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Glucocorticoids mediate stress induction of the alarmin HMGB1 and reduction of the microglia checkpoint receptor CD200R1 in limbic brain structures

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Abstract

Exposure to stressors primes neuroinflammatory responses to subsequent immune challenges and stress-induced glucocorticoids (GCs) play a mediating role in this phenomenon of neuroinflammatory priming. Recent evidence also suggests that the alarmin high-mobility group box-1 (HMGB1) and the microglial checkpoint receptor CD200R1 serve as proximal mechanisms of stress-induced neuroinflammatory priming. However, it is unclear whether stress-induced GCs play a causal role in these proximal mechanisms of neuroinflammatory priming; this forms the focus of the present investigation. Here, we found that exposure to a severe acute stressor (inescapable tailshock) induced HMGB1 and reduced CD200R1 expression in limbic brain regions and pharmacological blockade of GC signaling (RU486) mitigated these effects of stress. To confirm these effects of RU486, adrenalectomy (ADX) with basal corticosterone (CORT) replacement was used to block the stress-induced increase in GCs as well as effects on HMGB1 and CD200R1. As with RU486, ADX mitigated the effects of stress on HMGB1 and CD200R1. Subsequently, exogenous CORT was administered to determine whether GCs are sufficient to recapitulate the effects of stress. Indeed, exogenous CORT induced expression of HMGB1 and reduced expression of CD200R1. In addition, exposure of primary microglia to CORT also recapitulated the effects of stress on CD200R1 suggesting that CORT acts directly on microglia to reduce expression of CD200R1. Taken together, these findings suggest that GCs mediate the effects of stress on these proximal mechanisms of neuroinflammatory priming.

Keywords

stress; glucocorticoid; neuroinflammation; priming; alarmin; HMGB1; CD200R1

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1. Introduction

Glucocorticoids (GCs) are one of the main endocrine effectors of the stress response, which comprises an array of physiological changes that support the fight/flight response that occurs in the face of aversive and/or threatening environmental stimuli (Sapolsky et al., 2000). Since Selye's pioneering studies on stress-induced thymic involution (Selye, 1950), a large number of studies have reinforced the perspective that stress and stress hormones are largely immunosuppressive (Webster Marketon and Glaser, 2008). However, this perspective stems in large part from studies of peripheral immune function. In the CNS though, GCs exhibit seemingly paradoxical effects on neuroinflammatory processes depending on the dose or level, timing, and duration of stressor/GC exposure. While it is evident that exogenous GCs exert anti-inflammatory effects in a vast number of inflammatory conditions (Boumpas et al., 1993), in the context of environmental challenge, the immunological effects of endogenous GCs are more nuanced (Sorrells et al., 2009). Indeed, the immediate effects of acute stressor exposure are largely anti-inflammatory due to the large rise in systemic GC levels, which are thought to constrain the neuroinflammatory effects of stress (Sorrells and Sapolsky, 2007). However, accumulating evidence now suggests that stress-induced GCs also *augment* the neuroinflammatory response to *subsequent* immune challenges that occur at a later time-point when GCs have diminished to near basal levels (Frank et al., 2016).

This phenomenon of stress-induced neuroinflammatory priming has now been demonstrated in a large number of studies using an array of stress paradigms, immune challenges and measures of inflammatory endpoints (Cheng et al., 2016; de Pablos et al., 2014; de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Fonken et al., 2018; Fonken et al., 2016; Frank et al., 2007; Frank et al., 2018a; Frank et al., 2018b; Frank et al., 2012; Franklin et al., 2018; Johnson et al., 2002; Johnson et al., 2003; Johnson et al., 2004; Munhoz et al., 2006; Sun et al., 2017; Wang et al., 2017; Weber et al., 2013; Weber et al., 2015; Wohleb et al., 2012; Wohleb et al., 2011). Of these studies, a subset have demonstrated that pharmacological blockade of GC signaling during stressor exposure attenuates stress-induced priming of the neuroinflammatory response to immune challenges, suggesting a causal role for GCs in stressor-induced priming (de Pablos et al., 2014; de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Frank et al., 2012; Munhoz et al., 2006; Sun et al., 2017; Wang et al., 2017). Consistent with these findings, we have found that surgical suppression (adrenalectomy) of the stress-induced GC response also blocked priming of the neuroinflammatory response (Frank et al., 2012). While these studies have demonstrated the necessity of GCs in stress-induced neuroinflammatory priming, a number of studies have also found that exogenous GC administration recapitulates the neuroinflammatory priming effects of stress, suggesting sufficiency as well (Fonken et al., 2018; Frank et al., 2014; Frank et al., 2010; Kelly et al., 2018; Kelly et al., 2012; Koo et al., 2018; Loram et al., 2011; Munhoz et al., 2010; O'Callaghan et al., 2015). Although these studies implicate a key role for GCs, they fail to clarify the proximal neuroimmune mechanism(s) by which stress-induced GCs elaborate their neuroinflammatory priming effects. This is the focus here.

One proximal mechanism of stressor-induced neuroinflammatory priming involves the production of the alarmin high mobility group box 1 (HMGB1). HMGB1 is considered to be

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the prototypical endogenous danger molecule (DAMP) and it exerts an array of cytokine, chemokine, neuroimmune and metabolic functions (Yang et al., 2015). Several studies have found that stressor exposure induces HMGB1 in the CNS (Cheng et al., 2016; Frank et al., 2018a; Frank et al., 2018b; Franklin et al., 2018; Lian et al., 2017; Weber et al., 2015), and pharmacological blockade of HMGB1 signaling in brain abrogates stressor-induced priming of microglia (Weber et al., 2015). In addition, exogenous administration of the disulfide form of HMGB1 is sufficient to recapitulate the priming effects of stressors (Frank et al., 2015; Lian et al., 2017) and disulfide HMGB1 directly primes microglia (Frank et al., 2015). A second proximal mechanism of neuroinflammatory priming produced by stressors involves downregulation of the microglial checkpoint receptor CD200R1, which constrains microglia activation when ligated by CD200 (Deczkowska et al., 2018). We recently found that stressor exposure reduces expression of CD200R1 in limbic brain structures as well as CNS microglia (Fonken et al., 2018; Frank et al., 2018a; Frank et al., 2018b), suggesting that stressors disinhibit microglia via reduction of CD200:CD200R1 signaling. In addition, we found that stressor induction of HMGB1 is a consequence of diminished CD200:CD200R1 signaling (Frank et al., 2018a).

Given these findings, the present investigation examined whether 1) GCs mediate the effects of stressor exposure on these proximal mechanisms of neuroinflammatory priming, 2) exogenous GCs are sufficient to recapitulate the effects of stressors on these mechanisms and 3) direct exposure of microglia to GCs is also sufficient to recapitulate the effects of stressors on these mechanisms.

2. Materials and Methods

2.1. Animals

Male Sprague-Dawley rats (60–90 d old; Envigo) were pair-housed with food and water available *ad libitum*. The colony was maintained at 22 °C on a 12 h light/dark cycle (lights on at 07:00 h). All experimental procedures were conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals and approvals from the University of Colorado Institutional Animal Care and Use Committee. Studies were restricted to male rats because prior studies of GC-, HMGB1-and CD200R1-mediated neuroinflammatory priming have not been conducted in female rats

2.2. Pharmacological inhibition of stress-induced GC signaling

Rats were injected subcutaneously with vehicle (100% propylene glycol; MilliporeSigma, cat#: P4347) or the GC receptor antagonist RU486 (50 mg/kg; MilliporeSigma, cat#: M8046). These dosing parameters were based on our prior work demonstrating that this dose and route of RU486 administration blocks stress-induced neuroinflammatory and microglia priming (Frank et al., 2012). RU486 or vehicle was injected 24 h prior to stressor exposure to minimize the effects of injection stress on neuroimmune endpoints. It is important to note that RU486 has a long half-life (20 – 30 h)(Sarkar, 2002) and thus should be bioactive at the time of stress exposure. 24 h post-injection, rats were exposed to inescapable tailshocks (IS; see section 2.4.) or served as home cage controls (HCCs). Immediately after termination of the stressor, rats were anesthetized, perfused with saline

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and whole brain flash frozen in isopentane for micropunching of brain regions. This immediate time-point post-stress was selected based on our prior work demonstrating robust inductions of HMGB1 and reductions in CD200R1 in hippocampus and amygdala (Frank et al., 2018a). A limitation of RU486 is that it also functions as a progesterone receptor antagonist (Sarkar, 2002), which confounds the effects of this pharmacological approach. Thus, an alternative experimental approach (bilateral adrenalectomy; ADX) was added to confirm the effects of RU486.

