Published in final edited form as:

Soc Neurosci. 2018 December ; 13(6): 710–717. doi:10.1080/17470919.2018.1456957.

Social transfer of alcohol withdrawal-induced hyperalgesia in female prairie voles

Andre T. Walcott¹, Monique L. Smith¹, Jennifer M. Loftis^{1,2,3}, and Andrey E. Ryabinin¹

¹Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR 97239, USA

²Research & Development Service, VA Portland Health Care System, Portland, OR, USA

³Department of Psychiatry, Oregon Health & Science University, Portland, OR, USA

Abstract

The expression of pain serves as a way for animals to communicate potential dangers to nearby conspecifics. Recent research demonstrated that mice undergoing alcohol or morphine withdrawal, or inflammation, could socially communicate their hyperalgesia to nearby mice. However, it is unknown whether such social transfer of hyperalgesia can be observed in other species of rodents. Therefore, the present study investigated if the social transfer of hyperalgesia occurs in the highly social prairie vole (*Microtus ochrogaster*). We observe that adult female prairie voles undergoing withdrawal from voluntary two-bottle choice alcohol drinking display an increase in nociception. This alcohol withdrawal-induced hypersensitivity is socially transferred to female siblings within the same cage and female strangers housed in separate cages within the same room. These experiments reveal that the social transfer of pain phenomenon is not specific to inbred mouse strains and that prairie voles display alcohol withdrawal and social transfer-induced hyperalgesia.

Keywords

Empathy; nociception; alcohol withdrawal; animal housing

Introduction

Pain is considered to be a “biopsychosocial” phenomenon (Gatchel, Peng, Peters, Fuchs, & Turk, 2007; Lumley et al., 2011), because the experience of pain is dramatically influenced by social and environmental factors. Moreover, the relationship between pain and the social environment is bidirectional, such that the experience of persistent pain negatively affects not only the patient, but also individuals that are in close contact. For example, it is well documented that spouses of chronic pain patients report increased pain (Block, Kremer, & Gaylor, 1980; Mohamed, Weisz, & Waring, 1978; Saarijärvi, Rytökoski, & Karppi, 1990; Shanfield, Heiman, Cope, & Jones, 1979). However, we do not currently have a neurobiological explanation for this type of phenomena.

Pain is influenced by a variety of social and sensory cues, and is communicated to nearby conspecifics via several sensory modalities. For example, rodents demonstrate heightened responses to painful stimuli (hyperalgesia) following visual observation of a cage mate experiencing pain (Langford et al., 2006; Li et al., 2014). Recent studies also demonstrate that mice experiencing pain induce an indistinguishable state of hypersensitivity in mice housed and tested within the same room, and that this “social transfer” of pain is likely communicated via olfactory cues (Laviola et al., 2017; Smith, Hostetler, Heinricher, & Ryabinin, 2016).

As a social cue, pain is beneficial in providing a warning through multiple sensory modalities to conspecific animals about potential injury. For example, mice that witnessed other mice being attacked by biting flies, displayed self-burying behaviors when exposed to flies that were unable to bite (Kavaliers, Choleris, & Colwell, 2001). In another experiment, non-stressed rats could discriminate between odors produced by stressed and non-stressed rats and avoid the odor produced by the stressed rats (Mackay-Sim & Laing, 1981).

Recently, neuroanatomical experiments demonstrated activation of the anterior cingulate and anterior insula during the social transfer of hyperalgesia (Smith, Walcott, Heinricher, & Ryabinin, 2017), regions which have been implicated in the experience of pain and empathy in humans (Bernhardt & Singer, 2012; Gu et al., 2012; Singer et al., 2004). The involvement of this neurocircuitry suggests that the social transfer of hyperalgesia may be a phenomenon related to empathy. Furthermore, female mice displayed a stronger effect in the social transfer of pain compared to male mice (Smith et al., 2016), which matches stronger empathy responses observed in females versus males in humans (Christov-Moore et al., 2014; O’Brien, Konrath, Grühn, & Hagen, 2013).

