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Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses

Matthew G. Frank*, Brittany M. Thompson, Linda R. Watkins, and Steven F. Maier

Department of Psychology and Neuroscience, University of Colorado, Boulder, CO, USA

Abstract

Acute and chronic stress sensitizes or “primes” the neuroinflammatory response to a subsequent proinflammatory challenge. While prior evidence shows that glucocorticoids (GCs) play a pivotal role in stress-induced potentiation of neuroinflammatory responses, it remains unclear whether stress-induced GCs sensitize the response of key CNS immune substrates (i.e. microglia) to pro-inflammatory stimuli. An *ex vivo* approach was used to address this question. Here, stress-induced GC signaling was manipulated *in vivo* and hippocampal microglia challenged with the pro-inflammatory stimulus LPS *ex vivo*. Male Sprague–Dawley rats were either pretreated *in vivo* with the GC receptor antagonist RU486 or adrenal-ectomized (ADX). Animals were then exposed to an acute stressor (inescapable tailshock; IS) and 24 h later hippocampal microglia were isolated and challenged with LPS to probe for stress-induced sensitization of pro-inflammatory responses. Prior exposure to IS resulted in a potentiated pro-inflammatory cytokine response (e.g. IL-1 β gene expression) to LPS in isolated microglia. Treatment *in vivo* with RU486 and ADX inhibited or completely blocked this IS-induced sensitization of the microglial pro-inflammatory response. The present results suggest that stress-induced GCs function to sensitize the microglial proinflammatory response (IL-1 β , IL-6, NF κ B β) to immunologic challenges.

Keywords

Stress; Glucocorticoid; Microglia; Cytokine; Neuroinflammation; Priming

1. Introduction

The phenomenon of cross-sensitization between the neuroinflammatory sequelae of stress and pro-inflammatory immune activation has been well characterized (Anisman, 2009; Tilders and Schmidt, 1999). In particular, acute and chronic stress has been found to sensitize the neuroinflammatory response to both peripheral and central immunologic challenges (de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Frank et al., 2007; Johnson et al., 2002b, 2003, 2004; Munhoz et al., 2006; Wohleb et al., 2011). For example, exposure to a single session of inescapable tailshock (Johnson et al., 2002b) and to 14 daily sessions of unpredictable chronic stress (Munhoz et al., 2006) potentiate the increase in hippocampus

*Corresponding author. Address: Department of Psychology and Neuroscience, Campus Box 345, University of Colorado, Boulder, CO 80309-0345, USA. Tel.: +1 303 919 8116; fax: +1 303 492 2967; matt.frank@colorado.edu (M.G. Frank).

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and frontal cortex expression of pro-inflammatory mediators (e.g. interleukin-1 β ; IL-1 β) produced by a peripheral injection of lipopolysaccharide (LPS) given 24 h after the stressor regimen. Several studies have also shown that acute and chronic stress modulates the immunophenotype of microglia as indicated by up-regulation of MHCII (de Pablos et al., 2006; Frank et al., 2007), toll-like receptor 4 (TLR4) (Wohleb et al., 2011) and F4/80 antigen (Nair and Bonneau, 2006), suggesting that stress may shift the neuroimmune microenvironment towards a pro-inflammatory immunophenotype.

Stress-induced microglial activation and potentiation of neuroinflammatory processes has been blocked by a glucocorticoid (GC) receptor antagonist (de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Munhoz et al., 2006; Nair and Bonneau, 2006) suggesting that GCs may mediate stressor-induced sensitization of neuroinflammatory processes. However, it is important to note that in these studies it was not possible to determine which immunologic substrate(s), whether peripheral and/or central, was sensitized or primed by stress-induced GCs. This is because the pro-inflammatory challenge (LPS) was administered *in vivo*, and so sensitization of either the peripheral response that signals the brain, or processes in the brain (or both) could have been causal. We have previously shown that an acute stressor sensitizes the hippocampal microglial pro-inflammatory response to immune challenge *ex vivo* (Frank et al., 2007). In that study, hippocampal microglia were isolated 24 h post-stress, challenged with LPS *ex vivo* and pro-inflammatory cytokines measured 2 h later. Challenging microglia with LPS *ex vivo* serves two purposes. First, this *ex vivo* approach provides a clear test of whether microglia could be an immunologic substrate of stress-induced priming since it allows a definitive determination of whether microglia are, in fact, sensitized by the prior stressor. Secondly, LPS modulation of the HPA axis and GC signaling is necessarily excluded, thereby making it possible to separate the effects of stress-induced GCs from the effects of LPS-induced GCs on microglia pro-inflammatory responses. Therefore, an *ex vivo* experimental approach was taken in the present investigation to test whether GCs mediate stress-induced sensitization of microglia pro-inflammatory immune responses. To test this hypothesis, pharmacological (GR antagonist) and surgical (adrenalectomy) suppression of stress-induced GCs was utilized.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats (60–90 d old; Harlan Sprague–Dawley, Inc., Indianapolis, IN, USA) were pair-housed with food and water available *ad libitum*. The colony was maintained at 25 °C on a 12-h light/dark cycle (lights on at 07:00 h). All experimental procedures were conducted in accord with the University of Colorado Institutional Animal Care and Use Committee.

2.2. Reagents

RU486 (mifepristone; Sigma, St. Louis, MO), a GC receptor antagonist, was dissolved in 100% propylene glycol. LPS (*E. coli* serotype 0111:B4; Sigma) was dissolved in pyrogen free, sterile 0.9% saline.

2.3. Experimental design

2.3.1. Effect of RU486 on stress-induced potentiation of neuroinflammatory responses to LPS *in vivo*—Prior to proceeding with *ex vivo* studies, a preliminary study was conducted to verify the efficacy of RU486 to block stress-induced potentiation of neuroinflammatory responses *in vivo*. Animals were injected with RU486 (50 mg/kg, s.c.) or vehicle (100% propylene glycol) 1 h prior to onset of inescapable tailshock (IS). Home cage controls (HCC) were injected at the same time of day as were IS animals. All injections occurred between 08:00 and 10:00 h. Twenty four hours post-IS, IS and HCC animals were injected with LPS (10 µg/kg, i.p.) or vehicle. Thus, the design was a 2 × 2 factorial. Two hours post-LPS or vehicle, hippocampal pro-inflammatory cytokines were measured. Analysis was restricted to the hippocampus because we have shown that it is sensitive to IS-induced priming effects *in vivo* (Johnson et al., 2002b) and *ex vivo* (Frank et al., 2007) and of the CNS structures that are sensitive to IS-induced priming effects, it yields a sufficient number of microglia to conduct *ex vivo* experiments.

