Title

Empathic contagious pain and consolation in laboratory rodents: species and sex differences

Short title: Empathy for pain in laboratory rodents

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Abstract

Laboratory rodents are gregarious in nature and have a feeling of empathy when witnessing a familiar conspecific in pain. The rodent observers express two levels of empathic responses: empathic contagious pain (ECP) and empathic consolation (EC). Here we examined the sex and species difference of ECP and EC in male and female mice and rats. We observed no species difference in both ECP and EC, but significant species difference in general prosocial (allo-mouth and/or allo-tail sniffing) and non-social (self-grooming) behaviors. For sex difference, male mouse observers showed more allo-licking and allo-grooming behaviors toward a familiar conspecific in pain during and longer time increase in pain sensitivity after the PDSI than female mouse observers. However, no sex difference was observed in rats. Our results highlighted an evolutionary view of empathy that social animals including rodents also have the ability to feel, recognize, understand and share the other's distressing states.

MAIN TEXT

Introduction

Increasing lines of evidence from both clinical and basic research implicate an important role of social communication in modulation of pain (*1*, *2*, *3*). In practice, coping skills among couples and family members have been demonstrated to relieve pain under chronic conditions, probably through decreasing social stress and increasing social buffering (*4*, *5*). Recently, these findings raise some interesting questions and debates on the concept of pain. Some researchers indicated that pain should be redefined as a distressing experience associated with actual or potential tissue damage that involves not only sensory and emotional experience, but also cognitive and social components, highlighting the mediating roles of higher brain structures in social recognition and compassion of pain (*6*). However, so far less is known about the brain processing and neural mechanisms of one's social recognizing, understanding and sharing of

suffering in pain patients due to lack of theoretical framework, animal models and experimental tools in the field of pain research and management.

Empathy for pain is a concept referred to as an evolutionary behavior of social animals and humans associated with the ability to feel, recognize, understand and share the other's distressing (pain, social rejection and catastrophe) states through social communications and interactions (*7*, *8*). Empathy for pain is a vicarious feeling that is felt through social transfer or contagion from a distressing object to a witnessing subject. This process has been demonstrated to be mediated by central neural network mainly consisting of the anterior cingulate cortex (ACC) and anterior insular cortex that also mediates direct emotional feeling of pain (physical pain) in humans (*9*, *10*, *11*). Psychologically, witnessing distressing condition of others can motivate sympathy of a subject toward unfamiliar people, but may deeply activate a subject's empathic concern, consolation and desire to help toward his/her familiar social members (family, kin, friends, colleagues, *etc.*) (*8*, *12*, *13*). Meanwhile, witnessing or learning of one's family member in pain or distress may also result in a strong feeling of pain in one's heart through empathic contagion of pain across individuals (*7*, *9*). Social pain associated with social rejection, defeat and failure or loss of social connections may also activate the ACC and other brain structures (*14*), implicating an overlap of functional neural correlates that are associated with cognition, empathy for pain, social pain and physically emotional pain (*15*).

Do animals have a feeling of empathy? If yes, do animals share the same neural processing as humans do? This question is still on debate and requires to be answered by deep study and strong lines of experimental evidence. More recently, based upon the seed discovery of reciprocal enhancement of pain across dyadic mice both in pain through social interaction (*16*, *17*), we have developed a behavioral model of empathy for pain in rats (*18*, *19*, *20*). Experimentally, the behaviors associated with empathy for pain in rats can be recognized as two types: one is referred to as an observer's empathic consolation that is driven by social interacting with a demonstrator in pain (*18*, *21*), the other is referred to as empathic transfer of pain (contagious pain) from distressing object to witnessing subject (*18*, *19*, *20*). Briefly, the empathic consolation in rats has been identified as allo-licking and allo-grooming behaviors during 30-min priming dyadic social interaction (PDSI) between a naive cagemate observer (CO) and a familiar demonstrator (CD) in pain: (1) allo-licking can be defined as an observer's sustained licking action to a demonstrator's injury site; (2) allo-grooming can be defined as an observer’s head contact with the head or body of a demonstrator in pain, accompanied by a rhythmic head movement (*13*, *18*, for details see *22*). The bouts of allo-licking and allo-grooming behaviors can be captured by video camera recorder (VCR) and off-line analyzed qualitatively and quantitatively (see *22* and Methods below). While, empathic contagious pain, also referred to as empathic pain hypersensitivity in our previous reports, has been identified qualitatively and quantitatively as lowered pain threshold or increased pain sensitivity in the CO rats after the PDSI with a CD in pain (*18*, *19*, *20*). The empathic pain hypersensitivity remains unchanged for at least 5 h in time course measured immediately after the PDSI (*18*, *20*). Although allo-grooming behavior could be seen in both familiar and unfamiliar conspecifics during the PDSI, allo-licking behavior and empathic contagious pain could only be seen in familiar (CO) observer, suggesting that the establishment of familiarity among conspecifics is essential to induction of empathic responses to other’s pain in rats (*7*).

To answer the common questions whether there are species and sex differences in the model of empathy for pain in laboratory rodents, we further designed and studied the behavioral parameters associated with empathic contagious pain and consolation in both male and female mice and rats.

Results

2.1. Species and sex comparisons of empathic consolation behavior

2.1.1. Species comparisons of empathic consolation behavior

Under the experimental paradigm as shown in Fig.1, there was no species difference in latency, total time and counts of allo-licking and allo-grooming between mice and rats in either male or female (Table 1). Species difference was not revealed in allo-tail sniffing in terms of latency and total time between mice and rats in either male or female (Table 1). Although male mice had more counts than male rats ($p = 0.002$, Mann-Whitney U test), species difference was not seen between mice and rats of female for the counts of allo-tail sniffing (Table 1). As for the non-social behavior, rats of both sexes spent more time in self-licking and self-grooming than mice of both sexes (Table 1, mice vs. rats: $p = 0.017$ for male and $p = 0.016$ for female, Mann-Whitney U test) although counts showed no species difference. Moreover, rats of both sexes had shorter latency in self-grooming than mice of both sexes although statistical significance for species difference was only seen in male (Table 1, $p = 0.001$, Mann-Whitney U test). It was surprisingly noted that although allo-mouth sniffing could be seen in mice (Figs. 2C-D, Fig. 3C-D), no such behavior could be observed in rats during 30-min PDSI.

Taking the data of latency and time course together (Fig. 2), it was revealed that both mouse and rat observers of either male or female were likely to approach to the CD in pain as quickly as possible and spent more time on empathic consolation and prosocial behaviors than self-grooming behavior.

