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Endotoxin elicits ambivalent social behaviors

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Summary

The acute phase response to infection is reliably accompanied by decreases in social investigation; however, social behavior is commonly assayed in inescapable environments using unfamiliar social stimuli. In this experiment, male Wistar rats were raised from weaning with 2 familiar, same-sex conspecifics. In adulthood, rats were implanted with radiotelemetry devices that permitted localization in space, and were challenged with LPS treatments (150 mg/kg, i.p.) in a novel, semi-natural arena which afforded the treated (Focal) animal exclusive control of social exposure, and the ability to avoid social interactions. LPS reliably elicited thermoregulatory responses (transient hypothermia and fever) during the scotophase following injection, but did not yield changes in the proportion of time spent engaged in social interactions: both LPS- and saline-treated rats spent approximately 10% of the night with their familiar cagemates. Injection treatments markedly altered the spatial distribution of activity: LPS-treated rats exhibited significant increases in the amount of time spent as far as possible from their cagemates. The data suggest that sickness responses to LPS may give rise to a transient state of social ambivalence—characterized by a persistent motivation to engage in social contact, but also by increased avoidance of social environments. Selective maintenance of social motivation illustrates plasticity in the expression of sickness behaviors and may be adaptive in social species.

Keywords

Inflammation; Sickness behavior; Social behavior; Rat

1. Introduction

In response to infection, animals exhibit a suite of short-term changes in physiology and behavior, termed the acute-phase response (APR) and sickness behaviors, respectively (Hart, 1988; Dantzer, 2001). Physiological responses include cytokine production, production of acute-phase proteins, blood iron sequestration, stress hormone secretion, and fever; behavioral changes include anorexia, adipsia, anhedonia, decreases in exploratory behavior, and marked reductions in social motivation (Hart, 1988; Dantzer, 2001). The APR enhances short-term survival by complementing or potentiating several immunological processes (Kluger, 1978; Kluger and Rothenburg, 1979); the generalized behavioral inhibition accompanying the APR has been argued to conserve energy (Hart, 1988).

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Conflict of interest

None declared.

Although the adaptive value of fever is well established (Kluger et al., 1975; Hussein et al., 1982), its metabolic costs are severe. In humans, even modest hyperthermia (+1 °C) accrues substantial (13%) increases in metabolic rate (Dubois, 1948; Kluger, 1980). When compounded by transient anorexia, febrile responses can render animals in a state of severe negative energy balance (Hart, 1988).

In social species, some interactions between familiar adults (e.g., stationary side-by-side contact, huddling) conserve energy (Kauffman et al., 2003). In deer mice, huddling decreases oxygen consumption by 20–30% (Andrews and Belknap, 1986); in white-footed mice, social housing decreases metabolic rate by 16–33% (Glaser and Lustick, 1975); and in house mice, increases in food intake at an ambient temperature of 4 °C are markedly attenuated by social (groups of 2–5) housing (Prychodko, 1958). Huddling behaviors, however, are incompatible with the canonical, and widely reported *decrease* in social motivation that occurs during the APR (Bluthe et al., 1994; Dantzer, 2001).

The modal paradigm for assessing social motivation during the APR presents adult animals with the opportunity to interact with a novel, prepubertal conspecific for a brief interval (typically less than 5 min) in an inescapable environment (Bluthe et al., 1992; Fishkin and Winslow, 1997; Prendergast et al., 2007). Whereas this approach avoids sexual and aggressive confounds associated with adult social interactions, it omits essential aspects of the ecology of such interactions, such as: (1) familiarity with conspecifics and (2) the ability to seek refuge from social contact. Either of these factors stands to affect the strength and direction of sickness-induced changes in social behavior.

The goal of this study was to investigate whether sick animals approach or avoid social contact. We recently reported that LPS-treated rats initiated and received less affiliative contact, but spent more time huddling with their healthy cagemate (Yee and Prendergast, 2010). Therefore, we hypothesized that under conditions that more closely mimic the natural ecology of rat social interactions, namely, in which conspecifics are both familiar and avoidable, sick animals would maintain some degree of social contact in addition to exhibiting classic withdrawal from conspecifics.

