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Sexual activity modulates neuroinflammatory responses in male rats

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Abstract

Immune activity influences reproduction, however, the extent to which mating experience may inversely alter immune pathways is poorly understood. A few studies in humans suggest that mating triggers a circulating immune and hypothalamic-pituitary-adrenal axis response. In male rats, mating experience enhances neuroplasticity and improves cognitive function and affective-like behavior, independent of the physical activity component. Yet, the extent to which mating experience may influence immune responses in the brain remain unexplored. Here, we hypothesized that recent mating experience in male rats increases neuroinflammatory signaling (via lipopolysaccharide [LPS] stimulation, i.p.) and associated sickness behaviors (i.e., food intake, weight loss) relative to sexually-naïve controls. Virgin male rats were exposed to a sexually non-receptive (control) or sexually-receptive female for 30 minutes for six consecutive days. Immediately following the last mating experience, rats were administered a saline or LPS injection and euthanized four hours later. Mating increased *Tnfa* responses to LPS in the brain, which positively correlated with LPS-induced weight loss. Mating also increased peripheral corticosterone among saline-treated rats, but this corticosterone response was attenuated in the most proficient copulators (e.g., shortest latencies). Thus, recent mating experience may be a unique modulator of select stimulated inflammatory signals that are relevant to adaptive neuroimmune responses and behavior.

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Keywords

mating; corticosterone; cytokines; lipopolysaccharide; Tnf-alpha; microglia

1) INTRODUCTION

It is widely accepted that immune activation compromises reproduction (reviewed in Gustafsson et al. 1994). In fact, an immune challenge differentially affects subsequent mating behavior in males and females. Specifically, mating behavior is eliminated in hormonally-primed gonadectomized female rats following injection of lipopolysaccharide endotoxin (LPS; Rivier & Vale 1990). However, LPS does not disrupt mating behavior in males (Yirmiya et al. 1995), even though testosterone production is suppressed by LPS (Castellano et al. 2010). Despite this research, few studies have investigated the inverse relationship: the effects of sexual activity on immune function. Findings in humans suggest that specific markers of the innate immune system are activated by sexual experience in men. For example, the absolute number of peripheral blood leukocytes increases following sexual arousal or orgasm (Haake et al. 2004). In addition, promiscuous behavior in female primates increases leukocyte numbers (Nunn et al. 2000). However, in male hamsters, splenic natural killer cell activity and antibody response to antigen are suppressed after mating (Kress et al. 1989, Ostrowski et al. 1989), as well as innate immune function in male *Drosophila* (McKean & Nunney 2001). Reports on the effects of sexual experience on immune responses within the brain, specifically, are limited to one study in which brain mast cells increase following mating in male mice (Yang et al. 1999).

In parallel, a few studies in male rats indicate that sexual experience alters the brain. For example, mating broadly improves structural plasticity throughout the brain, as well as enhances cognitive function and reduces affective-like behavior (Glasper & Gould 2013, Glasper et al. 2015, Leuner et al. 2010). Furthermore, acute, but not repeated, mating activates the HPA axis in male rats (Leuner et al. 2010). The relationship between the peripheral immune consequences of mating and the aforementioned benefits to the brain has not been delineated. However, given the observed increases in glucocorticoids after acute mating in this model and the immunomodulatory actions of glucocorticoids in the brain (Bellavance & Rivest 2014), it is possible that the hypothalamic-pituitary-adrenal (HPA) axis may be one of many potential mediators of mating-related changes in both the immune and nervous systems. Likewise, exercise acutely stimulates the HPA axis (Stranahan et al. 2008), as well as increases muscular and circulating inflammatory cytokines, which is followed by elevations in anti-inflammatory molecules in the periphery and brain and increases in adult neurogenesis (Svensson et al. 2015). These studies illustrate the complexity of the interaction among the immune, endocrine, and nervous systems. Extrapolating these results suggests that, similar to the protective effects of physical exercise (reviewed in Cotman & Berchtold 2002), mating may broadly alter brain signaling pathways (e.g., brain-immune pathways) and regions of the brain outside of those directly responsible for sexual behavior and reward (e.g., hippocampus). It should be noted that the observed beneficial effects of mating on the brain are independent of the physical activity associated with sexual behavior (Glasper & Gould 2013). Therefore, mating may have unique

interventional potential in the context of disrupted neuroimmunology that may be distinct from the therapeutic and protective effects of physical exercise (reviewed in Hötting & Röder 2013, Park et al. 2015).