2.1.2. Sex comparisons of empathic consolation behavior

In mice, sex difference was distinctly seen in both empathic consolation and general prosocial behaviors in terms of time and counts but with latency being of no sex difference (Fig. 2A-F, Fig.3A-F, see Tables S1 and S2 for statistical analysis). Male mice spent more time and had more counts than female in allo-licking/allo-grooming and allo-mouth/allo-tail sniffing toward a CD in pain during the early 20 min PDSI (Fig.3A-F, see Tables S1 and S2 for statistical analysis), while there was no sex difference in self-licking/self-grooming in terms of latency, time and counts (Fig.2G-H, Fig.3G-H, see Tables S1 and S2 for statistical analysis).

In rats, no sex difference was seen in either empathic consolation or general prosocial behavior in terms of latency, time and counts (Fig.2I-L, Fig.3I-L, see Tables S1 and S2 for statistical analysis). Although female rats likely had relatively shorter latency than male ($p = 0.019$, Mann-Whitney U test), no sex difference was seen in time and counts of self-grooming behavior (Fig.2M-N, Fig.3M-N, see Tables S1 and S2 for statistical analysis).

2.2. Species and sex comparisons of empathic contagious pain

Similar to our previous reports on rats (*18*, *19*, *20*, *22*, also see Fig.5B-C here), empathic contagion of pain occurred as well in naive mouse observer after 30-min PDSI. Both male and female observer mice presented long-term mechanical pain hypersensitivity after 30-min PDSI with a CD in pain, being evidenced by significant leftward shift of stimulus-response functional curves from the baseline (Fig.4A-F, see Tables S1, S2 and S3 for statistical analysis). The empathic contagion of pain from a CD in pain to a naive CO did not disappear until 240 min in female and 300 min in male after PDSI in mice (Fig.4 and Fig.5A, see Tables S1, S2 and S3 for statistical analysis).

2.2.1 Species comparisons of empathic contagious pain

Generally speaking, no species difference in empathic contagious pain was found between mice and rats of either sex in terms of magnitude and time course under the same experimental condition, procedure and paradigm (Figs.4-5, see Tables S1, S2 and S3 for statistical analysis).

2.2.2. Sex comparisons of empathic contagious pain

No sex difference was found in empathic contagious pain between male and female observers in either mice or rats in terms of magnitude and time course under the same experimental condition, procedure and paradigm between 0-180 min period after PDSI (Fig.4 and Fig.5A, see Tables S1, S2 and S3 for statistical analysis). However, the empathic mechanical pain hypersensitivity in mouse observer was maintained relatively longer for about 60 min in male than in female (Fig.4 and Fig.5A, see Tables S1, S2 and S3 for statistical analysis). No sex difference was found in empathic contagious pain between male and female observers in rats during the whole time of observation (Fig.5B-C, see Tables S1 and S2 for statistical analysis).

Discussion

3.1. Evidence for evolutionary issue of empathy

From the evolutionary point of view, empathy has been proposed to be hierarchical in mammals that has evolved from very low stage (motor mimicry and emotional contagion) to relatively higher stage (empathic concern and consolation), and finally to the highest stage (perspective-taking, mentalizing, theory of mind and targeted-help) from lower animals to human beings (*8*). Although several emerging lines of evidence support existence of emotional contagion in lower mammals (*3*, *7*, *23*, 24, 25), answers to the questions about whether lower mammals are able to recognize, understand, share and care others are still controversial due to lack of enough direct experimentally supporting evidence (*13*, *18*, *26*). In a series of reports on the empathy for pain in rats and mice of the present study, our lab has provided with strong lines of experimental evidence supporting existence of both emotional contagion and empathic consolation in laboratory rodents (*7*, *18*, *19*, *20*, *22*). Before the coming of our findings, empathic consolation has only been observed in a special sub-species of wild rodents - socially monogamous, biparental prairie vole (*13*) although emotional contagious pain or observational fear learning have been increasingly evidenced (*3*, *7*, *8*). Taken together, it has been demonstrated experimentally that lower mammals such as rodents may have both lower stage (emotional contagion) and relatively higher stage (empathic concern and consolation) of empathy, supporting the rationality of theoretical Russian-doll model for the evolution of empathy in mammals (*8*). Moreover, the findings that social familiarity plays essential roles in induction of empathy for pain in rodents also support Darwin's assertion that “with all animals, sympathy is directed solely towards the members of the same community, and therefore towards known, and more or less beloved members, but not to all the individuals of the same species” (*7*, *12*).

3.2. Qualitative and quantitative assessment of empathy for pain in laboratory rodents

In the past century, study of empathy has been mostly performed in non-human primates and other non-laboratory animals outdoors (*8*, *23*, *24*, *25*). This has greatly limited the number of researchers joining the study and hindered the advances of empathy research in terms of bio-psychosocial-brain-behavioral paradigm (*7*, *23*, *24*, *25*). Therefore, discovering, developing and validating the laboratory animal models of empathy would be very important and critical for opening a new field of science - neuroscience of empathy. Here we have developed a state-of-the-art laboratory rodent model of empathy for pain in both mice and rats using a set of novel behavioral parameters for both qualitative and quantitative assessment. We have identified and validated two behavioral identities from laboratory rodent model of empathy for pain: (1) empathic consolation; (2) empathic contagious pain.

3.2.1. Are there species and sex differences in empathic consolation between mice and rats?

To make qualitative and quantitative assessment of empathic consolation, we successfully identified allo-licking and allo-grooming behaviors from the naive observer during PDSI with a

CD in pain. To see whether the observer's allo-licking and allo-grooming behaviors are selective or specific to the injury and pain of the object (CD), we also evaluated general prosocial behavior (allo-mouth and/or allo-tail sniffing) and non-social behavior (self-licking and self-grooming) in the observer (CO). In each type of targeted behaviors, four bio-parameters including latency, time course, total time and visit counts were quantitatively assessed. In the present study, it was clearly shown that there was no species difference between mice and rats for empathic allo-licking and allo-grooming behaviors in either male or female (Table 1), suggesting laboratory rodents can be motivated to perform empathic consolation when witnessing their familiars in painful or distressing condition. Mice and rats are likely sharing and caring as humans. Bio-parameter data showed that both mouse and rat observers began to approach toward the CD in pain in a short delay while witnessing and then spent longer time to lick the injury site and to groom the body of the injured partners. As contrast, the same animals had longer latency and less count in either self-licking/self-grooming or allo-tail and allo-mouth sniffing, suggesting that laboratory rodents have a strong ability to rapidly recognize and understand the distressing condition of others. And this process is likely to motivate visiting, sharing and caring of the injured object at the expense of loss of their time in exploring and self-grooming. Because self-grooming is predominant in rodents' usual behaviors (more than 40% of living time) (*27*, *28*), loss of self-grooming and gain of allo-licking and allo-grooming in time during PDSI highly implicate existence of prosocial and altruistic behaviors in observer rodents while witnessing a familiar in pain.