2. Methods

2.1. Animals and housing conditions

Beginning at weaning (21 days of age), 27 adult male Wistar rats (*Rattus norvegicus*) were housed 3 per cage until adulthood (>400 g) in clear, solid-bottom polycarbonate cages (46 cm × 25 cm × 20 cm) lined with wood shavings (Sani-Chips, Harlan, Indianapolis, IN, USA). Rats were provided *ad libitum* access to food (Harlan-Teklad 8604, Harlan) and filtered tap water throughout the experiment, in rooms maintained on a 14L:10D photocycle (lights off at 1400 h CST), with an ambient temperature of 20 ± 0.5 °C, and humidity of 53 ± 2%. All procedures conformed to USDA Guidelines for the Care and Use of Laboratory Animals and received prior approval from the University of Chicago IACUC.

2.2. Radiotelemetry

One rat from each triad was randomly selected as the Focal rat; the remaining 2 familiar cagemates were designated Stimulus rats. Focal rats were implanted i.p. with 3 small radiotransmitters (G2 model; Minimitter, Bend, OR) under deep surgical anesthesia (sodium pentobarbital, 40 mg/kg) one week prior to injection treatments and data collection; buprenorphine (0.05 mg/kg) analgesia was administered at 12 h intervals for 48 h after surgery. Animals were allowed to recover in isolation for 24 h, and then were returned to the group cage.

2.3. Behavioral testing environment

A novel cage environment was constructed for each triad (see schematic in Fig. 1). The housing structure was composed of 3 standard polycarbonate rat cages, each fitted with cagetops that held both food and water for the duration of the experiment, serially connected end-to-end by a 15 cm length of opaque PVC pipe (inner diameter: 5 cm). Tubes were inserted into holes drilled in the narrower wall of each cage and were fastened with silicone caulk. The housing structure was then arranged atop 3 separate Minimitter receiver boards, with one board under each component cage chamber. Each receiver board was configured to receive telemetric body temperature (T_b) data from a unique G2 radiotransmitter at 1 min intervals. Compiling data from the 3 receiver boards underlying each housing structure permitted continuous localization of the Focal rat in space at 1 min intervals.

Both Focal and Stimulus rats were allowed to move freely throughout the 3-cage structure for a habituation interval of 4 days. On the following afternoon (*Baseline* night), shortly (<30 min) before lights off, both Stimulus rats were fitted with plastic Elizabethan collars (E-collars) under light (3%) isoflurane anesthesia. Stimulus rats were then placed together into the proximal end cage (Fig. 1; henceforth referred to as the “Social” cage). E-collars were 7–8 cm in diameter and thus prevented Stimulus rats from entering the PVC tunnels to the middle and distal end cages (henceforth referred to as the “Vacant-Near” and “Vacant-Far” cages, respectively) without greatly inhibiting free movement within the Social cage. Focal rats, in contrast, were permitted free movement throughout all 3 cages. T_b data were collected throughout the dark phase (10 h) on the *Baseline* night. On the following afternoon (*Night 1*), shortly (<30 min) before lights off, focal animals were injected with either LPS (*Escherichia coli* lipopolysaccharide, serotype 0127:B8; Sigma; 150 μ g/kg, i.p.) or 0.5 ml sterile saline in a randomized, counter-balanced design, with successive injections separated by 7 days. Radiotelemetry data collection continued for 34 h after injections (i.e., until the end of *Night 2*). After *Night 2*, E-collars were removed but rats remained in the housing structure for the next 5 days.

2.4. Data analysis and statistics

Nocturnal position within the housing structure was determined for each Focal rat on the *Baseline* night, *Night 1*, and *Night 2*. T_b signals were recorded from the receiver boards positioned under the Social, Vacant-Near, and Vacant-Far cages, which allowed localization in space every minute. The proportion of time spent in each of the 3 cages was calculated as the number of T_b events in the given cage divided by the total number of 1 min bins in the 10 h scotophase. Cases in which more than one cage registered a T_b signal accounted for 14.6% of the total 1 min bins, and were excluded from all analyses. The resulting proportions were not normally distributed and were therefore transformed with an arcsine transformation appropriate for parametric analysis of proportions in which the number of sampled bins per timepoint (i.e., 60) remains constant.

