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Publication Date

2024-11-01

DOI

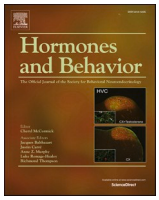
10.1016/j.yhbeh.2024.105653

Hormones and Behavior 166 (2024) 105653

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh



Prairie voles seek social contact with peer companions during immune challenge

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ARTICLE INFO

Keywords:
Sickness behavior
Social behavior
Peer affiliation
Social motivation
LPS
Prairie vole
Operant
Reward

ABSTRACT

Selection for group living has occurred across taxa, despite inherent risk of disease transmission. Behavioral and immune responses to sickness affect social interactions and can be altered by social contexts. However, the majority of research on sickness behavior has focused on species that do not form selective social relationships. Prairie voles (*Microtus ochrogaster*) form selective social relationships with mates and peers and provide a useful study system to examine effects of sickness on social seeking in established relationships. We used peripheral injections of lipopolysaccharide (LPS) of *E. coli* to stimulate the innate immune system and verified effects on activity, core temperature, and corticosterone concentrations for 6 h following treatment. We demonstrated that male and female same-sex pairs of prairie voles increase social contact when sick and that this increase persists when contact is initiated by the sick vole. Finally, we assessed social motivation following immune challenge using operant choice chambers equipped with two levers and side chambers. Voles worked to gain access to chambers with social and non-social rewards. While overall effort decreased following LPS injection, only immune-challenged voles worked significantly harder for their companion than for a non-social chamber. LPS treatment also increased proportion of rewards earned for the partner versus a stranger and again led to increased huddling behavior. Prior studies in other rodent species have shown decreased social interaction when sick; the present results demonstrate an alternative outcome of sickness in the context of dyadic bonds and lay the foundation for future work in peer companions.

1. Introduction

When sick, animals undergo marked behavioral changes. These changes—collectively termed "sickness behaviors"—can be strongly affected by and have effects on social interactions. Alterations in basic behaviors when sick, including feeding, movement, exploratory behavior, and mating, all impact the likelihood of social interactions. Social behavior, sickness behavior, and immune function are mediated by shared neuroendocrine-immune pathways (Ashley and Demas, 2017), further connecting these phenomena. Interactions between sickness behavior and immune function have historically been studied in rodents that do not form selective social bonds such as mice, rats, and degus (Bassi et al., 2012; Lopes et al., 2012; Nemzek et al., 2003). Selective peer relationships – termed "friendships" in humans and non-human primates – occur in multiple group-living species and have positive direct and indirect effects on fitness (Cheney et al., 2016; Massen et al., 2010). Little is known, however, about how sickness affects peer social behavior or established relationships between bonded individuals. Previous research has focused on social behavior and sickness in other social groupings. We sought to characterize changes in affiliative behavior and social motivation in sick prairie voles (*Microtus ochrogaster*) towards their healthy peer companions.

Immune responses allow individuals to fight pathogens. In mammals, the body's innate immune response promotes fever, increasing the body's core temperature to slow viral and bacterial replication and damage these pathogens (Demas and Nelson, 2012). Additionally, immune responses trigger the release of pro-inflammatory cytokines which orchestrate pathogen removal throughout the body. These large-scale alterations in physiology are energetically expensive (Carlton et al., 2014; Demas et al., 1997; Demas and Nelson, 2012). Sickness behaviors allow an organism to divert energy toward the immune response and increase the likelihood of an animal's survival. Herein, we use the term

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https://doi.org/10.1016/j.yhbeh.2024.105653
Received 5 July 2024; Received in revised form 21 September 2024; Accepted 10 October 2024
Available online 24 October 2024

"sick" to describe individuals undergoing an immune challenge.

The effects of sickness behaviors on social interactions vary widely: house mice living in groups withdraw from social interaction when sick (Lopes et al., 2016), zebra finches completely suppress sickness behaviors while in a group and maintain normal social behaviors (Lopes et al., 2012), while rhesus macaques increase affiliative behaviors (Schapiro, 2002). By impacting social interactions and social connectedness, sickness behaviors may have short-term direct effects on immune function leading to long-term consequences on survival (Archie et al., 2014; Meneses et al., 2018; Pyter et al., 2014). In species such as humans and prairie voles, which both form monogamous relationships, reductions in social connectivity lead to depressive-like behaviors (Grippo et al., 2007; Steptoe et al., 2004) and heightened pain responses (Okuda et al., 2022). In contrast, establishment and maintenance of social relationships promotes wound healing, longevity, and offspring survival across taxa from dolphins to hyenas to humans (Archie et al., 2014; Clutton-Brock, 2016; Detillion et al., 2004). The formation of enduring relationships is a rare feature among rodents. Social monogamy is highly prevalent in primates and carnivores, but occurs in only about 3 % of rodent species (Insel et al., 1995; Lukas and Clutton-Brock, 2013).

Among non-monogamous rodents such as rats and mice, there is little evidence for selective peer relationships, as novel conspecifics tend to be of greater interest to an individual rat or mouse (Hackenberg et al., 2021; Moy et al., 2004; Schweinfurth et al., 2017; Beery, 2018; Beery and Shambaugh, 2021). Prairie voles are socially monogamous rodents that form selective relationships with both mates and same-sex companions; thus they provide an excellent model for studying sickness behavior in the context of reproductive and non-reproductive social relationships (Insel et al., 1995; Kenkel et al., 2021; Klein and Nelson, 1999). In a laboratory environment, voles rapidly form 'partner preferences' for a familiar companion over an unfamiliar vole and, given the choice, prefer to huddle with this known companion (Beery, 2021; Carter and Getz, 1993). Once bonds have been formed, the social environment has strong, environment-specific effects on immune function (Klein and Nelson, 1999). For example, prairie voles which have undergone prolonged separation from a mate or a same-sex companion show reduced immune function (McNeal et al., 2014).