2.3.2. Effect of RU486 on stress-induced potentiation of hippocampal microglial responses to LPS *ex vivo*—Animals were injected with RU486 (50 mg/kg, s.c.) or vehicle (100% propylene glycol) 1 h prior to onset of IS. Twenty four hours post-IS, hippocampal microglia were isolated from IS and HCC animals and exposed to LPS for 2 h and pro-inflammatory cytokine gene expression assessed.

2.3.3. Effect of adrenalectomy (ADX) on stress-induced potentiation of hippocampal microglial responses to LPS *ex vivo*—Animals underwent ADX or sham surgery. Two weeks post-surgery, animals were exposed to IS or served as HCC. Twenty four hours post-IS, hippocampal microglia were isolated from IS and HCC animals and exposed to LPS for 2 h and pro-inflammatory cytokine gene expression assessed.

2.4. ADX surgery and verification

Bilateral ADX was aseptically performed under halothane anesthesia (Halocarbon Laboratories, River Edge, NJ, USA) as previously described (Der-Avakian et al., 2005). All removed tissue was examined immediately to ensure complete removal of the adrenal gland. Adrenal tissue was visually inspected to assess that the adrenal gland was intact. Sham-operated animals received the identical procedure, except that the adrenal glands were gently manipulated with forceps but not removed. Steroid replacement began for ADX animals immediately after surgery and continued for the remainder of the experiment. Steroid replacement was utilized because the rationale was to eliminate the IS-induced rise of CORT, not to eliminate basal levels. ADX animals received basal corticosterone (CORT; Sigma–Aldrich, St. Louis, Mo, USA) replacement in their drinking water since this method has been shown to mimic the normal circadian pattern of CORT secretion (Jacobson et al., 1988). CORT was initially dissolved in ethyl alcohol (EtOH) and diluted to a final concentration of 25 µg/ml in tap water to yield 0.2% EtOH. This concentration leads to normal basal levels. CORT-water also contained 0.9% saline. Sham animals received drinking water containing 0.2% EtOH and 0.9% saline. Rats were allowed 2 weeks to recover before exposure to IS. For ADX verification, cardiac blood was collected at the time of sacrifice and serum CORT measured.

2.5. Inescapable shock (IS)

Details of the present stressor protocol have been published previously and reliably potentiate pro-inflammatory cytokine responses in the hippocampus after peripheral immune challenge (Johnson et al., 2002b) as well as in isolated hippocampal microglia to LPS *ex vivo* (Frank et al., 2007). Briefly, animals were placed in Plexiglas tubes (23.4 cm in length $\times$ 7 cm in diameter) and exposed to 100 1.6 mA, 5 s tailshocks with a variable intertrial interval (ITI) ranging from 30–90 s (average ITI = 60 s). All IS treatments occurred between 09:00 and 11:00 h. IS animals were returned to their home cages immediately after termination of shock. HCC animals remained undisturbed in their home cages.

2.6. Tissue and blood collection

Animals were given a lethal dose of sodium pentobarbital. Animals were fully anesthetized and cardiac blood withdrawn within 3 min of injection. Cardiac blood was collected and animals were transcardially perfused with ice-cold saline (0.9%) for 3 min to remove peripheral immune leukocytes from the CNS vasculature. Brain was rapidly extracted and placed on ice and hippocampus dissected. For *in vivo* experiments, hippocampus was flash frozen in liquid nitrogen and stored at -80°C . For *ex vivo* experiments, hippocampal microglia were immediately isolated.

2.7. Ex vivo immune stimulation of hippocampal microglia with LPS

Hippocampal microglia were isolated using a Percoll density gradient as previously described (Frank et al., 2006). We have previously shown (Frank et al., 2006) that this microglia isolation procedure yields highly pure microglia (Iba-1+/MHCII+/CD163-/GFAP-). In the present experiments, immunophenotype and purity of microglia was assessed using real time RT-PCR. Microglia were suspended in DMEM + 10% FBS and microglia concentration determined by trypan blue exclusion. Microglia concentration was adjusted to a density of 5×10^3 cells/100 μl and 100 μl added to individual wells of a 96-well v-bottom plate. LPS was utilized to challenge microglia *ex vivo* as we have previously determined the optimal *in vitro* conditions under which LPS stimulates a microglia pro-inflammatory cytokine response (Frank et al., 2006). Cells were incubated with LPS (0.1, 1, 10, and 100 ng/ml) or media alone for 2 h at 37°C , 5% CO_2 . The plate was centrifuged at 1000g for 10 min at 4°C to pellet cells and cells washed $1 \times$ in ice cold PBS and centrifuged at 1000g for 10 min at 4°C . Cell lysis/homogenization and cDNA synthesis was performed according to the manufacturer's protocol using the SuperScript III CellsDirect cDNA Synthesis System (Invitrogen, Carlsbad, CA).

2.8. Real time RT-PCR measurement of gene expression

A detailed description of the PCR amplification protocol has been published previously (Frank et al., 2006). cDNA sequences were obtained from Genbank at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov). Primer sequences were designed using the Operon Oligo Analysis Tool (<http://www.operon.com/technical/toolkit.aspx>) and tested for sequence specificity using the Basic Local Alignment Search Tool at NCBI (Altschul et al., 1997). Primers were obtained from Invitrogen. Primer specificity was verified by melt curve analyses. All primers were designed to span exon/

exon boundaries and thus exclude amplification of genomic DNA (See Table 1 for primer description and sequences).

PCR amplification of cDNA was performed using the Quantitect SYBR Green PCR Kit (Qiagen, Valencia, CA). Formation of PCR product was monitored in real time using the MyiQ Single-Color Real-Time PCR Detection System (BioRad, Hercules, CA). Relative gene expression was determined by taking the expression ratio of the gene of interest to β -Actin.

2.9. Serum and hippocampus CORT assay

Cardiac blood was centrifuged (10 min, 14,000g, 4 °C) and serum collected. Hippocampus was sonicated using a tissue extraction reagent (Invitrogen) supplemented with a protease inhibitor cocktail (Sigma). Homogenate was centrifuged (10 min, 14,000g, 4 °C) and supernatant collected and stored at -20 °C. Total protein was quantified using a Bradford assay. CORT was measured using a competitive immunoassay (Assay Designs, Inc., Ann Arbor, MI) as described in the manufacturer's protocol.

