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Lumsden, E., et al. (2019). Antidepressant-relevant concentrations of the ketamine metabolite (2R,6R)-hydroxynorketamine do not block NMDA receptor function [Pharmacology]. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11). https://doi.org/10.1073/pnas.1816071116

Raver, C., et al. (2020) An amygdalo-parabrachial pathway regulates pain perception and chronic pain. *Journal of Neuroscience.* https://doi.org/10.1101/2020.01.10.902205

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ABSTRACT

Title: Social Interaction and Pain

Carleigh E. Jenne, Master of Science, 2020

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Chronic pain is the most common cause of disability. Progress in research to alleviate pain is hampered by the fact that metrics for studying pain in animal models are controversial. Rodents highly value social interactions, preferring them even over drugs of abuse or other hedonic rewards. Here, I tested the hypothesis that pain will reduce preference for social interaction, thereby offering a novel tool to quantify pain behaviors. After training rats to self-administer social interaction, I found that acute pain causes devaluation of social interaction. This devaluation was specific to social interaction, because after training rats to self-administer food, acute pain elicited no change in valuation for food self-administration. My findings display the importance of social interaction in pain behaviors, and suggest a novel metric for pain studies.

Social Interaction and Pain

by
Carleigh Jenne

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Chapter 1 Introduction

Pain affects over 23.5 million Americans and is the leading cause of disability in the United States (Nahin, 2015; Stewart, Ricci, Chee, Morganstein, & Lipton, 2003). Current treatments involve invasive procedures and management with medications. Opioids are one of the most prescribed options for treating pain, even though they typically fail to treat chronic pain. However, due to their addictive nature, opioids have contributed to the current opioid epidemic(Volkow & Collins, 2017{Epstein, 2018 #4). More than 11 million Americans misuse prescription opioids, and more than 2 million Americans have an opioid use disorder (Rudd, Aleshire, Zibbell, & Gladden, 2016; Volkow & Collins, 2017).

Animal models allow us to study mechanisms of pain, to manipulate genetics, and perform procedures to induce reproducible conditions (Akintola et al., 2019; Kim et al., 2014; J. S. Mogil, Chesler, Wilson, Juraska, & Sternberg, 2000; Jeffrey S. Mogil et al., 1999; Smeyne et al., 1994). A major limitation of these preclinical studies pertains to the ability to objectively quantify pain (Backonja & Stacey, 2004; Chapman et al., 1985; Gottrup, Nielsen, Arendt-Nielsen, & Jensen, 1998; J. S. Mogil, 2009). Pain research takes advantage of a variety of assays to quantify pain. These include testing reflexive behavior, such as the von Frey or hot plate tests, quantifying spontaneous behaviors, like facial grimace, licking, and shaking, and using operant conditioning paradigms (Mauderli, Acosta-Rua, & Vierck, 2000; J. S. Mogil, 2009; J. S. Mogil, Davis, &

Derbyshire, 2010; Neubert et al., 2005; Pitcher, Ritchie, & Henry, 1999). Most of these assays rely on subjective assessment by human experimenters to distinguish and quantify pain behaviors.

Human and animal studies have displayed the importance of social interaction in pain conditions. Therefore, an alternative approach to studying pain is to focus on the biopsychosocial aspects of pain (Langford et al., 2010; J. S. Mogil, 2015; Raber & Devor, 2002; Venniro & Shaham, 2020). Social support can help an individual feel less pain, but those in social isolation or in conflict may feel more pain (J. S. Mogil, 2015). Indeed, mice witnessing pain in cage-mates display pain-like behaviors (Langford et al., 2010). Social interaction, therefore, is an important aspect of behavior that needs to be considered when studying pain and could be contributing to the variability and complexity of studying pain.