Prior research has shown that sickness plays a role in pair bond formation in prairie vole mate partnerships (Klein and Nelson, 1999; Bilbo et al., 1999; Smith and Bilbo, 2021). When choosing between unfamiliar potential mates, healthy female voles prefer healthy males over sick males (Klein and Nelson, 1999). When co-housed with a healthy potential mate, activation of the immune system of a female decreases the time needed to form a pair-bond, unlike in males (Bilbo et al., 1999), suggesting that sickness may have different effects depending on sex and social context. While sickness impacts social bonding in prairie vole mate pairs, it remains unknown how selective same-sex relationships between long-term peer companions, such as those found in voles and primates, may be affected by immune stimulation.

We assessed how affiliative and social-seeking behaviors in peer relationships are affected by activation of the immune response. We used lipopolysaccharide (LPS) from *E. coli*—a glycoprotein component of the cell wall of *E. coli* used as a common immune stimulant—to stimulate the immune response in prairie voles. To establish its efficacy, we quantified locomotor activity, core body temperature, and corticosterone release following injection of LPS or saline control. We then characterized changes in affiliative social interactions within same-sex peer relationships following saline or LPS treatment, both in assays of huddling as well as operant quantification of social motivation for a familiar versus a novel peer or empty chamber. Across paradigms, LPS enhanced social seeking on the part of the sick individual.

2. Methods

2.1. Animals and housing

Prairie voles (*Microtus ochrogaster*) were bred in long day length conditions (14 h light, 10 h dark) at the University of California at Berkeley. Subjects had ad libitum access to food (LabDiet PicoLab 5LJ5) and water. Subjects were group-weaned at 21 days and males and females were placed into same-sex "companion" pairs with an age-matched littermate or non-littermate individual by day 28. New cohorts of animals were used for each of Experiments 1, 2, 3, and 4. Experiments shown in Fig. 1A and B and experiments 2 and 3 used both male and female subjects. Experiments shown in Fig. 1C as well as Experiment 4 used female subjects, as only females display seletive social motivation for familiar companions (Brusman et al., 2022; Vahaba et al., 2022; Beery et al., 2021). All procedures adhered to federal and institutional guidelines and were approved by the UC Berkeley Animal Care and Use Committee.

2.2. Experimental design and overview

2.2.1. Experiment 1: LPS effects on temperature and activity

To quantify changes in temperature following saline or LPS injection, core body temperature was recorded by iButtons in 7 females and 9

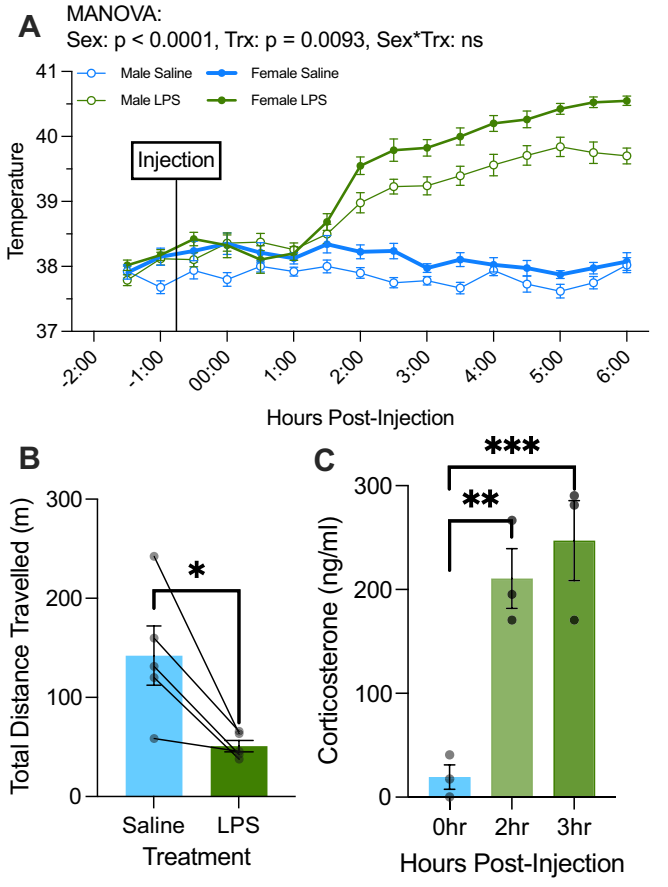

Fig. 1. Effects of LPS on physiological correlates of immune stimulation. A) Mean ± SEM of temperature following LPS or saline injection in adult female ($n = 7$) and male ($n = 9$) prairie voles. See Supplementary Fig. 1 for individual temperature traces in males and females. B) Mean ± SEM of distance traveled following LPS or saline injection in adult females in hours 3–6 after saline or LPS injection ($n = 6$/treatment). C) Mean ± SEM of corticosterone concentrations in the first three hours following LPS injection in adult females ($n = 3$/group).

males as described below in "Temperature and activity monitoring". At least 10 days after surgery, temperature was monitored following saline injection. 48 h later, the same individual was given an LPS injection. All animals received sequential saline and LPS injections across assays to avoid any long-term side effects of prior LPS exposure on behavior, activity (distance traveled) in the 6 h following saline or LPS injection was also assessed in 5 voles as described below.

2.2.2. Experiment 2: LPS effects on reciprocal social behavior in a companion relationship

To examine the effects of LPS on social interaction, free-moving social behavior was assessed in 11 same-sex pairs of voles (5 male-male, 6 female-female pairs) following injection with LPS or saline as described below (see "Reciprocal social interaction test"). Approach behaviors by each individual in a pair and huddling time between the individuals were quantified between PND 55 and PND 100 for a three-hour period 3–6 h after LPS or saline injection. During this time window, LPS-injected focal voles experience an innate immune response. The focal animal acted as its own control, receiving sequential saline or LPS injections before testing.