2.10. Statistical analysis and data presentation

All data are presented as mean $\pm$ SEM. Statistical analyses consisted of ANOVA followed by post hoc tests (Newman-Keuls) using Prism 5 (Graphpad Software, Inc., La Jolla, CA). Threshold for statistical significance was set at $\alpha = .05$. Four animals per experimental group were used in each experiment.

3. Results

3.1. Effect of the GR antagonist RU486 on stress-induced sensitization of the pro-inflammatory response to LPS in hippocampus

A preliminary study was conducted here to assess the efficacy of RU486 in blocking the acute IS-induced potentiation of hippocampal pro-inflammatory responses to LPS *in vivo*. The pro-inflammatory mediators, IL-1 β and NF κ B α , were measured in hippocampus to determine whether RU486 would block the IS-induced potentiation of the pro-inflammatory response to LPS. The results are shown in Fig. 1. The 3-way interaction effect between stress, RU486, and LPS on IL-1 β was statistically significant ($F = 41.15$, $df = 1, 24$, $p < .0001$). In animals not pretreated with RU486, LPS significantly increased cytokine expression in animals compared to Veh/HCC and Veh/IS animals, while prior IS potentiated the increase in IL-1 β produced by LPS (Fig. 1A). In animals administered RU486 prior to IS, the IL-1 β response to LPS was reduced to levels significantly below levels observed in LPS/IS animals pretreated with vehicle indicating that RU486 substantially blunted the potentiation of IL-1 β produced by IS. Interestingly, RU486 given 24 h before LPS in the HCC subjects increased the effect of LPS on IL-1 β compared to the effect of LPS in vehicle pretreated animals. The 3-way interaction effect between stress, RU486, and LPS on NF κ B α was also statistically significant ($F = 13.97$, $df = 1, 24$, $p < .001$). However, the pattern of data for NF κ B α (Fig. 1B) was different than for IL-1 β . In animals not pretreated with RU486, LPS significantly increased cytokine expression in animals compared to Veh/HCC and Veh/IS animals, while prior IS potentiated the increase in NF κ B α produced

by LPS. In animals administered RU486 prior to IS, the NF κ B α response to LPS was reduced to levels significantly below levels observed in LPS/IS animals pretreated with vehicle indicating that RU486 substantially blunted the potentiation of NF κ B α produced by IS. However, RU486 did not alter the effect of LPS in HCC animals compared to the effect of LPS in animals not treated with RU486. The 3-way interaction effect between stress, RU486, and LPS on hippocampal CORT levels was statistically significant ($F = 22.756$, $df = 1, 24$, $p < .0001$). Prior IS increased CORT levels compared to HCC animals regardless of RU486 treatment (Fig. 1C). In non-stressed animals, LPS increased CORT levels independent of RU486 treatment. Prior exposure to IS potentiated the CORT increase produced by LPS, and this was blocked by RU486 given before IS. These results validated the efficacy of RU486 to inhibit stress-induced potentiation of neuroinflammatory responses. Therefore, a subsequent experiment was conducted to examine whether hippocampal microglia could be a CNS immune substrate for these effects.

3.2. Effect of the GR antagonist RU486 on stress-induced sensitization of the microglial pro-inflammatory response to LPS ex vivo

Analysis of cDNA from hippocampal microglia showed robust expression of the microglia markers Iba-1, MHCII, and CD11b whereas the perivascular/meningeal macrophage marker CD163 failed to amplify by 35 cycles of PCR (data not shown). Likewise, the astrocyte marker GFAP failed to amplify by 35 cycles of PCR in all samples indicating that the microglia isolation procedure yielded a highly pure microglia population devoid of other CNS macrophages as well as astrocytes. Of note, CD163 can be upregulated on activated microglia under pathological conditions (Borda et al., 2008; Holfelder et al., 2011) and thus CD163 may not be a suitable marker to differentiate microglia from other CNS macrophages given that the stressor used here activates microglia (Frank et al., 2007). CD163 expression was assessed in hippocampal microglia from animals exposed to IS or HCC treatments and RU486 or vehicle treatments. Through 35 cycles of PCR, CD163 failed to amplify in isolated microglia from all animals in each treatment group (data not shown) indicating that highly pure microglia were obtained from each animal. To determine whether IS induced a “primed” immunophenotype in hippocampal microglia, MHCII expression was measured in HCC and IS treated animals. IS upregulated microglial MHCII expression, whereas Iba-1 was not significantly changed by IS (data not shown).

LPS applied to the isolated microglia increased IL-1 β (Fig. 2A) and NF κ B α (Fig. 2C) gene expression in a concentration dependent manner in all experimental groups. In order to determine whether RU486 given before IS blunted stress-induced sensitization of the microglial pro-inflammatory response to LPS challenge, area under the LPS concentration curve (AUC) was computed for each subject and the interaction between stress and RU486 treatment determined. In vehicle treated animals, IS significantly potentiated the microglial IL-1 β response, which was completely blocked by RU486 treatment (interaction, $F = 10.05$, $df = 1, 12$, $p < .01$). The IL-1 β response to LPS was significantly greater in IS animals not treated with RU486 compared to all other experimental groups, whereas RU486 treatment reduced the IL-1 β response to LPS in IS animals to levels observed in Veh/HCC and RU486/HCC animals. A similar interaction was observed with NF κ B α (interaction, $F = 17.09$, $df = 1, 12$, $p < .05$). Thus, the pharmacological inhibition of stress-induced GC

signaling suppressed the stress-induced potentiation of the microglia pro-inflammatory immune response. In light of experimental liabilities associated with RU486 pharmacodynamics and pharmacokinetics (see discussion), surgical suppression of endogenous CORT was employed as an alternate converging strategy to modulate stress-induced CORT.

3.3. Verification of the effect of ADX on stress-induced serum CORT levels

IS induced a substantial serum CORT response (Fig. 3), which persisted for at least 24 h post-stress. ADX completely blocked the serum CORT response observed 24 h post-IS. In sham surgery animals, IS induced a significant increase in serum CORT, which was significantly reduced in ADX animals (interaction, $F = 22.32$, $df = 1, 12$, $p < .01$) to levels comparable to sham/HCC and ADX/HCC animals. In these same animals, hippocampal microglia were challenged with LPS *ex vivo*.