When human and non-human animals are treated with LPS, a non-replicating cell wall component of Gram-negative bacteria, a well-coordinated suite of behavioral and physiological sickness responses ensue (Dantzer 2004). These adaptive “sickness behaviors” include anorexia, fever, lethargy, social withdrawal, adipsia, and anhedonia and are hypothesized to have evolved to aid in recovery from infection and to be necessary for host survival (Hart 1988). Mechanistically, a peripheral LPS injection stimulates peripheral immune signaling, which is transduced into the brain (Quan 2014) and results in the secretion of cytokines in situ by immune cells therein (e.g., microglia) (Layé et al. 2000). The principal cytokines mediating this neuroinflammatory response that cause sickness behaviors include interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α (TNF α) (Dantzer 2001). Thus, peripheral LPS administration is routinely used to assess functional inflammatory signaling pathways in the brain.

The present study aimed to extend the existing mixed immune findings after mating in the literature (reviewed above) into the brain by testing the extent to which recent mating experience in male rats alters inflammatory pathways specifically in the brain. We hypothesized that mating would augment LPS-induced neuroinflammatory responses.

2) MATERIALS AND METHODS

2.1 Animals

Sexually mature (i.e., young adult; Sengupta, 2013), but sexually-naïve, male and ovariectomized (OVX) female Sprague Dawley rats (2–3 months of age; Charles River, Wilmington, MA, USA) were acclimated to the colony for 7 d before initiation of the experiment (data were combined from 2 treatment-balanced replicate cohorts with $n=4$ male rats/group/cohort for a total of $n=8$ /group). Sample size was determined by power analysis ($\alpha=0.05$) based on variation among gene expression in previous LPS studies. Experimental manipulations were performed on males ($n=32$), while females ($n=32$) were used as stimulus animals only (e.g., sexual experience manipulations). Rats were provided unlimited access to food and water and maintained on a reverse 12:12 light:dark cycle (lights on 1900 h) at $20^{\circ}\text{C} \pm 4^{\circ}\text{C}$ and relative humidity of $50\% \pm 5\%$. Males were housed 2/cage, whereas females were individually-housed. Rats were pseudorandomized among treatments, with those housed together in the same experimental group. Rats were identified by markings placed on their tails and coded by an animal number so that the experimenter was blinded to treatment. All experiments were approved by the University of Maryland Institutional Animal Care and Use Committee and conformed to the guidelines provided by the National Institutes of Health for the care and use of laboratory animals.

2.2 Sexual Experience Paradigm

Sexual receptivity was induced in some OVX female rats by subcutaneous injection of estradiol (0.75 mg/gm body weight in sesame oil) 48 h and progesterone (1.5 mg/gm body

weight in sesame oil) 3 h before pairing with a male rat; other OVX females were not treated with hormones and thus were sexually non-receptive (Tsukahara et al. 2014). Male rats were placed in a novel cage with a non-receptive female (“no mating”) or a sexually-receptive female (“mating”) daily (1300–1600 h), during the dark cycle, for 6 consecutive days. The same sexually non-receptive and sexually-receptive OVX rats were used every 3rd day, and as a result, interacted with a novel male rat twice during the 6-day paradigm. Rats were allowed to interact (i.e., mate or socially investigate in the case of non-receptive females) for a total of 30 min starting from the first intromission (sexually-receptive female only) and were then returned to their home cages. If the male did not initiate sexual behavior with the sexually-receptive female within 30 min, the session was terminated, the maximal latency to behave was recorded (Agmo 1997), and the rat was returned to its home cage. Sexual behavior of naive male rats is more variable than sexually-experienced rats, therefore sexual behavior may vary greatly with inexperience (Agmo 1997). Interactions were monitored and videotaped in the dark under red-light illumination. Daily sexual behavior was hand-scored and analyzed for mounts, intromissions, and ejaculations, as previously described (Glasper et al. 2015, Glasper & Gould 2013, Agmo 1997).

2.3 Experimental Design

Immediately after the conclusion of sexual experience (i.e., following the end of the 30 minute session) on Day 6 (Figure 1), between 1330 h and 1530 h, pseudorandomized male rats were weighed and then injected i.p. with bacterial LPS (*E. coli*, 127:8B; 250 µg/kg; Sigma, Milwaukee, Wisconsin, USA) or 0.2 ml sterile saline (n=8/treatment) at 1400 h. Four hours later, male rats were re-weighed and brain tissues were collected (see below for details). Percent body mass loss over the 4 h was calculated (to the nearest g). As a complementary measure, food mass before and 4 h after injection of saline or LPS was also recorded in the second cohort (n=4/group) to calculate food intake.

