Brain, Behavior, & Immunity - Health 14 (2021) 100248

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Full Length Article

Immune receptor toll-like receptor 4 contributes to stress-induced affective responses in a sex-specific manner

Cana B. Quave, Steven J. Nieto, Colin N. Haile, Therese A. Kosten *

Department of Psychology, University of Houston, United States

ARTICLE INFO

Keywords:
TLR4
Predator odor
Sex differences
Depression
Anxiety
Anhedonia
Innate immune system
Rat

ABSTRACT

Stress activates innate immune Toll-like receptors (TLRs) and enhances susceptibility to depression, a condition that is more prevalent in females. The TLR4 receptor type is involved in inflammatory responses and its expression levels associate with depressive symptoms and their successful treatment. Yet, little preclinical research has examined the role of TLR4 in stress-induced affective responses to determine if these are sex-specific. One group per genotype of male and female *Tlr4* knockout (KO) and wild type (WT) rats were exposed to predator odor in a place conditioning apparatus with others exposed to saline. Affective behaviors evaluated included distance traveled and center time in an open-field apparatus, sucrose preference and fluid intake in a two-bottle test, and conditioned place aversion to the odor-paired compartment. Predator odor exposed rats showed conditioned place aversion to the odor-paired compartment, demonstrating predator odor was aversive. Such exposure led to anhedonia (decreased sucrose preference) across genotypes and sex. Predator odor exposure decreased distance traveled, an effect that was greater in KO rats, especially in females. *Tlr4* deletion also resulted in sex-specific effects on anxiety-like behavior. Compared to WTs, female KO rats showed lower center time after predator odor exposure whereas genotype did not affect this response in male rats. Across litters, fewer male KO and heterozygous rats and more WT rats were born whereas female rats showed the typical genotype distribution. Results suggest predator odor alters affective behaviors, consistent with the preclinical literature, and deletion of *Tlr4* enhances some stress-induced affective responses, often in a sex-specific manner.

1. Introduction

Major depressive disorder (MDD) is a debilitating and often recurring disease (Mueller et al., 1999) with a lifetime prevalence rate of over 20% among adults in the United States (Hasin et al., 2018). Women are about twice as likely as men to have MDD (Kessler et al., 1993) and more often show the classic signs of anhedonia, sadness, and feelings of worthlessness as well as altered sleep and feeding patterns (Frank et al., 1988; Kornstein et al., 2000; Zagni et al., 2001; Marcus et al., 2008). In contrast, depression in men is often accompanied by anger or addiction-like behaviors. Although men are less likely than women to seek treatment (Martin et al., 2013) and evidence suggests the sex gap is closing (Platt et al., 2020), the rate disparity in MDD may reflect, in part, the many sex differences seen in neurobiological systems that contribute to depression (Rubinow and Schmidt, 2019).

Historically, understanding the neurobiology of depression has focused on neural and hormonal systems activated by stress because depressive episodes are often precipitated by stressful life events. More recently, attention has turned to the role of the innate immune system in MDD (Maes, 1995; Frank et al., 2016; Liu et al., 2019) that communicates bi-directionally with neural and hormonal systems (Maier, 2003). Stress activates the innate immune system leading to the production of pro-inflammatory cytokines (Dhabhar and McEwen, 1997; Padgett and Glaser, 2003). Inflammatory cytokines affect brain systems known to contribute to MDD (Miller et al., 2008) and peripheral levels are increased in depressed patients (Levine et al., 1999; Dantzer et al., 2008; Rizavi et al., 2016; Zhang et al., 2018).

Toll-like receptors (TLRs) play a major role in the innate immune system and are considered pattern recognition receptors (PRRs) that are expressed by immune cells in addition to neural and glial cells (Vaure and Liu, 2014). TLRs promote the production of inflammatory factors such as cytokines (Akira et al., 2006). Toll-like receptor 4 (TLR4) is part of the mammalian family of TLRs that is activated by lipopolysaccharide (LPS) and is a particular focus of research in depression (Cheng et al., 2016).

* Corresponding author. Department of Psychology, TIMES, Health and Biomedical Sciences, Building 1, 4849 Calhoun Road, Houston, TX, 77204-6022, United States.
E-mail address: takosten@uh.edu (T.A. Kosten).

https://doi.org/10.1016/j.bbih.2021.100248
Received 17 March 2021; Received in revised form 23 March 2021; Accepted 28 March 2021
Available online 31 March 2021

TLR4s are also activated by damage- or danger-associated molecular proteins (DAMPs) that occur endogenously after psychological or physical stress (Franklin et al., 2018).

Whether there are sex differences in TLR4 function in humans (Jiang and Gilkeson, 2014) or rodents (Santos-Galindo et al., 2011) is not clear. In response to an immune challenge, men show higher TLR4 activation and greater pro-inflammatory cytokine production, whereas women have increased macrophage activation, phagocytic capacity, and IL-10 production (Klein and Flanagan, 2016). Yet, several innate and adaptive immune responses differ between the sexes (Marriott and Huet-Hudson, 2006) and are linked to depression (Bekhbat and Neigh, 2018; Rainville et al., 2018). Although few animal studies investigating the role of the innate immune system in depressive-like effects include females, greater behavioral and immune response to inflammatory challenges are seen in female rats compared to males (Tonelli et al., 2008).

Animal models of depression use stressors to induce depressive-like behaviors, such as anhedonia assessed by decreased sucrose preference (Papp et al., 1991), immobility in the forced swim test (Porsolt et al., 1977), and decreased activity in the open field test (Hall, 1936; Weiss et al., 1980). Stressors include chronic mild stress, inescapable shock, or learned helplessness models (Weiss et al., 1980; Willner, 1997; Maier and Watkins, 2005), all of which engage the innate immune system and associate with depressive-like effects (De_La_Garza, 2005; Kubera et al., 2011; Cheng et al., 2016). Predator odor exposure is considered an ethologically valid stressor devoid of physical stress that induces indices of anxiety, depression, and post-traumatic stress disorder (Cohen and Zohar, 2004; Whitaker et al., 2014; Wu et al., 2019). Predator odor exposure leads to enhanced affective responses that persist for weeks after a single exposure (Cohen et al., 2004; Edwards et al., 2013; Roltsch et al., 2014; Whitaker and Gilpin, 2015) and alters cytokine levels in the brains of rats and mice (Wilson et al., 2013b; Deslauriers et al., 2017). Yet, studies of predator odor exposure typically use male rodents only with some exceptions (Adamec et al., 2006; Mazor et al., 2009; Cozzoli et al., 2014). Interestingly, male and female rodents appear differentially vulnerable to the effects of predator odor stress, although this is task-dependent (Adamec et al., 2006; Albrechet-Souza et al., 2020).

Rodent studies that examined the role of TLR4 in the ability of stress to induce depressive-like effects employed male subjects only and show discrepant findings (Garate et al., 2011; Biesmans et al., 2016; Cheng et al., 2016; Couch et al., 2016; Deslauriers et al., 2017; Fu et al., 2019). One study that used predator odor stress showed that immune deficient and T-cell deficient male mice exhibited greater unconditioned fear and anxiety-like behaviors but depressive-like behaviors were not assessed (Cohen et al., 2006). Thus, the current study was designed to investigate if deletion of the *Tlr4* gene in rats alters the ability of predator odor stress to induce depressive-like behavioral responses and whether such effects are sex-specific. We predicted that *Tlr4* knockout (KO) rats would show decreased stress-induced depressive-like behaviors compared to wild type (WT) rats and that these effects would be greater in female vs. male rats. It is worth noting that this relatively new *Tlr4* knockout rat model (Ferguson et al., 2013) does not result in genotype differences in restraint stress-induced CRF modulation of GABAergic transmission in the amygdala (Varodayan et al., 2018) or in alcohol consumption (Harris et al., 2017).