It is interesting to note that there was a sex difference in visit counts and total time of allo-licking and allo-grooming as well as allo-mouth and allo-tail sniffing between male and female mice, however, no such sex difference was seen in rats. Unlike the results from humans and rodents that female are more empathic than male (*29*, *30*, *31*), in the current study, however, the male was likely to spend more time (three folds) than the female in mice to allo-lick and allo-groom the injured partner. Although the female observer mice had less time in allo-grooming but spent more time on allo-licking toward the BV-induced injury site in the CD object, the sex difference in empathic consolation in mice is not likely to be only caused by the sex difference in allo-grooming since general prosocial behaviors (allo-mouth and allo-tail sniffing) also had sex difference. Generally, the male has more consolation and more prosocial behaviors than the female in mice. Moreover, rats had equivalent amount of time and visit chance in allo-licking, allo-grooming and allo-tail sniffing between male and female. Although the underlying mechanisms of sex difference in the degree of empathic consolation and general prosocial behaviors in mice are not clear, the level of sex hormones, genetic background and other unknown factors should be considered. In mice, variability in empathic fear response has already been noted across different inbred strains (*32*, *33*).

3.2.2. Are there species and sex differences in empathic contagious pain between mice and rats?

As aforementioned, although mice and rats have different mechanical sensitivity to vF stimuli, standardized measurements revealed no species and sex differences in empathic contagious pain. Similar to our previous reports on male rats (*18*, *20*), the current data further showed that the rat observers had no sex difference in empathic mechanical pain hypersensitivity between male and female after PDSI with a CD in pain (Fig.5B-C). The paw withdrawal mechanical threshold (PWMT) of both sexes became lowered by more than 50% immediately after the PDSI, and the lowered PWMT was maintained unchanged until 300 min of observation. The relative long-term decrease in PWMT could be identified in both sides of hind paws and was paralleled between male and female in rat observers. Similarly, empathic mechanical pain hypersensitivity was also identified in the mouse observers of both sexes immediately after the PDSI by showing

leftward shift of the stimulus-response functional curves from the baseline (Fig.4). The leftward shift of the stimulus-response functional curves remained unchanged between male and female mice until 240 min after the PDSI. Although the functional curve in male mouse observers still remained leftward shifted, that in female mouse observers recovered to overlap with the baseline since 240 min after the PDSI, suggesting that the male is likely to have longer time course of empathic contagious pain than the female in mice. The mouse fitted vF intensity for the half maximal response that is equivalent to the PWMT in rats also showed a separation of time effect between male and female at 240 min after the PDSI. Although sex- and gender-difference in pain have been well established (*34*), the sex-difference in empathic contagious pain in mice is not likely to be attributed to the sex-difference in mechanical pain sensitivity because the baseline stimulus-response functional curves overlapped very well between male and female. Whether this sex-associated separation in time effect of empathic contagious pain in mice has some relationship with sex-difference in empathic consolation is unknown and requires to be further elucidated.

3.3. Laboratory rodent model of empathy for pain and its advantages in application

Empathy has been believed to be fundamental to prosocial, altruistic and moral behaviors in human beings (*8,35*). Impairment of empathy can definitely lead to deficits in social communication and sociability (attachment, bonding, prosocial reciprocity, altruism and morality) that may be fundamental to some psychiatric disorders such as autism spectrum disorder (ASD), psychopathy, misconduct, antisocial personality disorder and schizophrenia (*7*). Thus, development and validation of laboratory rodent model of empathy are of great significance in further understanding of the biological basis of empathy and its evolution. Moreover, the use of the empathy for pain model may also shed new light on the underlying mechanisms of deficits in social communication and sociability in the ASD and other psychiatric disorders.

Based upon the present results from species and sex studies, male rats and mice are highly recommended to be used as observer subjects for study of empathy for pain in laboratory rodents due to less empathic consolation (allo-licking/allo-grooming) identified in female mice. Because female are more sensitive to pain stimuli and more susceptible to chronic pain conditions than male in both human and animal subjects due to biopsychosocial variables (*34*), pain mechanisms in female are also more complex than male. Moreover, familiar conspecifics of the same sex for PDSI are also recommended because sexual behaviors could not be completely excluded if heterosexual cagemates were allowed.

As introduced in our previous reports (*18*), the selection of pain models for preparing a demonstrator in pain is also important and critical. The more visually distinctly visible the pain-related behaviors are displayed by the CD, the more empathic responses could be induced in the rat observers in terms of both empathic contagious pain and consolation (*18*). Namely, rat observers showed more consolation (allo-licking and allo-grooming) behaviors during PDSI with a CD treated with BV than with CFA (*18*). Meanwhile, rat observers have distinct empathic contagious pain after PDSI with a CD treated with BV and formalin but do not have empathic contagious pain after the same period of PDSI with a CD prepared with CFA and spared nerve injury (*18*). These results suggest important roles of visual information in the induction and maintenance of empathic contagious pain and consolation as suggested by a previous report (*16*). Moreover, blockade of the pain in the CD with lidocaine at the injury site can relieve empathic contagious pain in the observer, suggesting that the social transfer of pain is pain-selective and specific (*18*).

In summary, laboratory rodents are gregarious in nature and have a feeling of empathy when witnessing a familiar conspecific in pain. The advantages of the use of laboratory rodent

(rats and mice) model of empathy for pain are as follows: (1) laboratory rodents are fed in a SPF animal facility and tested in a standardized experimental environment that are safe in prevention of infectious disease transmission from animal to animal and from animal to experimenters; (2) biological control makes genetic background of laboratory rodents more clear and comparable than wild animals such as prairie vole; (3) attracting and recruiting more biologists and neuroscientists who are interested in biological basis of empathy to join the research; (4) unlike the "double pain paradigm" introduced by Mogil's lab (*16*), the laboratory rodent observer are under naive condition prior to and during PDSI that can completely exclude the distressing effects of tonic pain stimulation on observer itself and make neurobiological, endocrine and other biological assays possible in further tests; (5) the laboratory rodent model of empathy for pain has been validated to have both empathic consolation and empathic contagious pain that are useful paradigms for studying evolutionary issues of empathy in mammals (*7*, *8*); (6) our laboratory rodent model of empathy for pain has been approved to be mediated by top-down facilitation from the medial prefrontal cortex and the locus coeruleus -norepinephrine system (*19*, *20*) that are known to be also important brain structures involved in empathy for pain in humans (*9*, *36*); (7) our laboratory rodent model of empathy for pain will provide a novel bio-psychosocial-brain-behavioral paradigm that can be used in combination with other advanced techniques in neuroscience such as optogenetic, chemogenetic, *in-vivo* multi-electrode array recordings and other neuroimaging approaches in consciously socially interacting animals.

