

## Lipopolysaccharide facilitates partner preference behaviors in female prairie voles

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### Abstract

Exposure to proinflammatory cytokines (e.g., IL-1 $\beta$ ) or lipopolysaccharide (LPS) produces an acute activation of the immune response and results in a repertoire of behavioral patterns collectively termed sickness behaviors. Although nonspecific responses to pathogenic infection have traditionally been viewed as maladaptive effects of infection, sickness behaviors may have significant, adaptive value for the host. One set of adaptive behaviors affected by infection among mammals and birds is mate choice. In Experiment 1, female prairie voles exhibited the expected increase in blood corticosterone concentrations in response to a 0.1 cc i.p. LPS injection (50  $\mu$ g), indicating activation of the endocrine system. A separate cohort of females was injected with LPS or saline and paired for 6 h with a novel, previously unpaired male. Following the cohabitation period, LPS-injected females spent significantly more time ( $p < 0.05$ ) with the familiar partner when given a choice between familiar and unfamiliar males in a three-chamber apparatus designed to test partner preferences. Saline-injected females spent significantly more time with the unfamiliar male. In Experiment 2, males injected with LPS or saline spent equal amounts of time with familiar and unfamiliar females following a 6 h cohabitation with a naive female, and therefore, did not exhibit preferences. From a proximate perspective, this study provides evidence that sickness behaviors influence female, but not male, partner preference in prairie voles. © 1999 Elsevier Science Inc. All rights reserved.

**Keywords:** Partner preference; Lipopolysaccharide (LPS); Corticosterone; Sickness behavior

### 1. Introduction

Exposure of humans and nonhuman animals to lipopolysaccharide (LPS), a B-cell mitogen, or to pro-inflammatory cytokines [e.g., interleukin-1 (IL-1)] results in a cascade of linked physiological and behavioral responses that are collectively termed sickness behaviors [1]. Typical sickness behaviors include lethargy, anorexia, and reduced exploration, as well as decreased interest in social and reproductive activities [2]. Although nonspecific responses to pathogenic infection, such as anorexia and fever, have traditionally been viewed as maladaptive effects of infection, recent research has suggested that these responses may have significant, adaptive value for the host [1–3]. For example, high body temperature facilitates the destruction of pathogens. Prevention of fever increases the duration of infection, and may induce death [4]. Infected animals often become

anemic as well; iron is sequestered away from pathogens that require it for reproduction and treatment of the anemia may prolong the infection [3].

Sickness behavior is also associated with changes in sexual, parental, and other social interactions. Several studies have demonstrated that bacterial infection may either inhibit or facilitate mating [5–8]; however, the proximate mechanism underlying the change in mating behavior among infected animals remain unspecified. IL-1 $\beta$  secretion during an immune response may decrease sexual motivation by reducing sex steroid concentrations. Administration of IL-1 $\beta$  reduces gonadotropin secretion and subsequent sex steroid concentrations [9–11]. However, this neuroendocrine mechanism does not explain instances of enhanced reproduction among infected individuals [12–14].

On an ultimate level, infection rates and immune responses have been hypothesized to be critical in mate choice. According to one leading hypothesis, females choose mates based on characteristics that serve as general indicators of disease status or parasite load [15]. Infection of the female may also influence mate choice. For example, intracerebroventricular (i.c.v.) administration of IL- $\beta$  to female rats blocked their normal preference for gonadally in-

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tact males over castrated males in a two-choice test [8]. In contrast to females, IL-1 $\beta$  administration does not affect the social preference of male rats. Males prefer intact over castrated females in preference tests, regardless of whether they have been exposed to IL-1 $\beta$  or saline [8]. Additionally, when healthy animals are given a choice between healthy or infected conspecifics, healthy males prefer saline-injected females over infected females in preference tests, whereas healthy females do not distinguish between infected and healthy males [5]. Alternatively, sex differences may exist in sickness behaviors such as lethargy or sexual motivation, and thus obscure mate preferences. Taken together, the immune status of an individual may influence both proximate and ultimate features of reproductive success.

