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Partner loss in monogamous rodents: Modulation of pain and emotional behavior in male prairie voles

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Abstract

Objective—Pain is modulated by psychosocial factors and social stress-induced hyperalgesia is a common clinical symptom in pain disorders. To provide a new animal model for studying social modulation of pain, we examined pain behaviors in monogamous prairie voles experiencing partner loss.

Methods—After cohabitation with novel females, males (n=79) were divided into two groups on the basis of preference test scores. Half of the males of each group were separated from their partner (loss group), while the other half remained paired (paired group). Thus, males from both groups experienced social isolation. Open field tests, plantar tests, and formalin tests were then conducted on males to assess anxiety and pain-related behaviors.

Results—Loss males showing partner preferences (N=20) displayed a significant increase in anxiety-related behavior in the open field test (central area/total distance; paired, $13.65 \pm 1.58\%$ vs loss, $6.45 \pm 0.87\%$; $p < 0.001$), a low threshold of thermal stimulus in the plantar test (withdrawal latencies; paired, 9.69 ± 0.98 s vs loss, 6.15 ± 0.75 s; $p = 0.037$), and exacerbated pain behaviors in the formalin test (total number of lifts; paired, 40.33 ± 4.46 vs loss, 54.42 ± 1.91 ; $p = 0.042$) as compared to paired males (N=20). Thermal thresholds in the plantar test significantly correlated with anxiety-related behavior in the open field test ($r = 0.64$). No such differences were observed in the males that did not display partner preferences ($r = 0.15$).

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Conclusions—Results indicate that social bonds and their disruption, but not social housing without bonding followed by isolation, modulate pain and emotion in male prairie voles. The prairie vole is a useful model for exploring the neural mechanisms by which social relationships contribute to pain and nociceptive processing in humans.

Keywords

partner loss; pain; anxiety; social modulation; prairie vole

Introduction

Social separation or the sudden breakdown of a bonded partnership is a significant risk factor for mental and physical disease (1–2). Indeed, in humans, individuals with a lack of social contact experience an increased risk of developing cardiovascular disease (3), cognitive decline (4), and mortality in infants and older adults (5–7). Thus, psychosocial stress has various negative effects on health and welfare.

A similar phenomenon was also observed when studying social and psychological modulation of pain and there has been considerable research conducted on clinical populations. For instance, low social support and low job satisfaction in the work place had a statistically significant positive effect on the onset of back pain (8), a considerable portion of patients with high levels of pain vigilance fall into chronic postoperative pain (9), and lower socioeconomic status and a higher score of postnatal depression were risk factors for persistent pain after caesarean section (10). There are also considerable reports linking social stress with increased pain sensitivity (stress-induced hyperalgesia) under experimental pain testing conditions in healthy volunteers and patients (11–14). Thus, there is some evidence from human research that social support and psychosocial stress modulates pain, including stress-induced hyperalgesia. To our knowledge, there have been no studies examining the relationship of disruption of a strong social bond on pain modulation. In rodent studies, however, the social effects on pain remain ambiguous. It has been well established that exposure to stressful situations induced pain inhibition, hypoalgesia, through activation of endogenous pain inhibitory pathways in various animal models. One of the first pieces of evidence regarding the social effects on nociception in animal models was reported by Miczek, who showed that socially-defeated mice exhibited an elevation of the nociceptive threshold (15), and a similar antinociception was observed in rats (16) and hamsters (17). Social isolation also modulates pain perception. As for mice, withdrawal latencies in the hot-plate test increased under conditions of social isolation (18). By contrast, several animal studies have reported that some stressors, such as vibration (19) and forced swimming (20), elicit hyperalgesia instead of analgesia. Thus, contrary to clinical studies, most results from animal experiments provide evidence for antinociception by social stress, that is, stress-induced analgesia. Therefore, the molecular and cellular mechanisms underlying stress-induced hyperalgesia, which were seen in clinical patients, have been obscure because of a lack of appropriate animal models. On this point, it has been proposed that pain sensitivity is differently modulated depending on the quality and intensity of the stressor, that is, analgesia would predominate after an intense physical stressor and hyperalgesia after a moderate emotional stressor (21). Hence, we need to carefully select types of stimulation accompanied

by emotional reactions for induction of hyperalgesia in animals in order to better model the situation in humans.