2.4 Tissue Collection

Four hours after saline or LPS treatment, between 1730 and 1930 h, which corresponds to peak neuroinflammatory gene responses following LPS challenge (Pyter et al., 2014), rats were anesthetized using a ketamine/xylazine mixture (80mg/kg; 10mg/kg; i.p.) and transcardially perfused with 40 ml cold sterile 0.9% saline in order to flush circulating leukocytes from the brain microvasculature. Brains were extracted, placed in vials containing RNAlater® (Sigma), and stored at 4 °C until dissected for brain regions that regulate male sexual behavior (hypothalamus, nucleus accumbens; reviewed in Veening & Coolen 2014) and are involved in sickness behaviors (hippocampus, hypothalamus, frontal cortex; reviewed in Parnet et al. 2002) before processing for qPCR. Dissection of the nucleus accumbens was performed by slicing a 1-mm section posterior to the frontal cortex using a brain mold. Bilateral wedges encompassing the nucleus accumbens were removed via scalpel from the ventral side of slice with the anterior commissure as the point of the wedge and the bottom width of the wedge spanning ~2.5 mm. Blood was collected during transcardial perfusion via cardiac puncture (between 2–3 min after anesthetic administration) and centrifuged at 4 °C (3,000 rpm for 20 min). Plasma was obtained and stored at –80 °C until assayed for circulating levels of corticosterone.

2.5 Corticosterone Radioimmunoassay

The effects of LPS and mating experience on total circulating corticosterone concentrations were measured in samples of plasma by radioimmunoassay (RIA) using a Corticosterone Double Antibody RIA Kit (MP Biomedicals, Orangeburg, NY, USA). All samples were run in duplicate within the same assay and the intra-assay coefficient of variation was <16%. Due to experimental error, 1 sample (saline, no mating) could not be assayed.

2.6 Quantitative RT-PCR

Total RNA was extracted from the hippocampus, hypothalamus, nucleus accumbens, and frontal cortex using Qiagen RNeasy mini kits (Valencia, CA, USA). RNA concentrations were measured and 260/280 ratios were determined to be 1.8–2.0 (NanoDrop, Wilmington, DE, USA). Total RNA was reverse transcribed using SuperScript First-Strand kits (Invitrogen, Waltham, MA, USA) according to the manufacturer's protocol. One marker of microglia (*Cd11b*) and three cytokine (i.e., *Il-1 β* , *Il-6*, *Tnfa*) genes were chosen based on their established role in neuroinflammation-induced sickness-like behaviors. Rat TaqMan Gene Expression Assays were purchased from Applied Biosystems (Carlsbad, CA, USA) with probes labeled with 6-FAM and MGB (non-fluorescent quencher) at the 5' and 3' ends, respectively: *Il-1 β* (Rn00580432_ml), *Il-6* (Rn01410330_ml), *Tnfa* (Rn00562055_ml), *Cd11b* (Rn00709342_ml), *Gapdh* (Rn01775763_gl). The universal two-step RT-PCR cycling conditions used on the 7900HT Sequence Detection System (Applied Biosystems) were: 50°C (2 min), 95°C (10 min), 40 cycles of 95°C (15 s) and 60°C (1 min). Relative gene expression of individual samples (*to Gapdh*) run in duplicate was calculated by the comparative Ct method (2^{-CT}).

2.7 Statistical Analyses

Mating behaviors and percent copulation were analyzed using 1-way repeated measures ANOVAs using Graph Pad Prism version 7.00 for Windows (GraphPad Software, La Jolla, California, USA). All comparisons were followed by post-hoc Fisher's LSD tests or Student's t-tests based on *a priori* hypotheses. Statistical comparisons of corticosterone and sickness behaviors were analyzed using 2-way ANOVAs, while gene expression was compared using 2-way repeated measures ANOVA over space (i.e., brain region; Seltman 2013) using Statview version 5.0.1 software (Scientific Computing, Cary, NC, USA) when variance was normal. Nonparametric Mann-Whitney U tests were used when variance was not normally distributed. Pearson's correlations were used to determine the extent to which individual mating behaviors predicted the neural responses and how the neural responses related to sickness behavior (Statview 5.0). Data were determined to be statistically significant when $p < 0.05$ and are presented as mean + standard error of the mean (SEM) and are available upon request.