Previous research on sickness behaviors has been conducted on polygynous species such as laboratory rats (*Rattus norvegicus*) [16] and mice (*Mus musculus*) [17], in which the males do not contribute to the rearing of offspring. Polygynous individuals rarely develop preferences for their mate. Socially monogamous species must develop a partner preference to form a lasting pair bond. In prairie voles (*Microtus ochrogaster*), partner preference develops after a few hours in either sexual or nonsexual cohabitation with an opposite sex conspecific [18–22]. Reproductive behavior in monogamous prairie voles, in general, tends to be strongly influenced by intrinsic variables such as hormones, and extrinsic variables such as social factors [18]. Activation of the immune system alters hormones and may influence partner preference expression in male and female prairie voles. High corticosterone concentrations disrupt partner preference in female prairie voles and facilitate partner preference in male prairie voles [18]. Exposure to either IL-1 $\beta$  or LPS significantly elevates adrenocorticotrophic hormone (ACTH) and corticosterone concentrations in rats and mice [23, 24]. While LPS has no direct effect on ACTH production or adrenal output in rats and mice, the release of cytokines stimulates the release of corticotropin releasing hormone (CRH) from the hypothalamus, and the subsequent release of ACTH from the pituitary [25].

The goal of this study was to determine the effects of LPS administration on the formation and expression of social preferences in both male and female prairie voles. Male prairie voles significantly increase corticosterone concentrations in response to LPS [10]. Previously untested, we hypothesized that LPS would elevate corticosterone concentrations in female prairie voles. Elevated blood corticosterone concentrations should impair partner preference formation following a 6-h period of cohabitation in females, but either enhance or not alter partner preference in male prairie voles.

## 2. Materials and method

### 2.1. Animals

Sixteen female and 14 male prairie voles (*Microtus ochrogaster*) obtained from stock originally trapped near

Urbana, IL, were used in partner preference tests. An additional 12 females were used to determine blood corticosterone concentrations following treatment. All animals were adults between 60 days and 1 year of age, with a mean weight of 40 g. Animals were individually housed at least 2 weeks prior to the beginning of the experiment in polypropylene cages (27.8  $\times$  7.5  $\times$  13 cm) in colony rooms with a 16/8-h light/dark cycle (lights on at 0600 h EST) and relative humidity held constant at 50  $\pm$  5%. Food and water were available ad lib throughout the course of the experiment. Sentinel animals were housed in the colony room and screened regularly for the presence of common rodent diseases arising from parasitic, viral, bacterial, and fungal origins (all screens during the course of the experiment were negative).

### 2.2. Procedure

#### 2.2.1. Experiment 1

Females were randomly divided into two groups that received a 0.1-cc intraperitoneal (i.p.) injection of either sterile isotonic saline ( $n = 8$ ), or 50  $\mu$ g LPS (L-3755; Sigma Chemical Co., St. Louis, MO) suspended in saline ( $n = 8$ ), at 0900 h EST. Previous studies have quantified low and high doses of LPS administration in rodents (0.1  $\mu$ g/100 g and 100  $\mu$ g/100 g, respectively) [1,17,26–28]. Doses as low as 0.1  $\mu$ g in mice result in the production of cytokines and the activation of the immune system [17,28], while higher doses induce sickness behaviors such as anorexia, lethargy, and fever in a dose-dependent manner [1,26,27]. Male and female prairie voles are virtually identical phenotypically and in weight [29–31], and the present intermediate dose was selected because it significantly increases IL-1 $\beta$  and corticosterone concentrations in male prairie voles independent of significant locomotor impairments ([10]; personal observations). Following injections, each female was paired with a male conspecific for a 6-h cohabitation period in a standard polypropylene cage within the colony room. The cohabitation periods were monitored using time-lapse videotaping, and videotapes were scored by a research assistant unaware of the experimental conditions for time spent in physical contact during the final 3 h. Scoring only the final 3 h of cohabitation allowed preliminary habituations to the other animal to be excluded from the analyses, and therefore, provided a more accurate measure of time spent in contact. Immediately following cohabitation, partner preferences were assessed using a three-chamber apparatus consisting of plastic chambers (17  $\times$  20.5  $\times$  22 cm) connected in series by two plastic tubes (8  $\times$  5.5 cm). This apparatus has been described in detail previously [19]. Food and water were available ad lib in each of the three chambers. During each trial, two males, the cohabitating partner ( $n = 16$ ) and a novel stranger ( $n = 16$ ), were loosely tethered in one of the two peripheral chambers, with each male tethered in a single chamber. These males were matched in terms of age, size, and reproductive status. Males had access