To provide a new animal model for social modulation of pain, we focused on the prairie vole (*Microtus ochrogaster*) that is a socially monogamous rodent exhibiting a partner preference after pair-bonding and has been considered as a useful animal model for investigating the neurobiological mechanisms underlying the formation and maintenance of pair bonds (22–25). Unlike prairie voles, traditional experimental animals such as rats and mice do not have a socially monogamous mating strategy (26, 27). Given this characteristic, prairie voles, like humans, are highly sensitive to disruptions of their social bonds between males and females. Indeed, prairie voles expressed behavioral disturbances associated with depression and autonomic dysregulation following the loss of their bonded partner (28–30). Therefore, in the present study, we investigated the hypothesis that the disruption of established social bonds between male and female prairie voles (partner loss) would produce a behavioral hyperalgesia accompanied by emotional reactions, similar to the hyperalgesia reported in humans.

Materials and Methods

Animals

All animals were adult male and female prairie voles (100–160 days of age) from the laboratory-breeding colony at Kochi Medical School and Aichi Medical School originally derived from a colony at Emory University. Offspring were separated from breeding pairs at 21 days of age, and housed in same-sex sibling groups under standard laboratory conditions (14:10 h light-dark cycle, lights on at 07:30 h; 22 °C, 60 % humidity and free access to food and water). All experimental protocols were approved by the Animal Experiment Committee at Kochi Medical School and Aichi Medical School and followed the guidelines of the National Institute of Health. All efforts were made to minimize the number of animals used and their suffering.

General experimental procedure

The timeline of all procedures for the experiment is shown in Figure 1. Sexually naïve male voles were paired with unfamiliar sexually naïve females (Day 0). After 7 days of cohabitation with the females, the males were tested in a 2-hour partner preference test (PPT), conducted from 9:00 a.m. to 4:00 p.m., to evaluate pair-bond formation. Cage bedding was not changed during the cohabitation period in order to maintain their partner's olfactory cues. Immediately following the PPT, tested males were randomly divided into two groups: paired (maintained cohabitation with the partner) or loss (separated from the partner) groups. In the loss group, the male was moved without the female partner from the home cage to a new cage, the same size as the home cage, and was kept alone. All of the female partners were carried out of the experimental room in order to prevent the loss males from having sensory cues (i.e., visual, olfactory, and auditory cues) of their partners. In contrast, the paired male was transferred to a new cage along with the female partner, and was kept in a state of cohabitation. After that, the bedding in both housing conditions was not changed until the experiment was finished. During the paired/loss period, we explored

whether partner presence or absence would change the anxiety and pain-related behaviors in male prairie voles using a battery of behavioral tests: anxiety-related behavior was assessed by open field test (OT) at Day 11 and pain-related behavior by plantar test (PT) at Day 12 or formalin test (FT) at Day 13. Each test was performed in the following time: OT, 9:00 a.m. - 12:00 a.m.; PT, 1:00 p.m. - 5:00 p.m.; FT, 1:00 p.m. - 5:00 p.m. Subjects were returned to their previous home cages (paired males returned with their partners and loss males returned without their partners) immediately after each behavioral test. Males from both bonded and non-bonded groups experienced social isolation, controlling for the effect of the lack of social stimulation.

Partner preference test and evaluation of pair-bond formation

Pair-bond formation was evaluated by PPT using a three chamber apparatus made in reference to one, which has been previously reported (31). In short, the apparatus consists of one long box (75 cm length $\times$ 20 cm width $\times$ 30 cm height), divided into three equal-sized chambers: partner, neutral and stranger chambers by placing two slats. Fresh wood-chip bedding was added to cover the floor and 15 ml glass bottle for water supply was placed at each end of the apparatus. During the PPT, a test male was free to move throughout the apparatus for 2 hours, but a partner and a stranger female were tethered in the partner and stranger chamber located at each end of the apparatus. Each female was used twice, once as the partner in her own pair and again as the stranger in another pair to reduce animal numbers. Each PPT was video recorded and compressed as an MPEG file for later analysis. When replaying the MPEG files, the male's affiliative behaviors, including social proximity (sitting or lying in a female's chamber), huddling (physical contact with a female side by side) and allogrooming (licking the fur of the stimulus female), were quantified by a well-trained observer using a "snapshot" technique, i.e., every 30-s presence of any one of the behaviors was noted. Taking into consideration habituation of the testing animals to the apparatus, behaviors in the last 90 minutes of the test were observed. The data were scored as the ratio of affiliative behaviors to total observations, 2 observations/min. $\times$ 90 min.=180 observations. For example, when a male shows affiliative behaviors to the stimulus female in 20 observations the score is 0.11. In a pilot study, we examined the relationship between nesting behavior and PPT scores in male voles, with the result that the males made their nests on the female side where the males showed high PPT scores. In particular, more than 66% of males built their nests on the female side where the score was higher than 0.2 in the PPT, while males which built their nest with a female with scores lower than 0.2 was less than 43%. Thus, in the present study, we divided tested males into two groups according to a PPT score: males showing scores of 0.2 or more selectively to their partners were classified as bonding males (Bonded) while those with less than 0.2 to their partners were classified as non-bonding ones (Not bonded). Note that males which showed scores of 0.2 or more to both their partner and the stranger were not included in Bonded but in Not bonded.