To integrate social interaction in pain studies, I took advantage of a rodent model showing the protective effect of social reward on drug addiction (Venniro, Russell, Zhang, & Shaham, 2019; Venniro & Shaham, 2020; Venniro et al., 2018). Male and female rats voluntarily press a lever to open an automatic guillotine-style door and gain access to novel or familiar peer (Venniro & Shaham, 2020). Surprisingly, rats preferred this social interaction over self-administration of addictive drugs, including methamphetamine and heroin (Venniro & Shaham, 2020; Venniro et al., 2018). Although the main findings on the above-mentioned reports focused on the ability of social reward to abolish drug self-administration and drug craving (Venniro et al., 2019; Venniro & Shaham, 2020; Venniro et al., 2018), this behavioral model may offer a unique tool to

study pain, and to explore brain mechanisms of operant social reward and potential impairments in social reward in animal models of pain.

To this end, I tested the hypothesis that voluntary social interactions can be used as a novel measure for pain sensitivity.

Chapter 2 Materials and Methods

Animals

All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committees at the University of Maryland School of Medicine. We used 24 male Long Evans rats (150-175g), 16 "resident" and 8 "social partners" (Charles River). We singly housed 1 week prior to beginning experiments after 2 to 3 weeks of social housing. The social partners were novel to the resident on the first day of the experiment. Each experimental rat was paired with the same social peer. The social partners were used for two sessions a day (acted as social peers for two experimental animals).

Apparatus

We used 4 custom-made social-choice self-administration chambers. As reported elsewhere (Venniro & Shaham, 2020; Venniro et al., 2018)) each chamber is equipped with a house light located at the top of the chamber, a nose poke receptacle located 5-10 cm above the floor, two cue lights, and two retractable levers located 3-9 cm above the ground. The social interaction chambers contain a main operant chamber for the experimental animal (approximately 32 x 25 x 25 cm) and an additional chamber for the

social peer (approximately 18 x 18 x 25 cm). Each of the retractable levers can be associated with the opening of a retractable door that allows rats access to a social peer. The food associated lever was on the left side of the chamber with the nose poke receptacle, while the social associated lever was on the right side of the chamber with the guillotine door.

Social self-administration

Initially, we gave rats one 60-minute session to habituate to the apparatus. We trained rats to self-administer for access to their social partner during daily 60-min sessions (60 trials/session, 60 seconds social interaction) using a discrete-trial design. Each resident rat presses for time with a social partner. The session started with illumination of the social-paired house light, followed 10 seconds later by the insertion of the social-paired lever. We allowed resident rats a maximum of 60 seconds to press the lever before the lever retracted and the house light turned off. When the lever was successfully pressed, the active lever was retracted and the guillotine door that provided access to the social peer opened. The resident rat could interact with the social partner for 60 seconds. At the 60 second time point, the guillotine door closed. The number of times the guillotine door opened was recorded as rewards or successful trials. The number of active lever presses and inactive lever presses were also recorded(Venniro & Shaham, 2020; Venniro et al., 2018).

Food self-administration

The training procedure is similar to social self-administration and reported elsewhere(Venniro et al., 2019; Venniro & Shaham, 2020; Venniro et al., 2018). We

trained rats to self-administer for food delivery with daily 60 minute sessions (60 trials/session) using a discrete-trial design. The session started with illumination of the food-paired house light, 10 seconds later the food- paired lever was inserted. We allowed the rats a maximum of 60 seconds to press the lever before the lever retracted and the house light turned off. When the lever was successfully pressed, the active lever was retracted which led to the delivery of 1, 45 mg food pellets (TestDiet, Catalogue # 1811155, 12.7% fat, 66.7% carbohydrate, and 20.6% protein). The resident rat was given 60 seconds to eat the food pellet. At the 60 second time point, the next trial began. The number of times the food was delivered was recorded as rewards or successful trials. The number of active lever presses and inactive lever presses were also recorded.