to their own chambers only, and were not visible to each other. At the beginning of each trial, a female experimental animal was released into the center chamber and allowed access to all three chambers. The female could elect to spend time alone, with the cohabitating partner, or with a comparable stranger. Trials lasted for 3 h and were recorded using time-lapse videotaping. The amount of time that each female spent in physical contact with either male during the preference period was scored by viewing of time-lapse videotapes.

### 2.2.2. Experiment 2

Males were divided into two groups that received a 0.1-cc i.p. injection of either sterile isotonic saline ( $n = 6$ ), or 50  $\mu\text{g}$  LPS suspended in saline ( $n = 8$ ). Following injections, each male was paired with a female conspecific for a 6-h cohabitation period, with time spent in contact with the partner recorded as described in Experiment 1. Partner preferences for each male were assessed using the three-chamber apparatus described above, and time spent in contact with each female was recorded.

### 2.2.3. Steroid hormone RIA

A separate cohort of females received a 0.1-cc i.p. injection of either sterile isotonic saline ( $n = 6$ ), or 50  $\mu\text{g}$  LPS suspended in saline ( $n = 6$ ) at 0900 h EST. Immediately following injections, females were returned to their cages and paired with a novel, naive male for 2 h. Two hours postinjection, males were returned to their home cages, and all females were taken to a separate room for bleeding. This time was selected because it coincides with the period of maximum physiological effects of LPS [17,32]. Animals were lightly anesthetized with methoxyflurane vapors (Metofane, Schring-Plough, Union, NJ). Blood samples were drawn from the retro-orbital sinus, and handling was kept consistent and time kept to a minimum ( $<2$  min). Samples were allowed to clot for 1 h, the clot removed, and the samples centrifuged at 4°C for 30 min at 3500 rpm. Serum aliquots were aspirated and stored in sealable polypropylene microcentrifuge tubes at  $-80^{\circ}\text{C}$  until assayed for corticosterone by radioimmunoassay using an  $^{125}\text{I}$  RIA kit (ICN Biochemicals, Inc.) that had previously been validated for use in prairie voles [33]. This assay is highly specific; crossreaction with other steroids is  $<0.5\%$ . Samples were run in duplicate with a 5% interassay coefficient of variation. Due to unusually high basal blood corticosterone concentrations in prairie voles, the serum was diluted 1:2121 in assay buffer prior to the assay.

### 2.3. Statistical Analyses

An arcsine transformation was performed on the percentage of time each experimental animal spent in contact with the partner during the cohabitation period and the percentage of time spent in contact with either the familiar or unfamiliar animal during the preference trial. Time spent with either animal during the preference test was then analyzed

for males and females injected with LPS compared to males and females injected with saline using two-tailed  $t$ -tests. Males and females were further compared within each treatment group for the amount of time spent with each animal during the preference test by calculating a preference ratio of the time each experimental animal spent in contact with each of the stimulus animals over the total time in contact with either animal. Preference ratios were then analyzed using two-tailed  $t$ -tests. Mean differences in time spent in contact with the cohabitation partner during the final 3 h of the 6-h cohabitation period were analyzed for males and females using two-tailed  $t$ -tests. Mean differences in corticosterone concentrations in LPS-injected females versus saline-injected females were analyzed using a one-tailed  $t$ -test. All differences were considered statistically significant if  $p < 0.05$ .

## Results

### 3.1. Experiment 1

#### 3.1.1. Cohabitation

During the final 3 h of the 6-h cohabitation period, females injected with LPS spent significantly more of the total time in contact with the cohabitation partner than saline injected females,  $t(12) = 2.19$ ,  $p < 0.05$  (Fig. 1A).