2. Methods

2.1. Animals

Male and female Wistar rats (*Rattus norvegicus*) heterozygously expressing an inactive variant of the *Tlr4* gene were generated by and acquired from Gregg E. Homanics, PhD (University of Pittsburgh, Department of Anesthesiology) (Ferguson et al., 2013). All animals were obtained through breeding. The 91 rats employed in this study were derived from 19 litters comprised of 216 pups total, although only 193 (17 litters) were genotyped. There were two additional male KOs in the study that were born prior to our use of mLIMS transgenic mouse colony management system (Bioinforx; Madison, WI) used to keep track of breeding data. Litter information on these two rats is missing but their data are included in the behavioral analyses. No more than two pups per sex per genotype per litter were assigned to one of the two experimental groups. The group sizes used in the behavioral studies by sex, genotype, and odor-exposure group are seen in Table 1.

Rats were caged with their dam and littermates until weaning on postnatal day 21. Afterwards, they were group-housed by sex and genotype (2–4 to a cage) in amber polysulfone cages in a humidity- and temperature-controlled environment with *ad libitum* access to food and water except where noted differently. A 12-h light-dark cycle (lights on at 0700) was maintained throughout the study. Animals were at least 65 days of age at the start of testing. Procedures were approved by the University of Houston Institutional Animal Care and Use Committee in accordance with the NIH's *Guide for the Care and Use of Laboratory Animals*.

2.2. Genotyping

Prior to weaning, each rat was anesthetized with isoflurane to remove a 3-mm section from the tip of the tail. Tail snips were sent to TransnetYX Inc. (Cordova, TN) for genotyping.

2.3. Experimental procedure

Adult, wild type (WT) and knockout (KO), male and female rats were randomly assigned to either Odor Exposed or Non-Exposed groups as seen in Table 1. The experimental procedure timeline is shown in Fig. 1. First, baseline behavioral measures were obtained on two tests of anxiety- and depressive-like behaviors: 1) locomotor activity (distance traveled in cm) and center time (in sec) in the open field test (OFT); and 2) sucrose preference and total fluid intake in the two-bottle sucrose preference test (SPT). After baseline tests were conducted, some rats were subjected to a predator-associated odorant stressor (Exposed group) whereas the other rats were exposed to saline (Control group) in a place conditioning apparatus. A random number generator (random.org) was used to determine apparatus compartment pairing. Next, all rats were retested on OFT and SPT over the following 48 h. Finally, rats were tested for conditioned place aversion (CPA) by examining the change in time (sec) spent in the predator odor-paired compartment in the place conditioning apparatus at both 24-h and 10-days after predator odor (or saline) exposure. All tests were carried out during the animals' light hours under low red-light conditions in designated testing rooms except for SPT that was performed over 48-h in the animals' home cages. For tests occurring in designated testing rooms, animals were allowed to acclimate to the room in their home cages for at least 20-m. Behavioral apparatuses were cleaned thoroughly with 70% ethanol solution after each use.,

2.4. Predator odor-exposure

Apparatus. Testing took place in one of eight Med Associates three-compartment place preference apparatuses (MED-CPP-013AT, Med Associates Inc., St Albans, VT), each consisting of: 1) a black compartment

Table 1
Group sizes by Experimental Group, Genotype, and Sex.

Experimental Group	Genotype and Sex			
	Wild Type		Knockout	
	Male	Female	Male	Female
Odor Exposed	11	6	8	12
Non-Exposed	21	9	10	14

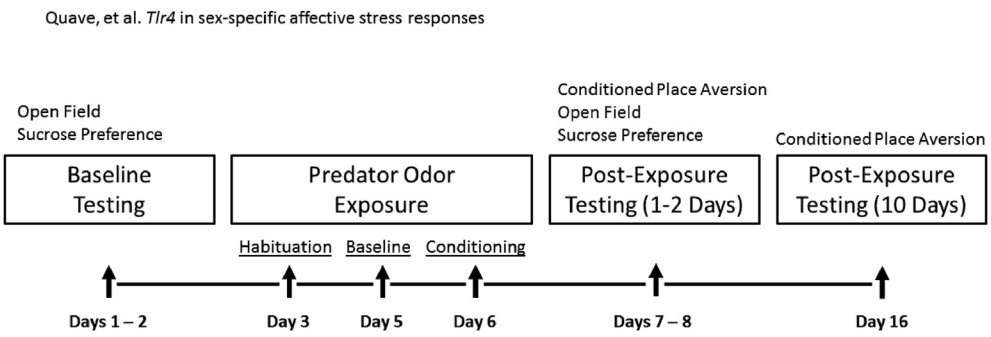

Fig. 1. Experimental Timeline. Animals underwent baseline testing on the open field (OFT) and sucrose preference tests (SPT) two days prior to predator odor exposure. Baseline SPT began two days prior to predator odor exposure and continued for 24 h (Days 1–2). Animals were exposed to the entire 3-chamber place conditioning apparatus twice over two days. Times spent in either of the main chambers of the apparatus were recorded as baseline (Days 3–5). Animals were then confined to one chamber of the apparatus and exposed to predator odor. Post odor SPT began immediately following conditioning (Day 6). Conditioned place aversion (CPA) was assessed at 24 h and post-predator odor exposure measures were obtained in OFT and SPT over the next two days (Days 7–8). A second CPA test occurred 10 days post-predator odor exposure (Day 16).

with metal bar flooring, 2) a white compartment with metal grid flooring, and 3) a gray center compartment with solid flooring connecting the black and white compartments (main compartments). Each of the main compartments measured 11.75″ L x 8.25" W x 8″ H (29.85 × 20.96 × 20.32 cm) and the center compartment measured 4.75″ L x 8.25″ W x 8″ H (12.06 × 20.95 × 20.32 cm). The three compartments were divided by vertical guillotine doors. Infrared sensors in each compartment tracked animal movement and recorded times in each compartment using the corresponding software (MED Test, v. 4.2.0.0, MED Associates, Fairfax, VT). Individual incandescent lights in each compartment had been manually adjusted to reduce inherent side preferences.

Procedure. Animals were individually exposed to the apparatus and baseline times (sec) in the two main compartments of the apparatus were recorded over the 15-m session. The following morning, animals were randomly assigned and confined to one distinct compartment by closing the vertical guillotine doors and exposed to saline-soaked filter paper contained in a plastic weigh boat placed beneath the floor of the apparatus for 15-m. That evening, each animal was placed in the opposite compartment and exposed to either bobcat urine- (Pmart, LLC, Sandy Point, ME) or saline-soaked filter paper for 15-m. Testing for conditioned place aversion (CPA) or avoidance of the odor-paired compartment was performed at 24-h post-exposure and again at 10-days post-exposure. In these tests, the vertical guillotine doors were lifted so that the rats had access to the entire apparatus. Time spent (s) on the odor-paired side over the 15-m test was tabulated. The measure of CPA was the time spent in the odor-paired compartment on the test day minus the time recorded on the baseline day.

2.5. Open field testing (OFT)

Animals were placed in a 43.2 cm × 43.2 cm (width) x 30.5 cm (height) open field box (Med Associates Inc., St. Albans, VT). The box consisted of white Plexiglas floors and clear Plexiglas walls, along the base of which were positioned arrays of infrared detectors. Animal movement was tracked using Med Associates Activity Monitor software (version 6.02, Med Associates Inc., St Albans, VT). Distance traveled (cm) and time spent (s) in the center zone (as determined by the software) were recorded over a 30-m testing period.

2.6. Two-bottle choice sucrose preference testing (SPT)

Animals were housed singly in their home cages and the lixit water system removed. For baseline measurement, access to two bottles with leak-proof OptiRat Plus lids (Animal Care Systems, Inc., Centennial, CO) containing water was given for four consecutive days. One water bottle was then replaced with a bottle containing a 1% sucrose solution and animals were given free choice between water and sucrose solution for a period of 48 h. Bottles were removed and weighed at 24- and 48-h. Bottle positions were switched at the 24-h weighing time to eliminate possible position preferences. After the second weighing, the standard lixit water access was restored. Sucrose preference was recorded by dividing the weight of the sucrose solution bottle by the weight of both bottles together (i.e., total fluid intake water). During sucrose preference testing, animals were housed singly and returned to *ad libitum* drinking and their original group housing conditions after testing was completed.