Formalin Injections

A solution 3% formalin (Sigma-Aldrich) was prepared in sterile saline, and filtered through 0.22 µm filter paper(Raver et al., 2020). Before each injection, the injected area was prepared by swabbing with 70% alcohol. 50 µl of this solution is injected subcutaneously (<30G needle) into the dorsal surface of the hind-paw. Formalin injections cause local irritation and increased grooming activity. Because these injections are made to evoke inflammatory pain, no analgesics were used. Behavioral assessments (described below) were performed 5 to 15 minutes after formalin injection into the hind-paw. Even with repeated injections, formalin does not produce persistent or chronic pain(Dubuisson & Dennis, 1977; Lee & Jeong, 2002).

Specific Experiments

Experiment 1A, effect of pain on social self-administration: before beginning either the tests, naïve rats were given 60 minutes for 1 day to habituate to the box. The next day, rats were trained on an FR1 schedule, when rats press the lever once, they gain access to a social peer. After stable social self-administration behavior (6-8 days), we increased the ratio required to receive the reward (FR3 – 3 days). At this point we divided the rats in two cohorts: one group continued under FR3 and trained the other group for social self-administration using the progressive ratio schedule. Once we achieved stable FR3 (group 1) or the PR (group 2) responding for 3 consecutive days, we administered a hind paw formalin injection. After 5 to 10 minutes of pretreatment, we exposed the rats to a FR3 session (group 1) or PR session (group 2) to compare active lever presses and breakpoints before and after the formalin injection. Additionally, we re-expose the rats to 2 more sessions of FR3 (group 1) or PR (group 2) after the injection to establish any long-lasting effects.

Experiment 1B, effect of pain on palatable food self-administration: we kept the rats of group 2 in the facility for 3 days before training them for palatable food self-administration. We trained the rats initially using FR1 reinforcement schedule (3 days) and progressively increased to FR3 (3 days) and PR (3 days). After this training phase, we injected formalin in the rats with a hind paw (the opposite hindpaw as used in Exp. 1A). After 5 to 10 minutes of pretreatment, we exposed the rats to a PR session. Additionally, we re-expose the rats to 1 more sessions of PR after the injection to establish any long-lasting effects.

Statistical analysis

We analyzed group data using GraphPad Prism version 8 (GraphPad Software, La Jolla CA). Data are presented as median values ± 95% confidence intervals (95% CI). We used a repeated measures one-way ANOVA and a Tukey's multiple comparisons test (see Results).

Chapter 3 Results

Self-administration Training

Exp. 1A: We trained rats (n=16) to social self-administration under FR1 (6-8 days) and FR3 (3 days) Figure 1A). Rats reliably self-administered social interaction under FR1 and they increased their active lever presses under FR3 (ANOVA p=<0.001, F=23.30; Figure 1B). To determine the effect of formalin injection to social self-administration, we compared active lever presses of group 1 (n=8) at FR3 two days before formalin injection, the day of formalin injection, and two days after the injection (ANOVA p=0.002, F=16.16)) (Figure 1C). The number of lever presses after formalin were reduced more than three-fold relative to baseline [from a mean of 48.31 (95% CI 29.20 to 67.43) to 14.38 (95% CI 3.820 to 24.93; p=0.003, Tukey's multiple comparisons test). After recovery, lever presses returned to baseline levels (44.75, 95% CI of 27.41 to 62.09, p=0.81 relative to baseline).

