ELSEVIER

Physiology & Behavior 87 (2006) 694 – 699

PHYSIOLOGY & BEHAVIOR

# Maternal aggression persists following lipopolysaccharide-induced activation of the immune system

Zachary M. Weil *, Stephanie L. Bowers, Eliot R. Dow, Randy J. Nelson

*Departments of Neuroscience and Psychology, and Institute of Behavioral Medicine Research, The Ohio State University, Columbus, OH 43210, USA*

Received 17 August 2005; received in revised form 28 November 2005; accepted 3 January 2006

**Abstract**

Lactating females direct aggressive behaviors towards intruders presumably to reduce the likelihood of infanticide of their pups. Infected animals display a constellation of responses that include lethargy, anorexia, and decreased social interactions. This suite of responses is referred to as sickness behavior, and is putatively part of an adaptive strategy to aid the organism in recovery from infection. Previous work has suggested that animals can suppress the behavioral symptoms of sickness in order to engage in adaptive behaviors. To test whether adaptive nest defense is affected by illness, dams received a peripheral injection of either saline or lipopolysaccharide (LPS [50, 400, or 1000 μg/kg]), a non-replicating component of bacterial cell walls that activates the immune system. Simulated infection with LPS reduced body mass and food intake in dams and interfered with litter growth in a dose-dependent manner. Generally, nest defense was unaffected by LPS; the proportion of dams displaying maternal aggression against a male intruder, as well as the latency and duration of aggressive encounters were only suppressed at the highest LPS dose tested. Further, LPS treatment also altered non-agonistic behavior during the aggression test as indicated by reduced social investigation of the intruder and an increased time spent immobile during the session. LPS administration also significantly increased serum corticosterone concentrations in lactating females. These findings suggest that maternal aggression is not suppressed by LPS-evoked immune activation at doses that attenuate other aspects of maternal and social behavior.


*Keywords:* Sickness behavior; Maternal behavior; Maternal aggression; LPS; Endotoxin; HPA axis

## 1. Introduction

Infanticide by male rodents is a common tactic to induce a lactating dam into estrus rapidly in order to facilitate mating [1,2]. In the presence of a strange male, females of many rodent species will spontaneously abort or resorb fetuses during early pregnancy, a strategy that has been hypothesized to minimize additional investment in pups that may eventually be killed by the intruder male [1]. During late pregnancy and after the pups are born, however, dams display intense aggressive behavior towards a strange male [3]. This so-called maternal aggression or 'nest defense' likely serves to protect the pups from infanticide [4]. These defensive behaviors appear to be the result of an evolutionary trade-off among the risks of injury to the mother and infants, as well as prior investment in the litter. The extent to which situational factors influence nest defense remains poorly understood.

Sick animals display a constellation of physiological characteristics that are collectively termed the acute phase response (APR) [5]. The APR includes induction of fever, suppression of gonadal hormone release, and potent activation of the hypothalamic-pituitary-adrenal (HPA) axis [6]. In addition, a strong behavioral component comprises the sickness response, including anorexia, adipsia, lethargy, and reduced social interactions [7]. Symptoms of the sickness response can be produced experimentally by injection of lipopolysaccharide (LPS; endotoxin), a non-replicating component of gram-negative bacterial cell walls. The proximate mediators of the APR are the proinflammatory cytokines interleukin-1 (IL-1), IL-6,

* Corresponding author. Department of Neuroscience, Ohio State University, Columbus, OH 43210 USA. Tel.: +1 614 538 9540; fax: +1 614 451 3116.
*E-mail address:* Weil.20@osu.edu (Z.M. Weil).

0031-9384/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2006.01.005

and tumor necrosis factor-alpha (TNFα) [8,9]. These polypeptide signaling molecules are produced peripherally by immune cells and in the central nervous system by perivascular and meningeal macrophages, a subset of neurons and glia [10].

Behavioral responses to endotoxin administration are somewhat plastic and can be altered by the immediate environmental or situational context [11,12]. For instance, nest building behavior was inhibited by LPS when animals were housed at mild ambient temperatures. When animals were maintained at temperatures sufficiently low (6 °C) to threaten pup survival, then nest-building was unaffected by LPS [13]. Seasonally breeding Siberian hamsters (*Phodopus sungorus*) use day length to time physiological and behavioral processes. These hamsters breed in long day lengths; production of offspring coincides with environmental conditions most conducive to survival [14]. When maintained under short days, Siberian hamsters display attenuated behavioral and physiological responses to endotoxin [12] This phenomenon has been attributed to the high cost of mounting a full APR that may be energetically incompatible with survival during the short days of winter when resources are presumably limited [15].