Open field test

Experimental subjects were exposed to the open field test to examine anxiety-related behaviors. Individual paired or loss males were released in a corner of the open square container (90 $\times$ 90 $\times$ 30 cm, 200 lux) at the start of each test and their movements were video recorded with a camera mounted on the ceiling for a total of 15 minutes. Subsequently, the

total walking distance in the entire field for locomotor activity and the distance in the central area (45×45 cm) for anxiety were analyzed using the ANY-maze tracking software (Stoelting Co, USA).

Plantar test

Thermal nociception of a male was evaluated by measuring hind paw withdrawal latency in response to radiant heat using a plantar test apparatus (Ugo Basile, Italy). The experimental males experienced 15 minute-habituation to the apparatus 1 day before testing. The latency was measured five times at 10-15 minute intervals, alternating between right and left sides. The median of the five measurements was adopted as the representative latency of each male. All measurements were performed by a well-trained observer, blind to the experimental conditions of the testing males.

Formalin test

Inflammatory pain behavior was assessed with the FT. After a 30-minute acclimation, voles received a subcutaneous injection of 20 μ l 5% formalin into the plantar surface of the right hind paw in the test apparatus, an open Plexiglas observation chamber. Pain behaviors induced by the formalin injection were video recorded through the transparent floor of the test chamber and were subsequently quantified as the duration of licking and the number of lifts made by the treated paw up to 60 minutes after the injection. The number of lifts was counted using 1-minute snapshots. All behaviors were recorded by a well-trained observer, blind to the experimental conditions.

Statistical analysis

For the data from the PPT, a two-way analysis of variance (ANOVA) was performed with factors (FEMALE TYPE: partner vs stranger) and SEPARATION: paired vs loss) within each bonds group (Bonded or Not bonded). All of the data from the OT and PT were compared using a two-way ANOVA with factors (BONDS: Bonded vs Not bonded and SEPARATION). In the FT, the total duration of licking, total number of lifts, and number of lifting behaviors at each point were analyzed by a two-way ANOVA with factors (BONDS and SEPARATION). A two-way ANOVA was followed by post-hoc Student's t-tests for a comparison of the two groups or by post-hoc Tukey-Kramer HSD tests for more than two groups when interactions were detected. The correlation between two values was statistically analyzed using Pearson's correlation coefficient. The data are presented as means + SEM. A probability value of $p < 0.05$ was considered to be statistically significant. All statistics were performed using the JMP statistics software package (SAS Institute, Cary, NC, USA).

Results

Pair-bond formation

Figure 2 displays the affiliative scores in the PPT. A two-way ANOVA revealed a significant main effect of FEMALE TYPE (Bonded, $F_{1,76}=164.45$; Not bonded, $F_{1,74}=25.84$; $p=0.001$), but not of SEPARATION (Bonded: $F_{1,76}=0.87$, $p=0.35$; Not bonded: $F_{1,74}=1.16$, $p=0.28$) or FEMALE TYPE $\times$ SEPARATION interaction (Bonded, $F_{1,76}=0.66$, $p=0.42$; Not bonded, $F_{1,74}=0.88$, $p=0.35$) on affiliative scores during the PPT. Post-hoc Tukey-Kramer's HSD tests

indicated that males in Bonded showed significantly higher scores with their partner than with a stranger ($p=0.001$), and conversely, in Not bonded showed higher scores with strangers than with their partners (pair, $p=0.025$; loss, $p=0.001$).

