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Stress-induced neuroinflammatory priming is time of day dependent

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ABSTRACT

Circadian rhythms are endogenous cycles of physiology and behavior that align with the daily rotation of the planet and resulting light–dark cycle. The circadian system ensures homeostatic balance and regulates many aspects of physiology, including the stress response and susceptibility to and/or severity of stress-related sequelae. Both acute and chronic stressors amplify neuroinflammatory responses to a subsequent immune challenge, however it is not known whether circadian timing of the stressor regulates the priming response. Here, we test whether stress-induced neuroinflammatory priming is regulated by the circadian system. As has been previously shown, exposure to 100 inescapable tails shocks (IS) increased hippocampal cytokines following a subsequent inflammatory challenge. However, this effect was limited to animals that experienced the stressor during the light phase. Rats exposed to stress during the dark phase did not alter inflammatory potential following lipopolysaccharide (LPS) challenge. To determine whether microglia might be involved in diurnal differences in neuroinflammatory priming, microglia were isolated 24 h after stress that occurred either during the middle of the light or dark phase. Only microglia isolated from animals stressed during the light phase demonstrated an exaggerated inflammatory response when treated *ex vivo* with LPS. To determine possible circadian dependency of microglia responsiveness to glucocorticoids – the likely proximal mediator for stress associated neuroinflammatory priming – microglia were isolated during the middle of the light or dark phase and treated *ex vivo* with corticosterone. Glucocorticoids treatment downregulated CX3CR1 and CD200R, two genes involved in microglial inflammatory "off" signaling; however, there was no effect of time of day on expression of either gene. Importantly, while absolute concentrations of corticosterone were comparable following IS during the light and dark phase, the magnitude of change in corticosterone was greater during the light phase. This work highlights the importance of studying circadian rhythms to elucidate biological mechanisms of stress.

1. Background

Exaggerated innate immune/inflammatory responses in the brain are implicated in the etiology of numerous psychiatric disorders including depression, PTSD, autism, and schizophrenia (Dantzer et al., 2008). Stress is a major predisposing factor in the development of these psychiatric disorders and a potential source of elevated inflammation in the brain. In this regard, both acute and chronic stressors amplify peripheral and central inflammatory responses to a subsequent immune challenge (Johnson et al., 2002, 2003; Frank et al., 2010; Wohleb et al., 2012). Glucocorticoids are implicated in mediating the effects of stress on inflammatory priming (Frank et al., 2010, 2012, 2014; Busillo et al., 2011). Although glucocorticoids are traditionally regarded as anti-inflammatory, a considerable number of studies demonstrate that glucocorticoids can simultaneously suppress ongoing inflammation while potentiating inflammatory responses to a later immune challenge (reviewed in Frank et al., 2015). The neuroinflammatory "priming" produced by stress is regulated in part by microglia, the predominant innate immune cell of the central nervous system (Frank et al., 2007, 2014). While glucocorticoids are recognized as a critical mediator through which stress sensitize microglia (Frank et al., 2010, 2012, 2014; Weber et al., 2015), the exact mechanisms involved in microglia priming are not fully understood.

Circadian rhythms are endogenous cycles of physiology and behavior that have periods of about 24 h (hours) and are aligned to the timing of the daily rotation of the planet. The presence of a functional circadian system is evolutionarily advantageous as

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organisms that are entrained to their environment have improved survival odds (Davidson et al., 2006). Circadian rhythms allow organisms (and individual cells) to predict consistent changes in environment and temporally compartmentalize incompatible processes (Karatsoreos and McEwen, 2014). The circadian system is critical for ensuring homeostatic balance and regulates many aspects of physiology including the immune system. For example, there are circadian differences in immune system activation (Halberg et al., 1960; Marpegan et al., 2009; Spengler et al., 2012) and disruption of the circadian system can lead to heightened peripheral and central inflammatory responses (Castanon-Cervantes et al., 2010; Fonken and Nelson, 2013; Phillips et al., 2015; Wright et al., 2015). Moreover, microglia express intrinsic circadian clock mechanisms and display altered immune potential over the course of the day (Fonken et al., 2015). The time-of-day at which an inflammatory challenge occurs affects stress-induced neuroinflammatory priming (Johnson et al., 2003). However, whether diurnal timing of the stressor affects neuroinflammatory priming has not been established. Responses to stressors and susceptibility to and/or severity of stress-related comorbidities are regulated by the circadian system (Cohen et al., 2015). This suggests that microglia may also be differentially sensitive to the effects of stressors throughout the day.

Here, we tested whether stress-induced inflammatory priming is time of day dependent. Furthermore, we examined the effects of stressor exposure and *ex vivo* corticosterone treatment on microglia inflammatory priming. Determining whether the hippocampus is more or less sensitive to the effects of stress at different times of day provides a novel platform through which it is possible to identify factors involved in stress associated neuroinflammatory priming. Better understanding of the mechanisms involved in imparting resilience or contributing to vulnerability to stress may help devise interventions to mitigate risk factors.

