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# Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses

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**Abstract**

Prior exposure to a stressor can potentiate CNS pro-inflammatory immune responses to a peripheral immune challenge. However, the neuroimmune substrate(s) mediating this effect has not been determined. The present investigation examined whether microglia serve as this neuroimmune substrate given that microglia are the primary immune effector cell in the CNS. The effect of inescapable shock (IS) on glial activation (MHC II, CD11b, Iba-1, and GFAP) and regulatory markers (CD200) in vivo, and microglia pro-inflammatory responses (interleukin-1β; IL-1β) to lipopolysaccharide (LPS) ex vivo, were assessed in rat hippocampus. IS upregulated the microglia activation marker MHC II 24 h post-IS, while the astroglia marker GFAP was unaffected. IS also downregulated the neuronal glycoprotein CD200, which functions to hold microglia in a quiescent state. Moreover, IS potentiated the pro-inflammatory response to LPS ex vivo 24 h post-IS in isolated hippocampal microglia. Finally, the behavioral controllability of shock was manipulated and the effect of escapable (controllable) shock was comparable to the effect of IS on hippocampal microglia responses to LPS ex vivo. The present results suggest that stress can activate microglia, thereby sensitizing the pro-inflammatory reactivity of microglia to immunogenic stimuli.


*Keywords:* Stress; CNS; Pro-inflammatory cytokines; Interleukin 1-β; Microglia; MHC II; CD200; Sensitization; Priming

## 1. Introduction

Both exposure to stressors and peripheral immune activation induce corresponding pro-inflammatory "cytokine signatures" in the CNS (Maier, 2003). Moreover, there can be cross-sensitization between the two, so that stress potentiates CNS cytokine responses to peripheral pro-inflammatory stimuli (Johnson et al., 2002), suggesting that stress and infection may induce neural processes that intersect at a shared neuroimmune substrate(s). While the neuroimmune substrate for the interaction between stress and infection is unknown, the microglial cell is a likely candidate as this cell type is the primary immune effector cell in the CNS mediating pro-inflammatory immune reactions. The present investigation examined whether microglia serve as a basis for stress-induced potentiation of pro-inflammatory responses to immunogenic stimuli.

Peripheral immune activation with immunogens such as lipopolysaccharide (LPS) signals the brain via a number of routes (Maier, 2003). When the signal from peripheral immune activation reaches the brain a complex neural cascade ensues, and interestingly, this cascade includes the *de novo* synthesis of pro-inflammatory cytokines such as interleukin (IL)-1β at both the protein (Hagan et al., 1993; Hillhouse and Mosley, 1993; van Dam et al., 1992) and mRNA level (Ban et al., 1992; Buttini and Boddeke, 1995; Laye et al., 1994). Within the brain, IL-1β mediates, in part, the induction of sickness behaviors in response to infection (Maier, 2003). Importantly, stressors also induce many components of this

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constellation of sickness behaviors (Maier and Watkins, 1998).

Not only can stressors and peripheral immune activation produce similar behavioral and physiological changes, but stressors can also induce pro-inflammatory cytokines in the CNS (Ishikawa et al., 2001; Minami et al., 1991; Nguyen et al., 1998; O'Connor et al., 2003; Shintani et al., 1995). This overlap in the neural processes produced by stress and infection could be responsible for the potentiated CNS pro-inflammatory cytokine response to a peripheral immune challenge produced by prior exposure to stressors (Johnson et al., 2002). Of note, central administration of the IL-1 receptor antagonist blocks stress-induced potentiation of CNS pro-inflammatory immune responses to a peripheral immune challenge, while exogenous IL-1β mimics the effects of stress (Johnson et al., 2004). These findings suggest that central IL-1β mediates stress-induced potentiation of the CNS immune response. Since (a) stress only transiently up regulates IL-1β in the CNS (O'Connor et al., 2003), but (b) stress-induced potentiation of the CNS immune response to LPS occurs at least 24 h after stress exposure (Johnson et al., 2002), stress would appear to prime or sensitize the CNS to respond in a heightened fashion to a subsequent peripheral immune stimulus. Taken together, the above findings suggest that stress and infection induce processes in the CNS that may converge upon a cell type, which plays a salient role in CNS pro-inflammatory cytokine reactions. Since microglia are a primary immune effector cell in the CNS and are a major source of CNS pro-inflammatory cytokines (Kreutzberg, 1996), microglia may serve as this neuroimmune substrate. Astrocytes might also play a role, but microglia are more reactive than astrocytes and the induction of IL-1β by peripheral immune activation is much more prominent in microglia than astrocytes (Aloisi, 2001). For these reasons, microglia are the focus of the present investigation.