2.7. Statistical analysis

For CPA data, a 2 × 2 x 2 Analysis of Variance (ANOVA) was employed to determine between groups interactions of sex (male or female), exposure condition (saline or predator odor), and genotype (WT or KO), respectively. For OFT and SPT, a series of 2 × 2 x 2 Analysis of Covariance (ANCOVAs) was employed to determine between groups interactions of sex, exposure condition, and genotype, respectively. Baseline measures in the OFT and SPT were used as a covariate in these statistical analyses. All data were analyzed using PROC GLM in SAS software version 9.4 (SAS Institute Inc., Cary, NC). Correlations across the various behavioral measures were made but none were significant so they will not be reported here. Graphical visualization of data was completed with GraphPad Prism 8 software (GraphPad Software, Inc., La Jolla, CA). Birth and litter data (sex ratio; genotype ratio) were analyzed by chi-square with Statistica software version 13 (Palo Alto, CA) using theoretical results as the expected values (i.e., 50%/sex; 25%/25%/50% for WT/KO/HET genotypes).

3. Results

3.1. Birth and litter data

Of the 193 pups born in the 17 litters bred and genotyped, 100 were male and 93 were female resulting in a male/female sex ratio of 51.8%, p

Table 2
Numbers of pups per sex per genotype.

Genotype				
Sex	Wild Type	Knockout	Heterozygous	Total
Male*	45	16	39	100
Female	22	25	46	93
Total	67	41	85	193

*$p < 0.00002$.

> 0.10. The proportions of pups per sex per genotype are shown in Table 2. The proportions of WT, KO, and heterozygous (HET) female rats born were what would be expected from breeding HET dams with HET sires, $p > 0.10$. On the other hand, male pups born to these litters showed a disproportionately higher number of WTs and lower numbers of KOs and HETs, chi-square = 21.66; df = 2; $p < 0.00002$.

3.2. Baseline behaviors

There are several sex differences in baseline (i.e., pre-predator odor exposure) behaviors as seen in Table 3. Female rats show greater distance traveled and spend less time in the center in the open field test (OFT). These statements are supported by significant main effects of Sex for both distance traveled, F(1, 87) = 10.31, $p = 0.002$, and time spent in the center of the open field, F (1,87) = 7.38, $p = 0.005$. There are no significant main effects of Genotype ($p = 0.809$; $p = 0.0947$) nor a Sex X Genotype interaction for distance traveled ($p = 0.774$) or time in the center of the open field ($p = 0.846$). Female rats also ingest more total fluid and show greater sucrose preference compared to male rats in the two-bottle sucrose preference test (SPT). These statements are supported by main effects of Sex on total fluid intake, F(1, 92) = 17.48; $p < 0.0001$, and sucrose preference, F(1, 92) = 10.22, $p = 0.002$. There are no significant main effects of Genotype ($p = 0.467$; $p = 0.149$) nor Sex X Genotype interactions ($p = 0.923$; $p = 0.777$) for total fluid intake or sucrose preference, respectively. To compensate for these baseline differences in OFT and SPT, these measures were used as co-variates in the ANCOVA.

3.3. Conditioned place aversion

The aversive effects of predator odor exposure (i.e., decreased time spent in the odor-paired compartment after exposure compared to baseline time) were assessed in the conditioned place aversion (CPA) procedure at both 24-h and 10-days post-exposure. Results are shown in Fig. 2. There are no main effects of Exposure ($p = 0.225$), Sex ($p = 0.316$), or Genotype ($p = 0.876$), or their interactions (p's > 0.05) on CPA when tested 24-h after conditioning sessions as seen in Fig. 2A–B. When CPA was measured 10-days post-exposure, there is a significant main effect of Exposure, F(1, 79) = 8.91; p = 0.004. Exposed animals spend significantly less time in the odor-paired compartment on the test relative to baseline (i.e., show greater CPA) compared to non-exposed controls as seen in Fig. 2C–D. There are no other significant main effects or interaction effects (p's > 0.10).

3.4. Open field test

The effects of predator odor exposure on distance traveled (cm) and center time (s) in the open field test (OFT) are shown in Fig. 3. For distance traveled, there are significant main effects of Exposure, F (1,87) = 6.76; $p = 0.011$, Sex, F (1,82) = 10.93; $p = 0.001$), and Genotype, F (1,87) = 7.48; $p = 0.007$, but not in their interactions (p's > 0.05). Exposed animals travel less distance than non-exposed animals when measured post-odor exposure as seen in Fig. 3A–B. Females show greater distance traveled compared to males and KO animals travel less distance than WT animals. For time in the center of the open field, there are significant main effects of Exposure, F (1,82) = 14.89; $p = 0.0002$), Sex, F (1,82) = 8.67; $p = 0.004$), and a Sex X Genotype interaction, F (1,82) = 5.15; $p = 0.026$). All other main effects and interactions are not significant (p's > 0.10). Exposed animals spend less time in the center zone compared to non-exposed animals and female rats spend less time in the center zone relative to male rats as seen in Fig. 3C–D. Female KO rats with predator odor exposure spend the least amount of time in the center compared to the three other groups (Fig. 3C). This statement is supported by Tukey *post hoc* tests (p's = 0.014 and 0.001).

Table 3
Baseline measures in open field (OFT) and sucrose preference (SPT) tests by sex.

Measure	Females		Males	
	Mean	SEM	Mean	SEM
Distance Traveled (cm; OFT)*	8993.10	506.31	7025.56	344.99
Time in Center (sec; OFT)*	703.94	36.81	901.58	54.38
Total Fluid Intake (mL; SPT)**	91.39	5.53	63.53	3.15
Sucrose Preference (% of total intake; SPT)*	95.16%	0.96%	88.97%	1.31%

*$p < 0.01$; **$p < 0.0001$.

3.5. Sucrose preference test

The effects of predator odor exposure on total fluid intake (water + sucrose in mL) and on sucrose preference (% sucrose intake out of total fluid intake) in the sucrose preference test (SPT) are shown in Fig. 4. There is a significant main effect of Exposure, F (1,87) = 6.29; $p = 0.014$, on total fluid intake as seen in Fig. 4A and B. Exposed animals ingested lower total fluid relative to non-exposed controls. There are no significant main effects of Sex ($p = 0.395$), Genotype ($p = 0.541$), or their interactions (p's > 0.05) on total fluid intake. There is a significant main effect of Exposure, F (1,87) = 6.83; $p = 0.011$, on sucrose preference as seen in Fig. 4C and D. Exposed animals show lower sucrose preference compared to non-exposed controls. There are no significant main effects of Sex ($p = 0.817$), Genotype ($p = 0.557$), or their interactions (p's > 0.05) on sucrose preference.

4. Discussion

The results of the present study demonstrate that predator odor exposure is aversive and alters affective responses, some of which depend upon sex and genotype. Across genotypes and sex, this stress causes anhedonia as measured by decreased sucrose preference and total fluid intake. A reduction in distance traveled also occurs due to predator odor exposure, particularly in *Tlr4* KOs and in females that may reflect a greater depressive-like effect. Female KO rats spend the least amount of time in the center of the open field after predator odor exposure compared to male rats. This demonstrates a sex-specific effect of predator odor stress on anxiety-like behavior in rats in which the absence of the *Tlr4* gene leads to an increased anxiety-like response to stress in females. The lack of effect of predator odor exposure by *Tlr4* gene deletion in males may reflect, in part, that significantly fewer male KO rats were born. Thus, we confirm that predator odor exposure is aversive and show that this stress differentially alters affective behaviors that, in some cases, depend on the presence of the *Tlr4* gene and on sex.