#### 3.1.2. Partner preference

Female prairie voles injected with LPS spent significantly more time with familiar partners than unfamiliar animals,  $t(14) = 3.552$ ,  $p < 0.003$  (Fig. 1B). LPS-injected females also spent more time with familiar partners than saline injected females,  $t(14) = -2.776$ ,  $p < 0.015$ . Conversely, saline-injected females spent significantly more time with the unfamiliar animal than the familiar partner,  $t(14) = -2.376$ ,  $p < 0.05$ , and spent significantly more time with the unfamiliar animal than did LPS-injected females,  $t(14) = 2.2$ ,  $p < 0.05$ . We detected no significant differences in locomotor activities among the females.

#### 3.1.3. Steroid hormone concentrations

Female prairie voles injected with LPS exhibited significantly higher corticosterone concentrations than females injected with saline,  $t(9) = 2.053$ ,  $p < 0.05$  (Fig. 2).

### 3.2. Experiment 2

#### 3.2.1. Cohabitation

LPS and saline-injected males spent an equivalent amount of time in contact with the cohabitation partner during the last three hours of cohabitation,  $t(12) = 1.28$ ,  $p < 0.223$  (Fig. 3A).

#### 3.2.2. Partner preference

LPS and saline-injected males spent equivalent amounts of time with familiar,  $t(12) = 0.592$ ,  $p < 0.56$ , and unfamiliar females,  $t(12) = 0.267$ ,  $p = 0.79$  (Fig. 3B). Preferences ratio  $t$ -test comparisons within each drug group revealed no

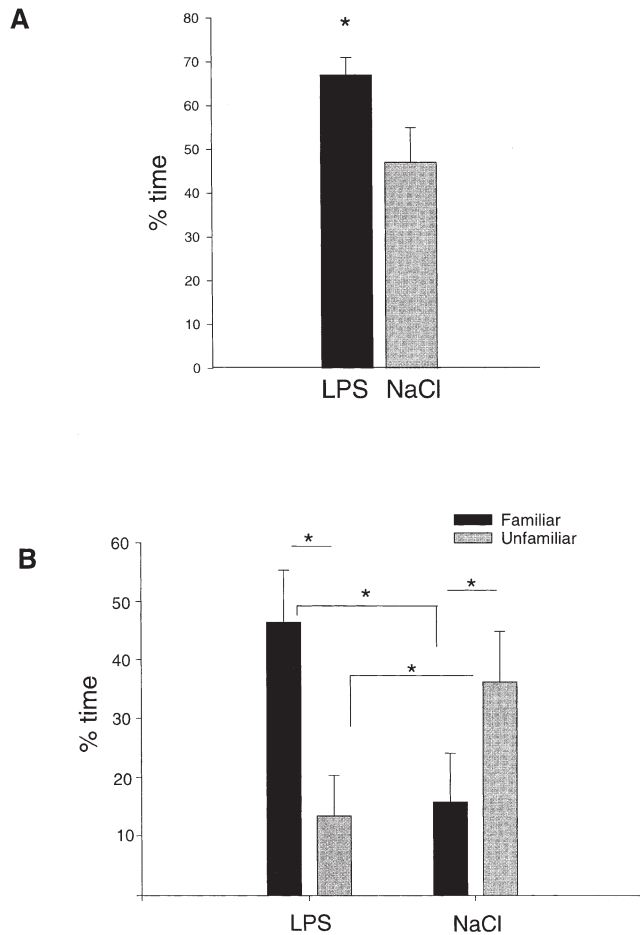


Fig. 1. (A) Mean percent time ( $\pm$ SEM) LPS and saline-injected females spent in physical contact with the cohabitation partner during the final 3 h of cohabitation. (B) Mean percent time ( $\pm$ SEM) LPS and saline-injected females spent in physical contact with the familiar partner and unfamiliar animal during the 3-h preference test.

differences as well. We detected no significant differences in locomotor activities among the males.

#### 4. Discussion

LPS administration in female prairie voles facilitated pair bonding and partner-preference expression. We initially hypothesized that the effects of LPS on partner preference may be mediated by changes in corticosterone concentrations. Infected female prairie voles exhibited the expected increase in blood corticosterone concentrations, but spent significantly more time with the familiar partner than the unfamiliar animal. In contrast, healthy females spent significantly more time with the unfamiliar animal. Six hours of nonsexual cohabitation failed to produce partner preferences in either healthy or infected male prairie voles.