2.2.3. Experiment 3: LPS effects on social preference of the sick focal vole

To assess whether voles seek increased contact following immune challenge, saline or LPS injected focal voles were tested for their behavior towards a tethered partner. Ten females and ten males were tested as focal voles in a three-chamber apparatus (Fig. 3B) as described below (Tethered Partner Huddling Test). One vole of the pair was given no injection and tethered to one side of the chamber. The focal animal acted as its own control, receiving sequential saline or LPS injections before testing days 2 and 5 (see Fig. 3A). Data were collected on an additional control group of individuals ($n = 4$ females) which only received a single injection of LPS to account for effects of injection order.

2.2.4. Experiment 4: LPS effects on social motivation

To quantify social motivation, nine female voles were housed with age-matched same-sex companions from weaning (PD21). One individual from each pair then underwent three phases of operant conditioning training and testing: 1) food reward training, 2) social training/habituation, and 3) social testing (see Fig. 4A and "Operant Conditioning Training and Testing" for details). During social testing, focal voles were injected with saline or LPS and tested a chamber with two levers. In one paradigm, one lever provided access to a chamber containing their companion and the other to an empty chamber (choice of partner vs. empty). In the other paradigm, the central chamber contained levers providing access to one chamber containing their companion and another containing a novel same-sex vole (choice of partner vs. stranger). Injections of saline or LPS were given three hours prior to testing to ensure that voles would be experiencing an innate immune response by the beginning of testing. Females were used for this experiment as prior studies have shown that female prairie voles work harder to access a peer or mate partner while males work harder for access to any female (Beery et al., 2021; Brusman et al., 2022; Vahaba et al., 2022).

2.3. Immune stimulation

To stimulate the innate immune response, voles were injected with lipopolysaccharide from *E. coli* (LPS; Sigma Aldrich L4391; 1.2 mg/kg) dissolved in sterile saline, or were given a saline (vehicle, 0.9 % sodium chloride) injection as control. LPS dose was determined by referring to other studies on rodents (Carrizo et al., 2023; Givalois et al., 1994; Lopes et al., 2016).

2.4. Temperature and activity measurement

Core body temperature was measured using iButtons (Maxim Integrated; DS1925L-F5#) implanted into the abdominal cavity. iButtons were set to record temperature every 15 min. Implantation was performed between 55 and 85 days of age, after subjects reached ≥30 g body weight. iButtons were placed in 90 % ethanol for at least 10 min before implantation to ensure sterilization. Subcutaneous injections of buprenorphine (0.05 mg/kg) and meloxicam (1 mg/kg) were administered to each subject before surgery. iButton implantation was performed under isoflurane anesthesia. A ventral incision was made and the iButton was placed in the intraperitoneal space. The muscle wall was closed with dissolvable sutures, after which the skin was closed with dissolvable sutures and VetBond glue (3 M No. 1469C). Subcutaneous injections of buprenorphine and meloxicam were repeated 12–24 h after surgery, and recovery was monitored daily for three days. For 24 h following the surgery, individuals were separated from their previous companion via a divider. iButtons were removed upon sacrifice of the animal.

To measure activity, distance traveled was quantified over 6 h following injection with saline or LPS. Voles were individually placed into a square arena (30 cm × 30 cm) with bedding and wet food available and videos were recorded. Distance traveled was quantified using Ethovision XT (Noldus Information Technology).

2.5. Corticosterone EIA

Serum corticosterone was quantified by enzyme immunoassay (Enzo Life Sciences, ADI-900-097; sensitivity 26.99 pg/mL). This assay has been previously validated for work in voles (Anacker et al., 2016). Trunk blood was collected following decapitation under isoflurane within two minutes of the researcher's initial contact with cage, three hours after initial injection with LPS. Samples were centrifuged at 3500 RCF for 20 min at 4 °C, and stored at −80 °C. Samples were thawed, centrifuged, and diluted 1:500 with assay buffer. Given the naturally high levels of corticosterone in prairie voles, this dilution factor was necessary to obtain results that fell on the standard curve of the EIA. Samples were plated in duplicate with six standards (32–20,000 pg/mL) and reference samples. Mean %CV intra-assay was 8.5 %.

2.6. Reciprocal social behavior test (Experiment 2)

Social interest of a focal vole was assessed during free-moving social interaction in a square arena (50x50cm, Fig. 2B). Both voles in a companion pair were placed in the apparatus 3 h after treatment for a period of 3 h. Voles were identified via RFID tag or fur marking. The bottom of the apparatus was covered in ½ inch of bedding (Envigo Teklad Laboratory Grade Sani-Chips #7090).

2.7. Tethered partner huddling test (Experiment 3)

Social interest expressed by the healthy or sick focal vole towards their healthy partner was assessed in a three-chamber apparatus (20 cm × 30 cm × 75 cm, Fig. 3B). In this setup, only the injected "focal", was able to move freely. The other vole of the pair, referred to as the "partner" was tethered to one end of the chamber using a zip-tie collar attached to a chain and clipped to the side of the apparatus. Each focal vole could choose to huddle with or avoid their tethered companion, yet still access food. A mix of 25 g food and 15 g water was used to make a wet food mixture so voles could access food and water without the need for a sipper bottle in the apparatus. Two containers of wet food were placed in the apparatus, one within reach of the tethered vole and one in a neighboring chamber. Videos of each test were scored using Boris (see "Behavioral Scoring" for detail).