2. General methods

2.1. Animals

Male Sprague-Dawley rats (~3 months old; Harlan Sprague-Dawley, Inc., Indianapolis, IN, USA) were pair-housed with food and water available *ad libitum* at an ambient temperature of 22 ± 2 °C. Rats were given one month to acclimate to colony conditions before experimentation began. All rats were maintained on a 12:12 light cycle with lights on either at 0700 or 2100 h [lights on is Zeitgeber time (ZT) 0]. All experimental procedures were conducted in accordance with the University of Colorado Institutional Animal Care and Use Committee.

2.2. Experimental design

2.2.1. Experiment 1: does stress at different times of day of differentially modulate neuroinflammatory priming to a later peripheral immune challenge?

Rats received 100 trials of inescapable tail shock (IS) over 2 h during either the middle of the light phase (Zeitgeber time 5–7; ZT5–ZT7) or dark phase (ZT15–ZT17). Half of the rats were maintained on a reverse light/dark cycle (that was staggered 2 h) in order for the stress and tissue collection to occur together for the separate time points. 24 h later rats were injected intraperitoneally (i.p.) with 10 μg/kg of lipopolysaccharide (LPS; *Escherichia coli* serotype 0111:B4; Sigma) or saline (vehicle control). This dose of LPS was selected because it results in a sub-threshold hippocampal pro-inflammatory response (Johnson et al., 2002). Because LPS elicits a greater response during the light phase it is important to equate times of day for LPS injection. Thus, a 36 h time point was also included in which IS occurred 12 h prior to IS in the 24 h time point groups. Because the reverse light/dark cycle room was not exactly 12 h out of phase with the standard light/dark cycle room (due to animal care requirements), middle of the light phase stress occurred from ZT3 to ZT5 and dark phase stress occurred from ZT17 to ZT19. All of the groups (light and dark phase and 24 and 36 h time points) received LPS injections together which corresponded to ZT6 in standard light/dark cycle and ZT16 in the reverse light/dark room. Three hours following LPS injection hippocampal tissue was collected and cytokine protein and gene expression evaluated (see Fig. 1 for schematic of experimental design). Gene and protein expression were evaluated in the hippocampus because IS specifically potentiates hippocampal pro-inflammatory processes to peripheral LPS (Johnson et al., 2002). This experiment had 8 groups (see Fig. 1): control with saline during the light phase, control with saline during the dark phase, control with LPS during the light phase, control with LPS during the dark phase, stress during the light phase with LPS during the light phase (24 h later), stress during the light phase with LPS during the dark phase (36 h later), stress during the dark phase with LPS during the light phase (36 h later), and stress at during the dark phase with LPS during the dark phase (24 h later). In order to minimize animal use and because our laboratory has previously demonstrated that 24 h post-IS rats do not exhibit elevated levels of pro-inflammatory cytokines (Johnson et al., 2003), we did not include IS/saline groups at ZT6 and ZT16.

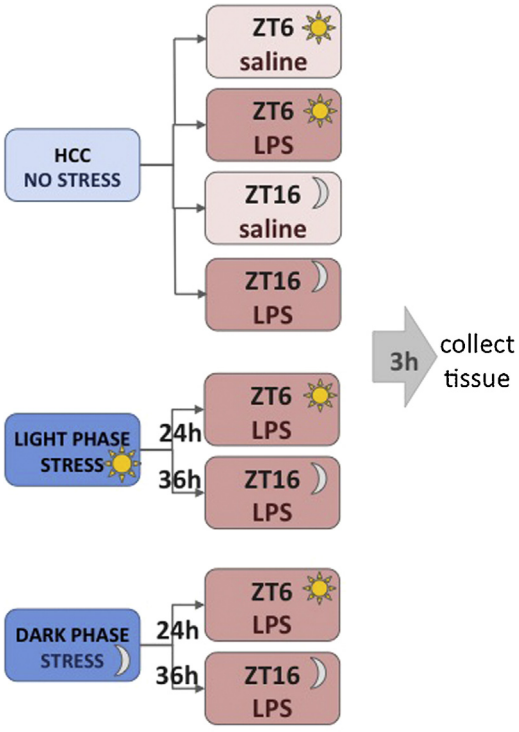

Fig. 1. Experimental design schematic.

2.2.2. Experiment 2: are microglia differentially sensitized by stress exposure during the light versus dark phase?

Rats received 100 trials of IS either during the middle of the light phase (ZT5–ZT7) or dark phase (ZT15–ZT17). 24 h later microglia were isolated from IS and HCC animals and treated *ex vivo* with LPS for 3 h. Cytokine mRNA expression was measured in microglia using qPCR to evaluate microglia sensitization.