgesia). This effect is similarly specific to same-sex male dyads, not being observed in female-female or male-female pairings, and was replicated using a noxious stimulus of a wholly different modality.

The relevant mechanism of social communication between the mice is currently unknown. Previous studies have shown that urinary odors, perhaps through hormonally derived pheromones, are powerful signals for behavior in rodents.[7] Therefore, the most obvious route for the observed threat communication is olfactory. However, the social communication of pain producing the empathy effect we described previously was visual,[22] and that sensory modality may also subserve the modulations seen here. In the writhing test, there are 2 potential sources of visual information: the abdominal constrictions themselves, and facial expressions of pain that we have shown to reliably accompany those constrictions.[21] However, it is much more difficult to imagine visual transmission of information in the radiant heat paw-withdrawal test, in which the pain behavior (a simple withdrawal from the stimulus) occurs within a fraction of a second and is not associated with a facial expression recognizable to the experimenter.[21]

Social Stress-Induced Analgesia

We have suggested that the unimpeded proximity of an unfamiliar male may result in a form of social stress-induced analgesia (SIA) in the test mouse.[22] Because the mice are drawn from separate cages and placed together in a novel environment, dominance hierarchies are not in place, and the 30-minute acclimation period prior to injection gives little time to settle the issue of dominance. Note that the testing apparatus is a neutral environment, novel to both members of the dyad, and thus there is no implied dominance like that found in a resident-intruder paradigm.[37] As a result, the presence of an unfamiliar and potentially dangerous conspecific likely initiates a stress response that may in turn affect pain sensitivity in the injected test mouse. Indeed, social stimuli have long been known to induce SIA in rodents. The most studied example is SIA from social defeat (the result of an aggressive encounter by a conspecific) in male rodents, which involves both opioid and nonopioid mechanisms.[26,35] Cross-species threat stimuli involving predators (or their odors) also produce SIA in rodents,[18,23] and the underlying neurochemistry is known to vary by sex.[19,20] We demonstrate here that the presence of a gonadally intact stranger male—who in the natural environment would represent a rival for territory, resources, and females—may also serve to activate the same descending analgesic circuitry that produces defeat and predator SIA, even prior to an adversarial encounter. That the presence of an

unaffected stranger male mouse—social threat—is stressful to the injected test mouse has been previously demonstrated by the increased number of fecal boli emitted in this situation compared to similar testing in isolation, with familiar males, or when both mice are injected with acetic acid (see Fig S4B in reference 22). Normal social interactions between unfamiliar mice are, of course, common and in fact preferred by even adult males over nonsocial options.[39,41] Indeed, we have shown that stress levels in the habituation period (ie, before acetic acid injection) are equivalent across different social testing conditions (see Fig S4B in reference 22). What may make the difference in this case is the fact that 1 of the mice in the dyad is in pain, and thus vulnerable.

That castration of the writher or the nonwrither eliminates the observed pain inhibition suggests that removal of gonadal hormones abrogates the social threat. Pheromones contained in urine have been shown to specifically promote intermale aggression in hormonally intact mice.[7,29] The absence of such aggression-promoting pheromones may therefore result in reduced social threat perception and normal pain responding.

SIA or Avoidance of Pain Behavior Display?

It is important to note that we cannot here distinguish whether the social stimulus reduces pain sensitivity in the observed mouse (ie, produces true SIA), or simply reduces the display of pain behavior (consciously or otherwise) without any reduction in perceived pain. The inhibition of writhing behavior was not reversed by naloxone (10 mg/kg, ip) in our hands (data not shown), likely ruling out an opioid-mediated SIA, but nonopioid forms of SIA would be unaffected by this antagonist. If pain behavior is interpreted by conspecifics as indicating vulnerability, this could invite aggression. Therefore, males may have evolved the tendency to suppress the overt display of pain when in the presence of a potentially threatening conspecific. It is also obviously adaptive to hide evidence of vulnerability from predators, but it is not at all clear why only males would choose to do so. Note that the SIA and display-avoidance explanations of the current phenomenon are not mutually exclusive, since an ideal means of inhibiting pain display would be to activate endogenous analgesia circuitry such that there is less pain to display, as has been postulated.[11]

Social Stress-Induced Hyperalgesia

Although SIA has been far more extensively studied, stress has also been shown to modulate pain sensitivity in the opposite direction, and there is growing interest in the phenomenon of stress-induced hyperalgesia (SIH).[16] A number of models have also been used to produce SIH, such as novelty exposure,[40] repeated cold,[30] restraint,[38] repeated forced swimming,[31] and social-defeat stress.[1] It should be noted that many of these models are also used to produce SIA; the determining factor appears to be the repetitiveness of the stressor or the time elapsed poststress. Generally, models that induce SIH involve chronic exposure to the stressor or behavioral testing days (rather than minutes) after the stressor, thus implying that SIH may be the result of more psychological stress (versus more acute physical stress in SIA models). Indeed, this observation is in line with evidence from human literature detailing strong comorbidity between mood disorders and chronic pain.[2] By limiting physical contact in our paradigm, we have likely eliminated the acute stress evoked by the potential for actual physical aggression, perhaps instead triggering psychological stress from the mere presence of an unfamiliar stranger male, who still represents competition and potential aggression.