Both *Tlr4* KO and WT rats show conditioned place aversion (CPA) to the odor-paired compartment when measured 10-days after exposure. This confirms prior work demonstrating that predator odor exposure is aversive when assessed several days after exposure (Edwards et al., 2013; Whitaker and Gilpin, 2015; Albrechet-Souza and Gilpin, 2019). Along with studies that show enhanced stress hormone reactivity in response to predator odor (Wilson et al., 2013a; Roltsch et al., 2014; Whitaker and Gilpin, 2015; Whitaker et al., 2016; Albrechet-Souza and Gilpin, 2019; Albrechet-Souza et al., 2020), our findings support the notion that predator odor is a stressor for rodents. Like other stressors, predator odor exposure should induce depressive-like effects. Indeed, we find evidence of anhedonia in the sucrose preference test (SPT), consistent with prior studies that employed chronic unpredictable stress (Papp et al., 1991). However, results from mouse studies with predator odor exposure are inconsistent; findings of no effect in SPT in male or female mice (Adamec et al., 2006) as well as a decrease (Calvo-Torrent et al., 1999) and an increase in sucrose intake in male mice (Burgado et al., 2014) have been reported. Several methodological or species differences, as have been noted by others (Pruett et al., 2008), may explain the discrepancies. Alternatively, these disparities may reflect that the link between stress and consummatory behavior is bi-directional, sometimes leading to decreases and other times leading to increases in consumption (Maniam and Morris, 2012).

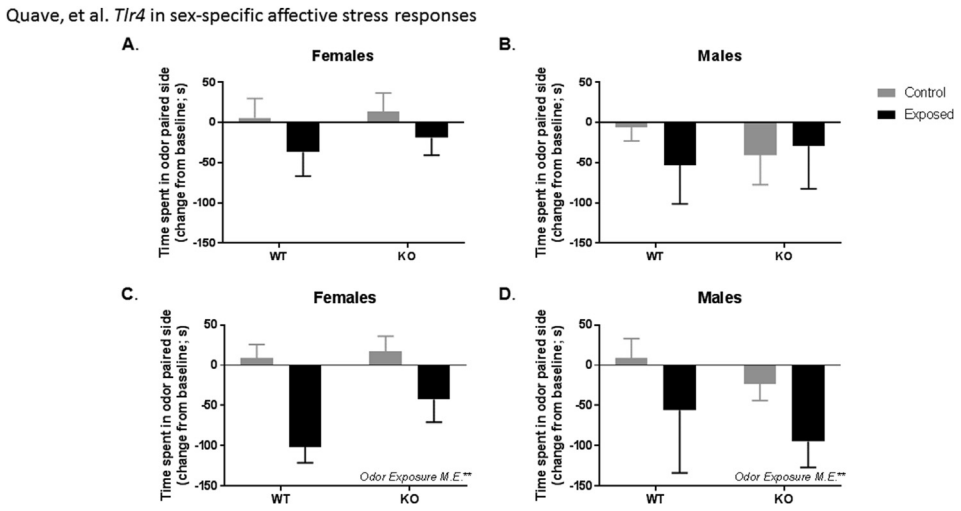

Fig. 2. Predator odor exposure induces delayed and persistent avoidance behavior in the CPA test. Predator odor exposure animals did not display conditioned place aversion (CPA) at 24 h post-exposure ($p > 0.10$) (A, B). At 10 days post-exposure, predator odor-exposed animals showed CPA and spent significantly less time on the side of the apparatus previously paired with predator odor ($p = 0.004$) (C, D). There were no Sex or Genotype effects at either test time. *M.E.*, main effect; $^{**}p < 0.01$.

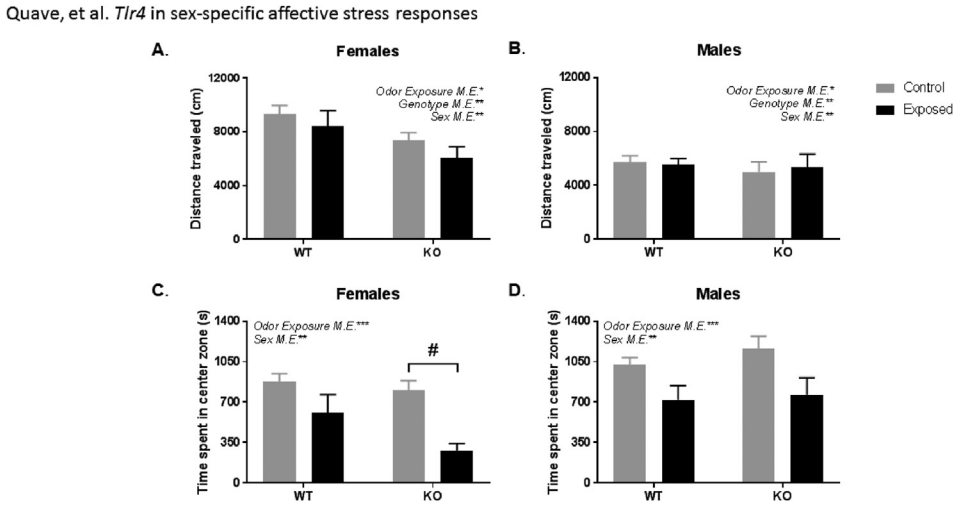

Fig. 3. Predator odor exposure suppresses locomotion and decreases time spent in the center zone in the OFT. Predator odor-exposed rats showed decreased distance traveled during the open field test (OFT; $p = 0.011$). Overall, females traveled greater distances than males ($p = 0.001$) and WT animals traveled greater distances than KO animals ($p = 0.007$) (A, B). Time spent in center zone in the OFT was reduced in predator odor-exposed groups ($p = 0.0002$). Males spent more time in the center zone than females ($p = 0.004$). Of the predator odor exposed animals, female KOs spent the least amount of time in the center zone (#, Sex X Genotype interaction; $p = 0.026$) (C, D). Data presented in figure represent actual values; data were analyzed by ANCOVA to take into account significant sex differences in baseline values. *M.E.*, main effect; #, interaction effect, $p < 0.05$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

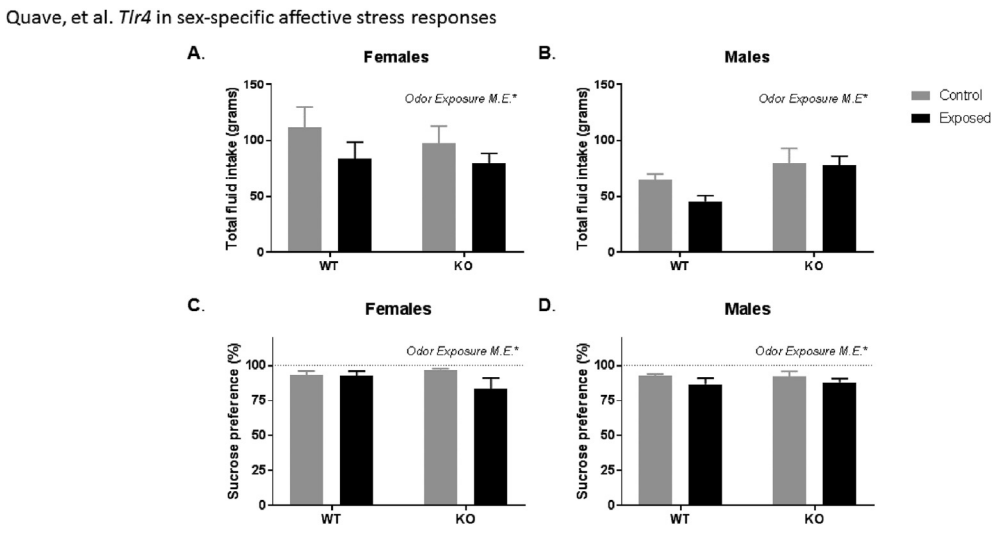

Fig. 4. Predator odor exposure reduces total fluid consumption and sucrose preference in the SPT. Predator odor-exposed animals consumed less total fluid (water + sucrose solution) than non-exposed animals ($p = 0.014$) (A, B) and showed decreased preference for the sucrose solution in the sucrose preference test (SPT; $p = 0.011$) (C, D). Data presented in figure represent actual values; data were analyzed by ANCOVA to take into account significant sex differences in baseline values. *M.E.*, main effect; $^{*}p < 0.05$.