3) RESULTS

3.1 Mating Behavior

Young adult virgin male rats reliably engaged in sexual behavior when exposed to a sexually-receptive female. Greater than 65% of males copulated with the receptive female on

Day 1, with greater than 90% copulation (i.e., intromission) by Day 6 (Figure 2A). Since 100% of the males exposed to sexually-receptive females mounted every day, and successfully copulated (i.e., intromitted) on 3 or more days, with males differing on days in which they did not successfully mate, all males were included in all analyses. No male rats exposed to non-receptive females engaged in any copulatory behaviors. No significant differences in the percentage of males copulating across days were observed ($F_{3,43}=2.5$, $p=0.07$; Figure 2A). Proficiency of sexual behavior increased over time for all dependent measures (i.e., latency to mount, intromit, and ejaculate measures, number of mounts, intromissions, and ejaculations; Figure 2B–G).

Latency. Main effects of time were observed for latency to mount ($F_{2,29}=10.1$, $p=0.0005$), to intromit ($F_{3,50}=4.6$, $p=0.005$), and to ejaculate ($F_{4,56}=10.1$, $p<0.0001$). Latencies to mount and ejaculate on Day 1 were significantly longer than on Days 2–6 ($p<0.01$ in both cases; Figure 2B&F). Latency to intromit on Day 1 and Day 2 did not differ significantly ($p>0.05$), however, Day 1 latency to intromit was significantly longer than Days 3–6 ($p<0.05$ in each case; Figure 2D).

Quantity. Main effects of time were observed for number of mounts ($F_{3,52}=23.5$, $p<0.0001$), intromissions ($F_{4,53}=18.2$, $p<0.0001$), and ejaculations ($F_{4,58}=17.2$, $p<0.0001$). Numbers of mounts and ejaculations were lower on Day 1 compared to Days 2–6 ($p<0.001$ and $p<0.01$, respectively; Figure 2C&G). Number of intromissions did not significantly differ on Days 1 and 2 ($p=0.06$), however, the number of intromissions significantly differed between Day 1 and Days 3–6 ($p<0.01$, respectively; Figure 2E).

3.2 Circulating corticosterone

Overall, both mating ($F_{1,27}=16.95$, $p<0.005$) and LPS treatment ($F_{1,27}=4.80$, $p<0.05$) independently increased circulating corticosterone concentrations (Figure 3A). Mating experience-induced elevations in corticosterone concentration were driven primarily by rats treated with saline ($p<0.05$), whereas LPS-induced increases were observed within both mated and unmated rats ($p<0.05$ for both).

However, within the saline-treated males that mated, those with a higher frequency of mating behaviors (i.e., mounts, intromissions), characteristic of reproductive proficiency, on the day of the injection (Day 6) tended to correlate with reduced corticosterone (data not shown; $p=0.07$ and 0.06 , respectively). Indeed, when the number of mounts from the two most recent mating experiences (Days 5 and 6) were averaged, it was significantly associated with reduced corticosterone (Figure 3B, $p<0.05$). Likewise, the number of intromissions on Days 5 and 6 tended to be associated with reduced corticosterone (Figure 3B, $p=0.07$). In contrast, all LPS-treated rats displayed statistically comparable corticosterone concentrations (Figure 3A) and recent mating behavior did not modulate this response ($p>0.05$, in all cases).

3.3 Brain qPCR

Overall, LPS treatment significantly increased inflammatory gene expression among the majority of the markers and over all brain regions examined (Table 1). Mating experience significantly increased *Tnfa* mRNA as analyzed across all brain regions (Figure 4A;

$F_{3,45}=5.1$, $p<0.005$). In addition, LPS treatment and mating interacted to increase *Tnfa* ($F_{3,45}=3.8$, $p<0.05$), driven primarily by LPS-treated rats ($F_{3,24}=5.3$, $p<0.01$). While mating did not statistically alter the other genes examined (Table 1), recent mating behaviors predicted expression of multiple genes after LPS. Specifically, within LPS-treated rats with mating experience, proficiency in specific sex behaviors on Day 6 (i.e., last day of mating followed immediately by LPS treatment) significantly predicted higher gene expression of numerous neuroinflammatory markers, including *Tnfa* as well as *Il-1 β* , *Il-6*, and *Cd11b*. For example, shorter latencies to intromit on the final day of mating tended to predict elevations in frontal cortex *Tnfa* (Figure 4B; $p=0.06$) and predicted elevations in frontal cortex and hippocampal *Il-6* ($p<0.05$ in both cases; data not shown), nucleus accumbens *Il-1 β* (Figure 4B; $p<0.05$), and frontal cortex ($p<0.05$; data not shown), hypothalamic ($p<0.05$; data not shown), and hippocampal *Cd11b* (Figure 4B, right; $p<0.01$) after LPS administration. Additionally, a greater number of ejaculations predicted higher frontal cortex and hippocampal *Il-1 β* mRNA (data not shown; $p<0.05$ and $p<0.005$, respectively). Similarly, shorter latencies to mount predicted higher hippocampal *Cd11b* (Figure 4B; $p<0.05$). For each of these correlations, mating behavior consistent with mating mastery (i.e., shorter latencies, more ejaculations) predicted higher inflammatory gene expression following LPS treatment (Figure 4B). Of note, less recent mating behavior relative to LPS treatment (e.g., averaging mating behaviors over the 6 days of mating) did not have significant predictive value for LPS responses in the brain (analyses not shown; $p>0.05$). Despite these numerous correlations, mating did not significantly influence absolute *Il-1 β* , *Il-6*, and *Cd11b* gene expression nor their gene expression analyzed across the brain (Table 1).