Social stimuli are also able to modulate animals' response to simulated infection. For instance, male, but not female, rats suppress the symptoms of sickness behavior in order to mate [11]. In contrast, exposure to an estrous female potentiated the expression of proinflammatory cytokines in the brain and altered the sickness response in adult male house mice [16] Similarly, LPS-injected male mice fail to initiate aggressive behavior towards an intruder in a resident–intruder aggression paradigm, but display normal defensive aggression if provoked [17]. Taken together, it appears that sickness responses can be altered when presumably adaptive to do so and that social factors may serve as particularly potent modulators. The present study was designed to test whether lactating mice rendered sick with LPS would override the normal suppression of social interaction and engage in protective aggressive behavior towards an unfamiliar male intruder.

## 2. Materials and methods

### 2.1. Animals

Fifty adult female CD-1 mice were used in this study. Mice were procured from Charles River Laboratories (Wilmington, MA) at 6–8 weeks of age, allowed one week to acclimate, then paired with an unmanipulated CD-1 male. Following impregnation females were individually housed in polycarbonate cages (28×17×12 cm) with ad libitum access to food (Harlan-Teklad #8640, Madison, WI) and filtered tap water throughout the experiment. Animals were housed in colony rooms maintained on a 14 : 10 light dark cycle (lights on at 0100 h EST) and temperatures of 20±4 °C and relative humidity of 50%±10%. The pups' date of birth was considered postpartum day 0 (PND0). On PND3, litters were randomly winnowed to 6 to decrease variability in maternal aggression [18]. The Ohio State University Institutional Lab Animal Care and Use Committee approved all animal protocols in accordance with National Institutes of Health guidelines.

### 2.2. Procedure

On PND5-6 dams were randomly assigned to receive either a 0.1 ml i.p. injection of sterile saline ($n=21$) or one of three i.p. doses of LPS; 50 (Low, $n=8$), 400 (Moderate, $n=17$) and 1000 μg/kg (High, $n=7$). Injections were administered at 1400 h, 1 h prior to lights out. At the same time dams, litters, and the total amount of food in the cage were weighed. Cages were then returned to the colony room.

Three hours later, dams and pups were transferred to an adjoining procedure room illuminated with photographic red light. The pups were gently removed from the cage and placed in a small container and covered with bedding. Pups were removed from the cage during the aggression tests in order to

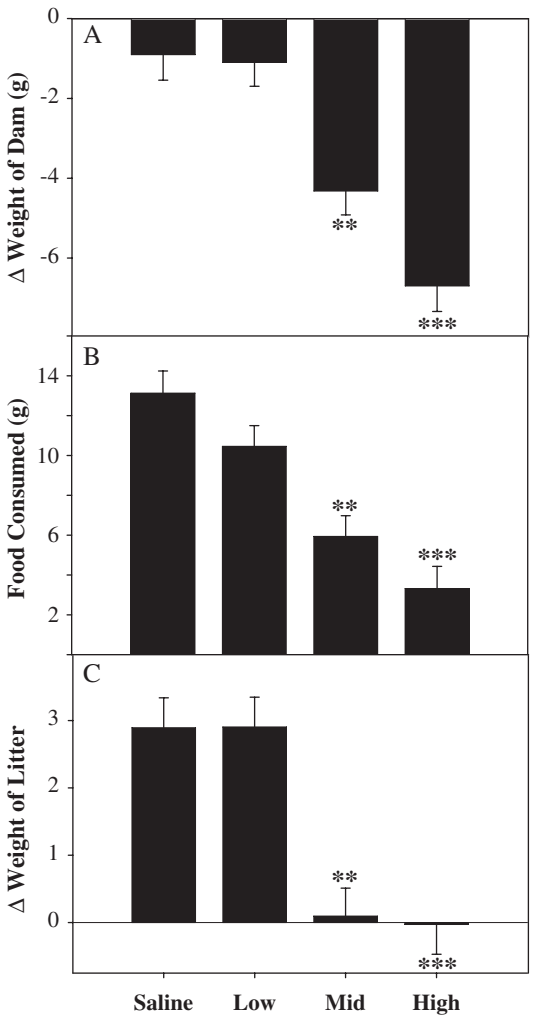


Fig. 1. LPS-induced sickness responses in lactating females. Data are presented as mean (±S.E.M.). Treatment with LPS significantly reduced the lactating female's body weight (A) and food intake (B) by 24 h following treatment. At high doses, LPS administration also interfered with litter growth (C). **Significantly different from saline and low groups $p<0.001$. ***Significantly different from saline and low groups $p<0.0001$.