Currently, the social transfer of hyperalgesia has only been demonstrated using inbred mouse strains. It is unknown whether social transfer can be observed in other rodent species. Of particular interest would be the examination of this phenomenon in species with unique social phenotypes like the prairie vole (*Microtus ochrogaster*). Similar to humans, and in contrast to mice, prairie voles develop long-term attachments between adult individuals, i.e., pair bonds (Carter & Getz, 1993). Moreover, neurochemical mechanisms mediating the development of pair bonds in prairie voles also have been shown to play a role in social attachments in humans, demonstrating homologies in mechanisms regulating social behaviors between humans and prairie voles (Aragona et al., 2006; Insel & Hulihan, 1995; Lee, Macbeth, Pagani, & Young, 2009; Pitkow et al., 2001; Walum et al., 2008; Wang et al., 1999). Therefore, to test whether social transfer of hyperalgesia can be observed beyond inbred mouse strains, the current studies explored this phenomenon in prairie voles.

Materials and Methods

Subjects

Adult female prairie voles (n=60) ranging from 74-121 days at the start of the experiment were used from our breeding colony at the VA Portland Health Care System (VAPORHCS) Veterinary Medical Unit. Animals were weaned at 21 days and housed in same-sex sibling groups in cages (27×27×13 cm), with females and males housed in different rooms. All

subjects had access to cotton nestlets and *ad libitum* access to water and a diet of mixed rabbit chow (LabDiet Hi-Fiber Rabbit; PMI Nutrition International, Richmond, IN), corn (Nutrena Cleaned Grains; Cargill, Inc., Minneapolis, MN), and oats (Grainland Select Grains; Grainland Cooperative, Eureka, IL) throughout the duration of the experiment. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the VAPORHCS.

Housing Conditions

Voies were housed in a social housing cage (27×27×13 cm) with a wire mesh divider down the center of the cage, which kept each of the paired voies in one half of the cage (Fig. 1). These cages allow the monitoring of individual fluid consumption, while simultaneously allowing for olfactory, visual, and auditory contact between animals. Previous studies indicated that these cages do not strongly affect social or drinking behaviors (Anacker, Loftis, & Ryabinin, 2011; Curtis, 2010; Walcott & Ryabinin, 2017).

Two-Bottle Alcohol Drinking and Withdrawal

The protocol for alcohol drinking and withdrawal was modeled after previous studies in mice (Smith et al., 2016; 2017), with the exception that all voies were socially housed as described above, rather than isolated (as in the mouse studies). Voies on each side of the mesh were given continuous access to two 25 mL glass tubes with metal sippers attached to rubber stoppers. Animals that received access to ethanol had one bottle containing tap water and one bottle containing increasing concentrations of unsweetened ethanol (3-10% v/v) dissolved in tap water. Fluid levels were measured every 24 hours and the locations of the bottles were switched every day to prevent side bias. Voies were given continuous access to two bottles for two weeks. Once a week, the ethanol bottles were removed and replaced with bottles containing water. During the first week of drinking all voies received 3% ethanol for 2 days, 6% ethanol for 2 days, and 10% ethanol for 1 day followed by a 24-hour withdrawal period (WD1). In the second week, all voies received 10% ethanol for 6 days followed by a second 24-hour withdrawal period (WD2). Average alcohol preference over water for each vole was calculated by dividing the total volume of alcohol consumed by the total volume of fluid consumed. Additionally, the average daily alcohol consumption was calculated by dividing the grams of alcohol consumed (the density of alcohol multiplied by the v/v concentration multiplied by the volume consumed) by the weight of each vole in kilograms (g/kg).

Mechanical Sensitivity

Mechanical sensitivity was measured using the von Frey up-down technique (Chaplan, Bach, Pogrel, Chung, & Yaksh, 1994). We focused on mechanical sensitivity as a test of nociception because our previous study in mice demonstrated similar social transfer when we measured either mechanical sensitivity, thermal sensitivity, or nocifensive behaviors to a chemical irritant (Smith et al., 2016). Responses were elicited by mechanical stimulation by von Frey hairs (0.04 to 6.0g of plastic fibers) to the plantar surface of left hindpaw. Hindpaw withdrawal, shaking, or licking from the fiber stimulation was considered a response. This method uses stimulus oscillation around the response threshold to determine the median 50% threshold of the response. As previously described in mice (Smith et al., 2016), voies