2.3 Surgical suppression of stress-induced GC secretion

ADX was aseptically performed under isoflurane anesthesia as previously described (Frank et al., 2012). Adrenal tissues were visually inspected to confirm that all removed adrenal glands were intact. Sham-operated rats received the identical procedure, except that the adrenal glands were gently manipulated with forceps, but not removed. Corticosterone (CORT; MilliporeSigma, cat#: 27840) replacement began for ADX rats immediately after surgery and continued for the remainder of the experiment. CORT replacement was utilized because the rationale was to eliminate the IS-induced rise of CORT, but not eliminate basal levels. ADX rats received basal CORT 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 100% ethyl alcohol (EtOH) and diluted to a final concentration of 25 μg/ml in tap water to yield 0.2% EtOH. This concentration of CORT leads to normal fluctuations in basal levels across the light/dark cycle. Thus, ADX rats exhibit a pattern of serum CORT levels across the light/dark cycle that mimics the pattern observed in non-ADX HCC rats. CORT-water also contained 0.9% saline to compensate for the loss of aldosterone. Sham rats received drinking water containing 0.2% EtOH and 0.9% saline. Rats were allowed 2 weeks to recover from surgery before exposure to IS. Hippocampal CORT levels were measured to verify the effects of ADX on stress-induced CORT (see section 2.10)

2.4. Exogenous CORT administration

Rats were injected subcutaneously with vehicle (100% propylene glycol) or CORT (2.5 mg/kg). Two hours post-injection, rats were saline-perfused, whole brains were removed and flash frozen in isopentane for micropunching of brain regions. These dosing parameters were selected because we have previously shown that this dose and route of administration results in serum CORT levels that reproduce the pattern of serum CORT levels observed during and after exposure to IS (Fleshner et al., 1995). We have also found that this dose and route of CORT administration primes the neuroinflammatory and microglial responses to an immune challenge (Frank et al., 2010). The duration of IS exposure (~ 2h) served as the basis for selecting the time-point post-injection for euthanasia.

2.5. Inescapable tail-shock (IS)

Details of the stressor protocol have been published previously and this protocol reliably potentiates pro-inflammatory cytokine responses in rat hippocampus after peripheral immune challenge (Johnson et al., 2003) as well as in isolated rat hippocampal microglia to LPS *ex vivo* (Frank et al., 2007). Briefly, rats were placed in Plexiglas tubes (23.4 cm in length × 7 cm in diameter) and exposed to 100–1.6 mA, 5 s tail-shocks with a variable inter-

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trial interval (ITI) ranging from 30 – 90 s (average ITI = 60 s). All IS treatments occurred between 09:00 and 11:00 h. IS rats were immediately euthanized after termination of shock. HCC rats remained undisturbed in their home cages.

2.6. Tissue collection

Animals were given a lethal dose of sodium pentobarbital. Rats were fully anesthetized and then transcardially perfused with ice-cold saline (0.9%) for 3 min to remove peripheral immune leukocytes from the CNS vasculature. For micropunching of hippocampal and amygdalar sub-regions, whole brain was flash frozen in isopentane. All tissue samples were stored at −80 °C. For *in vitro* experiments, whole brain microglia were immediately isolated.

2.7. Hippocampal and amygdala micropunching

Brains were sectioned at 50 μm increments on a Leica cryostat at −20 °C until the region of interest was reached. Tissue punches were then excised from discrete regions of dorsal hippocampus (CA1, CA3, and dentate gyrus) and basolateral (BLA) and central nucleus (CEA) of the amygdala using a brain punch tool (1 mm diameter × 1 mm depth). Tissue punches (2 per region per hemisphere) were stored at −80 °C until assayed. One hemisphere was used for assay of gene expression and one for protein.

2.8. Microglia isolation and stimulation with CORT in vitro

Whole brain microglia were isolated from adult male rats using a Percoll (GE Healthcare, cat#: 17089101) density gradient as previously described (Frank et al., 2006). This procedure of isolating cells takes ~1.5 h. We have previously shown (Frank et al., 2006) that this microglia isolation procedure yields highly pure microglia (Iba-1+/CD11b+/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.

2.9. CORT stimulation in vitro

CORT was dissolved in 100% EtOH to yield a 20 mM solution. CORT was serially diluted in media (DMEM + 10% FBS) to yield 1 μM, 0.1 μM and 0.01 μM concentrations. Microglia concentration was adjusted to a density of 4×10^4 cells/90 μl of media and 90 μl added to individual wells of a 96-well v-bottom plate. CORT (10 μl) was added to wells to yield final concentrations of 100 nM, 10 nM and 1 nM. All CORT conditions had a final EtOH concentration of 0.005%. CORT concentrations were derived from our prior work (Fonken et al., 2016). The media control well had 10 μl of 0.05% EtOH in media added to yield 0.005% EtOH. Cells were incubated for 3h at 37 °C at 5% CO_2. The plate was centrifuged at 1000 × g for 10 min at 4 °C to pellet cells. Supernatant was collected for protein assays. Cells were washed 1x in ice cold PBS to remove trace media and centrifuged at 1000 × g for 10 min at 4 °C. Cells were lysed using the CellsDirect Resuspension and Lysis Buffer (ThermoFisher, cat#: 11739010). Lysate was stored at −80 °C until cDNA synthesis, which was performed using the SuperScript IV Reverse Transcriptase kit (ThermoFisher, cat#: 18090050).

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2.10. Real time RT-PCR measurement of gene expression

Total RNA was isolated from hippocampal and amygdalar micropunches using TRI Reagent (MilliPore Sigma, cat#: 93289) and a standard method of phenol:chloroform extraction (Chomczynski and Sacchi, 1987). Total RNA was quantified using a NanoDrop 2000 spectrophotometer (TheroFisher). cDNA synthesis was performed using the SuperScript II Reverse Transcriptase kit (ThermoFisher, cat#: 18064014). 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/tools/oligo-analysis-tool.aspx) and tested for sequence specificity using the Basic Local Alignment Search Tool at NCBI (Altschul et al., 1997). Primers were obtained from ThermoFisher. Primer specificity was verified by melt curve analyses. All primers were designed to span exon/exon boundaries and thus exclude amplification of genomic DNA. Primer sequences were as follows: Beta-actin (*Actb*), F: TTCCTTCCTGGGTATGGAAT, R: GAGGAGCAATGATCTTGATC; *Cd200*, F: CTCTCTATGTACAGCCCATAG, R: GGGAGTGACTCTCAGTACTAT; *Cd200r1*, F: TAGAGGGGGTGACCAATTAT, R: TACATTTTCTGCAGCCACTG; CCAAT/enhancer binding protein, beta (*Cebpb*), F: TTCGGGACTTGATGCAATCC, R: CCCGCAGGAACATCTTTAAG. PCR amplification of cDNA was performed using the Quantitect SYBR Green PCR Kit (Qiagen, cat#: 204145). Formation of PCR product was monitored in real time using the CFX96 Touch Real-Time PCR Detection System (BioRad). Relative gene expression was determined using β-Actin as the housekeeping gene and the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.11. CORT and HMGB1 ELISA

Hippocampal and amygdalar micropunches were sonicated using a tissue extraction reagent (TheroFisher, cat#: FNN0071) supplemented with a protease inhibitor cocktail (MilliporeSigma, cat#: P2714). Homogenate was centrifuged (10 min, 14,000 × g, 4 °C) and supernatant collected and stored at −80 °C. Total protein was quantified using a Bradford assay. CORT was measured using a competitive immunoassay (Enzo LifeSciences, cat#: ADI-900–097) as described in the manufacturer's protocol. CORT levels are presented as pg/mg total protein. HMGB1 protein was measured using a standard colorimetric sandwich ELISA (LifeSpan Biosciences, cat#: LS-F4039). HMGB1 protein was quantified as pg/mg total protein.

2.12. Statistical analysis and data presentation

All data are presented as mean + SEM. Statistical analyses consisted of ANOVA followed by post-hoc tests (Tukey's) using StatView. For the effect of RU486, a 2 (vehicle vs RU486) × 2 (HCC vs IS) × 5 (brain region) mixed ANOVA was conducted. For the effect of ADX, a 2 (sham vs ADX) × 2 (HCC vs IS) × 5 (brain region) mixed ANOVA was conducted. For the effect of exogenous CORT, a 2 (vehicle vs CORT) × 5 (brain region) mixed ANOVA was conducted. For *in vitro* experiments, four replications were conducted on whole brain microglia isolated from 4 adult rats and a one-way ANOVA was conducted. Threshold for statistical significance was set at $\alpha = 0.05$. Sample sizes are provided in figure captions.

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3. Results

3.1. RU486 modulates the effects of stress

Here, pharmacological blockade of GC signaling was employed to determine a causal role for GCs in IS-induced mechanisms of priming. A major concern with using RU486 is the potential for altering GC negative feedback on the HPA axis, thereby altering the CORT response to IS. Thus, we examined the effect of RU486 on stress-induced CORT in hippocampal and amygdalar sub-regions. While the 3-way (drug × stress × brain region) and 2-way (drug × stress) interaction was not significant, the main effect of IS on CORT levels was significant ($df = 1, 28$; $F = 135.8$, $p < 0.0001$), indicating that RU486 failed to alter the IS-induced increase in CORT in any of the brain regions (Fig. 1A).