Effect of partner presence/absence on anxiety

During the OT, although the total distance traveled did not differ among groups ($F_{3,75}=0.19$, $p=0.91$; Figure 3B), significant BONDS $\times$ SEPARATION interaction was noted in the distance traveled in the center of the field ($F_{1,75}=5.66$, $P=0.020$; Figure 3C) and post-hoc analysis detected a significant difference in Bonded. Hence, paired males in the Bonded group displayed a significantly higher percentage of distance traveled in the central area (central area/total distance) than loss males, but not in Not bonded (BONDS $\times$ SEPARATION interaction: $F_{1,75}=9.83$, $P=0.003$; paired vs loss, $p=0.001$). Furthermore, in the percentage of distance traveled in the central area, a significant difference in paired males between Bonded and Not bonded was also detected ($p=0.014$; Figure 3D).

Effect of partner presence/absence on thermal nociception

Thermal thresholds were determined using the PT. Figure 4 illustrates paw-withdrawal latencies of the paired and loss males in Bonded and Not bonded. A two-way ANOVA revealed a significant SEPARATION effect ($F_{1,34}=8.90$, $P=0.005$), but no significant BONDS $\times$ SEPARATION interaction ($F_{1,34}=2.11$, $p=0.16$). A Tukey-Kramer's HSD post hoc comparison showed that the latency of paired males was significantly longer than loss in Bonded ($P=0.037$).

Effect of partner presence/absence on inflammatory pain behavior

Inflammatory pain was induced by 5% formalin injection into the paw. Injected voles showed mainly two kinds of pain behaviors: licking and lifting of the affected paw. There was no group difference in the total duration of licking ($F_{3,37}=0.66$, $p=0.58$; Figure 5A). A significant SEPARATION effect in the total number of lifts was detected by a two-way ANOVA ($F_{1,37}=5.65$, $P=0.023$), and a Tukey-Kramer's HSD post hoc also revealed that loss males exhibited a significantly greater total number of lifts than paired males in Bonded ($p=0.042$; Figure 5B). Additionally, changes in the number of lifts per 5 minutes up to 60 minutes after the formalin injection are shown in Figure 5C. Statistical analysis showed that loss males in Bonded kept the affected paw lifted during the FT, whereas all other groups gradually decreased over the test period (post hoc at each time point; 35-40 min, Bonded paired vs Bonded loss, $p=0.032$).

Correlation between anxiety and nociception

Dot-plots and linear correlations between anxiety and nociception are presented in Figure 6 (6A: % central area in the OT vs latency of withdrawal response in the PT and 6B: % central area in the OT vs licking duration in the FT). A statistically significant positive correlation was found between the increase in % central area (decrease in anxiety level) and the increase in latency of withdrawal response (increase in threshold of thermal nociception) (Pearson's correlation coefficient $r = 0.64$, $p = 0.008$). Although it was not statistically significant, there

was a weak negative correlation between % central area in the OT vs licking duration in the FT (Pearson's correlation coefficient $r = -0.37$, $p = 0.071$).

Discussion

In humans, a sudden separation (e.g. bereavement) from the bonded partner causes emotional dysregulation (32) and increases the risk of developing mental disease (33). Prairie voles, monogamous rodents, also experience a similar phenomenon under experimental conditions of partner loss (28, 34). Here, we extend this line of research to assess the effect of social bonds and their disruption on pain perception and provide a new animal model for social modulation of pain that parallels human findings on stress-induced hyperalgesia since male prairie voles separated from their bonded female partner show behavioral hyperalgesia accompanied by emotional reactions.

Male voles in the present study, which had been separated from their female partners for 4 days after 7 days of cohabitation, displayed a significant decrease in the ratio of central/total locomotion in the OT (vs paired males, Figure 3), indicative of a state of anxiety in rodents (35) including voles (36). Importantly, this anxiogenic effect of partner loss was not found in males that showed no preference for their female partners in the PPT, suggesting that the effect is selective for males that established bonds with female partners and not an effect of social isolation *per se*. Our results may explain previous work reporting that anxiety-related behavior on the elevated plus-maze was largely unchanged (albeit passive depressive-like behavior increased) in male prairie voles by separation from a female partner for 4 days (28). In that study, subject males were not evaluated on having a preference for their partners after cohabitation, and therefore it is possible that partner preference was not induced by cohabitation in some males. Indeed, in the present study, there is no significant difference in the anxious state between paired and loss males when data from Bonded and Not bonded were combined (Figure 3C and 3D). Since the Not bonded male did not show a response when isolated, this is clear that the effect is due to the psychosocial stress of loss of a bonded partner and not social isolation.