Author Manuscript

Author Manuscript

Author Manuscript

were allowed to acclimate to a Plexiglas box located on top of a wire mesh testing rack for 2 days for 40 minutes prior to the start of the experiment. Prior to experimental treatment, basal mechanical thresholds were measured (baseline) and animals were assigned to treatment groups in a counterbalanced manner. Testing occurred 24-hours after the start of each withdrawal session. Before the start of each mechanical test session, voles were put into the Plexiglas box for 10-20 minutes to acclimate. All testing sessions occurred during the light cycle, but testing occurred in a room only lit by a dim red lamp. Testing occurred in a separate room from the housing room. In our previous experiments in mice, when the animals were tested in the same room in which they were housed, the experimenter performing the von Frey test was blind to the conditions of the group (Smith et al., 2016). Due to our decision to perform the test in a separate room from housing rooms in order to avoid potential visual mimicry, the current study was run in a semi-blinded fashion. During both experiments, the investigator was aware whether animals came from the Co-Housed or Separate room. The investigator was aware whether the animals belonged to the EtOH or H₂O Co-Housed group in Experiment 2, but not in Experiment 1.

Statistical Analysis

Mechanical sensitivity was analyzed by repeated-measures ANOVA design using group (EtOH/Co-housed, H₂O/Co-housed, and H₂O/Separate) as the between-subjects factor and mechanical threshold test (week/WD session) as the repeated measure. Significant outcomes were followed by a Tukey's *post hoc* test. Significance threshold was set at $p < 0.05$ for all analyses. All statistical analyses were performed using GraphPad Software Prism 6. The number of animals in each group was determined by previous mouse studies (Smith et al., 2016; 2017). One animal was removed from all analyses due to incomplete mechanical threshold measurements during the baseline test.

Results

The social transfer of alcohol-withdrawal induced hypersensitivity within the same cage

To determine whether alcohol-withdrawal would affect sibling prairie voles within the same cage, we examined three different groups of female prairie voles. As described above, females in the *EtOH/Co-housed* group (n=10) were exposed to increasing concentrations of ethanol (3-10%) for two weeks, followed by a 24-hour withdrawal period. Females in the *H₂O/Co-housed* group (n=9) were housed in the same cage as the females in the *EtOH/Co-housed* group, but were separated by a mesh divider. *H₂O/Co-housed* females were given access to only water for two weeks and mechanical threshold was measured weekly. Lastly, *H₂O/Separate* females (n=10) were socially housed in a separate room in mesh divided cages with another *H₂O/Separate* female and received access to only water. All groups were tested weekly for mechanical threshold on the same day, in a separate room from their housing cages.

EtOH/Co-housed females voluntarily consumed 7.3 ± 0.7 g/kg (Fig. 2A) and had a $48 \pm 4.1\%$ preference (Fig. 2B) for alcohol over the two-week period. A repeated-measures ANOVA comparing mechanical sensitivity over time revealed a significant main effect of week ($F_{2,52} = 28.01$, $p < 0.0001$) and treatment ($F_{2,26} = 5.60$, $p = 0.01$). There was no

significant interaction between week and treatment ($F_{4,52} = 1.36$, $p = 0.26$; Fig. 2C). To test our *a priori* hypothesis if there were treatment differences over time, we included the *post-hoc* results showing that females in the EtOH/Co-housed and H₂O/Co-housed group showed a significant increase in mechanical sensitivity from baseline after WD1 and WD2. Meanwhile females in the H₂O/Separate group showed no significant difference in mechanical sensitivity after WD1 and WD2 relative to baseline. These findings confirm that 24-hour withdrawal from alcohol leads to mechanical hypersensitivity in female prairie voles that is socially transferred to female siblings housed in the same cage.

The social transfer of alcohol-withdrawal induced hypersensitivity between different cages

We next examined whether social transfer occurs between non-sibling animals housed in separate cages within the same room. Therefore, with a different set of female voles we conducted the same social transfer paradigm as above, with the exceptions that: (1) animals in this experiment received the same treatment as their cage mate, and (2) animals in the three treatment groups were tested separately, to eliminate visual mimicry between voles in different treatment groups.

Females in the EtOH/Co-housed group self-administered on average 7.3 ± 0.7 g/kg of alcohol (Fig. 3A) and showed an average $60 \pm 3.0\%$ preference (Fig. 3B) for alcohol over the two-week period. Analysis of the mechanical threshold after each withdrawal revealed a mean effect of week ($F_{2,54} = 12.74$, $p < 0.0001$), treatment ($F_{2,27} = 19.44$, $p < 0.0001$), and a significant interaction between treatment and week ($F_{4,54} = 4.58$, $p = 0.003$; Fig. 3C). A Tukey's *post hoc* analysis revealed that females in both EtOH/Co-housed ($n=10$) and H₂O/Co-housed ($n=10$) groups showed a significant decrease in mechanical threshold after WD1 and WD2. Meanwhile, females in the H₂O/Separate ($n=10$) group showed no hypersensitivity at any point in time. These data further indicate that alcohol-withdrawal induced hypersensitivity is socially transferred not only within the same cage, but also between female prairie voles housed in different cages within the same room.