Materials and Methods

Animals

Male and female C57BL/6 mice and Sprague-Dawley albino rats, purchased from the Laboratory Animal Center of the Fourth Military Medical University (FMMU), were used in this study. Both mice and rats with age of postnatal week 4-5 were translocated from the FMMU to Tangdu Hospital SPF animal facility in which 4-6 animals of the same species and the same sex were co-housed in each cage for another 2-3 weeks so as to familiarize with each other as cagemates (Fig.1). The newly regrouped animals were fed under standard conditions with a light-dark cycle (08:00-20:00) and adjustable room temperature (25±2 ℃) and air humidity (55-65%). Both water and food pellets were available *ad libitum*. This study was fully in accordance with the recommendations of the ARRIVE guidelines (*37*), the U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines, the EU Directive 2010/63/EU for animal experiments, the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978), and the ethical guidelines for investigations of experimental pain in conscious animals of the International Association for the Study of Pain were also critically followed (*38*). The number and suffering of animals were greatly minimized as required.

Experimental design and procedures

Because, as aforementioned in the Introduction, the behaviors associated with empathy for pain in rodents can be experimentally classified into two types: an observer's empathic consolation that is driven by a demonstrator in pain during the PDSI (*18*, *21*) and the empathic contagious pain identified immediately after PDSI (*18*, *19*, *20*, *22*). The behavioral assays were carried out in a timeline as shown in Fig.1 (for details see our published protocol *22*).

Establishment of familiarity

After arrival at the hospital SPF animal facility, 4 mice or 4-6 rats of the same sex were regrouped and co-housed in each cage for more than 2 weeks (Fig.1, for protocol details see *22*). To avoid social conflicts among adult animals, the time for regrouping should be 3-4 weeks

after birth and the number of animals to be co-housed should be limited to less than four for mice (more aggressive when stranger adults meet) and four to six for rats (less aggressive when stranger adults meet).

Habituation to experimental procedures

The subjects to serve as an observer should be trained by acclimatizing to hand handling, experimental environment and VCR equipment once daily at least for three days before formal procedures for testing (Fig.1, for protocol details see *22*). Hand handling was a very important procedure in this study because it could buffer social stress that may block empathy for pain (*17*, *18*, *20*).

Preparation of a demonstrator in pain

The selection of pain models for preparing a demonstrator in pain is another critical step for induction of empathy for pain in a witnessing observer during and after the PDSI (*18*, *22*). As demonstrated by our pioneering work (*18*), the induction of empathy for pain in an observer rat would be determined by the observability or visibility of spontaneous pain-related behaviors displayed or expressed by a familiar demonstrator in pain. Among the animal models of pain tested, the bee venom (BV) test, the formalin test and the acetic acid test that can induce long-term robust spontaneous pain-related behaviors such as paw flinching, paw licking and lifting or abdominal writhing have been demonstrated to be effective to induce both empathic consolation and empathic contagious pain, whereas, the complete Freund's adjuvant (CFA) and the spared nerve injury (SNI) models that induce less spontaneous pain-related behaviors are not effective in this paradigm (*18*, *21*). Since the BV test is both a scientifically well-established and human-rodent co-experienced type of pain (*39, 40, 41, 42*), it was used in the whole experiment of this study. Briefly, the cagemate demonstrator (CD) received a subcutaneous (s.c.) injection of BV solution (25 μl for mice and 50 μl for rats, 0.4% lyophilized whole venom of *Apis millifera* dissolved in physiological saline) into the left hind paw just before the start of the VCR recording of the PDSI and then re-united with the naive observer in the testing box (for details see *22*).

Quantitative sensory test with von Frey filaments

The mechanical pain sensitivity test setting includes a supporting platform and a nontransparent plastic testing box (10.5 cm x 10.5 cm x 15.8 cm) that is necessary to prevent any visual information from coming during testing. The supporting platform (160 x 30 x 40 cm) is equipped with metal mesh. The pore size of the mesh (0.5 cm x 0.5 cm) is preferably such that both mice and rats can move freely on the surface without getting caught. Because the mechanical pain sensitivity for paw withdrawal reflex was quite different between mice and rats, different quantitative method was used in this study. For both mice and rats, the mechanical pain sensitivity of the observer was measured prior to (1 day before for baseline) and after the PDSI (immediate, 30, 60, 120, 180, 240, 300 min). For mice who are likely to have high mechanical pain sensitivity and more active in locomotion in nature, an ascending series of calibrated von Frey (vF) filaments with intensities ranging from 0.16 to 1.40 g (1.60 to 13.72 mN) were used to induce paw withdrawal reflex from minimum (0) to maximum (100%). With the increasing intensity, each stimulus should be continued 1-2 seconds for 5 repetitions in 5 seconds apart, avoiding the same site. A sharp paw withdrawal or lift-up after a stimulus was considered a positive response and should be recorded. The averaged percent response (%) of a mouse to 5 stimuli of each intensity was calculated and the pooled stimulus-response functional curves were plotted. Comparing to the baseline, leftward shift of the stimulus-response functional curve was defined as hypersensitivity (hyperalgesia or allodynia), while rightward

shift of the curve was defined as hyposensitivity (analgesia) (*43*). Finally, the fitted vF intensity of half maximal response was obtained by Bliss method (*44*), serving as relative mechanical threshold for mice. For rats who have relatively low mechanical pain sensitivity and inactive in locomotion in nature, a series of calibrated vF filaments with bending force intensities ranging from 2.00 to 60.00 g (19.60 to 588.00 mN) were used to induce paw withdrawal reflex. The paw withdrawal mechanical threshold (PWMT), namely the bending force of a vF filament that enabled 50-60% response to 10 stimuli, was calculated. For details see our published protocol (*22*).

PDSI and VCR recording

Priming dyadic social interaction (PDSI) has been defined as a preemptive condition that allows full body contact, social communication and interaction between a naive observer and a demonstrator in pain for 30-min (*7*). A naive observer meant that the subject animal had no experience of pathologically tissue or nerve injury at all but only had experienced physiologically stroking stimulus by vF filaments one day before the PDSI (*7*). Briefly, a VCR (Sony, FDR-AX40, Japan) setting was arranged in a right top-down vertical view over the testing box (19 x 19 x 30 cm for mice and 40 x 30 x 15 cm for rats) which was used as an arena for 30-min PDSI (for details see *22*).