Predator odor stress decreases locomotor activity in the open-field test (OFT) in the present study consistent with a study in male mice (Deslauriers et al., 2017). Similarly, chronic predator odor stress blocks the increase in distance traveled upon the second exposure to an open field and decreases center zone distance in male mice (Burgado et al., 2014). Decreased locomotor activity can be interpreted as a depressive-like effect (Hall, 1936; Weiss et al., 1980). These results would therefore be consistent with our findings in the SPT. We also see that this stress affects center time in the OFT, a behavior thought to reflect anxiety. Other studies also show anxiety-like effects of predator odor exposure (Cohen et al., 2004; Wilson et al., 2013a; Whitaker and Gilpin, 2015; Deslauriers et al., 2017) although another study does not (Albrechet-Souza et al., 2020). Depression and anxiety are often co-morbid (Spijker et al., 2020) and are linked to alterations in the innate immune system in humans (Maes, 1995; Wu et al., 2015; Frank et al., 2016; Liu et al., 2019). Because predator odor affects cytokine levels in rats and mice (Wilson et al., 2013b; Deslauriers et al., 2017), such effects may promote these enhanced affective behavioral responses.

The link between TLR4 with depression and co-morbid anxiety is demonstrated by the finding that TLR4 expression levels correlate with severity of certain symptoms, specifically anxiety and weight loss, in patients with major depressive disorders (MDD) (Keri et al., 2014; Hung et al., 2015). In preclinical research, chronic mild stress increases *Tlr4* expression in the prefrontal cortex of male rats (Garate et al., 2011) and deletion of *Tlr4* decreases cytokine responses in hippocampus after stress in male mice (Cheng et al., 2016). Based on this literature, we predicted that deletion of the*Tlr4* gene would lessen the ability of predator odor stress to induce depressive- and anxiety-like effects. However, our results in the OFT show the opposite: an enhanced depressive-like response seen as decreased distance traveled in KO rats of both sexes and an enhanced anxiety-like response in female KO rats. Male immune deficient and T-cell deficient mice exposed to predator odor exhibited greater unconditioned fear and anxiety behaviors (Cohen et al., 2006). Similarly, another study demonstrated that elimination of *Tlr4* decreased novelty-associated exploratory behavior and social interaction in the absence of baseline anxiety-like differences in male mice (Li et al., 2016). Yet, a separate study of male and female *Tlr4* deficient mice showed anxiety-like behavior and decreased social interaction in the absence of an experimental stressor, with a greater effect on anxiety in males (Femenia et al., 2018). Additionally, heightened anxiety behavior was observed in male mice with intracerebroventricular administration of a TLR4 antagonist (Okun et al., 2011). Other studies show that decreased Tlr4 activity associates with reduced stress-induced affective responses. Male mice with *Tlr4* deletion exhibit decreased learned helplessness (Cheng et al., 2016) and inhibition of Tlr4 activity attenuates immobility in both forced swim and tail suspension tests after chronic mild stress (Fu et al., 2019). Finally, another study finds no relation between Tlr4 expression and behavior in the forced swim test in male rats (Garate et al., 2011). Despite correlational evidence in humans suggesting TLR4 mediates symptoms of anxiety and depression, the effects of TLR4 manipulation on anxiety- and depressive-like behaviors sometimes diverge in experimental animal studies that suggests a more nuanced role for TLR4 in affective responses that may vary depending on the type of stressor.

Tlr4 activity is increased by LPS administration and this reduces locomotor activity and center crossings in an open field in both male and female rats (Tonelli et al., 2008). Enhanced depressive-like effects in forced swim and tail suspension tests are also found in male mice when LPS is combined with chronic unpredictable stress (Couch et al., 2016). However, no effect of LPS combined with predator odor stress is seen in the OFT with male mice (Deslauriers et al., 2017). It is difficult to reconcile these discrepant findings in studies that examined responses to manipulations that either decreased or increased TLR4 activity, although they are likely due to procedural and species differences. Further, unlike our study that employed rats of both sexes wherein we found some sex-specific effects, much of the previous research was conducted with male rodents only. Although our results contradicted our original hypothesis, our findings stand in general agreement with most studies using knock-out, knock-down and antagonism strategies to target TLR4.

No genotype effect was seen in sucrose preference or in total fluid intake in the present study, consistent with a lack of effect of LPS administration in combination with chronic restraint stress in male rats (Biesmans et al., 2016). However, LPS administration combined with chronic mild stress did decrease sucrose preference in male mice (Couch et al., 2016). LPS alone attenuated saccharin preference in male rats, an effect that is reversed by chronic treatment with a tricyclic antidepressant drug (Yirmiya, 1996). Conversely, inhibition of Tlr4 activity attenuated sucrose preference after chronic mild stress in male mice (Fu et al., 2019). The sucrose concentration used in the present study may have been too high to uncover genotype or sex differences in this stress-induced affective response.

We predicted female rats would exhibit greater stress-induced affective behavioral responses than male rats. Indeed, this effect was seen in both measures of the OFT (distance traveled and center time) in the present study. This prediction is based on the fact that women with MDD are more likely than men to show signs of anhedonia and sadness (Frank et al., 1988; Kornstein et al., 2000; Zagni et al., 2001; Marcus et al., 2008). Our expectation that deletion of the *Tlr4* gene would attenuate these stress-induced affective responses to a greater extent in females is based on findings of gender differences in the immune system and depression. Women show greater inflammatory and cellular immune responses, including cytokine production and T cell activation, in response to infections or antivirals compared to men (Standberry et al., 2002; Nalbandian and Kovats, 2005; Klein et al., 2010) that may reflect gonadal hormone differences (Gillgrass et al., 2005). An inflammatory challenge leads to greater depressive responses (Moieni et al., 2015) and antiviral treatments are more likely to cause depression (Udina et al., 2012) in women compared to men. Women also show higher rates of autoimmune diseases (Whitacre, 2001; Ortona et al., 2014) and the risk of developing a MDD is greater among those with autoimmune diseases (Siegert and Abernethy, 2005; Menard et al., 2017; Zhang et al., 2017). Interestingly, the pattern of sex differences in affective responses to stress seen in humans contrasts with that observed in rodents, where male animals often show greater stress sensitivity than females (Cohen and Yehuda, 2011). Further understanding of the biological mechanisms of stress reactivity in humans and rodents will likely provide insight into the occurrence of these sex differences both within and between species.

The lack of sex differences in the SPT induced by predator odor exposure in the present study contrasts with another report wherein female rats exhibited greater anhedonia in sucrose preference tests than males after a single prolonged stress, an effect that was not due to gonadal hormones (Pooley et al., 2018). We did not monitor estrous stage in the current study, but the anxiety- and depressive-like responses recorded at baseline prior to predator odor exposure confirm prior reports of sex differences (see Table 3). Like other research, we find sex differences in sweet solution intake (Sclafani et al., 1987; Marshall et al., 2017). This may reflect that the sensitivity to or the discrimination of the taste of sweet solutions is lower in female rats (Curtis et al., 2004). Sex differences in open field behaviors have also been documented (Gray and Buffery, 1971; Archer, 1975; Beatty and Fessler, 1976). Male rats show less ambulation and rearing in open field compared to female rats (Masur et al., 1980) and spend more time in the center of the arena (Archer, 1975). Our findings are consistent with these prior reports.

Results of the present study provide further support for the role of the innate immune system, particularly the TLR4 receptor, in stress-induced depressive-like effects particularly in females. However, contrary to our prediction, deletion of the *Tlr4* gene enhanced rather than attenuated stress-induced affective responses in some cases. This hypothesis was based on the evidence that depressive symptoms in both humans and rodents associate with activation of the innate immune system, specifically TLR4 activity. Thus, the lack of the *Tlr4* gene would presumably attenuate the behavioral responses to the predator odor stress. Yet, the

literature on Tlr4 activity and behavior is mixed; both activation and elimination of this receptor can lead to similar responses as discussed above. This may reflect that adaptive behavioral responses depend upon the integrity of the innate immune system as suggested by Cohen and colleagues (Cohen et al., 2006). This integrity may be disrupted by either increasing TLR4 activity via LPS administration or by deleting the gene or may relate to compensatory effects in other systems. That the anxiety-like effect of predator odor exposure is not altered by *Tlr4* gene deletion in males may reflect, in part, the lower than expected number of male KO rats born, likely owing to unforeseen reproductive effects of *Tlr4* deletion. TLR4s present in the female uterine epithelium mediate an immune response to seminal fluid and may affect successful fertilization (Schjenken et al., 2015; Ezz et al., 2019). While TLR4 is also present in sperm, the role of the receptor in reproductive processes remains uncharacterized (Sahnoun et al., 2017; Fang et al., 2020). The presumed detrimental reproductive effects of Tlr4 deletion in male rats may have led to obtaining KOs with overcompensation in other systems, perhaps in the expression of other DAMP-responsive TLRs (Park et al., 2003; Ivanov et al., 2007). Nonetheless, our findings suggest that the efficacy of pharmacological treatments for depression and co-morbid anxiety may vary by sex differentially by stress exposure.