prevent injury. Removal of the pups immediately prior to the aggression test does not prevent the expression of maternal aggressive behaviors [19]. A previously group-housed, sexually naïve, CD-1 male intruder was placed in the females' home cage with the dam for 15 min. Previous work has shown that virgin males are likely to engage in infanticdal behaviors and as such are potent stimuli for the induction of maternal aggression

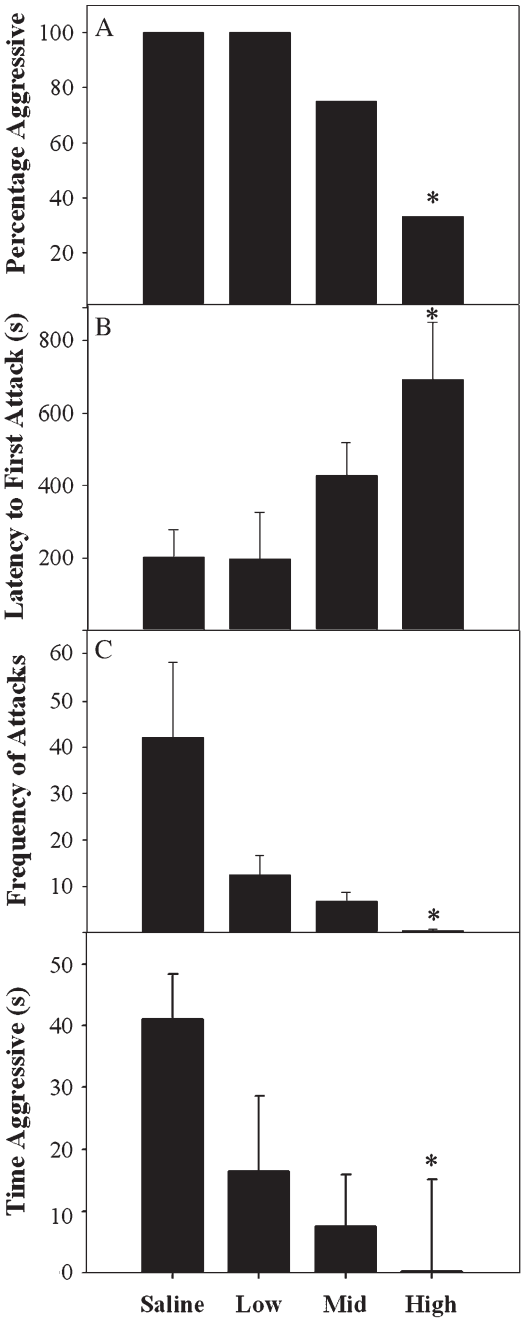


Fig. 2. LPS administration reduced aggressive behavior in the high-dose LPS groups only. Treatment with LPS reduced the percentage of lactating females exhibiting at least one aggressive encounter (number of animals exhibiting at least one attack divided by the total number of sessions (A), increased the latency to the first attack (mean±S.E.M.); (B), and decreased the total time spent engaged in aggressive encounters (C). *Significantly different from saline group $p<0.05$.