Given that glucocorticoids are endogenous anti-inflammatory mediators (Barnes 1998), the relationship between circulating corticosterone and neuroinflammation was assessed. Indeed, circulating corticosterone was negatively correlated with frontal cortex *Cd11b* mRNA in both LPS-treated and saline-treated rats (Supplementary Figure 1A). However, corticosterone predicted lower hippocampal *Cd11b* mRNA (data not shown; $\beta=0.4$, $R^2=0.2$, $p<0.05$), driven precisely by the unstimulated saline control group (Supplementary Figure 1B; $p<0.01$). Corticosterone concentrations did not predict other neuroinflammatory markers (analyses not shown).

3.4 Sickness Behavior

Overall, LPS treatment induced weight loss (Figure 5A; $F_{1,24}=50.6$, $p<0.0001$) relative to saline. Mating approached statistical significance for inducing weight loss ($p=0.1$), particularly for LPS-treated rats with mating experience relative to no mating controls ($p=0.08$). Indeed, within the LPS-treated rats, weight loss after LPS injection was greater in rats with higher *Tnfa* across the four brain regions examined (for example, see hypothalamus in Figure 5B), a finding that was characteristic of more efficient mating. Furthermore, this relationship between weight loss and higher neuroinflammation extended beyond *Tnfa* to include other cytokines (*Il-1 β* and *Il-6*; data not shown), similar to the relationship observed between more efficient mating and various higher cytokines (see above). Food intake after treatment did not vary by mating experience, nor was a significant reduction in 4-h food intake after LPS detectable (Figure 5C).

4) DISCUSSION

Recent mating experience significantly increased baseline corticosterone and, in line with our prediction, increased select neuroinflammatory responses to a peripheral immune challenge in adult male rats. Furthermore, both the corticosterone and neuroinflammatory responses were proportionate to mating proficiency.

The mating experience-induced increases in circulating corticosterone observed within saline-treated rats after their 6th mating interaction were similar to elevations in corticosterone previously observed 30 min after a single acute mating experience in young adult male rats (Leuner et al. 2010). In birds, baseline and stress-related corticosterone are likewise elevated in breeding individuals (Apfelbeck et al. 2017). This consistency among vertebrates suggests that this glucocorticoid response may facilitate reproductive activity or success (Breuner et al. 2008). However, this acute elevation in corticosterone subsides after 14 d of mating in male rats (Leuner et al. 2010), suggesting that the corticosterone response to mating experience diminishes sometime between 6 and 14 days of daily mating exposures, and is potentially inversely related to the concurrent increasingly proficient reproductive behavior. Indeed, in the present study, greater reproductive proficiency was correlated with lower corticosterone within mated males. This relationship was likely masked by the LPS-induced increases in corticosterone within the mated LPS-treated rats. A similar pattern is observed in female rats, in which corticosterone responses to an acute stressor are lower among dams that have conceived young compared to nulliparous females without sexual experience (Workman et al. 2016), however, it is possible that these observed effects are due to pregnancy and not copulation. It is important to note that plasma was collected between two and three minutes following injection of an overdose with ketamine/xylazine anesthesia. Many types of anesthesia can increase peripheral levels of corticosterone (Arnold & Langhans 2010, Khalili-Mahani et al. 2015), therefore, it is possible that our sampling method may have increased basal levels of corticosterone (e.g., 100 ng/ml in saline-injected, no mating rats). Nevertheless, among saline-treated rats, peripheral corticosterone was higher in males that had mated, compared to non-mated counterparts. Finally, circulating corticosterone fluctuates along a consistent circadian rhythmic pattern and it is possible that mating could act as a secondary zeitgeber, thus altering the cross-sectional corticosterone comparison. However, all rats were housed and assessed for behavior in a constant 12:12 light-dark cycle and secondary zeitgebers are masked by this primary light zeitgeber (Mrosovsky 1996).