As noted, exposure to IS induces HMGB1, while reducing *Cd200r1* expression, and both are necessary for IS-induced neuroinflammatory priming. Thus, we examined whether pharmacological blockade of GR signaling during IS would mitigate these effects of IS. We found that RU486 modulated the effect of IS on HMGB1 (2-way interaction; $df = 1, 28$; $F = 17.05$, $p = 0.003$; Fig. 1B) and *Cd200r1* (2-way interaction; $df = 1, 28$; $F = 73.27$, $p < 0.0001$; Fig. 1C). However, these interactions occurred independent of brain region. In vehicle-treated rats, IS exposure resulted in a robust increase in HMGB1 ($p < 0.001$) and a decrease in *Cd200r1* ($p < 0.001$). RU486 treatment significantly reduced HMGB1 ($p < 0.001$) and increased *Cd200r1* ($p < 0.001$) compared to vehicle in IS-exposed subjects. While RU486 failed to affect HMGB1 in HCCs, RU486 inexplicably reduced *Cd200r1* expression in HCCs ($p < 0.001$).We also examined the effect of stress and RU486 on *Cd200* expression, which functions as the cognate ligand for *Cd200r1*. We found that stress and RU486 failed to significantly alter *Cd200* expression (Suppl. Fig. 1A).

We previously demonstrated that exposure to IS induces robust increases in hippocampal *Cebpb* expession (Frank et al., 2018a), which functions as a transcriptional repressor of *Cd200r1* expression (Dentesano et al., 2012). Thus, given the effect of RU486 on stress-induced decreases in *Cd200r1*, we examined the effect on *Cebpb*. Consistent with the effects on *Cd200r1*, there was a significant 2-way interaction between RU486 treatment and IS with regard to *Cebpb* expression ($df = 1, 28$; $F = 8.74$, $p = 0.0063$). Post-hoc comparisons show that IS (vehicle-treated) significantly increased *Cebpb* expression compared to vehicle-treated HCCs ($p < 0.001$) and RU486-treated HCCs ($p < 0.001$). In IS-exposed subjects, RU486 significantly reduced *Cebpb* expression compared to vehicle-treated subjects ($p < 0.001$)(Fig. 1D).

3.2. ADX modulates the effects of stress

As noted above, RU486 functions not only as a GR antagonist, but also as a progesterone receptor (PR) antagonist. It is important to consider that progesterone is detectable in adult male rat hippocampus (Hojo and Kawato, 2018) and the PR is broadly expressed throughout the brain in most cell types (Brinton et al., 2008; Habib and Beyer, 2015). In addition, progesterone exerts an array of non-reproductive functions in the CNS (Brinton et al., 2008; Schumacher et al., 2014). Given the pervasive non-reproductive roles of progesterone in the CNS, results stemming from the use of RU486 as a GR antagonist present interpretive

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challenges. Therefore, we utilized ADX, which involves surgical suppression of stress-induced GCs, with basal CORT replacement, as a convergent experimental approach to confirm the effects of RU486.

To verify the effects of ADX, we measured GC levels in hippocampal and amygdalar sub-regions immediately after termination of the stressor. We found that the interaction between ADX and IS was significant ($df = 1, 28$, $F = 203.59$, $p < 0.0001$)(Fig. 2A) and this effect occurred independent of brain region. In surgery-control (sham) rats, IS induced a robust increase in CORT in all brain regions compared to HCCs (vs. sham, $p < 0.0001$; vs. ADX, $p < 0.0001$). However, ADX resulted in a complete abrogation of the CORT response to the stressor ($p < 0.0001$) indicating that ADX successfully suppressed the stress-induced rise in central GCs. Importantly, ADX failed to significantly affect basal GC levels in HCCs, indicating that CORT replacement in drinking water maintained basal CORT levels in brain.

Similar to the effects of RU486, ADX with basal CORT replacement modulated the effect of IS on HMGB1 (2-way interaction; $df = 1, 28$, $F = 7.94$, $p = 0.009$; Fig. 2B),*Cd200r1* (2-way interaction; $df = 1, 28$, $F = 18.88$, $p = 0.0002$; Fig. 2C) and *Cebpb* (2-way interaction; $df = 1, 28$, $F = 176.6$, $p < 0.0001$; Fig. 2D). However, these interactions did not vary as a function of brain region. In sham rats, IS exposure resulted in a robust increase in HMGB1 ($p < 0.001$), a decrease in *Cd200r1* ($p < 0.001$) and an increase in *Cebpb* ($p < 0.001$). While ADX treatment failed to affect HMGB1, *Cd200r1* and *Cebpb* in HCCs, ADX treatment significantly reduced HMGB1 ($p < 0.05$), increased *Cd200r1* ($p < 0.001$) and reduced *Cebpb* ($p < 0.001$) compared to sham treatment in IS-exposed rats. As with RU486, *Cd200* expression was not significantly altered by stress and ADX (Suppl. Fig. 1B).

3.3. Effects of exogenous CORT

The effects of RU486 and ADX provide convergent evidence that stress-induced GCs are necessary for the effects of stress on HMGB1, *Cd200r1* and *Cebpb*. These findings raise the possibility that GCs might be sufficient to recapitulate the effects of stress. Therefore, we examined the effect of exogenous CORT on these neuroimmune endpoints. Initially, we measured CORT levels in hippocampal and amygdalar sub-regions 2h after administration to verify that this treatment increased CORT levels. Indeed, exogenous CORT increased CORT levels uniformly in all brain regions (main effect; $df = 1, 18$, $F = 19.47$, $p = 0.0003$; Fig. 3A). Exogenous CORT also increased HMGB1 (main effect; $df = 1, 16$, $F = 5.04$, $p = 0.04$; Fig. 3B), decreased *Cd200r1* (main effect; $df = 1, 18$, $F = 16.96$, $p = 0.0006$; Fig. 3C) and increased *Cebpb* (main effect; $df = 1, 18$, $F = 6.53$, $p = 0.002$; Fig. 3D) across all brain regions. CORT failed to affect expression of *Cd200* (Suppl. Fig. 1C).

3.4. Effects of CORT on microglia in vitro

These effects of exogenous CORT prompted us to explore the possibility that CORT directly modultes microglial expression of HMGB1, *Cd200r1* and *Cebpb*. HMGB1 was undetectable in cell culture supernatants at all concentrations of CORT. However, CORT exposure resulted in a concetration-dependent decrease in *Cd200r1* ($df = 3, 12$, $F = 4.92$, $p = 0.02$) and a concentration dependent increase in *Cebpb* ($df = 3, 12$, $F = 9.44$, $p = 0.002$)(Fig. 4). CORT resulted in a significant decreae in *Cd200r1* at 10 nM ($p = 0.01$) and 100 nM ($p = 0.02$)

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compared to media control, while CORT increased *Cebpb* at 10 nM ($p = 0.005$) and 100 nM ($p = 0.006$) compared to media control.

4. Discussion

It has been a bedrock principle, since their discovery and characterization in the later 1940s (Saenger, 2010), that GCs are uniformly anti-inflammatory (Boumpas et al., 1993; Cain and Cidlowski, 2017). However, this principle has recently been challenged by several lines of evidence suggesting that GCs can, in some cases, exacerbate or prime inflammatory processes (Sorrells and Sapolsky, 2007). Indeed, this function of GCs has been well characterized in a number of stress paradigms demonstrating that GCs mediate the neuroinflammatory priming effects of stress (de Pablos et al., 2014; de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Frank et al., 2012; Munhoz et al., 2006; Sun et al., 2017; Wang et al., 2017). Moreover, administration of exogenous GCs recapitulates these priming effects of stress (Fonken et al., 2018; Frank et al., 2014; Frank et al., 2010; Kelly et al., 2018; Kelly et al., 2012; Koo et al., 2018; Loram et al., 2011; Munhoz et al., 2010; O'Callaghan et al., 2015). However, the neurobiological mechanism(s) by which GCs exert this function has largely been unexplored.