During the habituation phase, each focal vole was allowed to move freely in the chamber for 1 h while their companion was tethered. Following a rest day with no testing, the first focal vole of the pair (Focal A) was given a saline injection and the amount of time spent huddling with their tethered partner was measured for 6 h after injection. After an

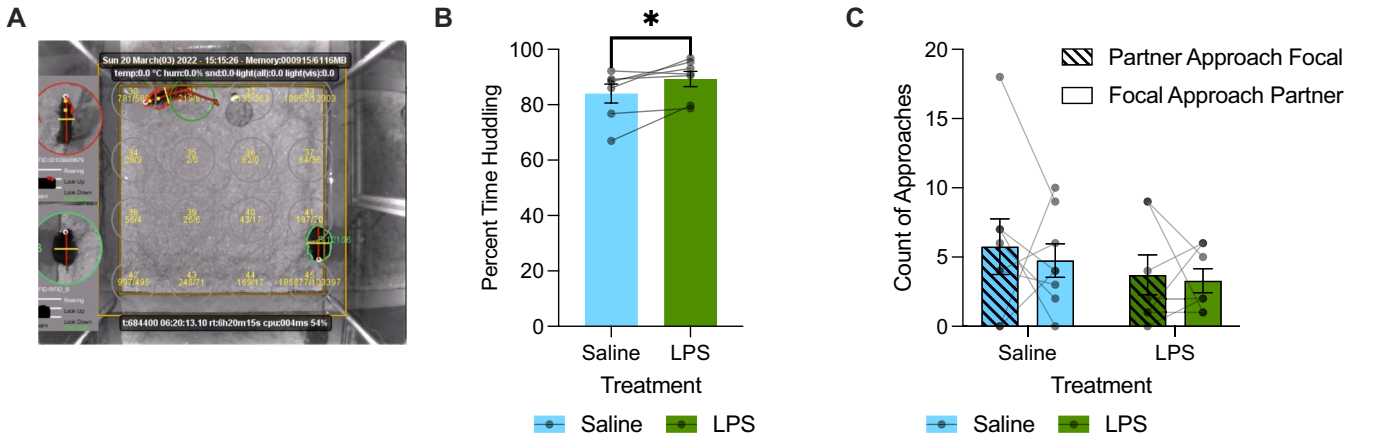

Fig. 2. Social dynamics in a reciprocal social behavior assay following saline or LPS treatment. A. Timeline of experiment 2. B. Dyadic social interaction assay apparatus. C. Huddling increased significantly following immune stimulation of one vole. There was no effect of sex, so males and females are shown together. D. Approach dynamics in advance of huddling bouts were consistent across treatments, suggesting contact was not principally driven by just the sick vole or its healthy companion (2-way ANOVA not significant).

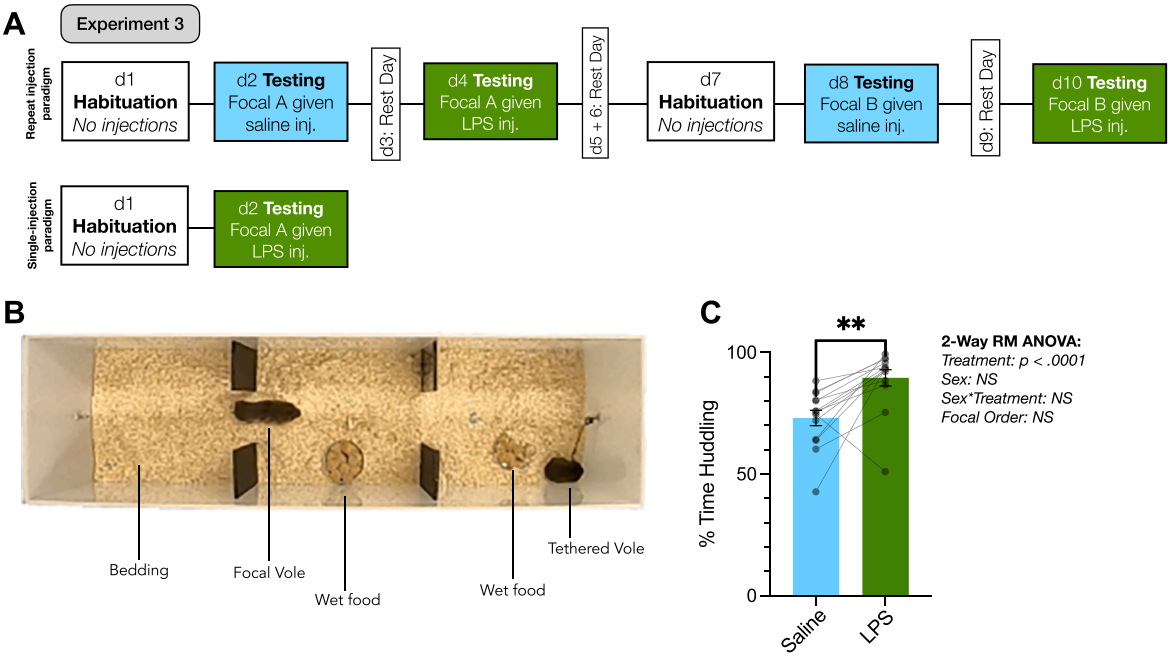

Fig. 3. Experiment 3 overview and results. A) Timeline of experiment 3. B) Social testing apparatus. Healthy or sick focal voles could freely roam the apparatus between empty chambers and a chamber occupied by their tethered same-sex partner. C) Time spent huddling with the tethered partner between hours 3–6 after injection in dually injected animals. Mean ± SEM. Huddling time was again significantly higher following LPS injection relative to saline injection; this time with huddling initiated by the saline or LPS-injected vole.

additional rest day, Focal A was given an LPS injection (1.2 mg/kg) and time spent huddling with their partner was again measured for the 6 h post-injection. Each pair then underwent two days without testing to allow attenuation of the immune response in Focal A. The three phases of testing listed above were then repeated with the original focal vole (Focal A) as the tethered partner, and with Focal A's partner (Focal B) as the new free-moving focal vole receiving injections (Fig. 3A).