Discussion

The present study demonstrated female prairie voles show mechanical hypersensitivity after an acute 24-hour withdrawal from several days of voluntary alcohol consumption. This withdrawal induced mechanical hypersensitivity is socially transferred to conspecifics. Females that were cohoused in the same cage displayed a level of hyperalgesia that roughly matched females that were experiencing the acute withdrawal. This display of hypersensitivity in females that were exposed to just water not only occurred when their cage mate was experiencing withdrawal, but it also occurred when other animals in different cages - within the same room - experienced alcohol withdrawal. Previous studies have shown that naïve mice acquire socially transferred hyperalgesia when housed in separate cages within the same room as conspecifics experiencing direct hyperalgesia (Smith et al., 2016; 2017). The present study is the first to show that socially transferred and alcohol-withdrawal induced hyperalgesia occurs in another species besides the traditional laboratory mouse models.

Until the current study, alcohol withdrawal has not been behaviorally demonstrated in prairie voles. The traditional way to measure alcohol withdrawal in mouse models is by measuring handling-induced convulsions or HICs (Crabbe, Janowsky, Young, & Rigter, 1980; Crabbe, Merrill, & Belknap, 1991). HICs require mice to be initially suspended in the air by their tails until a spontaneous convulsion occurs. Running this behavioral test in prairie voles is not feasible because the tail of a prairie vole is relatively short and extremely fragile. The von Frey mechanical threshold test provides an alternative and sensitive method to evaluate withdrawal- and socially-transferred hyperalgesia. The decreased mechanical threshold in prairie voles undergoing withdrawal provides additional evidence for the relationship between pain and alcohol withdrawal.

Importantly, our findings indicate that the social transfer of hyperalgesia initially observed in inbred mice, is also not specific to just this rodent species (Smith et al., 2016; 2017). In evolutionary terms, voles belong not only to a different genus, but a different family of rodents from mice and rats. Moreover, since prairie voles are genetically heterogeneous, this is the first demonstration that social transfer of hypersensitivity occurs between individuals of different genotypes. The present study also differs from the previous studies in that here the animals were housed in social conditions. Previous studies on social transfer of withdrawal-induced hypersensitivity used socially isolated mice housed in the same room. There is increased evidence that rodents can display empathy-like consolation behavior (Burkett et al., 2016; Rice & Gainer, 1962). Therefore, it was possible social housing would prevent or mask social transfer of hyperalgesia. Contrary to this possibility, we observed social transfer of hypersensitivity indicating consistency of this phenomenon across housing conditions. This finding is in agreement with the demonstration that olfactory cues are sufficient for transfer of hyperalgesia in mice (Smith et al., 2016).

The present study also differed from experiments in mice in the context in which the testing occurred. Thus, in the previous study, testing occurred in the same room in which the mice were housed, allowing for the possibility that the mice influenced each other's behavior during testing (for example, through visual mimicry (Langford et al., 2006)). In contrast, in the current experiments, testing occurred in a room that was separate from the housing room, removing the voles from any cues that were specific to the housing room. While previous studies in mice included experiments that suggested that visual mimicry does not play a role in social transfer of hyperalgesia (Laviola et al., 2017; Smith et al., 2016), the current experiments definitively eliminate this possibility. Since prairie voles were not able to observe each other during testing, hyperalgesia in the co-housed animals can only be explained by transfer of a hyperalgesic state, and not by visual mimicry.