Offline qualitative identification and quantitative analyses of social and non-social behaviors during PDSI

According to repeated observations of the VCR-based behaviors in a 30-min lapse of time, the behaviors were classified into three types: (1) empathic consolation behavior identified as allo-licking and allo-grooming that has been described earlier in our lab (*18*, *22*, also see Introduction); (2) general prosocial behaviors identified as allo-mouth sniffing and allo-tail sniffing (*23, 35*); (3) non-social behavior identified as self-licking and self-grooming that is an innate stereotyped and patterned behavior of rodents and other terrestrial mammals generated and controlled by the brain (*27*, *28*). For each type of behaviors, the latency for the observer subject to first perform a type of behaviors after initiation of the PDSI, the time course and total time the observer subject spent on a type of behaviors during 30-min period of PDSI, and the total counts the observer subject behaved for each type of behaviors during 30-min period of PDSI were quantitatively rated and statistically analyzed. Both social and non-social behaviors were captured by the VCR in real time, and qualitatively identified and quantitatively analyzed offline by one to two analyzers who were blind to the treatment of animals. Grooming of less than 1 s was excluded. Grooming directed toward the genitals was excluded in this study.

Statistical analysis

All data were presented as mean ± SEM. SPSS 25.0 was used for data analysis. In principle, parametric statistical analysis methods would be used if both normality test and equal variance test for samples passed, however, only non-parametric statistical analysis method would be used if either of the normality test or equal variance test failed (Table S1). Normality of the distribution was analyzed by Shapiro-Wilk test, while homogeneity of variance was analyzed by Levene test. Nonparametric two-tailed Mann-Whitney U test or parametric two-tailed *t*-test were used depending upon the results of the normality and homogeneity tests. Two-way ANOVA repeated measure (RM) with Bonferroni post hoc correction was used for time course data (Tables S2-S3). For within-time two-way ANOVA RM, Greenhouse-Geisser method was used if Mauchly's test of sphericity failed. For paired comparison, Wilcoxon Signed Rank Test, Friedman′s *M* test and Mann-Whitney *U* test (two-tailed) were used if Shapiro-Wilk test and Equal variance test failed (Tables S2-S3). Sample size was predicted with one-way ANOVA

Power Analysis (Table S4). $P < 0.05$ was considered as statistically significant. Graphs and plots in the illustrations were made by GraphPad Prism version 7.0a.

Supplementary Materials

Table S1. Detailed descriptions of the number of animals used and statistical analyses for each part of the experiments.

Table S2. Time effects of empathic consoling and empathic contagion of pain in mice and rats of both sexes.

Table S3. Sex comparisons of stimulus-response functional curves in mice.

Table S4. Sample size prediction by one-way ANOVA Power Analysis.

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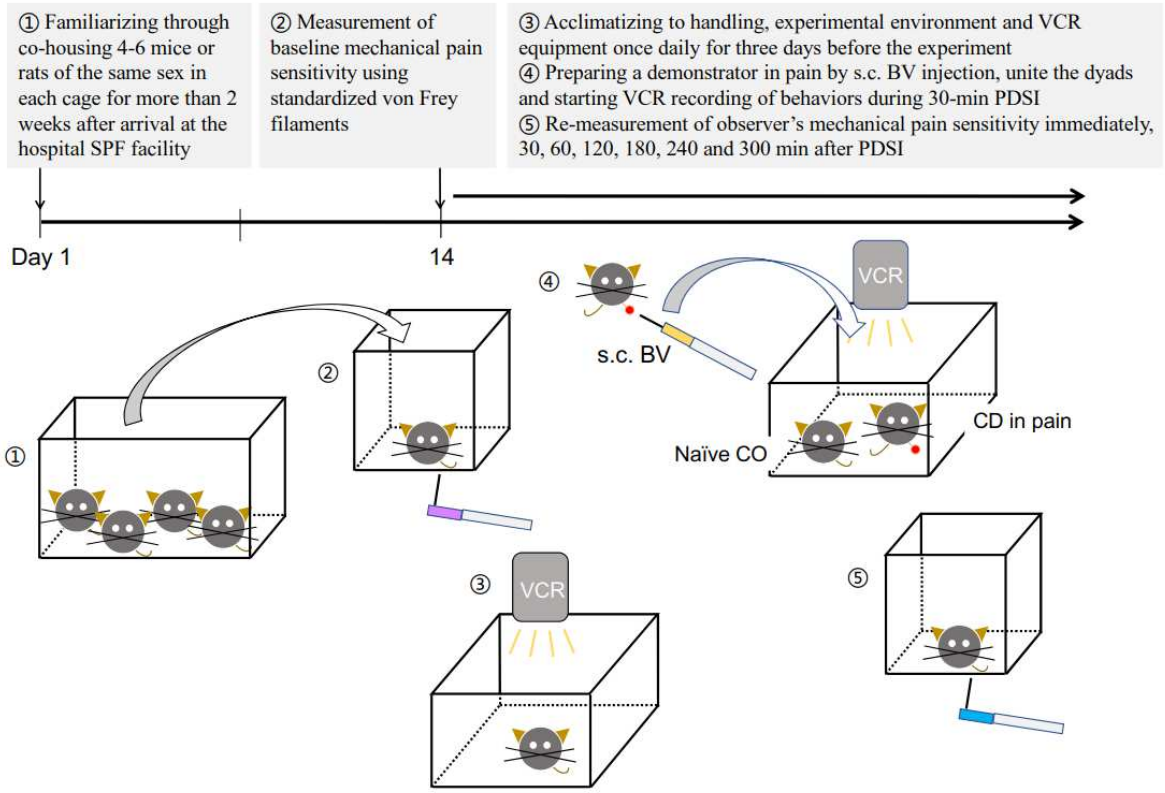
Author contributions: R.D. and W.J.L designed and performed the mouse and rat experiments, respectively, participated in data analysis and graph plotting. K.W.G contributed to the setup of VCR and initial identification of empathic consolation in mice. T.H. performed the partial male mouse experiments. C.L.L, Y.Y., N.W., and T.H. provided recommendations for experimental design and participated in some behavioral quantitative analysis. J.C. managed the whole procedure, integrated the whole data (illustrations and table) and wrote the manuscript. All authors participated in the discussion of the results and the final revision of the manuscript.

Competing interests: The authors declare that they have no competing interests.