In addition to permitting localization in space, actual T_b values were analyzed on *Night 1* in order to quantify febrile responses following LPS treatments. A repeated-measures ANOVA was performed on T_b values that were collected into 1 min bins and averaged in hourly intervals.

Comparisons of the proportion of time spent in each cage on each day and actual T_b values were performed using repeated measures ANOVA followed by post hoc Bonferroni corrected pairwise comparisons where appropriate. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Behavioral responses

LPS treatment did not affect the proportion of time spent in the Social cage ($F_{2,46} = 0.80$, $p > 0.05$). Both LPS- and saline-treated rats spent approximately 1 h of the *Night 1* scotophase in the Social cage (Fig. 1). However, LPS treatments significantly shifted the distribution of time spent in the Vacant-Near and Vacant-Far cages on *Night 1*. Focal rats injected with LPS spent significantly more time in the Vacant-Far cage ($F_{2,46} = 5.49$, $p < 0.01$), and significantly less time in the Vacant-Near cage ($F_{2,46} = 5.16$, $p < 0.01$). On *Night 2*, the proportions of time spent in the Vacant-Near and Vacant-Far cages were comparable between LPS- and saline-treated rats.

3.2. Febrile responses

T_b on *Night 1* differed significantly between LPS- and saline-treated rats ($F_{9,207} = 4.82$; $p < 0.0001$); LPS induced a transient hypothermia during hours 2 and 3 of the scotophase, which did not reach statistical significance, followed by a fever that lasted approximately 4–5 h (hours 6, 7, 9, and 10 of the scotophase; $p < 0.05$, all comparisons; Fig. 2), consistent with other reports using this LPS serotype/dosage in Wistar male rats (Yee and Prendergast, 2010).

4. Discussion

This experiment addressed whether the relations obtained between illness and social behavior would still manifest under conditions that more closely resemble those in which animals socially interact in nature, i.e., conditions that allow approach and avoidance of familiar conspecifics. In a recent report, LPS-treated rats initiated and received less affiliative contact, but spent more time huddling with their healthy cagemates (Yee and Prendergast, 2010). Based on these data we predicted that, rather than avoiding affiliative social contact, LPS-treated rats would remain in contact with familiar cagemates, but would decrease their approach behaviors directed towards cagemates. The present data indicate that LPS treatments sufficient to induce febrile responses did not yield decreases in the amount of time rats spent in social contact. This observation stands in marked contrast to the numerous reports of decreases in social motivation as measured in the home cage, novel juvenile paradigm (Bluthe et al., 1992; Fishkin and Winslow, 1997; Prendergast et al., 2007). Indeed, the proportion of time spent in the Social cage on the night of treatment was nearly identical in LPS- and saline-treated rats. However, LPS-treated rats reallocated their distribution of activity in the Vacant cages: LPS treatment increased the amount of time spent in the Vacant-Far cage (i.e., the area furthest from their cagemates), and decreased the amount of time spent in the Vacant-Near cage (more proximal to their cagemates). Thus, LPS-injected rats did not compensate for the increased time spent in the Vacant-Far cage by decreasing time spent in the Social cage, rather, they did so by selectively decreasing behavior in the Vacant-Near cage. The data suggest that when given the opportunity to titrate social exposure, LPS-treated rats do not alter the amount of social contact, but rather increase avoidance behaviors and decrease approach behaviors.

Social behavioral responses to simulated infection have largely been investigated through interactions between sick adults and novel juveniles. Decreased juvenile social investigation is a reliable behavioral indicator of illness, and has allowed numerous mechanistic insights (e.g., cytokines) into the control of sickness behavior (Bluthe et al., 1992, 1994). The use of juveniles as social stimuli circumvents issues normally associated with interacting adults, such as reproductive and aggressive behaviors, thus it is a convenient and useful assay for the behavioral symptoms of illness (Dantzer, 2001). However, sickness behaviors are plastic in the face of changing motivational contexts (Aubert et al., 1997). The persistent social

behavior reported here suggests that aspects of the social environment (familiarity of Stimulus animals, ability to titrate/control social interactions) also permit behavioral plasticity in the expression of social components of sickness behaviors.