Alcohol withdrawal can be accompanied by increased anxiety and disruption of sleep cycles in rodents and humans (Brager, Ruby, Prosser, & Glass, 2010; Landolt & Gillin, 2001; Rassnick, Heinrichs, Britton, & Koob, 1993). It could be theorized that the increase in mechanical sensitivity in the H₂O/Co-housed group was in part due to changes in anxiety or sleep cycles in these animals. Disruption of sleep increases corticosterone (CORT) levels and affects anxiety measures in rodents (LeGates et al., 2012; Silva et al., 2004). Our previous studies in mice have not found elevated plasma corticosterone (CORT) levels, and no changes behavior in elevated plus maze and acoustic startle tests following identical

procedures (Smith et al., 2016). Therefore, we reason that the social transfer observed between rodents is more specific to nociception, rather than explained by general anxiety, arousal or sleep disruption. Interestingly, Smith et al. (2016) have also shown that nociception can be socially transferred after inflammatory type of pain induced by complete Freund's adjuvant, indicating that the transfer is not limited to chronic withdrawal-induced hyperalgesia. It seems likely that similar mechanisms of social transfer are engaged in voles and in mice, but the exact nature of the transfer of hyperalgesia needs to be addressed in future studies.

Even though multiple treatments have been developed and used in preclinical models of pain, many treatment options have not been effective in clinical research. This translational problem could be due to the lack of the "social" aspect in the models of "biopsychosocial" phenomenon of pain. The prairie vole is one animal model that can provide an advantage over traditional laboratory rodents. For example, prairie voles show consolation behavior towards a conspecific that experienced a stressful situation. Interestingly, when meadow voles (a non-social species of voles) were tested they showed no consolation behavior and social buffering towards a stressed conspecific (Burkett et al., 2016; A. S. Smith & Wang, 2014). Prairie vole and human social relationships show similarity and are controlled by homologous biological mechanisms. Therefore, our future studies will investigate the biological mechanisms that play a role in the social transfer of hyperalgesia in prairie voles. Nevertheless, the current findings support that the use of prairie voles in pain research will help close the current translational gap.

As a cautionary note, our studies used a semi-blinded procedure because it was impossible to conceal the identity of housing arrangements of the Co-housed groups versus the Separate group. Indeed, early studies have noted that different raters using manual von Frey testing can deviate in their assessment of hyperalgesia (Chaplan et al., 1994). That said, three experimenters working independently in this laboratory have repeatedly observed the social transfer of hyperalgesia in mice or prairie voles. As an alternative interpretation, two potential scenarios can be theorized: an experimenter would not notice a deviation from threshold baseline due to repeated testing in the H₂O/Separate group or would notice a non-existent decrease in threshold in the H₂O/Co-Housed group. Our analysis of the limited hyperalgesia literature that mentions the terms "baseline" and "von Frey" in the publication abstracts identifies no deviation from baseline due to repeated testing across multiple mouse strains and in rats (Banik, Woo, Park, & Brennan, 2006; Macolino, Daiutolo, Albertson, & Elliott, 2014; E. E. Young et al., 2016). Interestingly, studies in mice indicating the use of non-bias procedures show a decrease from baseline in control groups equivalent to our H₂O/Co-Housed group (Macolino et al., 2014; Marquez de Prado, Hammond, & Russo, 2009). It is possible, however, that such deviation would be more difficult to detect in larger animals, such as rats (Nirogi, Goura, Shanmuganathan, Jayarajan, & Abraham, 2012). Therefore, we believe that our results were not influenced by our semi-blinded testing procedure. Nevertheless, future studies comparing Co-Housed and Separated animals will need to be specifically designed to avoid any potential testing bias.

Taken together, our studies expand existing examples of empathy-like behaviors in rodents. They indicate that such behaviors can be relatively resistant to influence by social context. In

addition, they further suggest that laboratory rodents housed in separate cages within the same room can influence each other's physiological states, and thereby prevent detecting differences between experimental groups and controls. This possibility needs to be taken into account when designing future animal experiments.

Acknowledgments:

This work was supported by NIH grants RO1 AA019793, RO1 AA025548 and T32 AA007468. This material is also the result of work supported with resources and the use of facilities at the Veterans Affairs Portland Health Care System (VAPORHCS) (Portland, OR; USA). Dr. Loftis is an employee of the VAPORHCS. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