We have previously demonstrated that the alarmin HMGB1 as well as the microglial checkpoint receptor CD200R1 play a mediating role in the neuroinflammatory priming effects of IS (Frank et al., 2018a; Weber et al., 2015). In the present study, we explored whether GCs mediate the effects of stress on these two mechanisms of neuroinflammatory priming. Our initial study utilized the GC receptor antagonist RU486 (mifepristone) to block GC signaling during stress exposure, and thus test whether GCs mediate the effects of stress on HMGB1 and CD200R1. Consistent with prior studies, we found that stress exposure induced a robust increase in HMGB1 protein (Cheng et al., 2016; Frank et al., 2018a; Frank et al., 2018b; Franklin et al., 2018; Lian et al., 2017; Weber et al., 2015) and a decrease in CD200R1 mRNA (Fonken et al., 2018; Frank et al., 2018a; Frank et al., 2018b) in both amygdalar and hippocampal sub-regions. We also examined the effect of stress on the transcription factor C/EBPβ, which serves to repress transcription of CD200R1 (Dentesano et al., 2012). Consistent with our prior study (Frank et al., 2018a), IS exposure induced a robust increase in C/EBPβ mRNA expression in all brain regions. Treatment with RU486 mitigated, but failed to fully block, all of these effects of stress. Of note, stress and RU486 failed to affect CD200 expression, which is the cognate ligand of CD200R1, which is consistent with our findings that stress selectively affects CD200R1 as part of this signaling dyad (Frank et al., 2018a). In addition, RU486 treatment failed to alter the CORT response to stress. Of course, brain CORT levels were only measured at a single time-point post-stress (immediately after), thus we cannot exclude the possibility that RU486 interfered with the CORT response during stress exposure. Clearly, if RU486 affected the CORT response during stress exposure, this might serve to confound the effects of RU486. Dalm et al. have recently demonstrated that single dose RU486 potentiates the CORT response to novelty, whereas multiple dosing suppresses the CORT response (Dalm et al., 2019). These findings illustrate the complex pharmacological actions of RU486, which might explain why RU486 failed to fully block the effects of stress on CD200R1, C/EBPβ and HMGB1. Furthermore, we found that RU486 treatment in home cage controls had anomalous effects (reduction) on

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CD200R1, but not on C/EBPβ and HMGB1. We previously found that RU486 had similar anomalous effects on proinflammatory cytokine expression in the hippocampus (Frank et al., 2012). It is unclear how RU486 exert these anomalous effects. Nevertheless, the effects observed here of RU486 suggest that GCs mediate, in part, the effects of stress on these mechanisms of neuroinflammatory priming. However, RU486 is not selective for the GC receptor, but also serves as an antagonist of the progesterone receptor, which is ubiquitously expressed throughout the CNS (Brinton et al., 2008; Habib and Beyer, 2015). In addition, progesterone exerts an array of non-reproductive functions in the CNS (Brinton et al., 2008; Schumacher et al., 2014). Clearly, these considerations warrant caution when interpreting results from the use of RU486. Therefore, we utilized an alternate approach, surgical suppression of the CORT response to the stressor (adrenalectomy; ADX) with basal CORT replacement, to validate the effects of RU486.

As with the RU486 study, we found that stressor exposure induced robust increases in CORT, HMGB1, and C/EBPβ concomitant with decreases in CD200R1 in all brain regions. Consistent with our prior study (Frank et al., 2012), ADX completely abrogated the CORT response to stress. Notably, brain CORT levels were similar in home cage control rats indicating that CORT replacement in drinking water maintained basal CORT levels in ADX rats. Consistent with the effects of RU486, ADX treatment mitigated the effects of stress on CD200R1, while ADX nearly completely blocked the effects of stress on HMGB1 and C/EBPβ. As with the RU486 study, stress and ADX failed to affect CD200 expression. Taken together, the effects of RU486 and ADX provide converging evidence that GCs mediate, in part, the effects of stress on these mechanisms of priming. However, as with RU486, ADX is not without its limitations. Most notably, ADX also suppresses the epinepherine response to stressors, and epinephrine also exerts effects on innate immune cells (Dhabhar et al., 2012). In light of these results, we examined the possibility that GCs might be sufficient to recapitulate these neuroimmune effects.

Here, exogenous CORT was administered at a dose that induces an endogenous CORT pattern that mimics the pattern induced by stress exposure (Fleshner et al., 1995). In addition, this dose of CORT primes the neuroinflammatory response to subsequent immune challenges (Frank et al., 2010). Consistent with these neuroinflammatory priming effects, exogenous CORT induced HMGB1 and C/EBPβ expression, while decreasing CD200R1 expression in all brain regions. These results suggest that CORT is sufficient to recapitulate the effects of stressors on these mechanisms of priming, although it is important to point out that the magnitude of these effects do not fully correspond with the magnitude of stressor effects. This might be due to the higher CORT levels observed in vehicle-injected animals, which might be attributable to injection-stress. Given these results, we examined whether CORT might directly induce these effects in isolated microglia and thus recapitulate the effects of stress. Indeed, consistent with our prior findings (Fonken et al., 2018), we found that CORT reduced CD200R1 expression in a concentration dependent fashion. In addition, we found that CORT induced C/EBPβ expression. We attempted to measure HMGB1 protein in supernatants, but it was undetectable. The present data do not illuminate the molecular mechanism(s) whereby GCs prime microglia or other cells. However, as microglia express GC receptors (Sierra et al., 2008), these data suggest that GCs might act directly on microglia to elaborate their effects. Interestingly, GCs, via the GC receptor, induce the

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expression and DNA binding activity of C/EBPβ at promoter elements (CCAAT) of target genes (Roos and Nord, 2012). As noted, the CD200R1 promoter contains this promoter element to which C/EBPβ binds and represses transcription of CD200R1 (Dentesano et al., 2012). This mechanism of GC action might mediate the effects observed here of GCs on C/EBPβ and CD200R1 in microglia.

CD200:CD200R1 signaling is one of several well-characterized microglial checkpoint mechanisms that serve to restrain the immune activity of microglia (Deczkowska et al., 2018). Via ligation by CD200, CD200R1 is thought to constitutively inhibit myeloid cell function (Gorczynski, 2005). CD200 is a membrane glycoprotein that is expressed ubiquitously in the CNS on neurons, endothelial cells and oligodendrocytes (Koning et al., 2009; Wright et al., 2000). Upon binding CD200R1, CD200 initiates an intra-cellular signaling cascade that results in general inhibition of myeloid cell function including pro-inflammatory cytokine responses to immune stimuli (Gorczynski et al., 2008; Jenmalm et al., 2006; Zhang et al., 2004). Indeed, disruption of CD200:CD200R1 signaling potentiates the microglial pro-inflammatory response to immune challenges (Costello et al., 2011; Denieffe et al., 2013), as well as exacerbates disease severity and progression in neuroinflammatory disease models (Hoek et al., 2000; Meuth et al., 2008; Wright et al., 2000). Consistent with these findings, we have recently demonstrated that exposure to IS disrupts CD200:C200R1 signaling, which results in dis-inhibition of microglia and priming of neuroinflammatory processes (Frank et al., 2018a). The present results suggest that GCs play a pivotal role in the stressor-induced attenuation of this microglial checkpoint mechanism.

A consequence of stressor-induced disruption of CD200:CD200R1 signaling is the elevation in brain levels of the alarmin HMGB1 (Frank et al., 2018a). We have found that HMGB1 mediates the neuroinflammatory priming effects of stressors (Weber et al., 2015) and that HMGB1 is sufficient to prime the neuroinflammatory as well as the microglial proinflammatory response to subsequent immune challenges (Frank et al., 2015). Interestingly, these priming effects of HMGB1 were contingent upon the redox state of HMGB1 such that only the disulfide form of HMGB1 induced priming. We have also found that stressor exposure induces the active release of HMGB1 from microglia (Weber et al., 2015). However, it is important to consider that all nucleated cells are a potential source of HMGB1 given its constitutive role as a DNA binding protein (Yang et al., 2013). In the context of these prior findings, the present set of findings suggest that stressor-induced GCs set in motion a cascade of neuroimmune events that culminates in a primed activation state in microglia. We propose that GCs initiate this cascade through induction of the transcription factor C/EBPβ, which represses transcription and expression of CD200R1. Subsequently, this repression of CD200R1 expression results in disruption of CD200:CD200R1 signaling and dis-inhibition of microglia immune reactivity. As a result of this dis-inhibition, we propose that microglia release HMGB1, which then acts in an autocrine and paracrine fashion to prime microglia through a number of receptors expressed by microglia including TLR2, TLR4 and RAGE (Yang et al., 2013). However, it is important to note that in the present study, we were not able to determine the cellular substrate(s) upon which CORT acts to induce HMGB1. In addition, CORT administration produced a small increase in HMGB1 relative to stress effects on HMGB1 suggesting that additional signals might be necessary to

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elaborate stress effects on HMGB1. As noted, all nucleated cells are a potential source of HMGB1 given its constitutive role as a DNA binding protein (Yang et al., 2013). Thus, the present findings do not exclude the possibility that alternate CNS cellular substrates serve as a source of HMGB1. That being said, we have previously found that exposure to inescapable tailshock induces the release of HMGB1 from hippocampal microglia *ex vivo* (Weber et al., 2015), which strongly implicates microglia as a key cellular substrate upon which CORT might act to induce the release of HMGB1. Of note, stress-induced secretion of HMGB1 in the CNS has not been demonstrated. However, we found that pharmacological blockade of HMGB1 signaling mitigated the neuroinflammatory priming effects of stress (Weber et al., 2015) suggesting that stress exposure induces the release of HMGB1 *in vivo*. We surmise that the stress-induced increases in hippocampal and amygdalar HMGB1 protein levels leads to the extra-cellular release of HMGB1. For release to occur, two cellular processes are thought to be necessary (Yang et al., 2015). First, HMGB1 must first translocate from nucleus to the cytoplasm. This translocation is mediated, in part, through hyperacetylation of HMGB1. Second, HMGB1 is then released into the extra-cellular space via a caspase-1 dependent mechanism or simply through cellular necrosis. As noted, stress-induced disruption of CD200:CD200R1 signaling leads to increases in HMGB1 levels and presumably release of HMGB1 (Frank et al., 2018a). However, it is unclear how modulation of CD200R1 signaling intersects with these cellular processes of HMGB1 translocation and release.