Declaration of competing interest

None.

Acknowledgments

Support was provided by NIAAA grant, U01-013476 (TAK), by a Summer Undergraduate Research Fellowship from University of Houston, and an undergraduate grant from Psi Chi International Honor Society in Psychology (CBQ). We thank Dr. Gregg Homanics for providing the original breeding pairs of heterozygous *Tlr4* rats.

References

Adamec, R., Head, D., Blundell, J., Burton, P., Berton, O., 2006. Lasting anxiogenic effects of feline predator stress in mice: sex differences in vulnerability to stress and predicting severity of anxiogenic response from the stress experience. Physiol. Behav. 88, 12–29.

Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. Cell 124, 783–801.

Albrechet-Souza, L., Gilpin, N.W., 2019. The predator odor avoidance model of post-traumatic stress disorder in rats. Behav. Pharmacol. 30, 105–114.

Albrechet-Souza, L., Schratz, C.L., Gilpin, N.W., 2020. Sex differences in traumatic stress reactivity in rats with and without a history of alcohol drinking. Biol. Sex Differ. 11, 1–11.

Archer, J., 1975. Rodent sex differences in the emotional and related behavior. Behav. Biol. 14, 451–479.

Beatty, W.W., Fessler, R.G., 1976. Ontogeny of sex differences in open-field behavior and sensitivity to electric shock in the rat. Physiol. Behav. 16, 413–417.

Bekhbat, M., Neigh, G.N., 2018. Sex differences in the neuro-immune consequences of stress: focus on depression and anxiety. Brain Behav. Immun. 67, 1–12.

Biesmans, S., Matthews, L.J.R., Bouwknecht, J.A., DeHaes, P., Hellings, N., Meert, T.F., Nuydens, R., VerDonck, L., 2016. Systematic analysis of the cytokine and anhedonia response to peripheral lipopolysaccharide administration in rats. BioMed Res. Int. 2016, 9085273.

Burgado, J., Harrell, C.S., Eacret, D., Reddy, R., Barnum, C.J., Tansey, M.G., Miller, A.H., Wang, H., Neigh, G.N., 2014. Two weeks of predatory stress induces anxiety-like behavior with co-morbid depressive-like behavior in adult male mice. Behav. Brain Res. 275, 120–125.

Calvo-Torrent, A., Brain, P.F., Martinez, M., 1999. Effect of predatory stress on sucrose intake and behavior on the plus-maze in male mice. Physiol. Behav. 67, 189–196.

Cheng, Y., Pardo, M., deSouza_Armini, R., Martinez, A., Mouhsine, H., Zagury, J.-F., Jope, R.S., Beurel, E., 2016. Stress-induced neuroinflammation is mediated by GSK3-dependent TLR4 signaling that promotes susceptibility to depression-like behavior. Brain Behav. Immun. 53, 207–222.

Cohen, H., Yehuda, R., 2011. Gender differences in animal models of posttraumatic stress disorder. Dis. Markers 30, 141–150.

Cohen, H., Ziv, Y., Cardon, M., Kaplan, Z., Matar, M.A., Gidron, Y., Schwartz, M., Kipnis, J., 2006. Maladaptation to mental stress mitigated by the adaptive immune system via depletion of naturally occurring regulatory CD4+CD5+ cels. J. Neurobiol. 66, 552–563.

Cohen, H., Zohar, J., 2004. An animal model of posttraumatic stress disorder: the use of cut-off behavioral criteria. Annals of N.Y. Acad. Sci. 1032, 167–178.

Cohen, H., Zohar, J., Matar, M.A., Zeev, K., Loewenthal, U., Richter-Levin, G., 2004. Setting apart the affected: the use of behavioral criteria in animal models of post traumatic stress disorder. Neuropsychopharmacology 29, 1962–1970.

Couch, V., Trofimov, A., Markova, N., Nikolenko, V., Steinbusch, H.W., Chekhonin, V., Schroeter, C., Lesch, K.-P., Anthony, D.C., Strekalova, T., 2016. Low-dose lipopolysaccharide (LPS) inhibits aggressive and augments depressive behaviours in a chronic mild stress model in mice. J. Neuroinflammation 13, 108.

Cozzoli, D.K., Tanchick-Nipper, M.A., Kaufman, M.N., Horowitz, C.B., Finn, D.A., 2014. Environmental stressors influence limited-access ethanol consumption by C57BL/6J mice in a sex-dependent manner. Alcohol 48, 741–754.

Curtis, K.S., Davis, L.M., Johson, A.L., Therrien, K.L., Contreras, R.J., 2004. Sex differences in behavioral taste responses to and ingestion of sucrose and NaCl solutions by rats. Physiol. Behav. 80, 657–664.

Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immunesystem subjugates the brain. Nat. Rev. Neurosci. 9, 46–56.

De_La_Garza, R., 2005. Endotoxin- or pro-inflammatory cytokine-induced sickness behavior as an animal model of depression: focus on anhedonia. Neurosci. Biobehav. Rev. 29, 761–770.

Deslauriers, J., van_Wijngaarde, M., Geyer, M.A., Powell, S., Risbrough, V.B., 2017. Effects of LPS-induced immune activation prior to trauma exposure on PTSD-like symptoms in mice. Behav. Brain Res. 323, 117–123.

Dhabhar, F.S., McEwen, B.S., 1997. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. Brain Behav. Immun. 11, 286–306.

Edwards, S., Baynes, B.B., Carmichael, C.Y., Zamora-Martinez, E.R., Barrus, M., Koob, G.F., Gilpin, N.W., 2013. Traumatic stress reactivity promotes excessive alcohol drinking and alters the balance of prefrontal cortex-amygdala activity. Transl. Psychiatry 3, e296.

Ezz, M.A., Marey, M.A., Elweza, A.E., Kawai, T., Heppelmann, M., Pfarrer, C., Miyamoto, A., 2019. TLR2/4 signaling pathway mediates sperm-induced inflammation in bovine endometrial epithelial cells in vitro. PLoS.

Fang, Y., Xia, W., Cai, W., Zhang, X., Zhang, J., Fu, X., Li, S., Fan, X., Sun, S., Wang, Z., Zhang, X., Zhu, S., Li, J., 2020. Effects of TLR4 overexpression on sperm quality, seminal plasma biomarkers, sperm DNA methylation and pregnancy rate in sheep. Theriogenology 142, 368–375.

Femenia, T., Qian, Y., Arentsen, T., Forssberg, H., Heijtz, R.D., 2018. Toll-like receptor-4 regulates anxiety-like behavior and DARPP-32 phosphorylation. Brain Behav. Immun. 69, 273–282.

Ferguson, C., McKay, M., Harris, R.A., Homanics, G.E., 2013. Toll-like receptor 4 (Tlr4) knockout rats produced by transcriptional activator-like effector nuclease (TALEN)-mediated gene inactivation. Alcohol 47, 595–599.

Frank, E., Carpenter, L.L., Kupfer, D.J., 1988. Sex differences in recurrent depression" are there any that are significant? Am. J. Psychiatr. 145, 41–45.

Frank, M.G., Weber, M.D., Watkins, L.R., Maier, S.F., 2016. Stress-induced neuroinflammatory priming: a liability factor in the etiology of psychiatric disorders. Neurobiol. Stress 4, 62–70.

Franklin, T.C., Xu, C., Duman, R.S., 2018. Depression and sterile inflammation: essential role of dange associated molecular patterns. Brain Behav. Immun. 72, 2–13.