The oscillation between highly social and highly non-social behavior following LPS may be characterized as ambivalent, in that rats appear to express a persistent motivation to engage in social contact, but this is interspersed with efforts to avoid social contact. Keeping a distance from family members in the wild may provide the adaptive benefit of preventing the spread of infectious disease (Kurzban and Leary, 2001; Kavaliers et al., 2003; Schaller and Duncan, 2007; Kavaliers and Choleris, 2011). However, the pro-social aspect of this ambivalent behavior may only be expressed in a permissive environment, one in which the Stimulus animals are sufficiently familiar and from which refuge can be sought; the social avoidance may only be measurable in a testing apparatus that affords different degrees of proximity to the social stimuli (i.e., the opportunity for the animal to be solitary and “near” versus solitary and “far”). The present data do not allow us to identify which component of the novel testing paradigm is most relevant to the experience of behavioral ambivalence. Familiarity of the Stimulus animal may increase their social attractivity to the Focal animal. Alternatively, or in addition, the ability to control social exposure may render social interactions less stressful as compared to those occurring in inescapable social environs. One methodological concern in the present work is that the fitting of Stimulus animals with E-collars that disallowed movement between cages may have altered their social attractivity. However, this seems unlikely, as baseline levels of social behavior by the Focal rats in this study (~15%) were comparable with those of Focal rats in an earlier report (Yee and Prendergast, 2010) in which Stimulus animals were untethered in a group-cage environment. A further potential limitation of the present work is that the telemetry data do not provide detailed insights into the precise nature of the social interactions that occurred between the Focal and Stimulus animals when in the Social cage together. However, in an earlier report in which the social environment was familiar but inescapable, Focal rats initiated and received fewer social contacts, but spent a greater amount of time huddling following LPS treatment (Yee and Prendergast, 2010), an outcome that prompts the conjecture that huddling may have occurred in the Social cage in the present study. However, this is ultimately an empirical issue that requires further study.

The use of testing paradigms that allow greater flexibility in behavioral responses to inflammatory stimuli (LPS, proinflammatory cytokines) may afford mechanistic insights into how the inflammatory response engages changes in social motivation and behavior. Indeed, in the current study, the pattern of thermoregulatory changes that accompanied changes in social behavior was different from that observed in an earlier study in which LPS-treated animals were unable to titrate their social exposure (Yee and Prendergast, 2010). Although male rats in the present study were group-housed, they exhibited T_b responses to LPS that mimicked those of socially isolated rats (c.f. Yee and Prendergast, 2010). An individual's ability to exert control over social exposure may have a profound impact on metabolic and behavioral components of the inflammatory response.

In conclusion, the present data provide a novel elaboration on the well-established decreases in social motivation that occur during the acute-phase response to LPS. When capable of titrating social contact, LPS-treated rats did not change the amount of time spent alone or in social contact; however, when alone, LPS-treated rats markedly altered their proximity to conspecifics, increasing the proportion of time spent as far away as possible from familiar rats. LPS-induced decreases in omnibus social motivation evident in paradigms involving novel social stimuli presented in inescapable environments may reflect a contextually forced resolution of this bivalent state—one in which avoidance overwhelms affiliation.

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Figure 1.

(Top) Percentage of time spent in each compartment of the housing structure by the Focal rat prior to (*Baseline*) and following (*Night 1*, *Night 2*) i.p. injection of 150 $\mu\text{g/kg}$ *E. coli* lipopolysaccharide (*LPS*) or 0.9% saline (*SAL*). * $p < 0.05$ vs. *SAL*. (Bottom) Schematic representation of three-cage housing structure. During acclimation, all rats could freely explore the enclosure. During *Baseline*, *Night 1*, and *Night 2* data collection intervals (see Section 2), Stimulus rats (*S*) were fitted with E-collars that restricted movement to within the “Social” cage, whereas the Focal rat (*F*) had free access to all cages.

Figure 2.

Core body temperature (T_b in °C) of focal male Wistar rats during the scotophase following i.p. injection of LPS (150 μ g/kg *E. coli* lipopolysaccharide) or 0.9% saline (SAL). * $p < 0.05$ vs. SAL.