It has also been proposed that the severity of the stressor differentially modulates pain sensitivity, such that more severe stressors evoke SIA, whereas less severe stressors evoke SIH.[40] This hypothesis is most clearly corroborated by human accounts of a complete lack of pain perception despite major injuries in sporting events, major accidents, or battle versus enhanced pain perception amongst those with anxiety disorders.[14] In humans, it has also been shown that fear (induced by electric shock) produces analgesia, whereas anxiety (induced by the threat of electric shock) induces hyperalgesia.[33] Our results appear to support these hypotheses, such that the immediate physical threat in the cylinders may have induced fear and therefore SIA, whereas reducing this threat by limiting contact or appraisal of the potential danger may have induced only anxiety (by the mere presence of a potential foe), thereby producing SIH.

That these phenomena are specific to male mice is not surprising. First, physical aggression is largely specific to males, denoted by the considerable involvement of testosterone in mediating aggressive behavior;[12] therefore, these paradigms may serve as sex-specific stressors. Second, crowding has been shown to be stressful in males, but not females;[4] the close proximity imposed by the testing apparatus thus may have also exerted sex-specific effects. Finally, there appears to be a basic sex difference in the behavioral response to stress, which evokes the canonical "fight-or-flight" response in both sexes, but females may secondarily activate a "tend-or-befriend" response[36] that in the current paradigm would mitigate against pain-related vulnerability in front of a conspecific being interpreted as stressful.

Conclusions

Studying the effects of such social stressors may be important to all social species, especially considering the robust social factors affecting pain sensitivity in humans, as well as recent evidence suggesting the impact of social factors in rodent pain models.[13,22,32] Furthermore, the observation that a similar social stressor may modulate pain in either direction suggests the involvement of different pathways in each case, and their study may lead to a better understanding of the underlying neural mechanisms of stress-induced changes in pain sensitivity. The present findings obviously have direct implications as well for the design of rodent-pain experiments, in which social effects on

pain behavior are largely unappreciated modulatory factors.[27]

Acknowledgments

Dale J. Langford and Alexander H. Tuttle are co-first authors. This research was supported by The Louise and Alan Edwards Foundation (J.S.M.) and a Research in Undergraduate Institutions grant from the National Science Foundation (W.F.S.). A.H.T. and D.B.F. were supported by a grant from the Arnold and Mabel Beckman Foundation. M.B. was supported by a grant from the Howard Hughes Medical Institute.

References

1. Andre J, Zeau B, Pohl M, Cesselin F, Benoliel JJ, Becker C: Involvement of cholecystokininergic systems in anxiety-induced hyperalgesia in male rats: Behavioral and biochemical studies. J Neurosci 25:7896-7904, 2005

2. Bair MJ, Robinson RL, Katon W, Kroenke K: Depression and pain comorbidity: A literature review. Arch Intern Med 163:2433-2445, 2003

3. Barfield RJ, Busch DE, Wallen K: Gonadal influence on agonistic behavior in the male domestic rat. Horm Behav 3:247-259, 1972

4. Brown KJ, Grunberg NE: Effects of housing on male and female rats: Crowding stresses males but calms females. Physiol Behav 58:1085-1089, 1995

5. Butler RK, Finn DP: Stress-induced analgesia. Prog Neurobiol 88:184-202, 2009

6. Callahan BL, Gil ASC, Levesque A, Mogil JS: Modulation of mechanical and thermal nociceptive sensitivity in the laboratory mouse by behavioral state. J Pain 9:174-184, 2008

7. Chamero P, Marton TF, Logan DW, Flanagan K, Cruz JR, Saghatelian A, Cravatt BF, Stowers L: Identification of protein pheromones that promote aggressive behaviour. Nature 450:899-902, 2007

8. Chesler EJ, Wilson SG, Lariviere WR, Rodriguez-Zas SL, Mogil JS: Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. Neurosci Biobehav Rev 26:907-923, 2002

9. Chesler EJ, Wilson SG, Lariviere WR, Rodriguez-Zas SL, Mogil JS: Influences of laboratory environment on behavior. Nat Neurosci 5:1101-1102, 2002

10. Crabbe JC, Wahlsten D, Dudek BC: Genetics of mouse behavior: Interactions with laboratory environment. Science 284:1670-1672, 1999

11. Fanselow MS: Conditioned fear-induced opiate analgesia: A competing motivational state theory of stress-induced analgesia. Ann N Y Acad Sci 467:40-54, 1986

12. Gandelman R: Gonadal hormones and the induction of intraspecific fighting in mice. Neurosci Biobehav Rev 4: 133-140, 1980