Hypothalamo–pituitary–adrenal (HPA) axis is an important component of stress responses and therefore HPA axis activity is used as an index for measuring the current stress status (37). Previous studies reported a significantly high level of basal plasma corticosterone and an increased weight of the adrenal glands in male voles with loss of a female partner for 5 days compared to males separated from a sibling partner (28, 34), indicating that loss of the bonded female partner chronically upregulates basal HPA axis in male voles. It is important to note that chronic upregulation of the HPA axis is thought to be critical for the pathogenesis of various psychiatric disorders, including anxiety and depressive disorders (38–40). In line with these hypotheses, male voles isolated from the bonded female partner showed increased anxiety behaviors in the present study (Figure 3). Although we did not measure corticosterone levels in the current study, future studies using contrasting Not bonded and Bonded will examine physiological measures such as corticosterone and other stress hormones.

Additionally, we report for the first time that partner-loss male prairie voles exhibited increased pain-related behaviors in the PT and in the FT, which are widely used for the detection of hyperalgesia in rats and mice (41, 42). With respect to thermal nociception, the loss males displayed shorter withdrawal latencies than paired males in the PT, indicating that thermal hyperalgesia was induced by partner loss (Figure 4). Accordingly, we observed that partner loss males showed increased licking and lifting of the affected paw in the FT as compared to paired males, indicating that partner loss exacerbated pain-related behaviors under inflammatory conditions (Figure 5). Historically, the involvement of stress in pain perception is well known as stress-induced analgesia in humans and animals (43), which provides the survival advantage of escaping from an enemy when in danger. This analgesia seems to emerge as a result of a response to an acute physical stressor: activation of the sympathetic-adrenal-medullary (SAM) axis, known as the canonical “fight and flight” response. As mentioned above, however, the HPA axis is also activated depending on the nature and/or parameters of the stressor and the activation seem to be involved in emotional dysregulation (38-40). Although HPA axis activity was not examined, we found positive correlations between an anxiety state and pain-related behaviors in prairie vole males in the present study. That is, males with higher anxiety levels after partner loss displayed an increase in pain-related behaviors (Figure 6). In concordance with our data, there is evidence of the existence of high comorbidity between mood disorders and chronic pain in clinical settings (44, 45). Therefore, direction of pain modulation by stress, i.e., stress-induced analgesia or hyperalgesia may be determined by the degree of SAM and/or HPA axes. This hypothesis should be investigated in future studies.

Here there is one important point that should be considered in the interpretation of our present data. Recently, positive effects of social support on various threats to physical and psychological well-being have received increasing attention in humans (44, 45) and similar effects in other mammals, which is termed social buffering (46), has been found, including in prairie voles (47, 48). Pain is no exception, and several human studies have focused on the effects of social support on pain perception, reporting that a social partner, e.g. a spouse or romantic partner, contributes to pain relief and associated aversive emotions. Interestingly, the social support effect on pain and emotion depends on the social relationships between partners (49-51). Indeed, paired males in Bonded displayed a significantly higher ratio of central/total locomotion (vs Not bonded) in an open field, an indication of anxiolysis, while a significant decrease in the ratio in loss males was not found between Bonded and Not bonded (Figure 3D). In other words, partner presence has a significant effect on the reduction of anxiety (anxiolysis) and the effect of enhanced anxiety by partner loss was weak. Taking into consideration these points of view, in the present study, differences in pain and anxiety behavior between paired and loss males might have arisen from both social stress (negative) and social support (positive) effects by the bonded females.

Interestingly, it is possible that many of the phenomena observed in the current study are linked through the oxytocin systems. Indeed, oxytocin is known to have both anxiolytic (52–54) and analgesic (55, 56) properties, and a recent study demonstrated a disruption in the oxytocin system in male prairie voles following partner loss (30). Thus a reduction in oxytocin signaling following loss of a partner could lead to both anxiety and hyperalgesia. Also of note, in the present study, we grouped animals that displayed a partner preference

(Bonded) and those that did not (Not bonded). The neurochemistry in the brain of these animals could be different, and the difference could make them have a predisposition to be more affected by loss of social support. Therefore, by selecting animals that bonded vs not, we could have selected for predispositions to the effect of social loss. Indeed, recent work has shown that a polymorphism in the Oxt gene leads to alteration in oxytocin receptor density and also robustly predicts OXTR expression in the brain as well as pair bonding behavior (57). It is possible that our animals in the Bonded represented are more susceptible to loss of social support due to genetic predisposition. This is an area for future research.