Time spent in contact with the companion was quantified from video recordings of the test. Voles were treated with saline or LPS three hours prior to the analysis window to ensure that voles were experiencing an innate immune response, if applicable, during analysis. Time in contact with a companion was analyzed from video recordings for 3 h (180 min) starting at 3 h after injection. See "Behavioral scoring" section for more detail.

2.8. Operant conditioning training and testing (Experiment 4)

Food reward training and social choice habituation were carried out following methods described in Vahaba et al. (2022). Briefly, voles underwent shaping and training on a fixed ratio 1 (FR-1) schedule to associate lever pressing with a food reward. Voles were tested on a fixed ratio schedule so that the relative "cost" of rewards accessed by the two levers remained the same throughout testing. Following three days of pressing without the need for manual reinforcement, voles were shifted to FR-4 (four presses per reward) for four days to increase the number of lever presses. Following food training, animals were habituated to FR-4 with a social reward for four days before the start of data collection.

Social testing was performed in a three-chamber lever-pressing apparatus (Fig. 4B: 30.5 cm × 24.1 cm × 21.0 cm; ENV-307A;

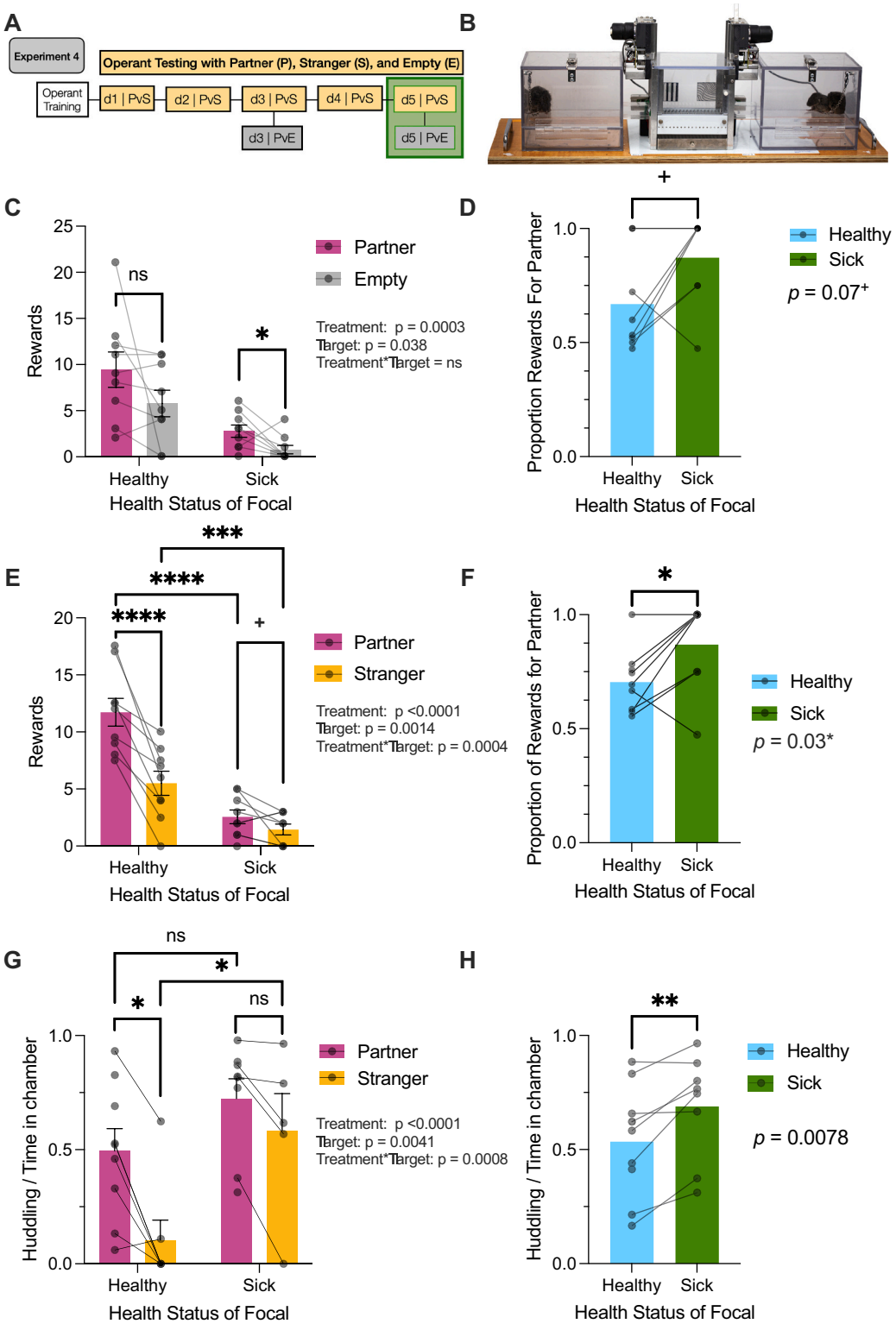

Fig. 4. Social motivation experimental design and results. A) Timeline of experiment 4. Stimuli in the side chambers are abbreviated as PvS: partner (companion) vs. stranger (novel vole), and PvE: partner vs. empty. B) Two-choice operant apparatus with stranger and partner voles tethered in the left and right chambers, respectively. The focal vole is huddling with its companion. C) Healthy voles expended the same effort to access a partner or an empty chamber. In contrast, sick voles showed significantly lower effort overall, though they expended more effort to access a partner over an empty chamber. D) Direct comparisons of the proportion of rewards directed at the partner shows the partner is somewhat more rewarding than an empty chamber both while healthy and sick, though there is a marginal increase in reward value of the partner when sick. E) Healthy voles worked harder to access their peer companion than a novel vole, as in prior studies. When sick, effort decreased, and this preference was no longer significant. However, the proportion of partner directed rewards out of all rewards was even higher in sick voles when non-pressers were excluded. F) Reward from social stimuli was significantly greater than reward from an empty chamber in sick voles but not in healthy voles G) When the doors were open, sick voles spent more of that access time huddling, both overall (C), and with the novel vole in particular as compared to time spent with the novel vole when healthy. H) Sick voles spent more total time huddling relative to access time as compared to healthy voles. + indicates $p < 0.1$.