3.4. Effect of ADX on stress-induced sensitization of the microglial pro-inflammatory response to LPS *ex vivo*

LPS increased IL-1 β (Fig. 4A), NF κ B α (Fig. 4C), IL-6 (Fig. 4E) and TNF α (Fig. 4G) gene expression in a concentration dependent manner in all experimental groups. LPS failed to modulate TLR4 (Fig. 4I) gene expression in a concentration dependent manner. To determine whether ADX blunted stress-induced sensitization of the microglial pro-inflammatory response to LPS challenge, area under the LPS concentration curve (AUC) was computed for each subject and the interaction between stress and ADX treatment determined. In sham surgery animals, IS significantly potentiated the microglial IL-1 β response, which was completely blocked by ADX (Fig. 4B) ($F = 6.92$, $df = 1, 12$, $p < .05$). Independent of stress condition, ADX significantly suppressed IL-1 β expression compared to sham/HCC animals. A similar interaction between stress and ADX was observed for NF κ B α (Fig. 4D) ($F = 9.94$, $df = 1, 12$, $p < .01$). Again, independent of stress condition, ADX significantly suppressed NF κ B α expression compared to sham/HCC animals. In sham surgery animals, IS significantly potentiated the microglial IL-6 response, which was completely blocked by ADX (Fig. 4F) ($F = 15.61$, $df = 1, 12$, $p < .01$), however, ADX did not significantly suppress IL-6 expression compared to sham/HCC animals. The interaction between stress and ADX was not significant for TNF α (Fig. 4H), although the main effect of ADX on TNF α expression was significant indicating that ADX reduced TNF α expression. In sham surgery animals, IS significantly reduced microglial TLR4 levels, which was completely blocked by ADX (Fig. 4J) ($F = 20.19$, $df = 1, 12$, $p < .001$). In HCC animals, ADX also resulted in a significant reduction in TLR4 expression compared to sham surgery.

4. Discussion

The data from the present set of experiments provide converging evidence that GCs mediate stress-induced sensitization of microglia to later pro-inflammatory challenges. Both pharmacological (RU486) and surgical (ADX) suppression of stress-induced GC signaling blocked the stress-induced sensitization of the proinflammatory response (i.e. IL-1 β) of microglia to a pro-inflammatory challenge. We have previously demonstrated that microglia are a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory

immune responses (Frank et al., 2007). A recent study by Wohleb et al. shows that repeated social defeat also potentiates the microglial pro-inflammatory response to LPS *ex vivo* (Wohleb et al., 2011). The present results suggest that microglia are a neuroimmune substrate of stress-induced GC action, which in turn “prime” microglia to over-respond to proinflammatory insults. These results are consistent with several studies showing that acute and chronic stressors sensitize the neuroinflammatory response to both peripheral and central immunologic challenges (de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Frank et al., 2007; Johnson et al., 2002b, 2003, 2004; Munhoz et al., 2006). It is important to note here that stress-induced potentiation of pro-inflammatory mediators does not necessarily translate into potentiation of behavioral phenotypes such as sickness responses because it is the balance of pro- and anti-inflammatory mediators that determines stress-induced behavioral outcomes. We have previously shown that exposure to a severe acute stressor (IS) as used here potentiates the sickness response to LPS administered 24 h post-stress (Johnson et al., 2003). Interestingly, administration of a single dose of GCs that mimics the GC response to IS (Fleshner et al., 1993) also potentiates the sickness response to LPS given 24 h post-stress (Hains et al., 2011). In addition, GCs are sufficient to sensitize the neuroinflammatory as well as the microglial response to pro-inflammatory challenges (Frank et al., 2010; Munhoz et al., 2010).

In prior studies of GC mediation of stress-induced neuroinflammatory sensitization (de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Munhoz et al., 2006), the pro-inflammatory challenge (i.e. LPS) was administered *in vivo*, which necessarily convolutes the GC effects of the stressor with the GC effects of LPS. Stress-induced GCs could modulate LPS-induced GC production (HPA axis; (Beishuizen and Thijs, 2003). Indeed, prior stressors such as inescapable shock do potentiate the later GC increase produced by LPS (Johnson et al., 2002a). In addition, stress-induced GCs could modify later LPS-induced signaling at the receptor level (GR; (Oakley and Cidlowski, 2011), plasma binding proteins (corticosteroid binding globulin; (Henley and Lightman, 2011), and/or metabolic enzymes (11 β - hydroxysteroid dehydrogenase Type I and II; (Seckl, 1997). In the present study, because microglia were exposed to LPS *ex vivo* and so LPS could not itself produce a GC response, the GC effects of stress could be studied independent of the GC effects of LPS. Here LPS was used *ex vivo* to probe the sensitization “status” of microglia. LPS is considered a pathogen associated molecular pattern or motif common to gram-negative bacteria, which is recognized and signals through the pattern recognition receptor TLR4 (Kawai and Akira, 2010). Importantly, TLR4 is expressed by microglia (Carpentier et al., 2008). The observation that stress-induced GCs sensitized the microglia response to LPS thus prompted us to explore whether stress-induced GCs modulated the expression of TLR4. Interestingly, exposure to acute stress downregulated TLR4 gene expression and surgical suppression of stress-induced GCs abrogated this effect of stress on TLR4 gene expression. This finding was unexpected given that LPS signaling is mediated by TLR4. However, LPS signaling also requires additional proteins. LPS typically must bind LPS-binding protein, which is then bound by CD14 and delivered to TLR4 complexed with MD2 (Kawai and Akira, 2010). It could be that stress-induced sensitization of microglia was mediated by stress-induced changes in one or more of these ancillary LPS signaling proteins. Interestingly, repeated social defeat stress up-regulated protein expression of CD14 and TLR4 on microglia

(Wohleb et al., 2011). Though speculative, one possible explanation for the present results is that the downregulation of TLR4 mRNA is compensatory for increased protein level. Further studies are warranted to investigate the effect of stress and GCs on TLR4 protein levels.