Glucocorticoids are the most potent endogenous anti-inflammatory signal and therefore may play a role in how sexual experience modulates neuroinflammatory responses. Indeed, here higher circulating corticosterone predicted lower expression of the microglial gene marker, *Cd11b*, in the frontal cortex of both control and LPS-treated rats. However, cytokine gene expression was unrelated to circulating corticosterone concentrations. Thus, the relationship between glucocorticoids and neuroinflammation in this context may be more related to stable microglial number than to acute cytokine signaling responses. Of note, unstimulated microglia also contribute to their microenvironment by maintaining neuronal health and plasticity and are associated with homeostatic and cognitive behaviors (e.g., sleep, stress responses, learning and memory; Yirmiya & Goshen 2011).

On the other hand, mating behaviors were associated with increased expression of a variety of LPS-stimulated neuroinflammatory genes in the present study. This suggests that neuroinflammatory changes were not driven by the glucocorticoid response to mating. The most consistently predictive reproductive behaviors were those from the day of the LPS injection (Day 6); cumulative reproductive totals or averages over all 6 days of mating exposures were less so. The observed relationship, that better copulatory proficiency on the day of LPS injection predicted increases in numerous inflammatory genes expressed in the brain, was absent in rats that received a saline injection. Thus, resting constitutive cytokine gene expression was unaffected by recent mating experience. In contrast, resting cytokine gene expression in the brain is observed to be altered in other contexts such as social distress (McKim et al. 2016), aging (Ye & Johnson 1999), enriched environment (Jurgens & Johnson 2012), high-fat diet (Guillemot-Legrise & Muccioli 2017), but not necessarily exercise (Olah et al. 2009). Part of the discrepancy between mating and these other manipulations may be the relatively short exposures to a receptive female (30–60 min/day for 6 days versus weeks-months, respectively).

The energetic trade-off of mounting an immune response at the expense of subsequent reproductive activity is well established (reviewed in Gustafsson et al. 1994). Inversely, the influence of sexual activity on subsequent inflammatory responses is poorly understood, and before the present work, virtually nonexistent with respect to brain inflammation and sickness behavior (but see mast cells Yang et al. 1999). However, a few reports on peripheral immune activity corroborate the present findings in the brain, and although peripheral inflammation was not assessed in the present study, it can mirror that of the brain (Hoogland et al. 2015), but see (Quan et al. 1998). In men, orgasm increases absolute circulating innate immune cells, while reducing adaptive immune cells and IL-6 (Haake et al. 2004). In male hamsters, suppressed antibody titers in the circulation are associated with one indication of mating proficiency (i.e., high number of ejaculations), as well as one indication of mating incompetence (i.e., few prolonged intromissions) (Ostrowski et al. 1989). Indeed, mating impairs bacterial clearance in *Drosophila* (McKean & Nunney 2001) and virtually all shrew-like male marsupials succumb to infections after intense sexual activity (Bradley et al. 1980).

The present neuroinflammatory results are consistent with our initial prediction, that mating would increase neuroinflammation. Although *Tnfa* was the lone cytokine for which absolute magnitude was elevated by LPS after mating, these data were supported by the many significant positive correlations between mating behaviors and other numerous proinflammatory cytokines throughout the brain. Treadmill exercise preceding brain ischemia in mice similarly elevated brain TNF α while reducing brain damage, suggesting that elevations specific to TNF α may not necessarily be detrimental in the context of ischemia (Ding et al. 2005). In contrast, exercise attenuates LPS-induced neuroinflammatory responses (Littlefield et al. 2015). A distinction between sexual behavior and general exercise is provided by the observation that the cognitive-enhancing effects of mating are lost following mating cessation (Glasper & Gould 2013), whereas withdrawal from treadmill running does not reverse the associated cognitive improvements (Radahmadi et al. 2015). Again, the duration of these interventions (brief mating versus prolonged exercise) may also contribute to the resulting discrepancies in neuroinflammatory responses. Other potential

pathways by which mating may modulate neuroinflammation are via oxytocin or via peripheral neural/humoral stimulation. However, oxytocin, which is released following copulation in male rats, has primarily been associated with reduced neuroinflammation (Karelina et al. 2011, Yuan et al. 2016), as opposed to the observed increased neuroinflammation. Thus, the more likely candidate pathway is that mating increases peripheral inflammatory signals, as described in previous studies above, which are then transduced into central inflammation (Quan 2014).