MedAssociates Inc., St. Albans, VT). Data were acquired using MED-PC-IV program running custom-coded training protocols. Subjects were tested in two social environments: "partner vs. stranger (PvS)" and "partner vs empty (PvE)" (Fig. 4A). In partner vs. stranger trials (days 1–5 of testing), subjects could work for access to a chamber containing their companion tethered at one end of the chamber, or a separate chamber containing a tethered unfamiliar "stranger" vole. Strangers were non-littermate individuals that the subject had not encountered before the start of testing and were re-used no more than once for each focal vole. In the partner vs. empty trials (after PvS trials on days 3 and 5), subjects could work for access to a chamber containing their companion or a separate empty chamber they could explore. Before testing began, subjects could explore the apparatus and the locations of the tethered stimulus voles with the motorized doors open for two minutes before testing began. Tests were run at FR-4 for 30 min and each reward (door opening) lasted one minute. When the doors closed, the subject was manually returned to the center chamber as necessary.

On days one and three of testing, the focal subject was given a saline injection three hours prior to the test. On day five, the focal subject was given a 1.2 mg/kg LPS injection three hours prior to the test.

Following testing, trained voles underwent extinction to ensure that any reward value of pressing the lever was not driving the motivation seen in the test. Voles were placed in the three-chamber apparatus and allowed to press for 30 min without chamber doors opening for 5–8 days. Extinction testing was stopped after two consecutive days of <10 presses.

2.9. Behavioral scoring

Video recordings from all multi-animal tests across experiments were scored using the Behavioral Observation Research Interactive Software (BORIS) Version 7.13 (Friard and Gamba, 2016). In free-moving trials, the approaching vole was defined as the individual initiating contact prior to huddling. Huddling was defined as side-by-side contact sustained for at least two seconds. Time in a given chamber was defined as the focal vole having its front two paws and head in that chamber. Videos of single voles were scored using EthoVision XT to compute distance traveled as a measure of activity.

2.10. Data analysis

Graphs were prepared and statistical analyses were performed using Prism (GraphPad Software, Version 9.4.1), and JMP (Version 17.0.0). Two-group comparisons were analyzed with *t*-tests or paired t-tests and labeled accordingly. Multilevel comparisons were analyzed by the following model types depending on the data: experiment 1 temperature series were analyzed by repeated measures MANOVA using JMP. Experiment 2 and 3 results were analyzed by 2-way repeated measures ANOVA. Experiment 4 was analyzed using mixed effects models (REML) with subject as a random variable. Model factors are articulated alongside the results of each experiment. All models for studies in which both subjects in a pair were tested as focals (experiments 3 and 4) were initially run with treatment [LPS or Saline], sex, and focal order [first, second] as main effects. Focal order was not significant in any models and was omitted from final models following a model selection approach. All tests were conducted two-tailed. Outliers were defined as values outside the global mean +/− 3 times the global standard error of the mean. This applied to two tests in experiment 3. Effect sizes for two-group comparisons were reported as Cohen's d, calculated using an online effect size calculator at Campbell Collaborations (Wilson, 2023). For ANOVAs and mixed effects models, Cohen's d was calculated using effect size calculator 6 at Psychometrica (Lenhard and Lenhard, 2017).

3. Results

3.1. Immune stimulation via LPS affected temperature, activity, and corticosterone

There was a main effect of immune challenge on body temperature ($p < 0.0093$; MANOVA) in both males and females (Fig. 1A). Sex differences in body temperature were also present regardless of treatment ($p < 0.0001$). While treatment itself was responsible for 34 % of the variation in temperature, sex differences contributed 2.4 % of variation seen in temperature. There was no significant interaction between sex and body temperature. Individual variation in the patterning and scale of this increase was also present (Supplementary Fig. S1). By 6 h post-injection, average core body temperature had increased by 2.4 °C in females and 1.4 °C in males, indicating a febrile response to LPS consistent with those found in other rodent models (Cai et al., 2016).

Activity, assessed as total distance traveled within the test chamber between three and six hours post-injection was significantly reduced in subjects treated with LPS as opposed to saline-treated subjects (Fig. 1B; $p = 0.017$, $t(8) = 0.299$, $d = 0.19$, males not tested). Corticosterone concentrations were elevated by 2–3 h after LPS injection but returned to baseline levels by 6 h post-injection, typical of the innate immune response in rodents (Fig. 1C; 0 h vs 2 h: $p = 0.0035$, $t(4) = 6.152$, $d = 5.02$; 0 h vs 3 h: $p = 0.0048$, $t(4) = 5.66$, $d = 4.62$; unpaired *t*-tests,). Corticosterone concentrations were measured in females only.

3.2. Social contact increases when sick

In a free-moving assay of dyadic interactions between a healthy vole and its saline or LPS-injected partner (Experiment 2; Fig. 2B), huddling time increased significantly following LPS treatment (Fig. 2C $n = 14$, $p = 0.036$, $t = 2.70$, df = 6, Cohen's d = 1.44). Only one animal per pair was used as a focal. There was no difference in bout duration between treatment groups ($p = 0.58$, $t(10) = -0.57$, Cohen's d: −0.33). Both the focal vole and its partner also approached each other prior to huddling at similar rates across treatments, with no one animal leading the social interactions in the free-moving environment (Fig. 2D). There were no sex differences in huddling time or approach dynamics.