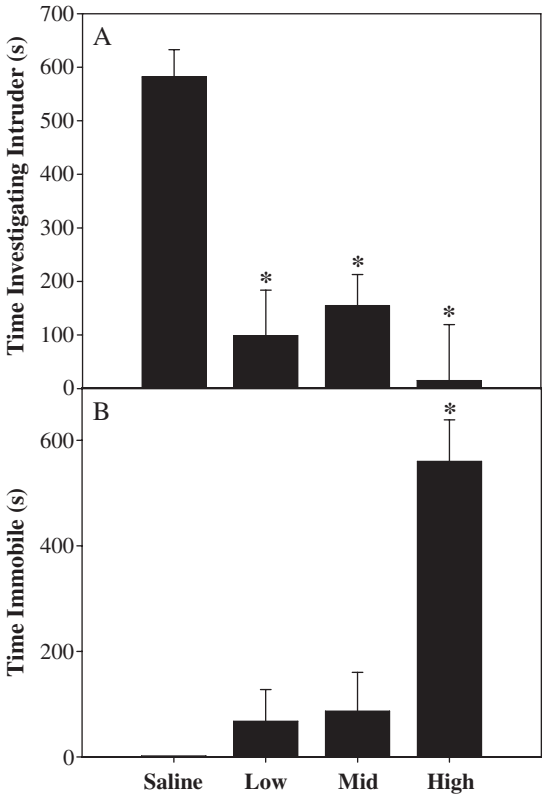


Fig. 3. Treatment with LPS altered non-aggressive behaviors during the aggression sessions as well. Specifically, LPS decreased the time dams spent socially investigating the intruder male (A) and significantly increased the time they spent immobile (B). *Significantly different from saline group $p<0.05$.

[20]. Aggression sessions were videotaped for subsequent behavioral analysis.

Immediately following the test session, females were anesthetized with isoflurane vapors and quickly bled from the retroorbital sinus. A subset of saline-injected dams ($n=8$) were bled without any other manipulation. Blood was allowed to clot at room temperature for at least 30 min, clots were removed, blood was spun at 2500 rpm for 30 min at 4 °C, and sera were stored at −70 °C until assayed for corticosterone concentration. Pups were then replaced in the nest and the cages were returned to the colony. Twenty-four hours later the dam, litter, and food were weighed again. Litters and dams were then euthanized.

### 2.3. Behavioral scoring

Aggression sessions were scored using Observer 5.0 software (Noldus Corp., Leesburg, VA) by a research assistant uninformed about the hypotheses or experimental conditions of the study. Aggressive behaviors of the lactating female were recorded including the latency to first attack, number of aggressive behaviors (operationally defined as biting, kicking, lunging or boxing with the intruder male), and total duration of aggressive behavior [2,21]. Females were considered to be aggressive if they exhibited at least one attack. If no aggressive encounters occurred, then a latency of 900 s (the total

duration of the test) was assigned. In addition, the duration of time spent investigating the intruder male (sniffing or grooming the intruder) and time spent immobile (operationally defined as ≥3 s with no observable movement) were also recorded.

### 2.4. Corticosterone RIA

Serum corticosterone concentrations were determined with a double antibody $^{125}$I radioimmunoassay kit (MP Biomedicals, Costa Mesa, CA). The assay was conducted according to the manufacturer's guidelines. The kit is highly specific and cross-reacts with other steroids >1%. Intraassay variance was <10% and the minimum detection threshold was 5 ng/ml.

### 2.5. Statistical analysis

Corticosterone and body weight data were analyzed by use of a one-way ANOVA for treatment. Significant results were followed by Tukey's HSD analyses for multiple comparisons. Data for percentage of animals exhibiting at least one aggressive behavior were analyzed with a chi-square test. Other behavioral data were not normally distributed and as such were analyzed with the nonparametric Kruskal–Wallis test followed by Dunn's method for multiple comparisons. Mean comparisons were considered significant if $p<0.05$. Due to technical difficulties with the videotapes, some of the aggression data were lost, and the sample sizes used for these analyses were reduced. However, because of the significant results and power analyses for these reduced sample sizes the remaining data and significant results are likely valid.

## 3. Results

### 3.1. Sickness responses

LPS treatment induced body weight losses in a dose dependent fashion ($F_{(3,30)}=19.568$, $p<0.0001$, Fig. 1A) such that animals injected with both Moderate and High doses of LPS lost significantly more weight than did saline-injected animals. Similarly, LPS treatment reduced food intake ($F_{(3,30)}=16.25$, $p<0.0001$, Fig. 1B) and this effect was also mediated by reductions in the Moderate and High dose groups. In addition, LPS treatment of the dams altered litter weights 24 h later ($F_{(3,29)}=14.417$, $p<0.0001$, Fig. 1C) such that pups in the saline-injected and Low groups gained weight over that period. However, pups in the Moderate group failed to add significant body mass and in the case of the High group actually lost weight during the 24 h following LPS treatment.