While neuroinflammatory *Tnfa* responses were modulated by sexual experience, significant modulation of sickness behaviors caused by these signals was not observed. This may be due, in part, to the low sample size of some behavioral data that were only accessed in the 2nd cohort (i.e., n=4/group for food intake), as behavior tends to be relatively variable, and/or the short duration between LPS treatment and behavior acquisition (i.e., 4 h). However, high *Tnfa* gene expression in the hypothalamus, which regulates energy homeostasis, correlated with greater weight loss after LPS. Furthermore, higher *Tnfa* after LPS treatment was associated with superior mating behavior and thus, it may be extrapolated that superior mating behavior predicts greater sickness behavior. Similarly in humans, moderately tall men (up to 185 cm) mount a greater immune response to antigen, as well as have higher reproductive success, than their shorter counterparts (Krams et al. 2014). A direct association between mating and sickness behavior was not observed in the present study. Finally, mating did not alter neuroinflammatory responses to the control saline injection, although it is possible that such effects may occur <4 h after mating or may have become habituated with multiple mating exposures.

This work implies that sexual experience may modulate select signaling pathways in the brain of male rats upon endotoxin challenge. Given the role that neuroinflammatory signaling is hypothesized to play in sickness behavior, as well as general mental health (Ekdahl et al. 2009, Schneider et al. 1998, Yirmiya & Goshen 2011, Ziv et al. 2006), further research focused on understanding how sexual experience may modulate behavior (e.g., cognition or mood) acutely, if not chronically, in both males and females is warranted. Additionally, the extent to which neuroinflammatory signaling may relate to mating-related changes in structural plasticity should be further explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

5) ACKNOWLEDGEMENTS/CONFLICT OF INTEREST DISCLOSURE

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HIGHLIGHTS

- Mating and LPS independently increased peripheral corticosterone levels in male rats
- Mating attenuated corticosterone in proficient maters (i.e., short latencies)
- LPS treatment and mating interacted to increase *TNF α* expression in the brain
- Mating behaviors were associated with increased neuroinflammatory gene expression after LPS
- Neuroinflammation correlated with weight loss in sexually experienced male rats

Figure 1.
Experimental design for testing how sexual experience modulates neuroinflammatory signaling in male rats.

Figure 2. Sexual behavior of young adult male rats exposed to a receptive female on 6 consecutive days.

Mean $\pm$ SEM **A)** percentage of rats that successfully copulated, **B)** latency to first mount (s), **C)** total number of mounts, **D)** latency to first intromission (s), **E)** total number of intromissions, **F)** latency to first ejaculation (s), **G)** total number of ejaculations for each day. $n=8/\text{group}$. * $p<0.05$, relative to Day 1.

Figure 3. Circulating corticosterone concentrations

. **A)** Mean + SEM plasma corticosterone concentrations (ng/ml) measured by radioimmunoassay in male rats 4 h post-LPS or saline injection (i.p.) with or without recent mating experience. **B)** Simple linear regressions between mating behaviors averaged from the last two days of mating (Days 5 & 6) and corticosterone 4 h post-saline injection. n =saline, no mating (7); saline mating (8); LPS, no mating (8); LPS, mating (8). # $p < 0.05$; * $p < 0.05$, relative to no mating.

Figure 4. Effects of mating experience on mRNA expression of inflammatory genes in brain regions that regulate sickness and sexual behavior

. **A)** Mean + SEM TaqMan quantitative gene expression of hippocampal, hypothalamic, nucleus accumbens, and frontal cortex *Tnfa* in male rats 4 h post-LPS or saline injection (i.p.) with or without recent mating experience. **B)** Representative examples of simple linear regressions correlating mating behaviors from the last day of mating (Day 6) with the expression of other inflammatory genes in relevant brain regions 4 h post-LPS injection. $n=8/\text{group}$. * $p<0.05$, mating repeated measures over brain region; # $p<0.05$, mating x injection interaction repeated measures over brain region.

Figure 5. Sickness behaviors 4 h post-saline/LPS injection

A) Mean + SEM percent change in food intake (g; n=4/group). **B)** Simple linear regression between hypothalamic *Tnfα* gene expression and weight loss 4 h post-LPS injection in male rats with recent mating experience. **C)** Mean + SEM body weight (g; n=8/group) in male rats 4 h post-LPS or saline injection (i.p.) with or without recent mating experience.

Table 1

Mean gene expression $\pm$ SEM (relative to *Gapdh*) of other Inflammatory markers In brain regions Involved In sickness or sexual behavior and ANOVA statistical analyses.