The present set of studies provides converging evidence that GCs mediate, at least in part, the effects of stressors on these mechanisms of neuroinflammatory priming. However, it is likely that other effectors of the stress response, such as catecholamines, might play a role as well. Studies have demonstrated that stress-induced catecholamines induce a primed immunophenotype in microglia (Wohleb et al., 2011) and that catecholamines are sufficient to prime microglia proinflammatory responses (Johnson et al., 2013). It is important to note that GCs regulate catecholamine synthesis and signaling (Pacak et al., 1993), and thus the interplay between these stress effectors is likely important for neuroinflammatory priming. However, the role of catecholamines in HMGB1 and CD200R1 mediated priming has not been examined.

A variety of data suggest that the induction of brain HMGB1 and the downregulation of CD200R1 are critical to the neuroinflammatory priming produced by stressors such as inescapable tailshock. Taken together, the present results suggest that stress-induced GCs mediate these key processes as GCs proved to be both necessary for IS-induced alterations in HMGB1 and CD200R1, as well as sufficient by itself to produce these outcomes. These data add to a growing set of findings that indicate that GCs cannot be viewed as simply anti-inflammatory, and that GCs can facilitate future neuroinflammatory responses to immune challenges at the same time as they suppress ongoing inflammation (Frank et al., 2013). It has been argued (Bolles and Fanselow, 1980) that external threats lead to a sequential set of adaptive responses. First, the threat produces defensive behaviors designed to deal with the threat—freezing, fight, or flight. Bolles and Fanselow argued that once the threat is overcome, there follows a recuperative phase in which wounds are healed and spent energy is restored. GCs, of course, produce energy such as that needed during the defensive phase, and it would be adaptive to blunt inflammatory and other immune responses during this

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phase of intense activity. However, it might be equally adaptive to potentiate inflammatory responses during the recuperative phase. It should be noted that exposure to stressors primes the sickness response to subsequent immune challenges (Fonken et al., 2018; Johnson et al., 2003; Wohleb et al., 2012) and the sickness response has been argued to be adaptive partly because it is energy conserving (Hart, 1988), a process critical to recuperation. Thus, GCs could at one and the same time serve both defense and delayed recuperation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ, 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402. [PubMed: 9254694]

Bolles RC, Fanselow MS, 1980 A perceptual-defensive-recuperative model of fear and pain. Behav Brain Sciences 3, 291–301.

Boumpas DT, Chrousos GP, Wilder RL, Cupps TR, Balow JE, 1993 Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. Ann Intern Med 119, 1198–1208. [PubMed: 8239251]

Brinton RD, Thompson RF, Foy MR, Baudry M, Wang J, Finch CE, Morgan TE, Pike CJ, Mack WJ, Stanczyk FZ, Nilsen J, 2008 Progesterone receptors: form and function in brain. Front Neuroendocrinol 29, 313–339. [PubMed: 18374402]

Cain DW, Cidlowski JA, 2017 Immune regulation by glucocorticoids. Nat Rev Immunol 17, 233–247. [PubMed: 28192415]

Cheng Y, Pardo M, Armini RS, Martinez A, Mouhsine H, Zagury JF, Jope RS, 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. [PubMed: 26772151]

Chomczynski P, Sacchi N, 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem 162, 156–159. [PubMed: 2440339]

Costello DA, Lyons A, Denieffe S, Browne TC, Cox FF, Lynch MA, 2011 Long term potentiation is impaired in membrane glycoprotein CD200-deficient mice: a role for Toll-like receptor activation. J Biol Chem 286, 34722–34732. [PubMed: 21835925]

Dalm S, Karssen AM, Meijer OC, Belanoff JK, de Kloet ER, 2019 Resetting the Stress System with a Mifepristone Challenge. Cellular and molecular neurobiology 39, 503–522. [PubMed: 30173378]

de Pablos RM, Herrera AJ, Espinosa-Oliva AM, Sarmiento M, Munoz MF, Machado A, Venero JL, 2014 Chronic stress enhances microglia activation and exacerbates death of nigral dopaminergic neurons under conditions of inflammation. J Neuroinflammation 11, 34 [PubMed: 24565378]

de Pablos RM, Villaran RF, Arguelles S, Herrera AJ, Venero JL, Ayala A, Cano J, Machado A, 2006 Stress increases vulnerability to inflammation in the rat prefrontal cortex. J Neurosci 26, 5709–5719. [PubMed: 16723527]

Deczkowska A, Amit I, Schwartz M, 2018 Microglial immune checkpoint mechanisms. Nat Neurosci 21, 779–786. [PubMed: 29735982]

Denieffe S, Kelly RJ, McDonald C, Lyons A, Lynch MA, 2013 Classical activation of microglia in CD200-deficient mice is a consequence of blood brain barrier permeability and infiltration of peripheral cells. Brain Behav Immun 34, 86–97. [PubMed: 23916893]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Dentesano G, Straccia M, Ejarque-Ortiz A, Tusell JM, Serratosa J, Saura J, Sola C, 2012 Inhibition of CD200R1 expression by C/EBP beta in reactive microglial cells. J Neuroinflammation 9, 165. [PubMed: 22776069]

Dhabhar FS, Malarkey WB, Neri E, McEwen BS, 2012 Stress-induced redistribution of immune cells--from barracks to boulevards to battlefields: a tale of three hormones--Curt Richter Award winner. Psychoneuroendocrinology 37, 1345–1368. [PubMed: 22727761]

Espinosa-Oliva AM, de Pablos RM, Villaran RF, Arguelles S, Venero JL, Machado A, Cano J, 2011 Stress is critical for LPS-induced activation of microglia and damage in the rat hippocampus. Neurobiol Aging 32, 85–102. [PubMed: 19286276]

Fleshner M, Deak T, Spencer RL, Laudenslager ML, Watkins LR, Maier SF, 1995 A long-term increase in basal levels of corticosterone and a decrease in corticosteroid-binding globulin after acute stressor exposure. Endocrinology 136, 5336–5342. [PubMed: 7588279]

Fonken LK, Frank MG, Gaudet AD, D'Angelo HM, Daut RA, Hampson EC, Ayala MT, Watkins LR, Maier SF, 2018 Neuroinflammatory priming to stress is differentially regulated in male and female rats. Brain Behav Immun.

Fonken LK, Weber MD, Daut RA, Kitt MM, Frank MG, Watkins LR, Maier SF, 2016 Stress-induced neuroinflammatory priming is time of day dependent. Psychoneuroendocrinology 66, 82–90. [PubMed: 26799851]

Frank MG, Baratta MV, Sprunger DB, Watkins LR, Maier SF, 2007 Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. Brain Behav Immun 21, 47–59. [PubMed: 16647243]

Frank MG, Fonken LK, Annis JL, Watkins LR, Maier SF, 2018a Stress disinhibits microglia via down-regulation of CD200R: A mechanism of neuroinflammatory priming. Brain Behav Immun 69, 62–73. [PubMed: 29104062]

Frank MG, Fonken LK, Dolzani SD, Annis JL, Siebler PH, Schmidt D, Watkins LR, Maier SF, Lowry CA, 2018b Immunization with Mycobacterium vaccae induces an anti-inflammatory milieu in the CNS: Attenuation of stress-induced microglial priming, alarmins and anxiety-like behavior. Brain Behav Immun 73, 352–363. [PubMed: 29807129]

Frank MG, Hershman SA, Weber MD, Watkins LR, Maier SF, 2014 Chronic exposure to exogenous glucocorticoids primes microglia to pro-inflammatory stimuli and induces NLRP3 mRNA in the hippocampus. Psychoneuroendocrinology 40, 191–200. [PubMed: 24485491]

Frank MG, Miguel ZD, Watkins LR, Maier SF, 2010 Prior exposure to glucocorticoids sensitizes the neuroinflammatory and peripheral inflammatory responses to E. coli lipopolysaccharide. Brain Behav Immun 24, 19–30. [PubMed: 19647070]

Frank MG, Thompson BM, Watkins LR, Maier SF, 2012 Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. Brain, behavior, and immunity 26, 337–345.

Frank MG, Watkins LR, Maier SF, 2013 Stress-induced glucocorticoids as a neuroendocrine alarm signal of danger. Brain, behavior, and immunity 33, 1–6.