Both male and female prairie voles form pair bonds in nature [22,34]. In the laboratory, variable amounts of time have been required for pair bond formation. Males typically require at least 24 h of nonsexual cohabitation to form part-

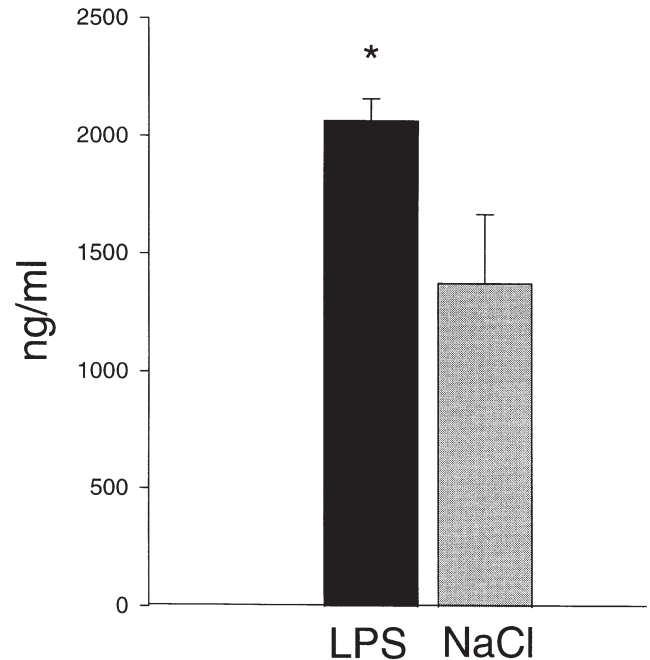


Fig. 2. Mean ( $\pm$ SEM) serum corticosterone (ng/ml) concentrations from females that were injected with LPS or saline, and immediately paired for 2 h with a novel male. Blood samples were collected 2-h postinjection.

ner preferences, although this process can be hastened if mating occurs [19,34]. Some labs report pair bonding in females within 3 h [18,19], although others report that females require  $\geq 24$  h for partner preferences to be expressed in the absence of mating [20,21,35,36]. In the present study, pair bonding apparently did not occur in healthy females within the 6 h of cohabitation. Mating was not consistently observed during cohabitation, and may be required for pair bonding to occur in this short period of time. Conversely, inter-laboratory variability in factors such as housing conditions and colony population characteristics may play a role in producing dissimilar results across studies.

LPS is a powerful activator of the immune system, and the involvement of LPS with central hormonal and neurotransmitter systems are proximate mechanisms through which LPS may facilitate pair bonding. Although the circuitry involved in processing of neural and/or humoral signals from the immune system remains largely unknown, LPS administration in rats increases dopamine release from the nucleus accumbens and locus ceruleus [37,38]. This involvement suggests a possible mechanism through which pair bonding may be rewarding. Furthermore, LPS significantly increases the release of the neuropeptides oxytocin (OT) and arginine vasopressin (AVP) from the posterior pituitary gland in rats [39–41], and there are high densities of OT receptors in the prefrontal cortex and nucleus accumbens of prairie voles, which are regions involved in the dopamine mesolimbic reward pathway [36]. A number of studies have implicated the involvement of OT and AVP in the mediation of complex social behaviors such as affiliation and parental care in prairie voles, as well as in the facil-