Microglia are typically described as the resident macrophage occupying the parenchyma of the CNS (Gehrmann et al., 1995). Under basal conditions, microglia exhibit a quiescent phenotype as indicated by a ramified morphology, downregulation of activation antigens such as major histocompatibility complex (MHC)II, and constitutive expression of macrophage antigens such as complement receptor 3 (CD11b) and ionized calcium binding adaptor protein-1 (Iba-1)(Ladeby et al., 2005). Under a multitude of CNS pathological conditions and systemic infectious processes, microglia undergo a phenotypic transformation characterized by a deramified morphology, upregulation of antigen presentation molecules (MHC II, CD80, CD86, and CD40), production of pro-inflammatory cytokines, generation of reactive oxygen and nitrogen species, and display of phagocytic activity (Streit et al., 1999). Microglia may display a subset of these phenotypes depending on the state of activation (Ladeby et al., 2005). Relevant to our interest in the relationship between stress and infection, microglia can enter an activation state, wherein they retain a ramified morphology, up regulate activation antigens such as MHC II, and yet are not secreting pro-inflammatory molecules (Betmouni et al., 1996; Walsh et al., 2001). However, when further stimulated in this state either through a peripheral or central immune challenge, microglia over-express pro-inflammatory cytokines such as IL-1β (Cunningham et al., 2005). In addition, a peripheral immune challenge in animals with chronic microglia activation show exaggerated sickness behavior (Combrinck et al., 2002). This "activated" or "primed" phenotype has been characterized in several neuropathologies, wherein the induction of a systemic infectious process exacerbates behavioral consequences of the disease state as well as potentiates pro-inflammatory cytokine production in the CNS (Perry et al., 2003). From these studies, Perry et al. (2003) developed a conceptual framework of microglia priming, in which a primary insult or disease process induces an "activated" microglia phenotype. Upon subsequent induction of a peripheral pro-inflammatory process (secondary insult), microglia exhibit a potentiated pro-inflammatory phenotype.

This phenomenon of microglial priming may underlie stress-induced potentiation of CNS pro-inflammatory responses to subsequent peripheral immune activation. Stress may function as the primary insult to activate microglia, thereby priming or sensitizing microglia to pro-inflammatory stimuli. Subsequent exposure to a peripheral pro-inflammatory stimulus may then result in a potentiated microglial pro-inflammatory response. There has been little study of whether stress might alter microglial activation. A recent report demonstrated that restraint stress activates microglia and induces microglia proliferation (Nair and Bonneau, 2006), however, we were unable to find any published reports on stress-induced sensitization of microglia pro-inflammatory responses. In the present investigation, we examined whether stress primes microglia. To test this hypothesis, the effect of uncontrollable stress on microglia activation markers was examined in vivo to determine whether stress shifts the state of microglia from quiescent to activated. In addition, the effect of controllable and uncontrollable stress on microglia responses to a pro-inflammatory stimulus was examined ex vivo to directly test whether microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory processes. The present study was restricted to examining hippocampal microglia given that stress potentiates hippocampal pro-inflammatory processes to peripheral LPS (Johnson et al., 2002) and the importance of this structure in pro-inflammatory cytokine-induced behavioral impairments (Rachal Pugh et al., 2001). We have developed a method for the rapid isolation of highly pure hippocampal microglia, which preserves the in vivo immunophenotype of these cells (Frank et al., 2006), thereby enabling us to examine stress-induced microglia priming.