References

- Anacker AMJ, Loftis JM, & Ryabinin AE (2011). Alcohol intake in prairie voles is influenced by the drinking level of a peer. *Alcoholism: Clinical and Experimental Research*, 35(10), 1884–1890. 10.1111/j.1530-0277.2011.01533.x
- Aragona BJ, Liu Y, Yu YJ, Curtis JT, Detwiler JM, Insel TR, & Wang Z (2006). Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nature Neuroscience*, 9(1), 133–139. 10.1038/nn1613 [PubMed: 16327783]
- Banik RK, Woo YC, Park SS, & Brennan TJ (2006). Strain and sex influence on pain sensitivity after plantar incision in the mouse. *Anesthesiology*, 105(6), 1246–1253. [PubMed: 17122588]
- Bernhardt BC, & Singer T (2012). The neural basis of empathy. *Annual Review of Neuroscience*, 35(1), 1–23. 10.1146/annurev-neuro-062111-150536
- Block AR, Kremer EF, & Gaylor M (1980). Behavioral treatment of chronic pain: the spouse as a discriminative cue for pain behavior. *Pain*, 9(2), 243–252. [PubMed: 7454386]
- Brager AJ, Ruby CL, Prosser RA, & Glass JD (2010). Chronic ethanol disrupts circadian photic entrainment and daily locomotor activity in the mouse. *Alcoholism: Clinical and Experimental Research*, 34(7), 1266–1273. 10.1111/j.1530-0277.2010.01204.x
- Burkett JP, Andari E, Johnson ZV, Curry DC, de Waal FBM, & Young LJ (2016). Oxytocin-dependent consolation behavior in rodents. *Science (New York, N.Y.)*, 351(6271), 375–378. 10.1126/science.aac4785
- Carter CS, & Getz LL (1993). Monogamy and the prairie vole. *Scientific American*, 268(6), 100–106. [PubMed: 8516669]
- Chaplan SR, Bach FW, Pogrel JW, Chung JM, & Yaksh TL (1994). Quantitative assessment of tactile allodynia in the rat paw. *Journal of Neuroscience Methods*, 53(1), 55–63. [PubMed: 7990513]
- Christov-Moore L, Simpson EA, Coudé G, Grigaityte K, Iacoboni M, & Ferrari PF (2014). Empathy: gender effects in brain and behavior. *Neuroscience and Biobehavioral Reviews*, 46 Pt 4, 604–627. 10.1016/j.neubiorev.2014.09.001 [PubMed: 25236781]
- Crabbe JC, Janowsky JS, Young ER, & Rigter H (1980). Handling induced convulsions in twenty inbred strains of mice. *Substance and Alcohol Actions/Misuse*, 1(2), 159–163. [PubMed: 7197806]
- Crabbe JC, Merrill CD, & Belknap JK (1991). Effects of convulsants on handling-induced convulsions in mice selected for ethanol withdrawal severity. *Brain Research*, 550(1), 1–6. [PubMed: 1888987]
- Curtis JT (2010). Does fertility trump monogamy? *Animal Behaviour*, 80(2), 319–328. 10.1016/j.anbehav.2010.05.014 [PubMed: 20823948]
- Gatchel RJ, Peng YB, Peters ML, Fuchs PN, & Turk DC (2007). The biopsychosocial approach to chronic pain: scientific advances and future directions. *Psychological Bulletin*, 133(4), 581–624. 10.1037/0033-2909.133.4.581 [PubMed: 17592957]
- Gu X, Gao Z, Wang X, Liu X, Knight RT, Hof PR, & Fan J (2012). Anterior insular cortex is necessary for empathetic pain perception. *Brain : a Journal of Neurology*, 135(Pt 9), 2726–2735. 10.1093/brain/aws199 [PubMed: 22961548]