13. Gioiosa L, Chiarotti F, Alleva E, Laviola G: A trouble shared is a trouble halved: Social context and status affect pain in mouse dyads. PLoS One 4:e4143, 2009

14. Gureje O: Comorbidity of pain and anxiety disorders. Curr Psychiatry Rep 10:318-322, 2008

15. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32:77-88, 1988

16. Imbe H, Iwai-Liao Y, Senba E: Stress-induced hyperalgesia: Animal models and putative mechanisms. Front Biosci 11:2179-2192, 2006

17. Jordan EG, Mogil JS: Mice, pain and empathy. Science 314:253, 2006

18. Kavaliers M: Brief exposure to a natural predator, the short-tailed weasel, induces benzodiazepine-sensitive analgesia in white-footed mice. Physiol Behav 43:187-193, 1988

19. Kavaliers M, Choleris E: Sex differences in *N*-methyl-D-aspartate involvement in κ opioid and non-opioid predator-induced analgesia in mice. Brain Res 768:30-36, 1997

20. Kavaliers M, Colwell DD: Sex differences in opioid and non-opioid mediated predator-induced analgesia in mice. Brain Res 568:173-177, 1991

21. Langford DL, Bailey AL, Chanda ML, Clark SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS: Coding facial expressions of pain in the laboratory mouse. Nat Methods 7:447-449, 2010

22. Langford DL, Crager SE, Shehzad Z, Smith SB, Sotocinal SG, Levenstadt JS, Chanda ML, Levitin DJ, Mogil JS: Social modulation of pain as evidence for empathy in mice. Science 312:1967-1970, 2006

23. Lester LS, Fanselow MS: Exposure to a cat produces opioid analgesia in rats. Behav Neurosci 99:756-759, 1985

24. Lindzey J, Wetsel WC, Couse JF, Stoker T, Cooper R, Korach KS: Effects of castration and chronic steroid treatments on hypothalamic gonadotropin-releasing hormone content and pituitary gonadotropins in male wild-type and estrogen receptor-α knockout mice. Endocrinology 139:4092-4101, 1988

25. Luttge WG: Activation and inhibition of isolation induced inter-male fighting behavior in castrate male CD-1 mice treated with steroidal hormones. Horm Behav 3:71-81, 1972

26. Miczek KA, Thompson ML, Shuster L: Opioid-like analgesia in defeated mice. Science 215:1520-1522, 1982

27. Mogil JS: Animal models of pain: Progress and challenges. Nat Rev Neurosci 10:283-294, 2009

28. More L: Intra-female aggression in the mouse (*Mus musculus domesticus*) is linked to the estrous cycle regularity but not to ovulation. Aggress Behav 34:46-50, 2008

29. Novotny M, Harvey S, Jemiolo B, Alberts J: Synthetic pheromones that promote inter-male aggression in mice. Proc Natl Acad Sci U S A 82:2059-2061, 1985

30. Ohara H, Kawamura M, Namimatsu A, Miura T, Yoneda R, Hata T: Mechanism of hyperalgesia in SART stressed (repeated cold stress) mice: Antinociceptive effect of neurotropin. Jpn J Pharmacol 57:243-250, 1991

31. Quintero L, Moreno M, Avila C, Arcaya J, Maixner W, Suarez-Roca H: Long-lasting delayed hyperalgesia after subchronic swim stress. Pharmacol Biochem Behav 67:449-458, 2000

32. Raber P, Devor M: Social variables affect phenotype in the neuroma model of neuropathic pain. Pain 97:139-150, 2002

33. Rhudy JL, Meagher MW: Fear and anxiety: Divergent effects on human pain thresholds. Pain 84:65-75, 2000

34. Richards AB, Morris RW, Ward S, Schmitz S, Rothmond DA, Noble PL, Woodward RA, Winslow JT, Weickert CS: Gonadectomy negatively impacts social behavior of adolescent male primates. Horm Behav 56:140-148, 2009

35. Rodgers RJ, Randall JI: Acute non-opioid analgesia in defeated male mice. Physiol Behav 36:947-950, 1986

36. Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RAR, Updegraff JA: Biobehavioral responses to stress in females: Tend-and-befriend, not fight-or-flight. Psychol Rev 107:411-429, 2000

37. Thurmond JB: Technique for producing and measuring territorial aggression using laboratory mice. Physiol Behav 14:879-881, 1975

38. Torres IL, Vasconcellos AP, Silveira Cucco SN, Dalmaz C: Effect of repeated stress on novelty-induced antinociception in rats. Braz J Med Biol Res 34:241-244, 2001

39. Van Loo PL, Van de Weerd HA, Van Zutphen LF, Baumans V: Preference for social contact versus environmental enrichment in male laboratory mice. Lab Anim 38: 178-188, 2004

40. Vidal C, Jacob J: Hyperalgesia induced by non-noxious stress in the rat. Neurosci Lett 32:75-80, 1982

41. Winslow JT: Mouse social recognition and preference. Curr Protoc Neurosci Unit 8:16, 2003