Frank MG, Weber MD, Fonken LK, Hershman SA, Watkins LR, Maier SF, 2015 The redox state of the alarmin HMGB1 is a pivotal factor in neuroinflammatory and microglial priming: A role for the NLRP3 inflammasome. Brain Behav Immun 55, 215–224. [PubMed: 26482581]

Frank MG, Weber MD, Watkins LR, Maier SF, 2016 Stress-induced neuroinflammatory priming: A liability factor in the etiology of psychiatric disorders. Neurobiology of stress 4, 62–70. [PubMed: 27981190]

Frank MG, Wieseler-Frank JL, Watkins LR, Maier SF, 2006 Rapid isolation of highly enriched and quiescent microglia from adult rat hippocampus: immunophenotypic and functional characteristics. J Neurosci Methods 151, 121–130. [PubMed: 16125247]

Franklin TC, Wohleb ES, Zhang Y, Fogaca M, Hare B, Duman RS, 2018 Persistent Increase in Microglial RAGE Contributes to Chronic Stress-Induced Priming of Depressive-like Behavior. Biol Psychiatry 83, 50–60. [PubMed: 28882317]

Gorczynski R, Boudakov I, Khatri I, 2008 Peptides of CD200 modulate LPS-induced TNF-alpha induction and mortality in vivo. J Surg Res 145, 87–96. [PubMed: 17981299]

Gorczynski RM, 2005 CD200 and its receptors as targets for immunoregulation. Current opinion in investigational drugs 6, 483–488. [PubMed: 15912961]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Habib P, Beyer C, 2015 Regulation of brain microglia by female gonadal steroids. The Journal of steroid biochemistry and molecular biology 146, 3–14. [PubMed: 24607811]

Hart BL, 1988 Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 12, 123–137. [PubMed: 3050629]

Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, Barclay AN, Sedgwick JD, 2000 Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 290, 1768–1771. [PubMed: 11099416]

Hojo Y, Kawato S, 2018 Neurosteroids in Adult Hippocampus of Male and Female Rodents: Biosynthesis and Actions of Sex Steroids. Frontiers in endocrinology 9, 183. [PubMed: 29740398]

Jacobson L, Akana SF, Cascio CS, Shinsako J, Dallman MF, 1988 Circadian variations in plasma corticosterone permit normal termination of adrenocorticotropin responses to stress. Endocrinology 122, 1343–1348. [PubMed: 2831028]

Jenmalm MC, Cherwinski H, Bowman EP, Phillips JH, Sedgwick JD, 2006 Regulation of myeloid cell function through the CD200 receptor. J Immunol 176, 191–199. [PubMed: 16365410]

Johnson JD, O'Connor KA, Deak T, Stark M, Watkins LR, Maier SF, 2002 Prior stressor exposure sensitizes LPS-induced cytokine production. Brain Behav Immun 16, 461–476. [PubMed: 12096891]

Johnson JD, O'Connor KA, Hansen MK, Watkins LR, Maier SF, 2003 Effects of prior stress on LPS-induced cytokine and sickness responses. Am J Physiol Regul Integr Comp Physiol 284, R422–432. [PubMed: 12399247]

Johnson JD, O'Connor KA, Watkins LR, Maier SF, 2004 The role of IL-1beta in stress-induced sensitization of proinflammatory cytokine and corticosterone responses. Neuroscience 127, 569–577. [PubMed: 15283957]

Johnson JD, Zimomra ZR, Stewart LT, 2013 Beta-adrenergic receptor activation primes microglia cytokine production. J Neuroimmunol 254, 161–164. [PubMed: 22944319]

Kelly KA, Michalovicz LT, Miller JV, Castranova V, Miller DB, O'Callaghan JP, 2018 Prior exposure to corticosterone markedly enhances and prolongs the neuroinflammatory response to systemic challenge with LPS. PloS one 13, e0190546. [PubMed: 29304053]

Kelly KA, Miller DB, Bowyer JF, O'Callaghan JP, 2012 Chronic exposure to corticosterone enhances the neuroinflammatory and neurotoxic responses to methamphetamine. Journal of neurochemistry 122, 995–1009. [PubMed: 22776046]

Koning N, Swaab DF, Hoek RM, Huitinga I, 2009 Distribution of the immune inhibitory molecules CD200 and CD200R in the normal central nervous system and multiple sclerosis lesions suggests neuron-glia and glia-glia interactions. J Neuropathol Exp Neurol 68, 159–167. [PubMed: 19151626]

Koo BB, Michalovicz LT, Calderazzo S, Kelly KA, Sullivan K, Killiany RJ, O'Callaghan JP, 2018 Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness. Brain Behav Immun 67, 42–46. [PubMed: 28782715]

Lian YJ, Gong H, Wu TY, Su WJ, Zhang Y, Yang YY, Peng W, Zhang T, Zhou JR, Jiang CL, Wang YX, 2017 Ds-HMGB1 and fr-HMGB induce depressive behavior through neuroinflammation in contrast to nonoxid-HMGB1. Brain Behav Immun 59, 322–332. [PubMed: 27647532]

Livak KJ, Schmittgen TD, 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408. [PubMed: 11846609]

Loram LC, Taylor FR, Strand KA, Frank MG, Sholar P, Harrison JA, Maier SF, Watkins LR, 2011 Prior exposure to glucocorticoids potentiates lipopolysaccharide induced mechanical allodynia and spinal neuroinflammation. Brain Behav Immun 25, 1408–1415. [PubMed: 21536123]

Meuth SG, Simon OJ, Grimm A, Melzer N, Herrmann AM, Spitzer P, Landgraf P, Wiendl H, 2008 CNS inflammation and neuronal degeneration is aggravated by impaired CD200-CD200R-mediated macrophage silencing. J Neuroimmunol 194, 62–69. [PubMed: 18164423]

Munhoz CD, Lepsch LB, Kawamoto EM, Malta MB, Lima Lde S, Avellar MC, Sapolsky RM, Scavone C, 2006 Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor-kappaB in the frontal cortex and hippocampus via glucocorticoid secretion. J Neurosci 26, 3813–3820. [PubMed: 16597735]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Munhoz CD, Sorrells SF, Caso JR, Scavone C, Sapolsky RM, 2010 Glucocorticoids exacerbate lipopolysaccharide-induced signaling in the frontal cortex and hippocampus in a dose-dependent manner. J Neurosci 30, 13690–13698. [PubMed: 20943909]

O'Callaghan JP, Kelly KA, Locker AR, Miller DB, Lasley SM, 2015 Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of Gulf War Illness. J Neurochem 133, 708–721. [PubMed: 25753028]

Pacak K, Kvetnansky R, Palkovits M, Fukuhara K, Yadid G, Kopin IJ, Goldstein DS, 1993 Adrenalectomy augments in vivo release of norepinephrine in the paraventricular nucleus during immobilization stress. Endocrinology 133, 1404–1410. [PubMed: 8396018]

Roos AB, Nord M, 2012 The emerging role of C/EBPs in glucocorticoid signaling: lessons from the lung. J Endocrinol 212, 291–305. [PubMed: 22045755]

Saenger AK, 2010 Discovery of the wonder drug: from cows to cortisone. The effects of the adrenal cortical hormone 17-hydroxy-11-dehydrocorticosterone (Compound E) on the acute phase of rheumatic fever; preliminary report. Mayo Clin Proc 1949;24:277–97. Clinical chemistry 56, 1349–1350.

Sapolsky RM, Romero LM, Munck AU, 2000 How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21, 55–89. [PubMed: 10696570]

Sarkar NN, 2002 Mifepristone: bioavailability, pharmacokinetics and use-effectiveness. Eur J Obstet Gynecol Reprod Biol 101, 113–120. [PubMed: 11858883]

Schumacher M, Mattern C, Ghoumari A, Oudinet JP, Liere P, Labombarda F, Sitruk-Ware R, De Nicola AF, Guennoun R, 2014 Revisiting the roles of progesterone and allopregnanolone in the nervous system: resurgence of the progesterone receptors. Prog Neurobiol 113, 6–39. [PubMed: 24172649]

Selye H, 1950 Stress and the general adaptation syndrome. Br Med J 1, 1383–1392. [PubMed: 15426759]

Sierra A, Gottfried-Blackmore A, Milner TA, McEwen BS, Bulloch K, 2008 Steroid hormone receptor expression and function in microglia. Glia 56, 659–674. [PubMed: 18286612]

Sorrells SF, Caso JR, Munhoz CD, Sapolsky RM, 2009 The stressed CNS: when glucocorticoids aggravate inflammation. Neuron 64, 33–39. [PubMed: 19840546]

Sorrells SF, Sapolsky RM, 2007 An inflammatory review of glucocorticoid actions in the CNS. Brain Behav Immun 21, 259–272. [PubMed: 17194565]

Sun R, Zhao Z, Feng J, Bo J, Rong H, Lei Y, Lu C, Zhang X, Hou B, Sun Y, Liu Y, Ma Z, Gu X, 2017 Glucocorticoid-Potentiated Spinal Microglia Activation Contributes to Preoperative Anxiety-Induced Postoperative Hyperalgesia. Molecular neurobiology 54, 4316–4328. [PubMed: 27339881]

Wang N, Ma H, Li Z, Gao Y, Cao X, Jiang Y, Zhou Y, Liu S, 2017 Chronic unpredictable stress exacerbates surgery-induced sickness behavior and neuroinflammatory responses via glucocorticoids secretion in adult rats. PloS one 12, e0183077. [PubMed: 28806788]

Weber MD, Frank MG, Sobesky JL, Watkins LR, Maier SF, 2013 Blocking toll-like receptor 2 and 4 signaling during a stressor prevents stress-induced priming of neuroinflammatory responses to a subsequent immune challenge. Brain, behavior, and immunity.