In conclusion, 4–6 days of separation from the bonded female partner in the monogamous male prairie vole leads to behavioral hyperalgesia, showing a certain degree of correlation to a state of anxiety, only in males showing partner preference, suggesting that social bonds modulate nociception as well as emotion, depending on the relationship characteristics of social bonds. Here we provide a useful animal model using prairie voles for understanding how the neurobiology of the social environment contributes to changes in pain behavior and nociceptive processing in humans.

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Abbreviations

PPT	partner preference test
OT	open field test
PT	plantar test
FT	formalin test
ANOVA	analysis of variance
SAM axis	sympathetic-adrenal-medullary axis
HPA axis	hypothalamo–pituitary–adrenal axis

Figure 1.

Experimental timeline. Male voles were paired with novel females for 7 days then tested in a 2-hour partner preference test (PPT) to evaluate pair-bond formation (Day 7). Immediately following the PPT, the males were divided into paired (stayed with the partner) or loss (separated from the partner) groups. From four to six days after the separation (Day 11-13), a battery of behavioral tests was conducted on males from both groups to determine whether partner presence and absence affected anxiety and pain-related behaviors. After an open field test at Day 11, all subjects received either a plantar test at Day 12 or a formalin test at Day 13. The number of tested males is presented in parenthesis. PPT, partner preference test; OT, open field test; PT, plantar test; FT, formalin test.

Figure 2.

Evaluation of pair-bond formation by the partner preference test. In both bonds (+) and bonds (-), partner preference was similar between paired and loss males: no significant difference was found between paired and loss males in both bonds (+) and bonds (-). In bonds (-), paired and loss tested males showed preferences for strangers rather than for partner females. $n=20$ for each group in bonds (+), $n=19$ for paired and $n=20$ for loss in bonds (-). Data are expressed as mean + SEM. *** $p < 0.001$, * $p < 0.05$ vs each stranger.

Figure 3.

Anxiety-related behavior in the open field test in paired and loss male prairie voles. Paired males exhibited less anxiety-related behavior than loss males in bonds (+) but not in bonds (-). Representative plots of the location in the open field of paired vs loss males in the bonds (+) group (A). Total distances traveled in the open field were not different among the groups (B). Paired males traveled more distance in the central area of the open field than loss males in bonds (+) but not in bonds (-) (C). The percentage of distance traveled in the central area (central area/total distance) of paired males was significantly higher than loss males in bonds

(+) and paired males in bonds (-), whereas no significant difference was found in bonds (-) (D). $n=20$ for each group in bonds (+), $n=19$ for paired and $n=20$ for loss males in bonds (-). Data are expressed as mean + SEM. *** $p < 0.001$ vs loss in the same group, # $p < 0.05$ vs paired males in bonds (-).

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Figure 4.

Paw-withdrawal latencies in the plantar test in paired and loss male prairie voles. Paired males in bonds (+) displayed longer withdrawal latencies as compared to loss males, while no significant difference was found in bonds (-). $n=8$ for each group in bonds (+), $n=10$ for paired and $n=12$ for loss in bonds (-). Data are expressed as mean + SEM. * $p < 0.05$ vs loss in the same group.

Figure 5.

Pain behaviors in the formalin test in male paired and loss prairie voles. The time spent licking (A) and the number of lifts recorded by every 1-minute snapshot (B, C) were measured during 60 minutes after subcutaneous formalin injection. Although significant differences were not seen in total licking duration, the total number of lifts in loss males significantly increased compared to paired males in bonds (+). In addition, loss males in bonds (+) frequently lifted their affected paw compared to other groups throughout the

formalin test. n=12 for each group in bonds (+); n=9 for paired and n=8 for loss in bonds (-). Data are expressed as mean + SEM. * $p < 0.05$ vs paired in the same group.

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Figure 6.

Dot-plots and linear correlations between anxiety and nociception in bonds (+) group, where significant differences were observed between paired and loss males: % central area in the open field test vs latency of withdrawal response in the plantar test (A) and % of central area in the open field test vs licking duration in the formalin test (B). n=16 in total (n=8 for each group) in A, n=24 in total (n=12 for each group) in B. paired, closed circles; loss, open circles. Pearson's correlation coefficients are expressed as r.