When the healthy partner was tethered and the saline or LPS-injected focal was free-moving (experiment 3; Fig. 3), two-way repeated measures ANOVA revealed a main effect of treatment [LPS/Saline] on huddling time ($p = 0.0029$, $F(1,12) = 13.82$, Cohen's d = 2.146) and no effect of sex ($p = 0.67$), or interaction between treatment and sex ($p = 0.10$). Immune stimulation also increased huddling in LPS trials relative to saline trials in the pooled mixed sample ($p < 0.0001$, $t(25) = 6.08$, $d = 2.34$, *t-test*), with no effect of injection sequence (i.e. LPS as the first versus second injection) found on huddling behavior in the three-hour interval assayed.

3.3. Social stimuli are rewarding to sick voles

In experiment 4 voles worked for access to two side-chambers, always containing their companion 'partner' in one side chamber, and containing either a novel 'stranger' or an 'empty chamber' on the other side, depending on experimental setup (Fig. 4B).

In the partner vs. empty (PvE) operant testing environment, a mixed effects model of health status [healthy/sick] and target [partner/empty chamber] on rewards attained with subject as a random effect showed main effects of health status and target (Fig. 4C, Health status: $p = 0.0003$, $F(1,16) = 20.86$, $d = 2.284$; Target: $p = 0.038$, $F(1,16) = 5.12$, $d = 1.13$). When healthy, voles did not differ in willingness to work to access an empty chamber or companion chamber ($n = 18$, $p = 0.1602$, $t(8) = 1.548$, $d = 0.73$, paired *t*-test). When sick, however, voles were significantly more willing to work for access to their partner than for the empty chamber (Fig. 4C, $p = 0.04$, $t(8) = 2.45$, $d = 1.15$, and relative rewards for the partner plotted as a proportion of total rewards

marginally when sick Fig. 4D ($n = 16$, $p = 0.069$, t(7) = 2.142, d = 1.07, paired *t*-test).

In the partner vs. stranger (PvS) operant testing environment, the same structure of mixed effects model revealed main effects of health status (Fig. 4E, $p < 0.0001$, F(1,8) = 75.12, d = 4.33), social target ($p = 0.0014$, F(1,8) = 22.91, d = 2.39), and an interaction between the two ($p = 0.0004$, (F(1,8) = 22.65, d = 2.9). When healthy, voles showed characteristic selective motivation for their companion's chamber versus the novel vole ($p = 0.0005$, t(8) = 5.71, d = 2.69 paired *t*-test). Sickness greatly reduced pressing effort and thus the number of rewards that were attained. When rewards for the partner were plotted as a proportion of total rewards, however, immune challenge enhanced the relative effort expended towards the partner (Fig. 4F; p = 0.038, t(7) = 2.54, Cohen's d = 1.27, paired *t*-test). Of note, one focal did not interact with the levers during their 'sick' trial. When working for access to the partner versus the stranger (PvS), voles again spent relatively more time huddling when they were sick than when they were healthy (Fig. 4H; $p = 0.0078$, t(7) = 3.69, d = 1.84, paired *t*-test), and were significantly more likely to huddle with the stranger than were healthy voles (Fig. 4G, $p = 0.0003$, Tukey's multiple comparisons).

Lever pressing was rapidly extinguished by testing in the operant choice apparatus with inactive levers that did not lead to door opening (Supplementary Fig. S2).

4. Discussion

The suite of changes in temperature, activity, and corticosterone concentrations seen in prairie voles after LPS injection was similar to responses to LPS across species (Klein and Nelson, 1999; Clutton-Brock, 2016; Jolink et al., 2022). Core body temperature differed between the sexes in the 3–6 h post-injection interval. A sex-dependent difference in temperature became more pronounced by hour 6 when female temperature continued to increase while male temperature declined. Female adult mice showed similar elevation of body temperature relative to males in these time ranges (Cai et al., 2016). Corticosterone concentrations, measured in a separate cohort of females, rose quickly in the first 3h post-LPS treatment. These results also parallel previous findings in prairie voles showing an increase in corticosterone 2–3 h after injection in males (Klein and Nelson, 1999) and females (Bilbo et al., 1999), as well as studies in mice and rats showing similar increases (Givalois et al., 1994; Kozak et al., 1994). Finally, a significant decrease in activity was noted in a separate cohort of females.

This suggests that a dose of 1.2 mg/kg LPS is sufficient to induce physiological, behavioral, and endocrine responses in adult prairie voles consistent with an innate immune response.

While these results are not surprising, they provide clear evidence that LPS can be used to stimulate physiological response to sickness in the prairie vole. This complements and extends prior research that has established suppression of testosterone and increases in circulating corticosterone and IL-1B three hours after LPS injection in male prairie voles (Klein and Nelson, 1999).

4.1. Sickness and social contact

Following validation of immune stimulation in response to LPS injection, we examined how social preference and social motivation change following immune challenge. Previous studies have shown that prior history of LPS exposure can impact behavior (Kelly et al., 2018). Because some studies used focals (focal B) whose partners had been previously treated with LPS, we explicitly tested for effects of focal order on outcomes and none were present for any study.

We saw a clear increase in social contact when sick across social contexts and behavioral assays. In a free-moving state, pairs of voles spent more time huddling when sick, but there were no significant differences in the frequency of approach leading up to contact (huddling) or departure from the huddle across conditions. This result suggests that the healthy vole did not avoid their partner, and that increased huddling resulted from more time in resting contact rather than more frequent approach. Instead of withdrawing from social behavior when sick, as seen in other rodents, voles increase time spent in contact when sick when in a free-moving context.