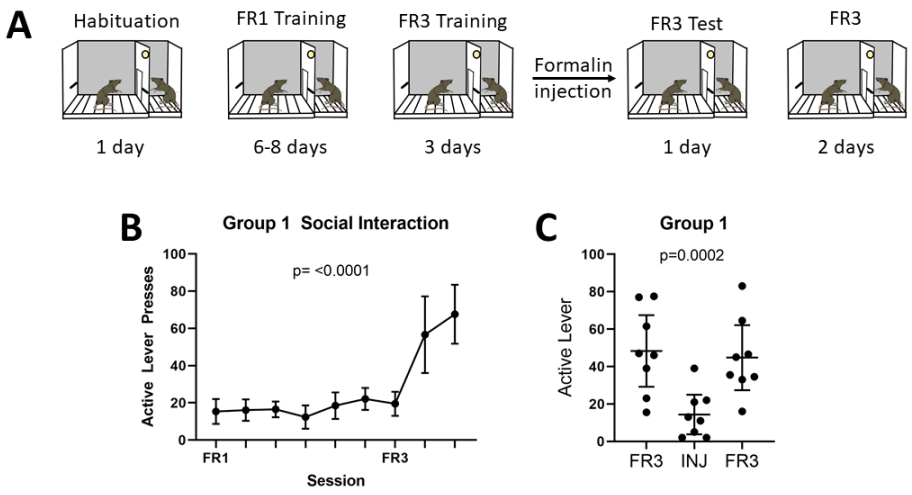

Figure 1: Group 1 Data, A, Timeline of experiments for Group 1 **B,** Active lever presses during social training through FR1 and FR3 training **C,** The number of active lever presses ±95% confidence intervals.

We trained group 2 (n=8) through to progressive ratio (PR) and analyzed breakpoint values (ANOVA p=0.0006, F=13.25; Figure 2A. The statistical analysis showed that rats reliably self-administered social interaction under FR1 and displayed a consistent learning curve by gradually increasing their lever presses and they increased their active lever presses under FR3 (ANOVA p=0.002, F=15.47; Figure 2B). Mean breakpoint values decreased 2.5-fold [from a mean of 16.72 (95% CI 9.554 to 23.88) to 6.625 (95% CI 3.137 to 10.11, p=0.00C08, TUKEY'S) (Figure 2C). After recovery, breakpoint values returned to baseline levels (16.13, 95% CI of 8.091 to 24.16, p=0.95 relative to baseline).

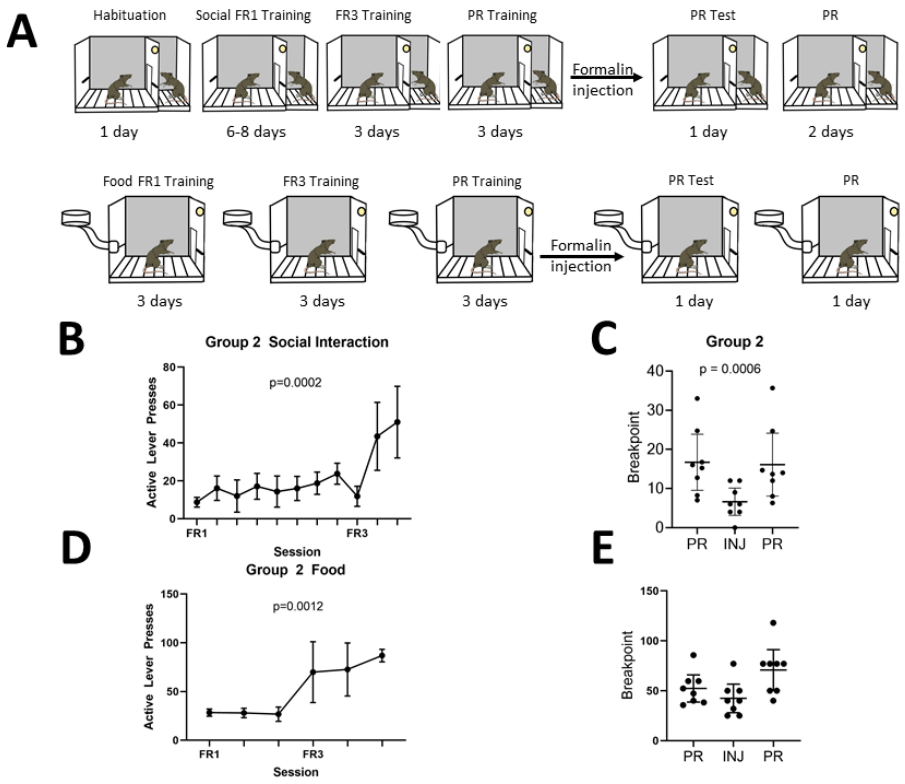

Figure 2: Group 2 Data, A, Timeline of the experiments for Group 2. **B** The number of active lever presses during social self-administration training **C,** The mean breakpoints ± 95% confidence intervals for social interaction **D,** The number of active lever presses during food self-administration training **E,** The mean breakpoint values ± 95% confidence intervals for food.