- Insel TR, & Hulihan TJ (1995). A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. *Behavioral Neuroscience*, 109(4), 782–789. [PubMed: 7576222]
- Kavaliers M, Choleris E, & Colwell DD (2001). Learning from others to cope with biting flies: social learning of fear-induced conditioned analgesia and active avoidance. *Behavioral Neuroscience*, 115(3), 661–674. [PubMed: 11439455]
- Landolt HP, & Gillin JC (2001). Sleep abnormalities during abstinence in alcohol-dependent patients. Aetiology and management. *CNS Drugs*, 15(5), 413–425. [PubMed: 11475945]
- Langford DJ, Cragger SE, Shehzad Z, Smith SB, Sotocinal SG, Levenstadt JS, et al. (2006). Social modulation of pain as evidence for empathy in mice. *Science (New York, N.Y.)*, 312(5782), 1967–1970. 10.1126/science.1128322
- Laviola G, Zoratto F, Ingiosi D, Carito V, Huzard D, Fiore M, & Macrì S (2017). Low empathy-like behaviour in male mice associates with impaired sociability, emotional memory, physiological stress reactivity and variations in neurobiological regulations. *PLoS ONE*, 12(12), e0188907 10.1371/journal.pone.0188907 [PubMed: 29200428]
- Lee H-J, Macbeth AH, Pagani JH, & Young WS (2009). Oxytocin: the great facilitator of life. *Progress in Neurobiology*, 88(2), 127–151. 10.1016/j.pneurobio.2009.04.001 [PubMed: 19482229]
- LeGates TA, Altimus CM, Wang H, Lee H-K, Yang S, Zhao H, et al. (2012). Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature*, 491(7425), 594–598. 10.1038/nature11673 [PubMed: 23151476]
- Li Z, Lu Y-F, Li C-L, Wang Y, Sun W, He T, et al. (2014). Social interaction with a cagemate in pain facilitates subsequent spinal nociception via activation of the medial prefrontal cortex in rats. *Pain*, 155(7), 1253–1261. 10.1016/j.pain.2014.03.019 [PubMed: 24699208]
- Lumley MA, Cohen JL, Borszcz GS, Cano A, Radcliffe AM, Porter LS, et al. (2011). Pain and emotion: a biopsychosocial review of recent research. *Journal of Clinical Psychology*, 67(9), 942–968. 10.1002/jclp.20816 [PubMed: 21647882]
- Mackay-Sim A, & Laing DG (1981). The sources of odors from stressed rats. *Physiology & Behavior*, 27(3), 511–513. [PubMed: 7335791]
- Macolino CM, Daiutolo BV, Albertson BK, & Elliott MB (2014). Mechanical allodynia induced by traumatic brain injury is independent of restraint stress. *Journal of Neuroscience Methods*, 226, 139–146. 10.1016/j.jneumeth.2014.01.008 [PubMed: 24486873]
- Marquez de Prado B, Hammond DL, & Russo AF (2009). Genetic Enhancement of Calcitonin Gene-Related Peptide-Induced Central Sensitization to Mechanical Stimuli in Mice. *Journal of Pain*, 10(9), 992–1000. 10.1016/j.jpain.2009.03.018 [PubMed: 19628434]
- Mohamed SN, Weisz GM, & Waring EM (1978). The relationship of chronic pain to depression, marital adjustment, and family dynamics. *Pain*, 5(3), 285–292. [PubMed: 724280]
- Nirogi R, Goura V, Shanmuganathan D, Jayarajan P, & Abraham R (2012). Comparison of manual and automated filaments for evaluation of neuropathic pain behavior in rats. *Journal of Pharmacological and Toxicological Methods*, 66(1), 8–13. 10.1016/j.vascn.2012.04.006 [PubMed: 22575456]
- O'Brien E, Konrath SH, Grün D, & Hagen AL (2013). Empathic concern and perspective taking: linear and quadratic effects of age across the adult life span. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*, 68(2), 168–175. 10.1093/geronb/gbs055
- Pitkow LJ, Sharer CA, Ren X, Insel TR, Terwilliger EF, & Young LJ (2001). Facilitation of affiliation and pair-bond formation by vasopressin receptor gene transfer into the ventral forebrain of a monogamous vole. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, 21(18), 7392–7396. [PubMed: 11549749]
- Rassnick S, Heinrichs SC, Britton KT, & Koob GF (1993). Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Research*, 605(1), 25–32. [PubMed: 8467387]
- Rice GE, & Gainer P (1962). "Altruism" in the albino rat. *Journal of Comparative and Physiological Psychology*, 55, 123–125. [PubMed: 14491896]
- Saarijärvi S, Rytökoski U, & Karppi SL (1990). Marital satisfaction and distress in chronic low-back pain patients and their spouses. *The Clinical Journal of Pain*, 6(2), 148–152. [PubMed: 2152010]

