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Divergent responses of inflammatory mediators within the amygdala and medial prefrontal cortex to acute psychological stress

Haley A. Vecchiarelli [a,b,c], Chaitanya P. Gandhi [a,b,c], J. Megan Gray [a,b,d], Maria Morena [a,b,d], Kowther I. Hassan [a,b], Matthew N. Hill [a,b,d,e,*]

[a] Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB T2N 4N1, Canada
[b] Mathison Centre for Mental Health Research and Education, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB T2N 4N1, Canada
[c] Department of Neuroscience, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB T2N 4N1, Canada
[d] Department of Cell Biology and Anatomy, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB T2N 4N1, Canada
[e] Department of Psychiatry, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB T2N 4N1, Canada

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ABSTRACT

There is now a growing body of literature that indicates that stress can initiate inflammatory processes, both in the periphery and brain; however, the spatiotemporal nature of this response is not well characterized. The aim of this study was to examine the effects of an acute psychological stress on changes in mRNA and protein levels of a wide range of inflammatory mediators across a broad temporal range, in key corticolimbic brain regions involved in the regulation of the stress response (amygdala, hippocampus, hypothalamus, medial prefrontal cortex). mRNA levels of inflammatory mediators were analyzed immediately following 30 min or 120 min of acute restraint stress and protein levels were examined 0 h through 24 h post-termination of 120 min of acute restraint stress using both multiplex and ELISA methods. Our data demonstrate, for the first time, that exposure to acute psychological stress results in an increase in the protein level of several inflammatory mediators in the amygdala while concomitantly producing a decrease in the protein level of multiple inflammatory mediators within the medial prefrontal cortex. This pattern of changes seemed largely restricted to the amygdala and medial prefrontal cortex, with stress producing few changes in the mRNA or protein levels of inflammatory mediators within the hippocampus or hypothalamus. Consistent with previous research, stress resulted in a general elevation in multiple inflammatory mediators within the circulation. These data indicate that neuroinflammatory responses to stress do not appear to be generalized across brain structures and exhibit a high degree of spatiotemporal specificity. Given the impact of inflammatory signaling on neural excitability and emotional behavior, these data may provide a platform with which to explore the importance of inflammatory signaling within the prefrontocortical-amygdala circuit in the regulation of the neurobehavioral responses to stress.

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1. Introduction

Despite the negative connotation typically associated with stress, the biological responses to stress generally represent essential, adaptive processes (McEwen, 2005). The mobilization of glucose, enhancement of sympathetic drive and anxiety, as well as the suppression of feeding and reproductive processes all represent adaptive responses that function to enhance the survival of an organism under conditions of threat or adversity (McEwen, 2005).

In the past two decades, it has become increasingly apparent that the immune system also acts to provide adaptation in response to acute exposure to stress. While it was initially demonstrated that stress results in a suppression of acquired immune responses, a large body of evidence has also revealed that acute stress exposure enhances many aspects of the innate immune response which are involved in wound healing mechanisms (i.e. leukocyte migration) or processes involved in pathogen clearance (i.e. inflammation) (Dhabhar, 2014; Dhabhar et al., 2012).

For example, acute stress, in both humans and rodents, results in a redistribution of leukocytes throughout the body, poising themselves to act in a way that promotes active surveillance and wound healing (Dhabhar, 2013). Numerous studies have examined peripheral levels of acute phase proteins, pro-inflammatory

* Corresponding author at: Department of Cell Biology and Anatomy, Cumming School of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB T2N 4N1, Canada.

E-mail address: mnhill@ucalgary.ca (M.N. Hill).

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cytokines and other inflammatory mediators after acute stress in humans and rodents. Generally, acute stress increases acute phase proteins, such as C-reactive protein (CRP) and α1-acid glycoprotein, as well as, pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α, in circulation or in peripheral organs such as the liver or spleen (Deak et al., 2005; Rohleder, 2014). However, these data can be somewhat inconsistent depending on type of stressor, duration of stressor, how long post-stressor analysis is performed, the methodology to examine different analytes, as well as, strain and sex of experimental animals (Deak et al., 2005).

Rodent studies, which have the additional advantage of allowing the examination of inflammatory molecules in the brain in response to acute stressors, have indicated that this inflammatory response to stress is not restricted to the periphery, and central indices of inflammation have also been documented following exposure to stress. However, as far fewer studies have investigated the neuroinflammatory response to stress, there is a general lack of clarity regarding the spatiotemporal nature of this response or the specificity of cytokines that respond to stress. For example, the majority of these studies have investigated the prototypical pro-inflammatory cytokines, IL-1β, IL-6 or TNF-α, and have focused on the hypothalamus, but occasionally the hippocampus or cortex. Similar to the studies examining the effects of stress on peripheral markers of inflammation, these studies are also somewhat inconsistent, depending on the criteria mentioned above regarding experimental variables and manipulations. Regardless of these inconsistencies, these studies generally agree with the peripheral findings and seem to indicate that there is a stress-induced increase in these pro-inflammatory cytokines in the hypothalamus, but not hippocampus, either at the level of mRNA or protein (Arakawa et al., 2011; Barnum et al., 2008; Blandino et al., 2009, 2006; Catanzaro et al., 2014; Deak et al., 2005, 2003; Hueston and Deak, 2014; Hueston et al., 2011; Jankord et al., 2010; Johnson et al., 2005; Minami et al., 1991; Nguyen et al., 2000, 1998; O'Connor et al., 2003; Plata-Salamán et al., 2000; Quan et al., 2000; Reyes et al., 2003; Shizuya et al., 1998, 1997).