Weber MD, Frank MG, Tracey KJ, Watkins LR, Maier SF, 2015 Stress Induces the Danger-Associated Molecular Pattern HMGB-1 in the Hippocampus of Male Sprague Dawley Rats: A Priming Stimulus of Microglia and the NLRP3Inflammasome. J Neurosci 35, 316–324. [PubMed: 25568124]

Webster Marketon JI, Glaser R, 2008 Stress hormones and immune function. Cell Immunol 252, 16–26. [PubMed: 18279846]

Wohleb ES, Fenn AM, Pacenta AM, Powell ND, Sheridan JF, Godbout JP, 2012 Peripheral innate immune challenge exaggerated microglia activation, increased the number of inflammatory CNS macrophages, and prolonged social withdrawal in socially defeated mice. Psychoneuroendocrinology 37, 1491–1505. [PubMed: 22386198]

Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF, 2011 beta-Adrenergic receptor antagonism prevents anxiety-like behavior and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

microglial reactivity induced by repeated social defeat. J Neurosci 31, 6277–6288. [PubMed: 21525267]

Wright GJ, Puklavec MJ, Willis AC, Hoek RM, Sedgwick JD, Brown MH, Barclay AN, 2000 Lymphoid/neuronal cell surface OX2 glycoprotein recognizes a novel receptor on macrophages implicated in the control of their function. Immunity 13, 233–242. [PubMed: 10981966]

Yang H, Antoine DJ, Andersson U, Tracey KJ, 2013 The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. J Leukoc Biol 93, 865–873. [PubMed: 23446148]

Yang H, Wang H, Chavan SS, Andersson U, 2015 High Mobility Group Box Protein 1 (HMGB1): The Prototypical Endogenous Danger Molecule. Mol Med 21 Suppl 1, S6–S12. [PubMed: 26605648]

Zhang S, Cherwinski H, Sedgwick JD, Phillips JH, 2004 Molecular mechanisms of CD200 inhibition of mast cell activation. J Immunol 173, 6786–6793. [PubMed: 15557172]

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1. Stressor exposure induced HMGB1 and reduced CD200R1.
2. The glucocorticoid receptor antagonist RU486 mitigated these effects of stress.
3. Adrenalectomy also mitigated these effects of stress.
4. Exogenous corticosterone (CORT) recapitulated these effects of stress.
5. CORT directly downregulated microglial expression of CD200R1.

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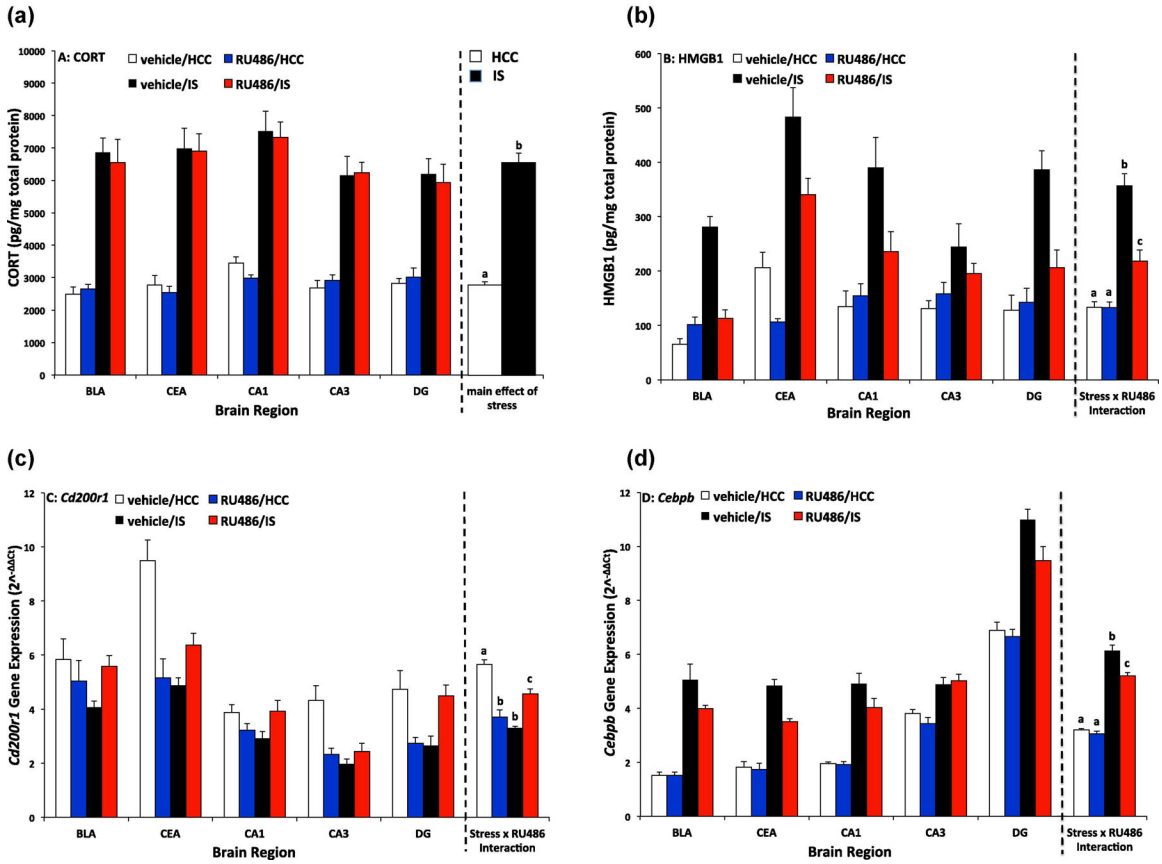

Fig. 1. RU486 modulates the effects of stress.

Rats were injected subcutaneously with vehicle (100% propylene glycol) or the GC receptor antagonist RU486 (50 mg/kg). Twenty-four hours post-injection, rats were exposed to inescapable tailshock (IS) or served as home cage controls (HCCs). Immediately after termination of stress, (**A**) CORT, (**B**) HMGB1, (**C**) *cd200r1* and (**D**) *cebpb* were measured in amygdalar (BLA and CEA) and hippocampal (CA1, CA3 and DG) sub-regions. Data are presented as the mean + SEM. N = 8 rats per experimental group. The 3-way interaction between brain region, drug treatment and stress was not significant. Thus, data are collapsed across brain region and the 2-way interaction between stress and RU486 treatment depicted to the right of the dotted line. Means designated with differing letters are significantly different ($p < 0.05$).

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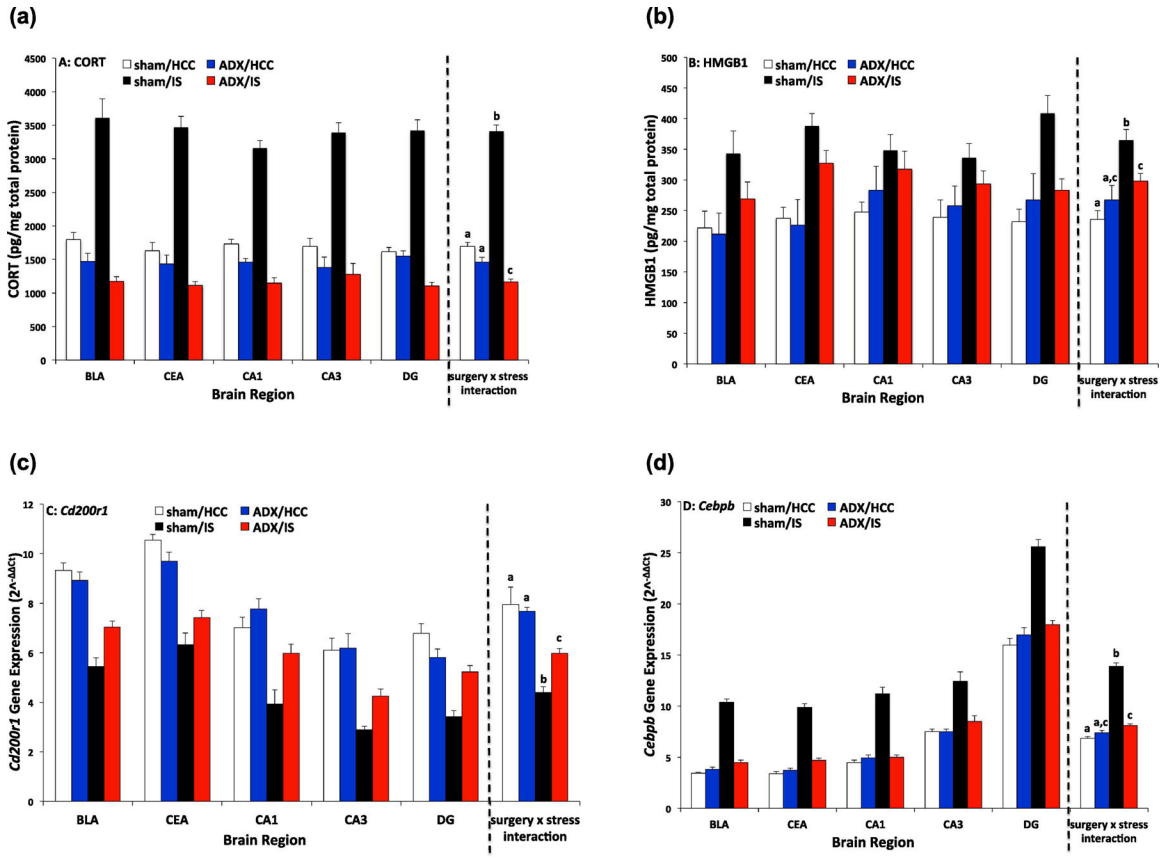

Fig. 2. ADX modulates the effects of stress.