Food Self-administration

Six days after the first formalin injection (when the effect of formalin is abolished)(Lee & Jeong, 2002), we trained group 2 (n=8) to self-administer palatable food pellets (Figure 2A). The statistical analysis of the training data reported that the rats consistently press under the FR1 schedule and increase their active lever presses under the FR3 (p=0.0012, F=25.83, Figure 2D). To determine the effect of formalin injection to palatable food

pellets self-administration, we compared active lever presses of break points the day before formalin injection, the day of formalin injection, and the day after the injection. Acute pain induced by formalin injections had no effect on mean breakpoint values. Mean baseline values were 52.33 at baseline (95% CI 38.67 to 66.00), and 70.75 (95% CI 50.28 to 91.22) after formalin ($p=0.35$; Figure 2E). This finding indicates that acute pain did not change the valuation of a food reward, suggesting that pain-evoked devaluation is specific to social rewards.

Chapter 4 Discussion

To determine how acute pain affects valuation of different rewards, we produced a pain condition in rats trained to self-administer social interaction, or palatable food. We found that after the formalin injections, rats strongly devalued social interaction, but not palatable food. These results suggest that rats, under pain conditions, value rewards differently. This finding suggests that a social interaction operant paradigm may be a valid index for pain sensitivity.

The relatively long training period required for this operant paradigm is disadvantageous for high-throughput pain studies. This is of particular concern for chronic pain studies, in which animals must be trained prior both before and after recovery from surgical procedures. We continue to refine our approaches to address this concern.

We recognize that these results need to be replicated, as only eight of the animals were tested with the critical progressive ratio training. We will continue working with our collaborators at NIDA to complete these experiments. This will not only provide an

increase in animal numbers, but also strengthen the validity of the results by replicating them at another laboratory.

We recognize that we did not include a control group of saline injections, to complement the formalin injections. The natural history of formalin injections, and an extensive publication record, attest to the fact that formalin injections invariably result in severe inflammatory pain (Dubuisson & Dennis, 1977; Lee & Jeong, 2002).

Previous studies tested several cues that may lead to devaluation of social interaction. Those studies repeatedly demonstrated that rats, when given the choice, choose social interaction over drug self-administration of addictive and rewarding substances like methamphetamine (Venniro et al. 2018). (Venniro et al., 2018) Only when social interaction was associated with delay and punishment, rats began to devalue this reward(Venniro et al., 2018). This was not unique to social interaction as the same findings were seen when using food as a reward. (Venniro et al., 2018). Our findings demonstrate pain is sufficient to devalue social interaction, whereas food is not. Thus, acute pain may be a more sensitive and informative metric for future pain studies.

In our current experiments, we present animals with each reward (social interaction or food) individually and then determine if pain causes devaluation of that reward. If presented with both rewards at the same time, we would expect the rats to choose social interaction over food administration before experiencing pain and switch to choosing food over social interaction after pain. If found to be true, this would significantly strengthen the argument that rats value rewards differently in pain conditions, specifically

social interaction. The next step in our studies is to perform a choice test between food and social interaction to determine if this is true.

Our main objective is to study chronic pain since this is what leads to so much disability in humans. Therefore, our next step is to use a chronic pain model, like chronic constriction injury of the sciatic nerve, to determine if chronic pain produces devaluation of social interaction(Kim et al., 2014). If this metric works for chronic pain, we can produce and increase our translational research to assist patients in the clinic.

Our results suggest that tests of social interaction devaluation may be a useful metric of pain perception. This method removes biased and subjective human observation and turns the data into quantitative objective results. With these changes, this paradigm could be extremely useful in the field of pain and provide many important data to further our findings in pain in hopes of bettering the treatments and understanding pain in humans.

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