Although the present results provide converging lines of evidence that GCs play a pivotal role in stress-induced priming of microglial pro-inflammatory responses, ambiguities associated with the use of RU486 and ADX should be noted. Though a highly effective GR antagonist, RU486 also antagonizes the progesterone receptor (Sarkar, 2002). Microglia express both GR and MR (Tanaka et al., 1997), however microglia do not express the progesterone receptor (Sierra et al., 2008) suggesting that the effects of RU486 observed here were mediated predominantly through the GR. Of note, we found that RU486 potentiated the LPS-induced increase in hippocampal IL-1 β in HCC animals. This finding was unexpected and not observed with NF- κ B α . The effect of RU486 on IL-1 β could be due to the long half-life of RU486 (20–30 h)(Sarkar, 2002), which may have blocked LPS-induced CORT signaling in the hippocampus, thereby ameliorating the anti-inflammatory effects of LPS-induced CORT. The long half-life of RU486 is an additional liability of this manipulation because of the potential effects on LPS-induced CORT responses 24 h post-stress. To obviate the liabilities associated with RU486, ADX was employed as an alternate converging strategy to modulate stress-induced GCs. ADX was as effective as RU486 in abrogating the effect of stress on microglia sensitization. However, in addition to removing the predominant source of endogenous GCs, ADX also removes a key source of peripheral mineralocorticoids (aldosterone) and catecholamines (epinephrine). Aldosterone has been found to modulate microglial expression of inducible nitric oxide synthase (Tanaka et al., 1997), however it is unknown whether aldosterone modulates neuroinflammatory responses. ADX animals were supplemented with basal replacement CORT in their drinking water, but not with aldosterone. Likewise, ADX also abrogates stress-induced release of catecholamines, which have been shown to play a pivotal role in the effects of acute and chronic stress on central pro-inflammatory cytokines.

We have shown that a severe acute stressor as used here induces pro-inflammatory cytokines in the CNS (hippocampus, hypothalamus and cortex) within hours of stressor termination (O'Connor et al., 2003) and is blocked by peripheral administration of β -adrenergic receptor antagonists (Johnson et al., 2005) suggesting that stress-induced catecholamines mediate, in part, the immediate effects of stress on central pro-inflammatory cytokines. Further, peripheral injection of a β -adrenergic receptor agonist was sufficient to induce central proinflammatory cytokines (Johnson et al., 2005). A subsequent study showed that central, but not peripheral β -adrenergic receptors mediate the effects of acute stress on central pro-inflammatory cytokines (Johnson et al., 2008). Similarly, Blandino et al. demonstrated that peripheral administration of a β -adrenergic receptor antagonist or the microglia inhibitor minocycline blocked the stress induced up-regulation of hypothalamic IL-1 β and CD14 (Blandino et al., 2009). Further, they demonstrated that CORT synthesis inhibition during stress potentiated the immediate stress effects on central proinflammatory cytokines. These results are consistent with a prior study showing that adrenalectomy potentiates the central proinflammatory cytokine response observed immediately after termination of the stressor (Nguyen et al., 1998). While these studies show that catecholamines play a pivotal role in the immediate effects of stress on central pro-inflammatory cytokines, a recent study by

Wohleb et al. showed that peripheral administration of a β -adrenergic receptor antagonist blocked stress-induced potentiation of the microglial pro-inflammatory response to LPS *ex vivo* (Wohleb et al., 2011). In addition, stress-induced up-regulation of microglial CD14 and TLR4 protein was blocked by prior treatment *in vivo* with a β -adrenergic receptor antagonist. Of note, microglia express α - and β -adrenergic receptors (Mori et al., 2002). Taken together, these studies suggest that catecholamines mediate, at least in part, the pro-inflammatory effects of stress in the brain. However, several studies have shown that catecholamines are sufficient to induce an anti-inflammatory immunophenotype in the CNS (Gleeson et al., 2010; McNamee et al., 2010a,b, 2009). An intriguing possibility is that catecholamines and GCs may work in concert to integrate stress-induced inflammatory immune responses. Indeed, a precedent for this possibility has been established in the field of learning and memory, which has found instances where GCs can amplify the effects of catecholamines on LTP (Joels et al., 2009).

The present results are consistent with a growing body of evidence demonstrating the permissive effects of stress and GCs in neuroinflammatory processes (Sorrells and Sapolsky, 2007). Several key parameters are pivotal in shaping the permissive function of stress and GCs. Of particular relevance to the present study, the timing of stress exposure relative to inflammatory challenge appears to play a pivotal role in whether a stressor is anti-inflammatory or pro-inflammatory. If a pro-inflammatory stimulus (LPS) is administered immediately before stress exposure, stress exhibits an anti-inflammatory effect, including inhibition of LPS-induced increases in brain IL-1 β (Goujon et al., 1995). However, if LPS is administered after stress exposure, stress potentiates the neuroinflammatory response to LPS (Johnson et al., 2002b). It is noteworthy that severe acute stress as used in the present investigation induces a rapid (within minutes of stress onset) and profound increase in serum GCs (Fleshner et al., 1993) in the absence of any pro-inflammatory challenge. A considerable literature has attempted to understand the physiological function of stress-induced GCs, in particular as they relate to host defense mechanisms (Munck and Naray-Fejes-Toth, 1994). It is problematic that GCs, which are universally considered anti-inflammatory and immunosuppressive (Boumpas et al., 1993; De Bosscher et al., 2003), would be elevated during a fight/flight emergency, when the chances of injury and infection increase. Notably, repeated social defeat stress increases the ability of splenic macrophages to kill bacteria (Bailey et al., 2007) suggesting that a fight/flight emergency may enhance an organism's innate immune response to immunological threats or dangers. An organism's innate immune response to pathogens involves coordinated and bidirectional communication between central and peripheral immune response systems (Blalock, 2005). As observed in the CNS, stressors also sensitize and potentiate the peripheral proinflammatory immune response to proinflammatory stimuli (Bailey et al., 2007, 2009; Johnson et al., 2002b, 2003; Powell et al., 2009; Stark et al., 2001). Further, stress modulates the activation state of peripheral macrophages. For example, social defeat stress upregulates the expression of the pattern recognition receptor TLR4 (Bailey et al., 2007), which is an immunophenotype also induced in microglia after social defeat stress (Wohleb et al., 2011). Thus, stress-induced hormones such as GCs and catecholamines may serve to integrate and facilitate the central and peripheral innate immune response to exogenous danger (i.e. pathogens, injury).