- Shanfield SB, Heiman EM, Cope DN, & Jones JR (1979). Pain and the marital relationship: psychiatric distress. *Pain*, 7(3), 343–351. [PubMed: 530740]
- Silva RH, Kameda SR, Carvalho RC, Takatsu-Coleman AL, Niigaki ST, Abílio VC, et al. (2004). Anxiogenic effect of sleep deprivation in the elevated plus-maze test in mice. *Psychopharmacology*, 176(2), 115–122. 10.1007/s00213-004-1873-z [PubMed: 15160262]
- Singer T, Seymour B, O’Doherty J, Kaube H, Dolan RJ, & Frith CD (2004). Empathy for pain involves the affective but not sensory components of pain. *Science (New York, N.Y.)*, 303(5661), 1157–1162. 10.1126/science.1093535
- Smith AS, & Wang Z (2014). Hypothalamic oxytocin mediates social buffering of the stress response. *Biological Psychiatry*, 76(4), 281–288. 10.1016/j.biopsych.2013.09.017 [PubMed: 24183103]
- Smith ML, Hostetler CM, Heinricher MM, & Ryabinin AE (2016). Social transfer of pain in mice. *Science Advances*, 2(10), e1600855–e1600855. 10.1126/sciadv.1600855 [PubMed: 27774512]
- Smith ML, Walcott AT, Heinricher MM, & Ryabinin AE (2017). Anterior Cingulate Cortex Contributes to Alcohol Withdrawal- Induced and Socially Transferred Hyperalgesia. *eNeuro*, 4(4), ENEURO.0087–17.2017. 10.1523/ENEURO.0087-17.2017
- Walcott AT, & Ryabinin AE (2017). Alcohol’s Effects on Pair-Bond Maintenance in Male Prairie Voles. *Frontiers in Psychiatry*, 8, 37–13. 10.3389/fpsyt.2017.00226 [PubMed: 28360866]
- Walum H, Westberg L, Henningsson S, Neiderhiser JM, Reiss D, Igl W, et al. (2008). Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 105(37), 14153–14156. 10.1073/pnas.0803081105 [PubMed: 18765804]
- Wang Z, Yu G, Cascio C, Liu Y, Gingrich B, & Insel TR (1999). Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles (*Microtus ochrogaster*): a mechanism for pair bonding? *Behavioral Neuroscience*, 113(3), 602–611. [PubMed: 10443786]
- Young EE, Bryant CD, Lee SE, Peng X, Cook B, Nair HK, et al. (2016). Systems genetic and pharmacological analysis identifies candidate genes underlying mechanosensation in the von Frey test. *Genes, Brain, and Behavior*, 15(6), 604–615. 10.1111/gbb.12302

Figure 1:

Schematic representation of the two experiments. In both experiments animals were socially-housed, separated by a mesh divider. In each experiment, one room contained both Co-Housed groups of voles and another room contained the H2O/Separate group. Mechanical sensitivity using von Frey fibers was performed in a different room (illustrated by a room with a rack below). In Experiment 1 (left), EtOH/Co-Housed and H2O/Co-Housed animals were housed in the same cage. In Experiment 2 (right), EtOH/Co-Housed and H2O/Co-Housed animals were housed in separate cages.

Figure 2:

Alcohol withdrawal's effect on mechanical threshold within same cages. Females in the EtOH/Co-housed group were exposed to increasing alcohol concentrations (3-10%) for two weeks, but showed no difference in (A) alcohol consumption or (B) alcohol preference between the day before and after alcohol withdrawal. (C) EtOH/Co-housed ($n=10$) and H₂O/Co-housed ($n=9$) females showed a significant decrease in mechanical threshold by the second withdrawal session when compared to females in the H₂O/Separate ($n=10$) group and baseline. Significant differences compared to the H₂O/Separate group ($p < 0.001$) are represented by ***. Significant changes from baseline ($p < 0.05$) are represented by #. Error bars indicated mean $\pm$ SEM. Mechanical threshold testing is represented by a dotted line (A, B). Mean basal responses of all groups are represented by a dashed line (C).

Figure 3:

Alcohol withdrawal's effect on mechanical threshold across cages. (A) Alcohol consumption and (B) alcohol preference ratio did not significantly differ between the day prior and the day after ethanol withdrawal. (C) Female prairie voles in the EtOH/Co-housed ($n=10$) and H₂O/Co-housed ($n=10$) groups showed a decrease in mechanical threshold from the H₂O/Co-housed ($n=10$) group and baseline after two withdrawal sessions. Significant differences compared to the H₂O/Co-housed group ($p < 0.001$) are represented by ***. Significant changes from baseline ($p < 0.001$) are represented by ###. Error bars indicated mean $\pm$ SEM. Mechanical threshold testing is represented by a dotted line (A, B). Mean basal responses of all groups are represented by a dashed line (C).