Tethering one animal in a pair allowed us to understand what components of increased social contact were driven by the sick individual. While both sick and healthy voles preferred to be in contact with their companion rather than remain on their own, sick focal voles spent more time huddling with their tethered companion relative to when they were healthy. Social contact is a common trait seen across small mammals which increases protection against predators and decreases costs of thermoregulation (Andrews et al., 1987; Clutton-Brock, 2016). However, the increased social contact we observed in sick voles contrasts with findings seen in traditional rodent models. Mice and rats both withdraw from social interaction when sick (Lopes et al., 2016). Instead, our results in prairie voles are more similar to the social seeking behavior observed in sick group-living rhesus macaques (Schapiro, 2002; Willette et al., 2007).

Previous work in prairie voles and seasonally-social meadow voles showed that, in females, the formation of new social bonds was hampered by a forced swim test—a common lab paradigm for inducing a stress response (DeVries et al., 1996; Anacker et al., 2016). A recent study in our lab also showed that prairie voles in established peer groups display reduced affiliative behavior following a stressor (N. Lee, unpublished data). A general "stress response", characterized by an increase in corticosterone concentrations following an unexpected change in environment, is part of the suite of neuroendocrine changes that occur when sick. Thus, our results suggest that affiliative behavior under stress is stressor-dependent.

Effects of social contact and connection on immune responses are clear and significant. In some species, social contact enhances immune responses. Rhesus macaques show substantial increases in IL-6 when social contact with a familiar individual is available (Schapiro, 2002) and social connection has been shown to increase antiviral responses across taxa, including humans and some rodents (Kappeler et al., 2015; Klein and Nelson, 1999). Perhaps, then, increased social seeking in prairie voles when sick has an adaptive function.

While the mechanism linking social contact and immune responses is unclear, research has shown clear relationships between social contact, central and peripheral oxytocin release, and immune responses (Wang et al., 2015). Oxytocin has strong modulatory effects on the immune response and is released both during an immune challenge (Matsunaga et al., 2000) and as a result of social touch (Lim and Hong, 2023; Okabe et al., 2015). Oxytocin secretion strengthens the innate immune response via activation of OXTRs on cytokines and has anti-inflammatory effects in peripheral organs. In summary, oxytocin has a protective effect on the body and brain during immune challenge. The link between social touch during disease and oxytocin release as an immunomodulator is an exciting area for further research.

4.2. Sickness and social motivation

Motivated social interaction, as opposed to social tolerance, activates reward circuits in the brain much like non-social reinforcers such as food and drugs. Social seeking and other appetitive behaviors are associated with dopamine release in the nucleus accumbens (Yu et al., 2016). Social seeking behavior, in contrast to social preference behavior, suggests that the individual "wants" or desires the social stimulus (Berridge and Kringelbach, 2008). In the context of the present study, our results suggest that sick voles desire access to their companion rather than tolerate or simply like social contact with their companion.

Following LPS injection, social stimuli remained rewarding: persistent but decreased pressing effort when sick suggests that voles were able to overcome some aspects of sickness-induced lethargy to approach a rewarding social stimulus. Increased selectivity towards a social

stimulus versus the opportunity for exploration of an empty chamber suggests that social interaction is relatively more rewarding than exploration when sick. When able to access a familiar conspecific or a novel individual, sick prairie voles showed a trend towards maintenance of selectivity towards familiar individuals and increased reward value of their companion. This is similar to findings in humans that showed that subjects moved away from novel individuals but towards a familiar "support figure" following immune stimulation by the influenza vaccine (Jolink et al., 2022). Whether reward value correlates with a functional role of social contact in prairie voles when sick remains an open question.

Our results on social motivation following saline injection also yield new insights into motivated social behavior when healthy. In the companion partner versus stranger experimental setup, our results replicated previous findings from our lab showing that female prairie voles work harder for access to a familiar peer companion than to a novel conspecific (Beery et al., 2021). In the partner versus empty experimental setup, healthy voles were not significantly more motivated to access their companion versus the empty chamber. In healthy voles, both the partner and empty chambers thus appear more rewarding than chambers containing a novel individual. These findings under control conditions provide an opportunity to assess how social and non-social rewards shift relative to each other under different conditions such as sickness, as in the present experiment. Seeking a familiar mate has been shown to elicit a distinct pattern of dopamine release relative to stranger seeking in prairie voles, providing evidence of the specificity of dopamine release (Pierce et al., 2024). Dopamine release patterns are thought to provide species with a flexible mechanism to fine tune social reward based on previous experience (Pierce et al., 2024). Further research into dopamine release dynamics while sick may provide insight into relative reward of a peer companion when a focal is healthy or sick. Additionally, further research into how social motivation may change after LPS administration in male peer-relationships is warranted. Previous studies have shown clear sex-differences in reward value of mates and peers in prairie voles, with no greater effort expended to access familiar individuals in males under control conditions (Vahaba et al., 2022), although this might change following immune challenge.

Further research is necessary to understand the neurobiological mechanisms that support social seeking when sick, whether huddling when sick leads to physiological outcomes that may be adaptive, and how healthy companions react to their sick partners in established relationships. Our results suggest that the prairie vole may be a uniquely appropriate non-primate model in which to study social-seeking behaviors when sick.

CRediT authorship contribution statement

Georgia K. Young: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Diana Chernyak:** Project administration, Methodology, Investigation. **Gautam A. Naik:** Methodology, Investigation. **Stephen Eun Song:** Visualization, Validation, Investigation. **Annaliese K. Beery:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Acknowledgements

We are grateful to the animal care staff for assistance with vole colony maintenance. George Bentley provided helpful feedback on study design and on an earlier version of this manuscript. Thank you to the members of the Beery and Bentley Labs for their feedback on study design and methods. This research was supported by a Greater Good Science Center fellowship to GY, and NIH R01MH132908 to AB.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2024.105653.

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