### 3.2. Maternal aggression

LPS altered the expression of maternal aggression at the highest dose of LPS only. Specifically, administration of LPS reduced the proportion of animals exhibiting at least one aggressive encounter in the highest dose only [$X^2_{(3,34)}=11.181$, $p<0.011$; Fig. 2A]. There was also a main effect of treatment on the latency to the first aggressive encounter (Kruskal–Wallis $H_{(3,34)}=8.673$, $p<0.05$, Fig. 2B). Specifically the High dose of LPS significantly reduced the percentage of dams behaving aggressively and increased the latency to first attack. In addition, treatment with LPS reduced the number of attacks by the mice injected with the highest dose of LPS [$H_{(3,34)}=12.8$, $p=0.005$, Fig. 2D]. Finally, administration of LPS altered the total time spent engaging in aggressive behaviors [$H_{(3,34)}=12.8$, $p=0.005$, Fig. 2C].

Non-agonistic behaviors during the aggression session were also altered by the administration of LPS. Specifically, LPS at all three doses dramatically reduced the time the dam spent socially investigating the intruder ($H_{(3,34)}=23.953$, $p<0.0001$, Fig. 3A). Additionally LPS induced a significant increase in the time spent immobile at the highest dose only ($H_{(3,39)}=9.514$, $p<0.023$, Fig. 3B) during the aggression session.

### 3.3. Corticosterone

LPS treatment significantly elevated corticosterone concentrations following the aggression session in both the Moderate and High groups ($F_{(3,25)}=4.759$, $p=0.008$, Fig. 4). Interestingly corticosterone concentrations do not show clear dose-dependency possibly due to a ceiling effect. Exposure to the intruder male increased corticosterone concentrations in saline injected animals relative to unmanipulated animals ($F_{(1,15)}=46.742$, $p<0.0001$).

## 4. Discussion

The primary goal of this experiment was to determine whether the induction of sickness behavior by LPS would affect maternal aggression. LPS administration at the doses used in this study clearly induced sickness responses as evidenced by weight loss in the pups and dams, and reduced food intake and social interactions. These symptoms are classical components of sickness behavior. Only the highest dose of LPS used in this

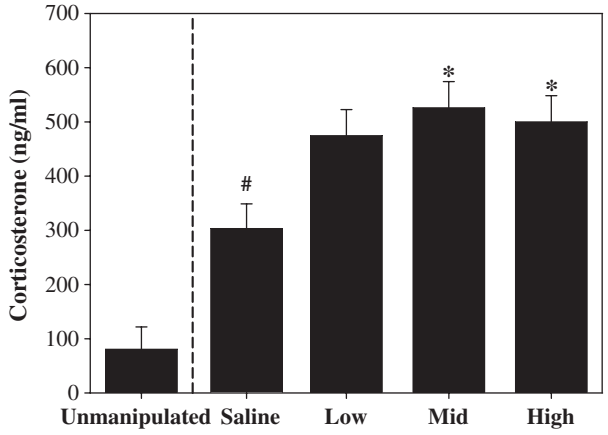


Fig. 4. LPS administration significantly elevated corticosterone concentrations in both the mid- and high-dose groups. Data are presented as mean (±S.E.M.). #Significantly different from unmanipulated group; *Significantly different from saline group; $p<0.05$.

study, a dose employed to induce sepsis and endotoxemia in this strain [22], suppressed maternal aggression. Consistent with our predictions lower doses of LPS that were sufficient to induce sickness behaviors did not suppress maternal aggression.

Sickness responses have been conceptualized as an adaptive mechanism to help individuals fight off infections. However, in this case the display of sickness behavior contrasted with the putative fitness gains associated with exhibiting nest defense. In other words, aggression and sickness appear to be not fully compatible behavioral states. Maternal behavior is unaffected by LPS when maternal care is critical to pup survival [13]. In the present study, animals injected with the two high doses of LPS showed decrements in maternal care such that their litters were not able to maintain normal growth. Maternal aggression was affected only by treatment with the highest dose, however, indicating that nest defense is generally resistant to the suppressive effects of immune activation. Furthermore, at all doses used in this study, social investigation of the intruder male was significantly reduced. Only animals administered the highest dose of LPS increased the time they spent immobile. Indeed, the dampening of maternal aggression in the animals receiving the highest dose of LPS is likely secondary to the overall behavioral depression and nearly complete immobility of those high-dose animals during the test session. Taken together, these results suggest that the fitness costs associated with infanticide partially prevented the expression of sickness behavior in order to engage in maternal aggression and that specific behaviors are differentially sensitive to suppression by LPS.