Fu, S., Wang, J., Hao, C., Dang, H., Jiang, S., 2019. Tetramethylpyrazine ameliorates depression by inhibiting TLR4-NLRP3 inflammasome signal pathway in mice. Psychopharmacology 236, 2173–2185.

Garate, I., Garcia-Bueno, B., Madrigal, J.L.M., Bravo, L., Berrocoso, E., Caso, J.R., Mico, J.A., Leza, J.C., 2011. Origin and consequences of brain Toll-like receptor 4 pathway stimulation in an experimental model of depression. J. Neuroinflammation 8, 2–14.

Gillgrass, A.E., Tang, V.A., Towarnicki, K.M., Rosenthal, K.L., Kaushic, C., 2005. Protection against genital herpes infection in mice immunized under different hormonal conditions correlates with induction of vagina-associated lymphoid tissue. J. Virol. 79, 3117–3126.

Gray, J.A., Buffery, A.W., 1971. Sex differences in emotional, cognitive behaviour in mammals including man: adaptive, neural bases. ACTA Psychol 35, 89–111.

Hall, C.S., 1936. Emotional behavior in the rat. III. The relationship between emotionality and ambulatory activity. J. Comp. Physiol. Psychol. 22, 345–352.

Harris, R.A., Bajo, M., Bell, R.L., Blednov, Y.A., Varodayan, F.P., Truitt, J.M., deGuglielmo, G., Lasek, A.W., Logrip, M.L., Vendruscolo, L.F., Roberts, A.J., Roberts, E., George, O., Mayfield, J., Billiar, T.R., Hackam, D.J., Mayfield, R.D., Koob, G.F., Roberto, M., Homanics, G.E., 2017. Genetic and pharmacological manipulation of TLR4 has minimal impact on ethanol consumption in rodents. J. Neurosci. 37, 1139–1155.

Hasin, D.S., Sarvet, A.L., Meyers, J.L., Saha, T.D., Ruan, W.J., Stohl, M., Grant, B.F., 2018. Epidemiology of adult DSM-5 major depressive disorder and its specifiers in the United States. JAMA Psychiatry 75, 336–346.

Hung, Y., Huang, K., Kang, H., Huang, G.Y., Huang, T., 2015. Antidepressants normalize elevated Toll-like receptor profile in major depressive disorder. Psychopharmacology 233, 1707–1714.

Ivanov, S., Dragoi, A., Wang, X., Dallacosta, C., Louten, J., Musco, G., Chu, W., 2007. A novel role for HMGB1 in TLR9-mediated inflammatory response to CpG-DNA. Blood 110, 1970–1981.

Jiang, W., Gilkeson, G., 2014. Sex differences in monocytes and TLR4 associated immune responses: implications for systemic lupus erythematosus (SLE). J. Immunother. Appl. 1, 1.

Keri, S., Szabo, C., Kelemen, O., 2014. Expression of Toll-Like Receptors in peripheral blood mononuclear cells and response to cognitive-behavioral therapy in major depressive disorder. Brain Behav. Immun. 40, 235–242.