Taken together, the present results support the notion that exposure to an acute stressor or fight/flight emergency induces GCs, which function, in part, to “prime” or alert the organism's CNS innate system to potential “danger” signals. These signals may include exogenous pathogens and/or pathogen associated molecular patterns or endogenous danger signals such as alarmins, which can be released in response to a wide array of stimuli including sterile injury. The notion explored here is that stress-induced GCs can function as an endocrine “warning signal” to the CNS innate immune system to induce a preparatory response (microglial sensitization) to subsequent proinflammatory stimuli, thereby potentiating an immune response to threats (i.e. injury and infection), which are more likely to occur during a fight/flight emergency.

References

- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 1997; 25:3389–3402. [PubMed: 9254694]
- Anisman H. Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. *J Psychiatr Neurosci.* 2009; 34:4–20.
- Bailey MT, Engler H, Powell ND, Padgett DA, Sheridan JF. Repeated social defeat increases the bactericidal activity of splenic macrophages through a Toll-like receptor-dependent pathway. *Am J Physiol Regul Integr Comp Physiol.* 2007; 293:R1180–R1190. [PubMed: 17596326]
- Bailey MT, Kinsey SG, Padgett DA, Sheridan JF, Leblebicioglu B. Social stress enhances IL-1 β and TNF- α production by *Porphyromonas gingivalis* lipopolysaccharide-stimulated CD11b $^{+}$ cells. *Physiol Behav.* 2009; 98:351–358. [PubMed: 19560480]
- Beishuizen A, Thijs LG. Endotoxin and the hypothalamo-pituitary-adrenal (HPA) axis. *J Endotoxin Res.* 2003; 9:3–24. [PubMed: 12691614]
- Blalock JE. The immune system as the sixth sense. *J Intern Med.* 2005; 257:126–138. [PubMed: 15656872]
- Blandino P Jr, Barnum CJ, Solomon LG, Larish Y, Lankow BS, Deak T. Gene expression changes in the hypothalamus provide evidence for regionally-selective changes in IL-1 and microglial markers after acute stress. *Brain Behav Immun.* 2009; 23:958–968. [PubMed: 19464360]
- Borda JT, Alvarez X, Mohan M, Hasegawa A, Bernardino A, Jean S, Aye P, Lackner AA. CD163, a marker of perivascular macrophages, is up-regulated by microglia in simian immunodeficiency virus encephalitis after haptoglobin-hemoglobin complex stimulation and is suggestive of breakdown of the blood-brain barrier. *Am J Pathol.* 2008; 172:725–737. [PubMed: 18276779]
- Boumpas DT, Chrousos GP, Wilder RL, Cupps TR, Balow JE. Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. *Ann Intern Med.* 1993; 119:1198–1208. [PubMed: 8239251]
- Carpentier PA, Duncan DS, Miller SD. Glial toll-like receptor signaling in central nervous system infection and autoimmunity. *Brain Behav Immun.* 2008; 22:140–147. [PubMed: 17920811]
- De Bosscher K, Vanden Berghe W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev.* 2003; 24:488–522. [PubMed: 12920152]
- de Pablos RM, Villaran RF, Arguelles S, Herrera AJ, Venero JL, Ayala A, Cano J, Machado A. Stress increases vulnerability to inflammation in the rat prefrontal cortex. *J Neurosci.* 2006; 26:5709–5719. [PubMed: 16723527]
- Der-Avakian A, Will MJ, Bland ST, Deak T, Nguyen KT, Schmid MJ, Spencer RL, Watkins LR, Maier SF. Surgical and pharmacological suppression of glucocorticoids prevents the enhancement of morphine conditioned place preference by uncontrollable stress in rats. *Psychopharmacology (Berl).* 2005; 179:409–417. [PubMed: 15821955]

- Espinosa-Oliva AM, de Pablos RM, Villaran RF, Arguelles S, Venero JL, Machado A, Cano J. Stress is critical for LPS-induced activation of microglia and damage in the rat hippocampus. *Neurobiol Aging*. 2011; 32:85–102. [PubMed: 19286276]
- Fleshner M, Watkins LR, Lockwood LL, Grahn RE, Gerhardt G, Meaney MJ, Laudenslager ML, Maier SF. Blockade of the hypothalamic-pituitary-adrenal response to stress by intraventricular injection of dexamethasone: a method for studying the stress-induced peripheral effects of glucocorticoids. *Psychoneuroendocrinology*. 1993; 18:251–263. [PubMed: 8391148]
- Frank MG, Baratta MV, Sprunger DB, Watkins LR, Maier SF. Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS proinflammatory cytokine responses. *Brain Behav Immun*. 2007; 21:47–59. [PubMed: 16647243]
- Frank MG, Miguel ZD, Watkins LR, Maier SF. Prior exposure to glucocorticoids sensitizes the neuroinflammatory and peripheral inflammatory responses to *E. coli* lipopolysaccharide. *Brain Behav Immun*. 2010; 24:19–30. [PubMed: 19647070]
- Frank MG, Wieseler-Frank JL, Watkins LR, Maier SF. Rapid isolation of highly enriched and quiescent microglia from adult rat hippocampus: immunophenotypic and functional characteristics. *J Neurosci Methods*. 2006; 151:121–130. [PubMed: 16125247]
- Gleeson LC, Ryan KJ, Griffin EW, Connor TJ, Harkin A. The beta2-adrenoceptor agonist clenbuterol elicits neuroprotective, anti-inflammatory and neurotrophic actions in the kainic acid model of excitotoxicity. *Brain Behav Immun*. 2010; 24:1354–1361. [PubMed: 20599496]
- Goujon E, Parnet P, Laye S, Combe C, Kelley KW, Dantzer R. Stress downregulates lipopolysaccharide-induced expression of proinflammatory cytokines in the spleen, pituitary, and brain of mice. *Brain Behav Immun*. 1995; 9:292–303. [PubMed: 8903847]
- Hains LE, Loram LC, Taylor FR, Strand KA, Wieseler JL, Barrientos RM, Young JJ, Frank MG, Sobesky J, Martin TJ, Eisenach JC, Maier SF, Johnson JD, Fleshner M, Watkins LR. Prior laparotomy or corticosterone potentiates lipopolysaccharide-induced fever and sickness behaviors. *J Neuroimmunol*. 2011; 239:53–60. [PubMed: 21907418]
- Henley DE, Lightman SL. New insights into corticosteroid-binding globulin and glucocorticoid delivery. *Neuroscience*. 2011; 180:1–8. [PubMed: 21371536]
- Holfelder K, Schittenhelm J, Trautmann K, Haybaeck J, Meyermann R, Beschoner R. De novo expression of the hemoglobin scavenger receptor CD163 by activated microglia is not associated with hemorrhages in human brain lesions. *Histol Histopathol*. 2011; 26:1007–1017. [PubMed: 21692033]
- Jacobson L, Akana SF, Cascio CS, Shinsako J, Dallman MF. Circadian variations in plasma corticosterone permit normal termination of adrenocorticotropin responses to stress. *Endocrinology*. 1988; 122:1343–1348. [PubMed: 2831028]
- Joels M, Krugers HJ, Lucassen PJ, Karst H. Corticosteroid effects on cellular physiology of limbic cells. *Brain Res*. 2009; 1293:91–100. [PubMed: 19332034]
- Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Greenwood BN, Fleshner M. Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines. *Neuroscience*. 2005; 135:1295–1307. [PubMed: 16165282]
- Johnson JD, Cortez V, Kennedy SL, Foley TE, Hanson H 3rd, Fleshner M. Role of central beta-adrenergic receptors in regulating proinflammatory cytokine responses to a peripheral bacterial challenge. *Brain Behav Immun*. 2008; 22:1078–1086. [PubMed: 18468841]
- Johnson JD, O'Connor KA, Deak T, Spencer RL, Watkins LR, Maier SF. Prior stressor exposure primes the HPA axis. *Psychoneuroendocrinology*. 2002a; 27:353–365. [PubMed: 11818171]
- Johnson JD, O'Connor KA, Deak T, Stark M, Watkins LR, Maier SF. Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain Behav Immun*. 2002b; 16:461–476. [PubMed: 12096891]
- Johnson JD, O'Connor KA, Hansen MK, Watkins LR, Maier SF. Effects of prior stress on LPS-induced cytokine and sickness responses. *Am J Physiol Regul Integr Comp Physiol*. 2003; 284:R422–432. [PubMed: 12399247]
- Johnson JD, O'Connor KA, Watkins LR, Maier SF. The role of IL-1beta in stress-induced sensitization of proinflammatory cytokine and corticosterone responses. *Neuroscience*. 2004; 127:569–577. [PubMed: 15283957]

- Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol.* 2010; 11:373–384. [PubMed: 20404851]
- McNamee EN, Griffin EW, Ryan KM, Ryan KJ, Heffernan S, Harkin A, Connor TJ. Noradrenaline acting at beta-adrenoceptors induces expression of IL-1beta and its negative regulators IL-1ra and IL-1RII, and drives an overall anti-inflammatory phenotype in rat cortex. *Neuropharmacology.* 2010a; 59:37–48. [PubMed: 20361987]
- McNamee EN, Ryan KM, Griffin EW, Gonzalez-Reyes RE, Ryan KJ, Harkin A, Connor TJ. Noradrenaline acting at central beta-adrenoceptors induces interleukin-10 and suppressor of cytokine signaling-3 expression in rat brain: implications for neurodegeneration. *Brain Behav Immun.* 2010b; 24:660–671. [PubMed: 20193756]
- McNamee EN, Ryan KM, Kilroy D, Connor TJ. Noradrenaline induces IL-1ra and IL-1 type II receptor expression in primary glial cells and protects against IL-1beta-induced neurotoxicity. *Eur J Pharmacol.* 2009; 626:219–228. [PubMed: 19818755]
- Mori K, Ozaki E, Zhang B, Yang L, Yokoyama A, Takeda I, Maeda N, Sakanaka M, Tanaka J. Effects of norepinephrine on rat cultured microglial cells that express alpha1, alpha2, beta1 and beta2 adrenergic receptors. *Neuropharmacology.* 2002; 43:1026–1034. [PubMed: 12423672]
- Munck A, Naray-Fejes-Toth A. Glucocorticoids and stress: permissive and suppressive actions. *Ann N Y Acad Sci.* 1994; 746:115–130. discussion 131–113. [PubMed: 7825870]
- Munhoz CD, Lepsch LB, Kawamoto EM, Malta MB, Lima Lde S, Avellar MC, Sapolsky RM, Scavone C. Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor-kappaB in the frontal cortex and hippocampus via glucocorticoid secretion. *J Neurosci.* 2006; 26:3813–3820. [PubMed: 16597735]
- Munhoz CD, Sorrells SF, Caso JR, Scavone C, Sapolsky RM. Glucocorticoids exacerbate lipopolysaccharide-induced signaling in the frontal cortex and hippocampus in a dose-dependent manner. *J Neurosci.* 2010; 30:13690–13698. [PubMed: 20943909]
- Nair A, Bonneau RH. Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. *J Neuroimmunol.* 2006; 171:72–85. [PubMed: 16278020]
- Nguyen KT, Deak T, Owens SM, Kohno T, Fleshner M, Watkins LR, Maier SF. Exposure to acute stress induces brain interleukin-1beta protein in the rat. *J Neurosci.* 1998; 18:2239–2246. [PubMed: 9482808]
- O'Connor KA, Johnson JD, Hansen MK, Wieseler Frank JL, Maksimova E, Watkins LR, Maier SF. Peripheral and central proinflammatory cytokine response to a severe acute stressor. *Brain Res.* 2003; 991:123–132. [PubMed: 14575884]
- Oakley RH, Cidlowski JA. Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. *J Biol Chem.* 2011; 286:3177–3184. [PubMed: 21149445]
- Powell ND, Bailey MT, Mays JW, Stiner-Jones LM, Hanke ML, Padgett DA, Sheridan JF. Repeated social defeat activates dendritic cells and enhances Toll-like receptor dependent cytokine secretion. *Brain Behav Immun.* 2009; 23:225–231. [PubMed: 18848983]
- Sarkar NN. Mifepristone: bioavailability, pharmacokinetics and use-effectiveness. *Eur J Obstet Gynecol Reprod Biol.* 2002; 101:113–120. [PubMed: 11858883]
- Seckl JR. 11beta-Hydroxysteroid dehydrogenase in the brain: a novel regulator of glucocorticoid action? *Front Neuroendocrinol.* 1997; 18:49–99. [PubMed: 9000459]
- Sierra A, Gottfried-Blackmore A, Milner TA, McEwen BS, Bulloch K. Steroid hormone receptor expression and function in microglia. *Glia.* 2008; 56:659–674. [PubMed: 18286612]
- Sorrells SF, Sapolsky RM. An inflammatory review of glucocorticoid actions in the CNS. *Brain Behav Immun.* 2007; 21:259–272. [PubMed: 17194565]
- Stark JL, Avitsur R, Padgett DA, Campbell KA, Beck FM, Sheridan JF. Social stress induces glucocorticoid resistance in macrophages. *Am J Physiol Regul Integr Comp Physiol.* 2001; 280:R1799–R1805. [PubMed: 11353685]
- Tanaka J, Fujita H, Matsuda S, Toki K, Sakanaka M, Maeda N. Glucocorticoid- and mineralocorticoid receptors in microglial cells: the two receptors mediate differential effects of corticosteroids. *Glia.* 1997; 20:23–37. [PubMed: 9145302]