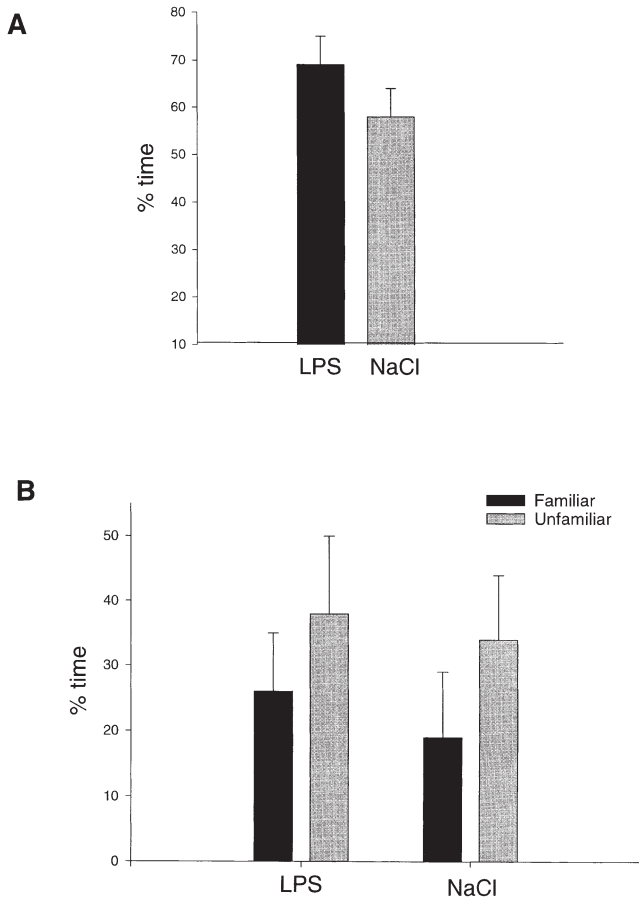


Fig. 3. (A) Mean percent time ( $\pm$ SEM) LPS and saline-injected males spent in physical contact with the cohabitation partner during the final 3 h of cohabitation. (B) Mean percent time ( $\pm$ SEM) LPS and saline-injected males spent in physical contact with the familiar partner and unfamiliar animal during the 3-h preference test.

itation of the consolidation of memory of socially familiar animals [20–22,34,36]. While best known for its role in milk let-down and muscular contractions during birth, the increase in the release of OT observed following genital and cervical stimulation in prairie voles has been implicated as one mechanism by which mating may facilitate pair bonding in females [42]. Similarly, AVP has been implicated for its role in pair bond formation and parental care in males [20,36]. It is unclear why facilitation of pair bonding did not occur in males following the release of AVP; however, it is likely that AVP is a reinforcement equivalent to mating in males, but that cohabitation times required to produce a preference are not significantly reduced enough to allow pair bond formation within 6 h.

From a proximate perspective, this study provides evidence that changes in physiology and behavior following exposure to LPS influence female preference. The use of a nonreplicating pathogen, such as LPS, eliminates the possibility of pathogen manipulation of host behavior, and suggests that the changes in social behavior observed are host-mediated behavioral modifications [10]. These results also

diminish the role of lethargy or decreased sexual motivation that have been factors in previous studies of partner preference in nonmonogamous species such as rats and mice [5,8]. It is unclear whether the cytokine-induced effects of ACTH on HPA axis function and subsequent release of corticosterone are dependent on the sex steroid environment. Previous studies in rats and mice have indicated that females may be more sensitive to the effects of endotoxin on the production of corticosterone [5,25]. It remains unknown whether this is also true in prairie voles, and should be investigated in future studies. It is possible that infected female prairie voles chose to spend more time with the familiar partner because it provided contact comfort or body heat during illness or fever, rather than because of a social preference. However, infected females did not alter their locomotor or activity levels from untreated females, and spent comparable time investigating the unfamiliar males, making this explanation unlikely.

From an ultimate perspective, this study provides a valuable glimpse into the effects that infection and subsequent sickness behaviors may have on sexual selection and mate choice. In many species, the debilitating effects of infection can cause profound declines in reproductive fitness unless counteracted by host behaviors that minimize adverse effects on fitness are employed, such as increased reproductive output. Individuals of some species increase reproduction during infection with parasites, including the great tit, *Parus major* [13], the common lizard (*Lacerta vivipara*) [14], a snail (*Lymnaea stagnalis*) [12], and some insects (e.g., *Acheta domesticus*) [43]. The present results suggest that female prairie voles may pair bond more quickly during infection to increase reproductive success with the advantages incurred through paternal assistance in raising young. Monogamous species often have higher parasite loads than polygynous species [44]. Although it is not certain whether monogamous animals may simply be exposed to pathogens more often, or are more susceptible to disease than polygynous species, the increased parasite burden of monogamous animals has been hypothesized to contribute to an increased need for biparental care [7,44]. Additional studies are needed to determine the extent to which facilitation of pair bonding as a sickness-associated behavior is an adaptive host response to an increased need for paternal contribution to the offspring in prairie voles.

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