Data availability: All data required to review the conclusions of this paper are contained in the paper and/or supplemental material. Additional data related to this article can be obtained from the authors.

562 **Figures and Tables**



563
564 **Figure 1** Timeline, experimental design, setup and protocol for the study of empathy for pain in mice
565 and rats. Abbreviations: BV, bee venom; CD, cagemate demonstrator; CO, cagemate observer; PDSI,
566 priming dyadic social interaction; s.c., subcutaneous; SPF, specific pathogen free; VCR, video camera
567 recorder.
568

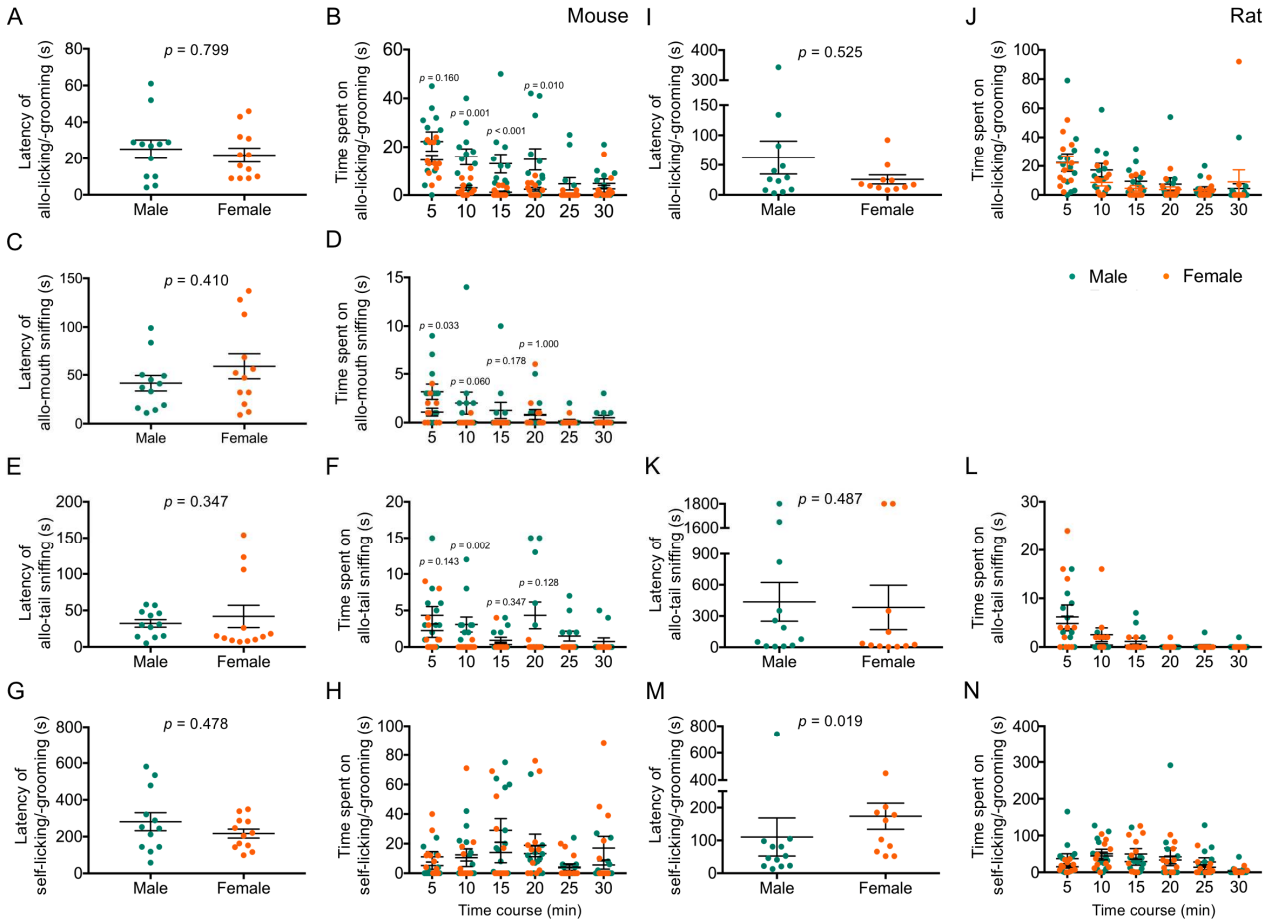


Figure 2 Sex and species comparisons of empathic consolation (allo-licking/allo-grooming), general prosocial (allo-mouth and/or allo-tail sniffing) and non-social (self-licking/self-grooming) behaviors between male and female observer mice (A-H) and rats (I-N) during 30-min priming dyadic social interaction with a cagemate demonstrator of the same sex in pain. Latencies and time courses spent by the cagemate observer on allo-licking/allo-grooming (**A-B** for mice and **I-J** for rats), allo-mouth sniffing (**C-D** for mice), allo-tail sniffing (**E-F** for mice and **K-L** for rats) and self-licking/self-grooming (**G-H** for mice and **M-N** for rats). $p < 0.05$ as statistical significance [Male (n=12) *vs*. Female (n=11-12) for each species] with two-tailed two-sample *t*-test or Mann-Whitney *U* test, for details see **Table S1-S2**]. Mean±SEM.