Rats underwent sham surgery or adrenalectomy (ADX) and given basal replacement corticosterone. Two weeks post-surgery, rats were exposed to inescapable tailshock (IS) or served as home cage controls (HCCs). Immediately after termination of stress, (**A**) CORT, (**B**) HMGB1, (**C**) *cd200r1* and (**D**) *cebpb* were measured in amygdalar (BLA and CEA) and hippocampal (CA1, CA3 and DG) sub-regions. Data are presented as the mean + SEM. N = 7–8 rats per experimental group. The 3-way interaction between brain region, surgical treatment and stress was not significant. Thus, data are collapsed across brain region and the 2-way interaction between stress and surgical treatment depicted to the right of the dotted line. Means designated with differing letters are significantly different ($p < 0.05$).

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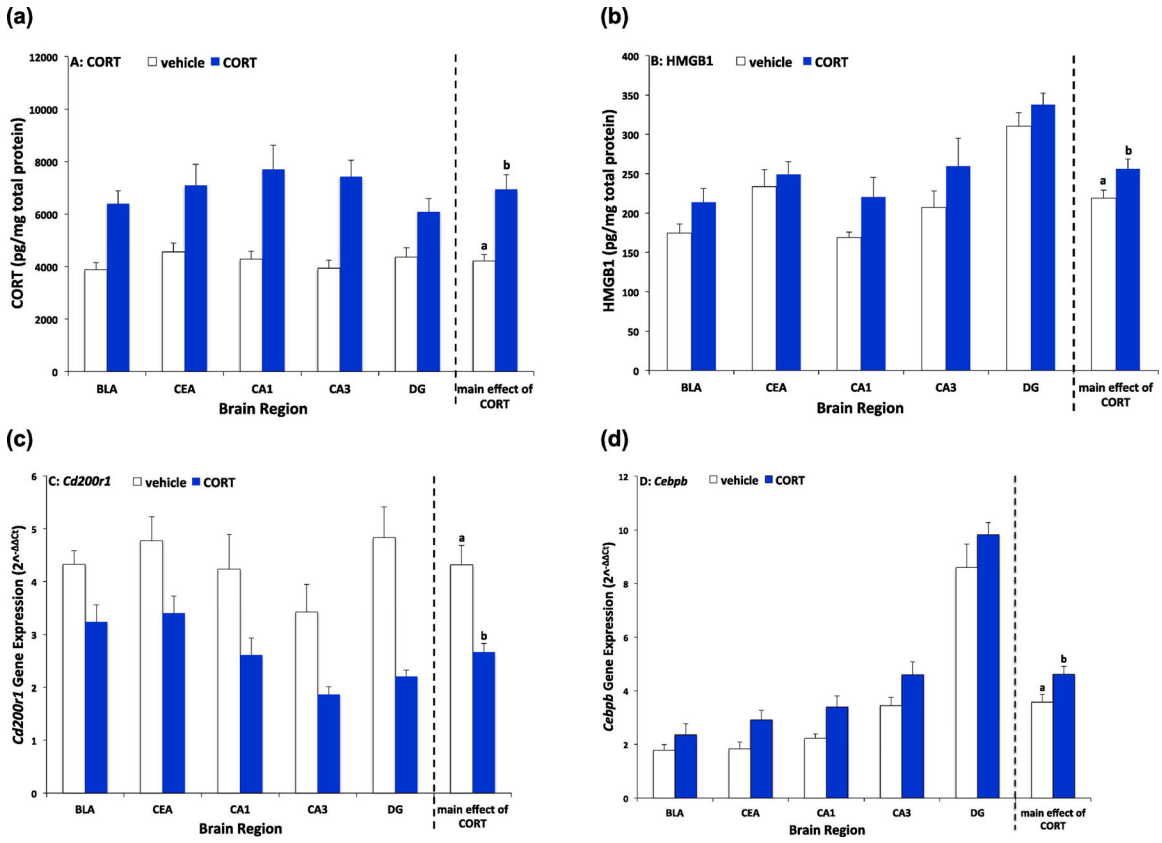

Fig. 3. Effect of exogenous CORT.

Rats were injected subcutaneously with vehicle (100% propylene glycol) or CORT (2.5 mg/kg). Two hours post-injection, (**A**) CORT, (**B**) HMGB1, (**C**) *cd200r1* and (**D**) *cebpb* were measured in amygdalar (BLA and CEA) and hippocampal (CA1, CA3 and DG) sub-regions. Data are presented as the mean + SEM. N = 8–9 rats per experimental group. The 2-way interaction between brain region and drug treatment was not significant. Thus, data are collapsed across brain region and the main effect of drug depicted to the right of the dotted line. Means designated with differing letters are significantly different ($p < 0.05$).

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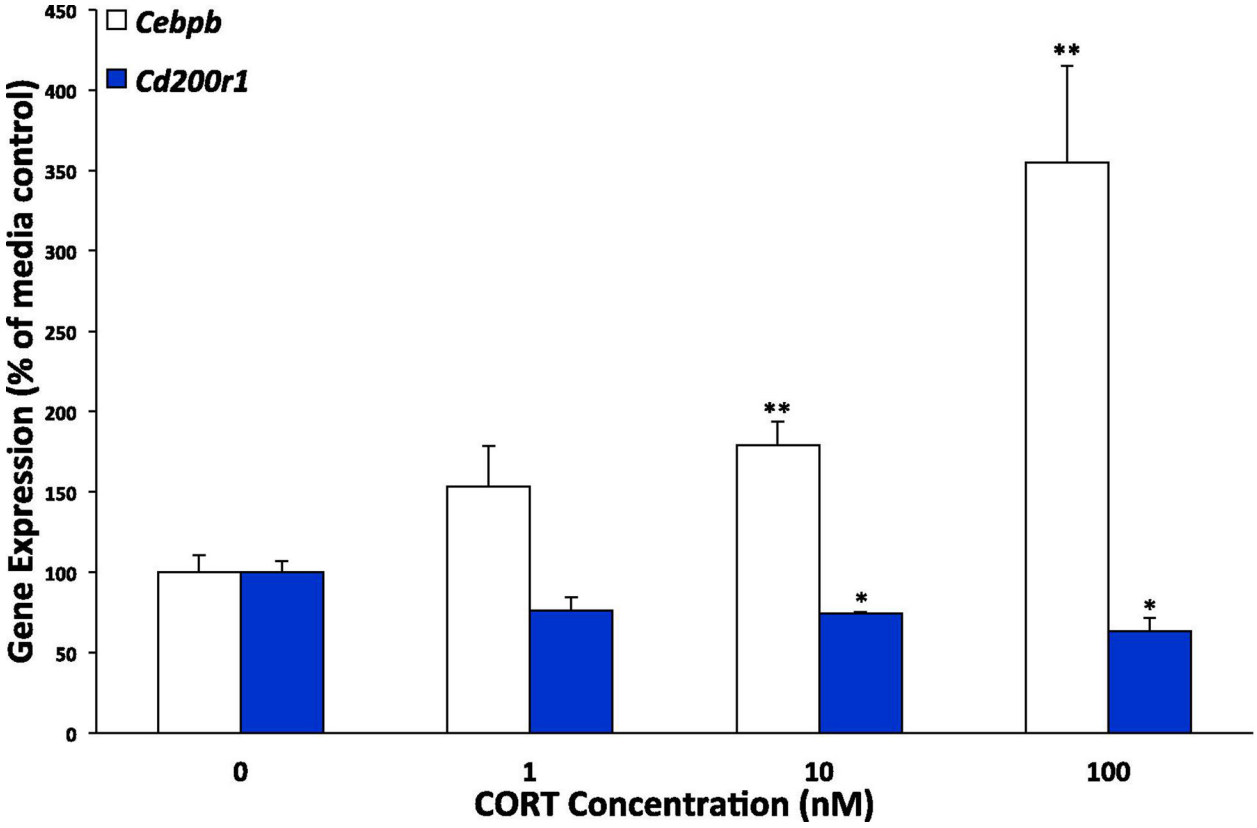

Fig. 4. *In vitro* effects of CORT on microglia.

Microglia were isolated from whole rat brain and directly exposed to CORT (0, 0.1, 1, 10 and 100 nM) for 3 h and, *cd200r1* and *cebpb* measured in cell lysates. Data are presented as the mean + SEM. N = 4 replicates. * $p < 0.05$, ** $p < 0.01$ compared to media control.