During lactation, females exhibited reduced anxiety-like behaviors in several behavioral paradigms including elevated plus maze [23] and acoustic startle [24]. The HPA axis responsiveness to stressors generally [25], and to endotoxin specifically [26] is attenuated in lactating rodents as compared to pregnant or nulliparous animals. Furthermore, the immunomodulatory effects of stress are blunted during lactation [26]. The hyporeactivity of the HPA axis in response to a male intruder can be reversed by the presence of pups, suggesting that the litter may serve as a gating mechanism for the perception of stressors [27]. The decreased HPA reactivity during lactation has been implicated in the control of maternal aggression. Indeed, lactating females often appear qualitatively unaware of the potential danger of engaging a male in aggressive behavior [3]. Further, intracerebroventricular infusions of corticotrophin-releasing hormone, a neuropeptide associated with fear and anxiety, suppresses maternal aggression [28]. Consistent with the results of this study, one of the prominent physiological responses to LPS is the potent and relatively persistent activation of the HPA axis; indeed, proinflammatory cytokines potently activate the HPA axis at all three neuroendocrine levels [29]. Glucocorticoids, the steroid hormone effectors of the HPA axis, then feed back to inhibit the production of cytokines and other acute phase proteins [30]. Endotoxin-induced sickness behavior has been associated with increased anxiety-like behaviors [31]. Although HPA responsiveness is attenuated in lactating rodents, rat dams did not show altered febrile responses to LPS relative to virgin animals [32]. Taken together, the blunted HPA response to LPS may have been permissive to the expression of maternal aggression during an acute phase response.

A 15-min test session only provides a brief assessment of the ability of a dam to protect her pups from infanticide. Prolonged or repeated testing may reveal individual differences in behavioral plasticity following infection. However, it appears that the presence of a strange conspecific is a sufficiently potent threat to maternal fitness that lactating mice will risk recovery from infection in order to defend their offspring aggressively. Interestingly, this same phenomenon does not occur in terms of providing nutrition for pups; as animals injected with the higher doses of LPS had litters that gained significantly less weight than saline-injected animals. The opportunity exists to compensate for the litter's weight loss by providing additional resources to offspring following recovery from infection. However, the loss of pups to infanticide represents the total loss of investment in the litter. It appears that a continuum exists wherein sickness behaviors gradually, but progressively, suppress other adaptive behaviors as the severity of infection increases.

It should be noted that while the aggressive behaviors were measured 3 h following administration of LPS the other maternal behaviors were measured over a 24-h period. Maternal responses between the injections and the aggression test were not specifically monitored. Further, variations in contact with the pups, as induced by LPS, may have been partially responsible for the alterations in maternal aggression [3].

In conclusion, lactating CD-1 females continue to display maternal aggression towards intruders when made sick with the middle dose of LPS even when other aspects of maternal care were inhibited. Additional research is necessary to discover the limits of behavioral plasticity of sickness responses in response to fitness threats.

## Acknowledgements

This work was supported by NIH grants MH 57535 and MH 66144 and NSF grant IBN 04-16897. Additional support was received from NIH grant P30NS045758. We thank Brenda Reader for valuable technical assistance and Drs. Brian Trainor and Lynn Martin for helpful comments on earlier versions of this manuscript.

## References

[1] Bruce HM. A block to pregnancy in the mouse caused by proximity of strange males. J Reprod Fertil 1960;1:96–103.
[2] Clutton-Brock TH. The Evolution of Parental Care. Princeton, New Jersey: Princeton Unversity Press; 1991.
[3] Lonstein JS, Gammie SC. Sensory, hormonal, and neural control of maternal aggression in laboratory rodents. Neurosci Biobehav Rev 2002;26:869–88.
[4] Wolff JO. Maternal aggression as a deterrent to infanticide in *Peromyscus leucopus* and *P. maniculatus*. Anim Behav 1985;33:117–23.