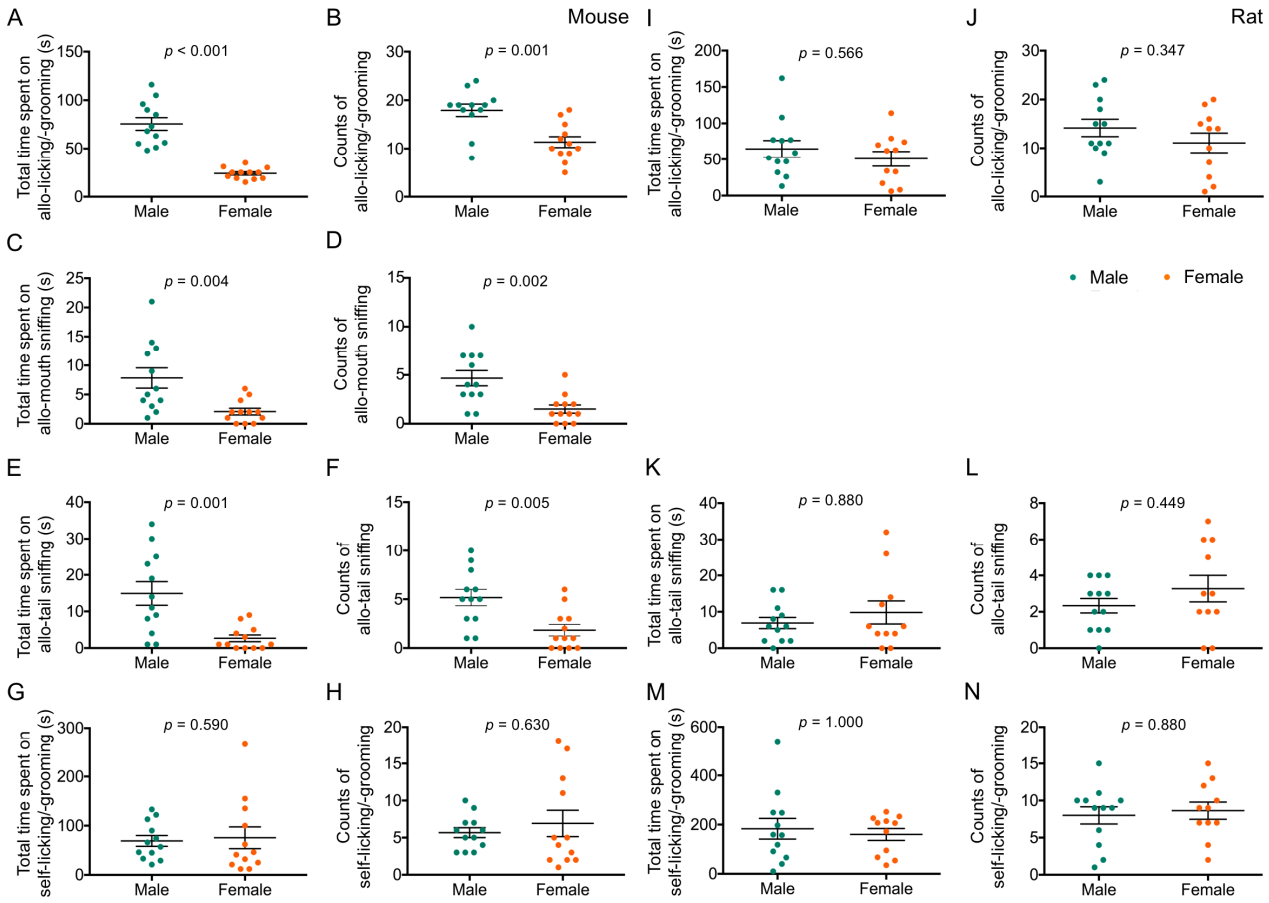


Figure 3 Sex and species comparisons of empathic consolation (allo-licking/allo-grooming), general prosocial (allo-mouth and/or allo-tail sniffing) and non-social (self-licking/self-grooming) behaviors between male and female observer mice (A-H) and rats (I-N) during 30-min priming dyadic social interaction with a cagemate demonstrator of the same sex in pain. Total time and counts spent by the cagemate observer on allo-licking/allo-grooming (**A-B** for mice and **I-J** for rats), allo-mouth sniffing (**C-D** for mice), allo-tail sniffing (**E-F** for mice and **K-L** for rats), and self-licking/self-grooming (**G-H** for mice and **M-N** for rats). $p < 0.05$ as statistical significance [Male (n=12) *vs*. Female (n=11-12) with two-tailed Mann-Whitney *U* test, for details see **Table S1**]. Mean±SEM.