Gene	Treatment	Hippocampus	Hypothalamus	Nucleus Accumbens	Frontal Cortex
<i>Cd11b</i>	NO MATING, saline	$8.2 \times 10^{-4} \pm 1.3 \times 10^{-4}$	$1.5 \times 10^{-3} \pm 2.8 \times 10^{-4}$	$1.6 \times 10^{-3} \pm 1.2 \times 10^{-4}$	$7.9 \times 10^{-4} \pm 1.3 \times 10^{-4}$
	NO MATING, LPS	$1.1 \times 10^{-3} \pm 1.4 \times 10^{-4}$	$2.6 \times 10^{-3} \pm 4.7 \times 10^{-4}$	$2.1 \times 10^{-3} \pm 2.9 \times 10^{-4}$	$9.7 \times 10^{-4} \pm 1.7 \times 10^{-4}$
	MATING, saline	$8.1 \times 10^{-4} \pm 2.0 \times 10^{-4}$	$1.4 \times 10^{-3} \pm 3.3 \times 10^{-4}$	$1.5 \times 10^{-3} \pm 7.8 \times 10^{-5}$	$5.9 \times 10^{-4} \pm 1.3 \times 10^{-4}$
	MATING, LPS	$1.2 \times 10^{-3} \pm 1.2 \times 10^{-4}$	$2.3 \times 10^{-3} \pm 1.0 \times 10^{-3}$	$2.3 \times 10^{-3} \pm 2.8 \times 10^{-4}$	$1.0 \times 10^{-3} \pm 2.0 \times 10^{-4}$
	Mating				
	LPS	*	*	*	p=0.08
	mating $\times$ LPS				
	NO MATING, saline	$6.0 \times 10^{-5} \pm 6.9 \times 10^{-6}$	$4.4 \times 10^{-5} \pm 6.1 \times 10^{-6}$	$4.5 \times 10^{-5} \pm 6.7 \times 10^{-6}$	$3.5 \times 10^{-5} \pm 4.6 \times 10^{-6}$
	NO MATING, LPS	$3.7 \times 10^{-4} \pm 7.1 \times 10^{-5}$	$3.4 \times 10^{-4} \pm 7.0 \times 10^{-5}$	$1.7 \times 10^{-4} \pm 1.9 \times 10^{-5}$	$1.4 \times 10^{-4} \pm 2.2 \times 10^{-5}$
	MATING, saline	$5.3 \times 10^{-5} \pm 6.6 \times 10^{-6}$	$4.0 \times 10^{-5} \pm 4.5 \times 10^{-6}$	$3.5 \times 10^{-5} \pm 4.2 \times 10^{-6}$	$3.0 \times 10^{-5} \pm 2.1 \times 10^{-6}$
<i>11-6</i>	MATING, LPS	$3.2 \times 10^{-4} \pm 6.8 \times 10^{-5}$	$3.8 \times 10^{-4} \pm 1.2 \times 10^{-4}$	$2.4 \times 10^{-4} \pm 4.6 \times 10^{-5}$	$1.4 \times 10^{-4} \pm 1.2 \times 10^{-5}$
	mating				
	LPS	*	*	*	*
	mating $\times$ LPS				
	NO MATING, saline	$2.8 \times 10^{-5} \pm 3.8 \times 10^{-6}$	$6.3 \times 10^{-4} \pm 1.8 \times 10^{-5}$	$3.0 \times 10^{-5} \pm 4.6 \times 10^{-6}$	$4.8 \times 10^{-5} \pm 6.9 \times 10^{-6}$
	NO MATING, LPS	$3.8 \times 10^{-4} \pm 6.9 \times 10^{-5}$	$9.5 \times 10^{-4} \pm 3.4 \times 10^{-4}$	$2.0 \times 10^{-4} \pm 2.8 \times 10^{-5}$	$2.2 \times 10^{-4} \pm 4.4 \times 10^{-5}$
	MATING, saline	$3.7 \times 10^{-5} \pm 5.1 \times 10^{-6}$	$8.4 \times 10^{-5} \pm 9.1 \times 10^{-6}$	$2.3 \times 10^{-4} \pm 3.1 \times 10^{-6}$	$3.4 \times 10^{-4} \pm 2.2 \times 10^{-6}$
	MATING, LPS	$3.8 \times 10^{-4} \pm 1.0 \times 10^{-4}$	$6.6 \times 10^{-4} \pm 1.3 \times 10^{-4}$	$2.1 \times 10^{-4} \pm 4.1 \times 10^{-5}$	$2.7 \times 10^{-5} \pm 4.1 \times 10^{-5}$
	mating				
	LPS	*	*	*	*
<i>11-1β</i>	mating $\times$ LPS				

* p<0.05; gray = p>0.05