Kessler, R.C., McGonagle, K.A., Swartz, M., Blazer, D.G., Nelson, C.B., 1993. Sex and depression in the national comorbidity survey I: lifetime prevalence, chronicity and recurrences. J. Affect. Disord. 29, 85–96.
Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. Nat. Rev. Immunol. 626–638.
Klein, S.L., Jedlicka, A., Pekosz, A., 2010. The Xs and Y of immune responses to viral vaccines. Lancet Infect. Dis. 10, 338–349.
Kornstein, S.G., Schatzberg, A.F., Thase, M.E., Yonkers, K.A., McCullough, J.P., Keitner, G.I., Gelenberg, A.J., Ryan, C.E., Hess, A.L., Harrison, W., Davis, S.M., Keller, M.B., 2000. Gender differences in chronic major and double depression. J. Affect. Disord. 60, 1–11.
Kubera, M., Obuchowicz, E., Goehler, L., Brzeszcz, J., Maes, M., 2011. In animal models, psychosocial stress-induced (neuro)inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35, 744–759.
Levine, J., Barak, Y., Chengappa, K.N., Rapoport, A., Rebey, M., Barak, V., 1999. Cerebrospinal cytokine levels in patients with acute depression. Neuropsychobiology 40, 171–176.
Li, C., Yan, Y., Cheng, J., Xiao, G., Ju, J., Zhang, L., Zhou, Y., 2016. Toll-like receptor 4 deficiency causes reduced exploratory behavior in mice under approach-avoidance conflict. Neurosci. Bull. 32, 127–136.
Liu, J.-F., Wu, R., Li, J.-X., 2019. Toll of mental disorders: TLR-mediated function of the innate immune system. Neurosci. Bull. 35, 771–774.
Maes, M., 1995. Evidence for an immune response in major depression: a review and hypothesis. Progress. Neuropsychopharmacol. Biol. Psych. 19, 11–38.
Maier, S.F., 2003. Bi-directional immune-brain communication: implications for understanding stress, pain, and cognition. Brain Behav. Immun. 17, 69–85.
Maier, S.F., Watkins, L.R., 2005. Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, and corticotrophin-releasing factor. Neurosci. Biobehav. Rev. 29, 829–841.
Maniam, J., Morris, M., 2012. The link between stress and feeding behaviour. Neuropharmacology 63, 97–110.
Marcus, S.M., Kerber, K.B., Rush, A.J., Wisniewski, S.R., Nierenberg, A., Balasubramani, G.K., Ritz, L., Kornstein, S.G., Young, E.A., Trivedi, M.H., 2008. Sex differences in depression symptoms in treatment-seeking adults: confirmatory analyses from the sequenced treatment alternatives to relieve depression study. Comprenhen. Psychiatry 49, 238–246.
Marriott, I., Huet-Hudson, Y.M., 2006. Sexual dimorphism in innate immune responses to infectious organisms. Immunol. Res. 34, 172–192.
Marshall, A.T., Liu, A.T., Murphy, N.P., Maidment, N.T., Ostlund, S.B., 2017. Sex-specific enhancement of palatability-driven feeding in adolescent rats. PloS One 12, e0180907.
Martin, L.A., Neighbors, H.W., Griffith, D.M., 2013. The experience of symptoms of depression in men vs women. JAMA Psychiatry 70, 1100.
Masur, J., Schutz, M.T., Boerngen, R., 1980. Gender differences in open-field behavior as a function of age. Dev. Psychobiol. 13, 107–110.
Mazor, A., Matar, M.A., Kaplan, Z., Kozlovsky, N., Zohar, J., Cohen, H., 2009. Gender-related qualitative differences in baseline and post-stress anxiety responses are not reflected in the incidence of criterion-based PTSD-like behaviour patterns. World J. Biol. Psychiatr. 10, 856–869.
Menard, C., Pfau, M.L., Hodes, G.E., Russo, S.J., 2017. Immune and neuroendocrine mechanisms of stress vulnerability and resilience. Neuropsychopharmacology 42, 1–19.
Miller, A.H., Maletic, V., Paison, C.L., 2008. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depressions. Biol. Psychiatr. 65, 732–741.
Moieni, M., Irwin, M.R., Jevtic, I., Olmstead, R., Breen, E.C., Eisenberger, N.I., 2015. Sex differences in depressive and socioemotional responses to an inflammatory challenge: implications for sex differences in depression. Neuropsychopharmacology 40, 1709–1716.
Mueller, T.I., Leon, A.C., Keller, M.B., Solomon, D.A., Endicott, J., Coryell, W., Warshaw, M., Maser, J.D., 1999. Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. Am. J. Psychiatr. 156, 1000–1006.
Nalbandian, G., Kovats, S., 2005. Understanding sex biases in immunity: effects of estrogen on the differentiation and function of antigen-presenting cells. Immunol. Res. 31, 91–106.
Okun, E., Griffioen, K.J., Mattson, M.P., 2011. Toll-like receptor signaling in neural plasticity and disease. Trends Neurosci. 34, 269–281.
Ortona, E., Pierdominici, M., Maselli, A., Veroni, C., Aloisi, F., Shoenfeld, Y., 2014. Sex-based differences in autoimmune diseases. Annali dell'Istituto superiore di Sanita 50, 286–290.
Padgett, D.A., Glaser, R., 2003. How stress influences the immune response. Trends Immunol. 24, 444–448.
Papp, M., Willner, P., Muscat, R., 1991. An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology 104, 255–259.
Park, J.S., Svetkauskaite, D., He, Q., Kim, J., Strassheim, D., Ishizaka, A., Abraham, E., 2003. Involvement of Toll-like receptors 2 and 4 in cellular activation by high mobility group Box 1 protein. J. Biol. Chem. 279, 7370–7377.
Platt, J.M., Bates, L.M., Jager, J., McLaughlin, K.A., Keyes, K.M., 2020. Changes in the depression gender gap from 1992 to 2014: cohort effects and mediation by gendered social position. Soc. Sci. Med. 258, 113088.
Pooley, A.E., Benjamin, R.C., Sreedhar, S., Eagle, A.L., Robison, A.J., Mazei-Robison, M.S., Breedlove, S.M., Jordan, C.L., 2018. Sex differences in the traumatic stress response: the role of adult gonadal hormones. Biol. Sex Differ. 9, 32.
Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.
Pruett, S., Hebert, P., Lapointe, J., Reagan, W., Lawton, M., Kawabata, T.T., 2008. Characterization of the action of drug-induced stress responses on the immune system: evaluation of biomarkers for drug-induced stress in rats. J. Immunot. 4, 25–38.
Rainville, J.R., Tsyglakova, M., Hodes, G.E., 2018. Deciphering sex differences in the immune system and depression. Front. Neuroendocrinol. 50, 67–90.
Rizavi, H.S., Ren, X., Zhang, H., Bhaumik, R., Pandey, G.N., 2016. Abnormal gene expression of proinflammatory cytokines and their membrane-bound receptors in the lymphocytes of depressed patients. Psychiatr. Res. 240, 314–320.
Roltsch, E.A., Baynes, B.B., Mayeux, J.P., Whitaker, A.M., Baiamonte, B.A., Gilpin, N.W., 2014. Predator odor stress alters corticotropin-releasing factor-1 receptor (CRF1R)-dependent behaviors in rats. Neuropharmacology 79, 84–89.
Rubinow, D.R., Schmidt, P.J., 2019. Sex differences and the neurobiology of affective disorders. Neuropsychopharmacology 44, 111–128.
Sahnoun, S., Sellami, A., Chakroun, N., Mseddi, M., Attia, H., Rebai, T., Lassoued, S., 2017. Human sperm Toll-like receptor 4 (TLR4) mediates acrosome reaction, oxidative stress markers, and sperm parameters in response to bacterial lipopolysaccharide in infertile men. J. Assist. Reprod. Genet. 34, 1067–1077.
Santos-Galindo, M., Acaz-Fonseca, E., Bellini, M.J., Garcia-Segura, L.M., 2011. Sex differences in the inflammatory response of primary strocytes to lipopolysaccharide. Biol. Sex Differ. 2, 7.
Schjenken, J.E., Glynn, D.J., Sharkey, D.J., Robertson, S.A., 2015. TLR4 signaling is a major mediator of the female tract response to seminal fluid in mice. Biol. Reprod. 93 (68), 61-13.
Sclafani, A., Hertwig, H., Vigorito, M., Feigin, M.B., 1987. Sex differences in polysaccharide and sugar preferences in rats. Neurosci. Biobehav. Rev. 11, 241–251.
Siegert, R.J., Abernethy, D.A., 2005. Depression in multiple sclerosis: a review. J. Neurol. Neurosurg. Psychiatry 76, 469–475.
Spijker, J., Muntingh, A., Batelaan, N., 2020. Advice for clinicians on how to treat comorbid anxiety and depression. JAMA Psychiatry 77, 645–646.
Standberry, L.R., Spruance, S.L., Cunningham, A.L., Bernstein, D.I., Mindel, A., Sacks, S., 2002. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. N. Engl. J. Med. 247, 1652–1661.
Tonelli, L.H., Holmes, A., Postolache, T.T., 2008. Intranasal immune challenge induces sex-dependent depressive-like behavior and cytokine expression in the brain. Neuropsychopharmacology 33, 1038–1048.
Udina, M., Castellvi, P., Moreno-Espana, J., Navines, R., Valdes, M., Forns, X., Langohr, K., Sola, R., Vieta, E., Martin-Santos, R., 2012. Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. J. Clin. Psychiatr. 73, 1128–1138.
Varodayan, F.P., Khom, S., Patel, R.R., Steinman, M.Q., Hedges, D.M., Oleata, C.S., Homanics, G.E., Roberto, M., Bajo, M., 2018. Role of TLR4 in the modulation of central amygdala GABA transmission by CRF following restraint stress. Alcohol Alcohol 53, 642–649.
Vaure, C., Liu, Y., 2014. A comparative review of Toll-like receptor 4 expression and functionality in different animal species. Front. Immunol. 5, 316.
Weiss, J.M., Bailey, W.H., Pohrecky, L.A., Korzeniowski, D., Grillione, G., 1980. Stress-induced depression of motor activity correlates with regional changes in brain norepinephrine but not in dopamine. Neurochem. Res. 5, 9–22.
Whitacre, C.C., 2001. Sex differences in autoimmune disease. Nat. Immunol. 2, 777–780.
Whitaker, A.M., Farooq, M.A., Edwards, S., Gilpin, N.W., 2016. Post-traumatic stress avoidance is attenuated by corticosterone and associated with brain levels of steroid receptor co-activator-1 in rats. Stress 19, 69–77.
Whitaker, A.M., Gilpin, N.W., 2015. Blunted hypothalamo-pituitary adrenal axis response to predator odor predicts high stress reactivity. Physiol. Behav. 147, 16–22.
Whitaker, A.M., Gilpin, N.W., Edwards, S., 2014. Animal models of post-traumatic stress disorder and recent neurobiological insights. Behav. Pharmacol. 25, 398–409.
Willner, P., 1997. The chronic mild stress procedure as an animal model of depression: valid, reasonably reliable and useful. Psychopharmacology 134.
Wilson, C.B., Ebenezer, P.J., McLaughlin, L.D., Francis, J., 2013a. Predator exposure/psychosocial stress animal model of post-traumatic stress disorder modulates neurotransmitters in the rat hippocampus and prefrontal cortex. PloS One 9, e89104.
Wilson, C.B., McLaughlin, L.D., Nair, A., Ebenezer, P.J., Dange, R., Francis, J., 2013b. Inflammation and oxidative stress are elevated in the brain, blood, and adrenal glands during the progression of post-traumatic stress disorder in a predator exposure animal model. PloS One 8, 76146.
Wu, M.-K., Huang, T.-L., Huang, K.-W., Huang, Y.-L., Huang, Y.-Y., 2015. Association between toll-like receptor 4 expression and symptoms of major depressive disorder. Neuropsychiatric Dis. Treat. 5, 1853–1857.
Wu, Y.-P., Gao, H.-Y., Ouyang, S.-H., Kurihara, H., He, R.-R., Li, Y.-F., 2019. Predator stress-induced depression is associated with inhibition of hippocampal neurogenesis in adult male mice. Neural. Regenerat. Res. 14, 298–305.
Yirmiya, R., 1996. Endotoxin produces a depressive-like episode in rats. Brain Res. 711, 163–174.
Zagni, G., Shakhar, K., Ben-Eliyahu, S., 2001. Sex and gender differences in central nervous system-related disorders. Neurosci. J. 2016, 1–13.
Zhang, H.X., Yu, Y.Q., Li, Y.Y., Lu, M.F., Shi, S.X., Ji, J.L., Wang, L.W., 2018. Difference in proinflammatory cytokines produced by monocytes between patients with major depressive disorder and healthy controls. J. Affect. Disord. 234, 305–310.
Zhang, L., Fu, T., Yin, R., Zhang, Q., Shen, B., 2017. Prevalence of depression and anxiety in systemic lupus erythematosus: a systematic review and meta-analysis. BMC Psychiatr. 17, 70.