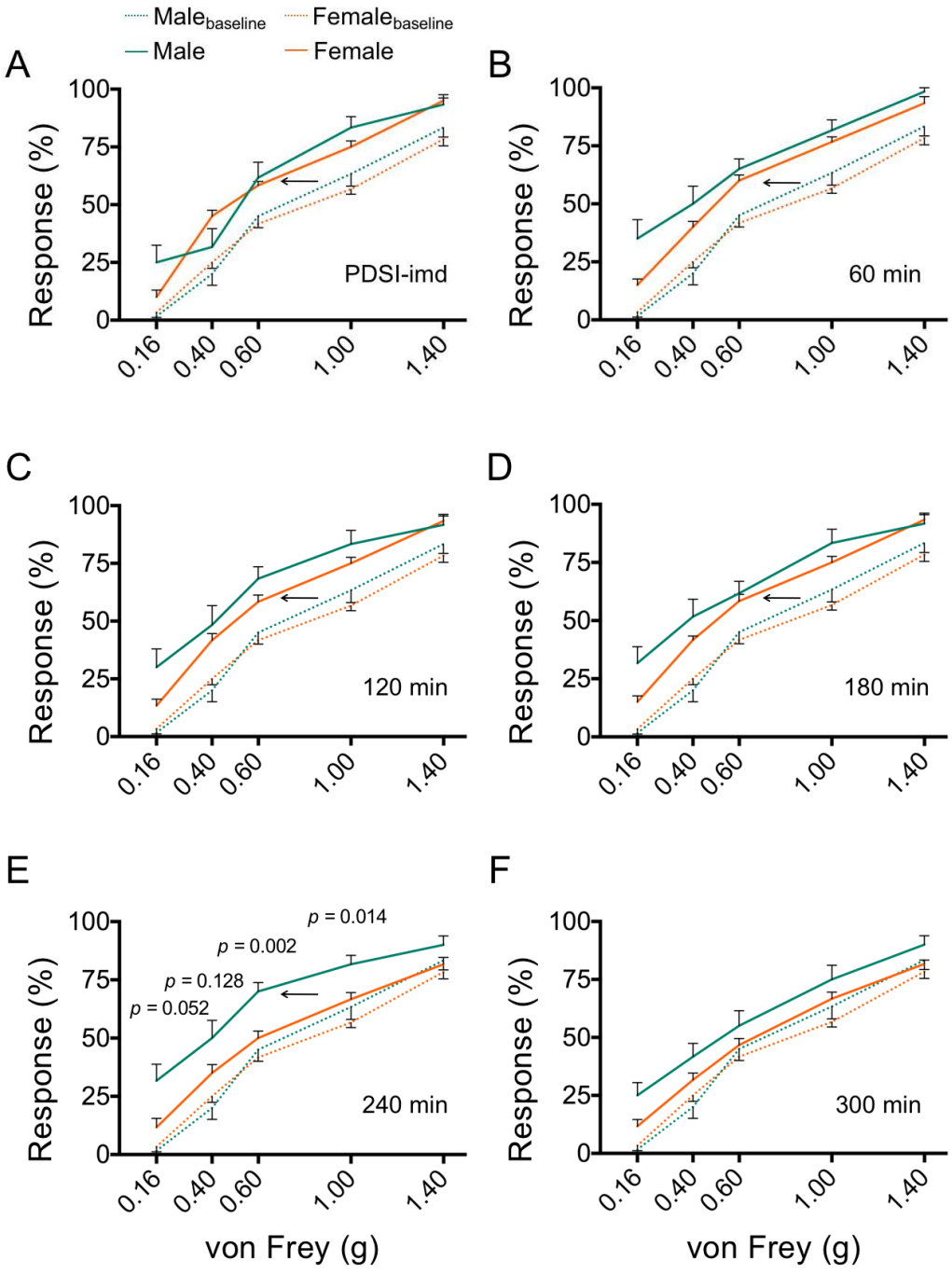


Figure 4 Sex comparisons of the stimulus-response functional curves for mechanical pain sensitivity in mice prior to (Baseline, dashed) and immediately (PDSI-imd, **A**), 60-min (**B**), 120-min (**C**), 180-min (**D**), 240-min (**E**) and 300-min (**F**) after priming dyadic social interaction (PDSI) with a cagemate demonstrator of the same sex in pain. $p < 0.05$ as statistical significance [Male (n=12) *vs*. Female (n=12) with two-tailed Mann-Whitney *U* test, for details see **Table S2-S3**]. BL, baseline. Mean±SEM.

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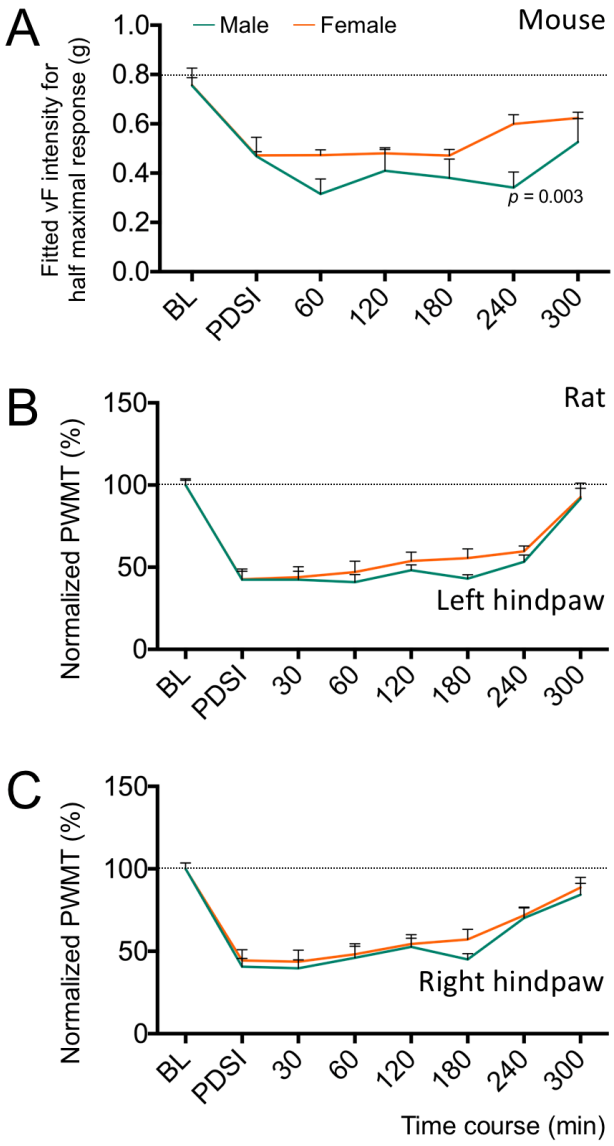

601
602 **Figure 5** Sex comparisons of changes in mechanical pain sensitivity in mice and rats prior to (BL) and
603 immediately, 30, 60, 120, 180, 240 and 300 min after priming dyadic social interaction (PDSI) with a
604 cagemate demonstrator of the same sex in pain. (A) Time courses of changes in von Frey (vF) intensity
605 (g) for half maximal response fitted from the stimulus-response functional curves of **Figure 4** in mice
606 by Bliss method. (B-C) Time courses of normalized paw withdrawal mechanical threshold (PWMT)
607 measured in the left (B) and right (C) hindpaws of rats. BL, baseline. $p < 0.05$ as statistical significance
608 [Male (n=8-12) *vs*. Female (n=12) with two-tailed Mann-Whitney *U* test, for details see **Table S2-S3**].
609 Mean±SEM.
610
611
612

613 Table 1 Cross-species comparisons of empathic consolation, general social and non-social behaviors in
614 subjects of the same sex

	Male			Female		
Allo-licking/-grooming	**Mouse**	**Rat**	***P* value**	**Mouse**	**Rat**	***P* value**
Latency (s)	**25.25 ± 5.07**	**62.50 ± 27.74**	***p* = 0.630**	**21.92 ± 3.84**	**25.82 ± 7.48**	***p* = 0.880**
Total time (s)	**75.50 ± 6.52**	**64.67 ± 11.62**	***p* = 0.425**	**24.00 ± 1.71**	**50.91 ± 10.25**	***p* = 0.079**
Counts	**17.92 ± 1.29**	**14.17 ± 1.79**	***p* = 0.143**	**11.33 ± 1.12**	**11.09 ± 2.02**	***p* = 0.928**
Allo-tail sniffing						
Latency (s)	**32.25 ± 5.23**	**436.67 ± 186.17**	***p* = 0.101**	**41.83 ± 15.31**	**382.82 ± 213.49**	***p* = 0.347**
Total time (s)	**14.92 ± 3.23**	**6.92 ± 1.54**	***p* = 0.089**	**2.67 ± 0.92**	**9.82 ± 3.16**	***p* = 0.051**
Counts	**5.17 ± 0.83**	**2.33 ± 0.40**	***p* = 0.006**	**1.83 ± 0.59**	**3.27 ± 0.73**	***p* = 0.151**
Self-licking/-grooming						
Latency (s)	**281.17 ± 48.87**	**110.42 ± 58.05**	***p* = 0.001**	**216.75 ± 24.46**	**173.91 ± 39.87**	***p* = 0.190**
Total time (s)	**69.08 ± 10.93**	**183.42 ± 42.01**	***p* = 0.017**	**75.58 ± 22.10**	**160.09 ± 24.36**	***p* = 0.016**
Counts	**5.67 ± 0.67**	**8.00 ± 1.16**	***p* = 0.095**	**6.92 ± 1.78**	**8.64 ± 1.15**	***p* = 0.260**
No. of animals	**n=12**	**n=12**		**n=12**	**n=11**	

615 Notes: All the data were expressed as mean ± SEM.Two tailed two-sample*t* test or Mann-Whitney *U*
616 test was used depending upon the results ofnormality and variance tests. $p < 0.05$ was considered as
617 statistically significant.
618