[5] Avitsur R, Yirmiya R. The immunobiology of sexual behavior: gender differences in the suppression of sexual activity during illness. Pharmacol Biochem Behav 1999;64:787–96.
[6] Exton MS. Infection-induced anorexia: active host defence strategy. Appetite 1997;29:369–83.
[7] Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 1988;12:123.
[8] Kent S, Bluthe RM, Kelley KW, Dantzer R. Sickness behavior as a new target for drug development. Trends Pharmacol Sci 1992;13:24–8.
[9] Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci 2002;25:154–9.
[10] van Dam AM, Brouns M, Louisse S, Berkenbosch F. Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? Brain Res 1992;588:291–6.
[11] Avitsur R, Yirmiya R. Cytokines inhibit sexual behavior in female rats: I. Synergistic effects of tumor necrosis factor alpha and interleukin-1. Brain Behav Immun 1999;13:14–32.
[12] Bilbo SD, Drazen DL, Quan N, He L, Nelson RJ. Short day lengths attenuate the symptoms of infection in Siberian hamsters. Proc R Soc Lond Ser B-Biol Sci 2002;269:447–54.
[13] Aubert A, Goodall G, Dantzer R, Gheusi G. Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. Brain Behav Immun 1997;11:107–18.
[14] Prendergast BJ, Nelson RJ, Zucker I. Mammalian seasonal rhythms: behavior and neuroendrocrine substrates. In: Pfaff DW, editor. Hormones, Brain, and Behavior, vol. 2. San Diego, Ca: Academic Press; 2002. p. 93–156.
[15] Nelson RJ. Seasonal immune function and sickness responses. Trends Immunol 2004;25:187–92.
[16] Weil ZM, Bowers SL, Pyter LM, Nelson RJ. Social interactions alter proinflammatory cytokine gene expression and behavior following endotoxin administration. Brain Behav Immun 2006;20:72–9.
[17] Cirulli F, De Acetis L, Alleva E. Behavioral effects of peripheral interleukin-1 administration in adult CD-1 mice: specific inhibition of the offensive components of intermale agonistic behavior. Brain Res 1998;791:308–12.
[18] Maestripieri D, Alleva E. Maternal aggression and litter size in the house mouse. Ethology 1990;84:27–34.
[19] Svare B, Betteridge C, Katz D, Samuels O. Some situational and experiential determinants of maternal aggression in mice. Physiol Behav 1981;26:253–8.
[20] Lonstein JS, De Vries GJ. Sex differences in the parental behavior of rodents. Neurosci Biobehav Rev 2000;24:669–86.
[21] Gammie SC, Nelson RJ. Maternal aggression is reduced in neuronal nitric oxide synthase-deficient mice. J Neurosci 1999;19:8027–35.
[22] Standiford TJ, Kunkel SL, Lukacs NW, Greenberger MJ, Danforth JM, Kunkel RG, et al. Macrophage inflammatory protein-1 alpha mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. J Immunol 1995;155:1515–24.
[23] Bitran D, Hilvers RJ, Kellogg CK. Ovarian endocrine status modulates the anxiolytic potency of diazepam and the efficacy of gamma-aminobutyric acid-benzodiazepine receptor-mediated chloride ion transport. Behav Neurosci 1991;105:653–62.
[24] Toufexis DJ, Rochford J, Walker CD. Lactation-induced reduction in rats' acoustic startle is associated with changes in noradrenergic neurotransmission. Behav Neurosci 1999;113:176–84.
[25] Windle R, Wood S, Shanks N, Perks P, Conde G, da Costa A, et al. Endocrine and behavioural responses to noise stress: comparison of virgin and lactating female rats during non-disrupted maternal activity. J Neuroendocrinol 1997;9:407–14.
[26] Shanks N, Windle RJ, Perks P, Wood S, Ingram CD, Lightman SL. The hypothalamic-pituitary-adrenal axis response to endotoxin is attenuated during lactation. J Neuroendocrinol 1999;11:857–65.
[27] Deschamps S, Woodside B, Walker CD. Pups presence eliminates the stress hyporesponsiveness of early lactating females to a psychological stress representing a threat to the pups. J Neuroendocrinol 2003;15:486–97.
[28] Gammie SC, Negron A, Newman SM, Rhodes JS. Corticotropin-releasing factor inhibits maternal aggression in mice. Behav Neurosci 2004;118:805–14.
[29] Turnbull AV, Rivier C. Regulation of the HPA axis by cytokines. Brain Behav Immun 1995;9:253–75.
[30] Goujon E, Laye S, Parnet P, Dantzer R. Regulation of cytokine gene expression in the central nervous system by glucocorticoids: mechanisms and functional consequences. Psychoneuroendocrinology 1997;22(Suppl 1):S75–80.
[31] Lacosta S, Merali Z, Anisman H. Behavioral and neurochemical consequences of lipopolysaccharide in mice: anxiogenic-like effects. Brain Res 1999;818:291–303.
[32] Martin SM, Malkinson TJ, Veale WL, Pittman QJ. Fever in pregnant, parturient, and lactating rats. Am J Physiol 1995;268:R919–23.