In addition to these acute effects on cytokine production, stress exposure also appears to sensitize neuroimmune responses to subsequent inflammatory challenges in the hypothalamus and hippocampus or increase levels of danger associated molecular pattern receptors and inflammasomes (Frank et al., 2013). It is believed that this sensitization occurs in order to more robustly respond to a future inflammatory insult (Frank et al., 2013). While this priming of the immune system following exposure to stress likely represents a highly adaptive response conserved across species to ensure appropriate pathogen clearance and wound healing following predatory attacks, the possibility also exists that these changes may relate to some of the neurobehavioral, and potentially pathological, responses to stress. For example, cytokines have been well established to have profound effects on emotional behavior, such as increasing anxiety, reducing social interaction and suppressing exploratory behavior (Anisman, 2009), which parallels many of the effects of stress exposure itself. More so, there is a well established relationship between stress exposure and the onset of inflammatory diseases, as well as between inflammation and the manifestation of neurobehavioral indices of stress (Dantzer et al., 2008; Reber, 2012). Accordingly, understanding the nature of the relationship between stress and inflammatory processes is germane to our exploration of the interaction of these processes.

As mentioned, the majority of research examining the effects of stress on markers of central inflammation have been restricted to the hypothalamus, and quite surprisingly there has been a glaring paucity in research examining the effects of stress on cytokine levels within discrete regions important for the regulation of stress and emotional behavior, such as the amygdala and medial prefrontal cortex. More so, there is almost no information regarding the temporal nature of the effects of stress on central inflammatory mediators, particularly with respect to the analysis of both mRNA and protein. Finally, as most studies have limited their analysis to prototypical pro-inflammatory cytokines (such IL-1β, IL-6 and TNF-α), it is not known if the effects of stress on cytokine levels are specific to this class of molecules or if there are differential effects of related signaling molecules, such as anti-inflammatory cytokines and chemokines. Accordingly, the current study provides a detailed and comprehensive analysis of both the mRNA and protein of a multitude of inflammatory mediators within corticolimbic brain structures involved in the regulation of stress (amygdala, hippocampus, hypothalamus and medial prefrontal cortex). Using complimentary techniques, our data indicate that acute exposure to psychological stress produces region-specific changes in inflammatory molecules, with the most prominent findings indicating that the pattern of these changes are highly divergent between the amygdala and medial prefrontal cortex.

2. Methods

2.1. Animals

All experiments utilized adult (apx. 70 post-natal days old/300 g), male, Sprague Dawley rats from Charles River (St. Constant, QC, Canada). Animals were allowed to acclimate for at least one week prior to experimental testing. Rats were kept on a 12:12 h light/dark cycle and had *ad libitum* access to food and water. All experiments were conducted during the light phase of the cycle. All animal protocols were approved by the University of Calgary Animal Care Committee and Canadian Council for Animal Care.

2.2. Experiment 1: Spatiotemporal mapping of acute psychological stress-induced cytokine production

2.2.1. Design

Animals in the stress groups were exposed to 120 min of restraint stress using a clear, Plexiglass restrainer within the first 2 h of the light cycle. The basal groups were kept in their home cages prior to tissue collection. Rats were decapitated either, immediately (0 h), 1 h, 2 h, 4 h or 24 h post-stress termination ($n = 8$ for all time points). Due to circadian fluctuations in cytokine levels (Nakao, 2014), we added an additional basal group for the 4 h post-stress termination time-point to allow for appropriate comparison with basal cytokine levels at this time-point (tissue harvested between 4 and 6 h following lights on). Accordingly, there were two basal groups, 0B, which was compared to the 0 h, 1 h, 2 h and 24 h stress groups (as all analysis was performed within the first 4 h of the light cycle) and 4B, which was compared to the 4 h post-stress group. A timeline schematic can be found in Fig. 1A.

Corticolimbic brain regions (amygdala, hippocampus, hypothalamus and medial prefrontal cortex) were excised using gross tissue dissection, as previously described (Hill et al., 2010), and immediately flash frozen on dry ice and stored for processing at −80 °C. Vacutainer blood collection tubes containing K2 ethylenediaminetetraacetic acid (EDTA) were used to collect trunk blood. Post collection, blood was held on ice for no more than 30 min and then centrifuged for 20 min at 10,000 *g* at 4 °C. Plasma was aliquoted and stored at −20 °C for corticosterone measurement and −80 °C for other measures.

2.2.2. Corticosterone ELISA

Plasma corticosterone levels were measured using a commercially available enzyme linked immunosorbent assay (ELISA) kit

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