- Tilders FJ, Schmidt ED. Cross-sensitization between immune and non-immune stressors. A role in the etiology of depression? *Adv Exp Med Biol.* 1999; 461:179–197. [PubMed: 10442174]
- Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF. Beta-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. *J Neurosci.* 2011; 31:6277–6288. [PubMed: 21525267]

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Fig. 1.

Effect of the GR antagonist RU486 on stress-induced sensitization of the pro-inflammatory response to LPS in hippocampus. Animals were pretreated with RU486 (50 mg/kg s.c.) or vehicle (100% propylene glycol). One hour post-injection, animals were exposed to inescapable tailshock (IS) or served as home cage controls (HCC). Twenty four hours post-IS exposure, animals were injected with LPS (10 μ g/kg i.p.) or vehicle (0.9% saline). Two hours post-LPS or vehicle injection, hippocampal IL-1 β (A), NF κ B α (B) and CORT (C) levels were measured. Means with different letters are significantly different ($p < .05$). Data are presented as the mean $\pm$ SEM.

Fig. 2.

Effect of the GR antagonist RU486 on stress-induced sensitization of the microglial pro-inflammatory response to LPS *ex vivo*. Animals were pretreated with RU486 (50 mg/kg s.c.) or vehicle (100% propylene glycol). One hour post-injection, animals were exposed to inescapable shock (IS) or served as home cage controls (HCC). Twenty four hours post-IS exposure, microglia were isolated from hippocampus and challenged with LPS (0, 0.1, 1, and 100 ng/ml) for 2 h and microglial pro-inflammatory gene expression measured. LPS increased IL-1 β (A) and NF κ B1 α (C) gene expression in a concentration dependent manner in all experimental groups. To determine whether RU486 blunted stress-induced sensitization of the microglial pro-inflammatory response to LPS challenge, area under the LPS concentration curve (AUC) was computed for each animal and means compared for IL-1 β (B) and NF κ B1 α (D). Means with different letters are significantly different ($p < .05$). Data is presented as the mean $\pm$ SEM. Data in A and C are expressed as relative gene expression (RFU, relative fluorescence units) and as a percentage of HCC/vehicle/media (0 LPS) levels.

Fig. 3.

Verification of the effect of adrenalectomy (ADX) on stress-induced serum corticosterone (CORT) levels. Two weeks post sham surgery or ADX, animals were exposed to inescapable tailshock (IS) or served as home cage controls (HCC). Twenty four hours post-stress exposure, serum CORT levels were measured in animals concomitant with isolation of hippocampal microglia. Means with different letters are significantly different ($p < .05$). Data is presented as the mean $\pm$ SEM.

Fig. 4.

Effect of adrenalectomy (ADX) on stress-induced sensitization of the microglial pro-inflammatory response to LPS *ex vivo*. Two weeks post sham surgery or ADX, animals were exposed to inescapable tailshock (IS) or served as home cage controls (HCC). Twenty four hours post-IS exposure, microglia were isolated from hippocampus and challenged with LPS (0, 0.1, 1, and 100 ng/ml) for 2 h and microglial pro-inflammatory gene expression measured. LPS increased IL-1 β (A), NF κ B α (C), IL-6 (E) and TNF α (G) gene expression in a concentration dependent manner in all experimental groups. LPS failed to modulate TLR4 (I) gene expression in a concentration dependent manner. To determine whether ADX blunted stress-induced sensitization of the microglial pro-inflammatory response to LPS challenge, area under the LPS concentration curve (AUC) was computed for each animal

and means compared for IL-1 β (B), NF κ B1 α (D), IL-6 (F), TNF α (H) and TLR4 (J). Means with different letters are significantly different ($p < .05$). Data is presented as the mean $\pm$ SEM. Data in A, C, E, G and I are expressed as relative gene expression (RFU, relative fluorescence units) and as a percentage of HCC/vehicle/media (0 ng LPS) levels.

Table 1

Primer description and sequence.

Gene	Primer sequence 5' → 3'	Function
β-Actin	F: TTCCTTCCTGGGTATGGAAT R: GAGGAGCAATGATCTTGATC	Cytoskeletal protein (Housekeeping gene)
CD 163	F: GTAGTAGTCATTCAACCCTCAC R: CGGCTTACAGTTTCCTCAAG	Macrophage antigen not expressed by microglia
Glial fibrillary acidic protein (GFAP)	F: AGATCCGAGAAACCAGCCTG R: CCTTAATGACCTCGCCATCC	Astrocyte antigen
Interleukin (IL)-1β	F: CCTTGTGCAAGTGTCTGAAG R: GGGCTTGGAAGCAATCCTTA	Pro-inflammatory cytokine
IL-6	F: AGAAAAGAGTTGTGCAATGGCA R: GGCAAATTCCTGGTTATATCC	Pro-inflammatory cytokine
Ionized calcium binding adapter protein (Iba-1)	F: GGCAATGGAGATATCGATAT R: AGAATCATTCTCAAGATGGC	Microglia/macrophage antigen
Major histocompatibility complex (MHC)II	F: AGCACTGGGAGTTTGAAGAG R: AAGCCATCACCTCCTGGTAT	Microglia/macrophage antigen
NFκB inhibitor, α (NFKBIA)	F: CACCAACTACAACGGCCACA R: GCTCCTGAGCGTTGACATCA	Induced by NFκB to inhibit NFκB function
Tumor necrosis factor (TNF)α	F: CAAGGAGGAGAAGTTCCCA R: TTGGTGTTTGCTACGACG	Pro-inflammatory cytokine