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Research report

Social interaction modulates the neuroinflammatory response to global cerebral ischemia in male mice

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ABSTRACT

Social isolation is a risk factor for cardiovascular and cerebrovascular diseases, although the underlying mechanisms remain underspecified. Considering the potential of microglia to become sensitized by stressors and their role in neuroinflammation, we hypothesized that social isolation primes microglia, resulting in an exaggerated neuroimmune response to experimental cerebral ischemia. First, major histocompatibility complex II (MHC II) gene expression, an indicator of microglial priming, was compared between mice that were socially isolated or pair-housed. MHC II increased in the hippocampus and cortex of socially isolated mice, which is suggestive of isolation-induced microglial priming. In experiment 2, isolated and pair-housed mice underwent ~8 min of global cerebral ischemia. Hippocampal mRNA expression of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) was significantly increased among both isolated and pair-housed ischemia groups relative to sham controls. Hippocampal expression of interleukin 1 beta (IL-1β) and cortical TNF-α, IL-1β and IL-6, were significantly increased 24-h post ischemia in isolated mice, but not pair-housed mice, relative to controls. Ischemia-induced increases in microglial cell body area and percent area fraction of ionized calcium binding adaptor molecule 1 (Iba-1) positive staining were also observed in isolated, but not pair-housed mice, relative to controls. For experiment 3, brain sections from socially isolated and pair-housed mice underwent 15 min of oxygen glucose deprivation (OGD), an *ex vivo* model of cerebral ischemia. IL-6 gene expression was significantly elevated following OGD only in hippocampi from mice that had been socially isolated, indicating that isolation prior to ischemia is sufficient to modulate the neuroinflammatory response. Together, these data suggest microglial priming as a possible mechanism underlying the detrimental effects of social isolation on cerebral ischemia outcome.

1. Introduction

Social isolation presents as a risk factor for cardiovascular and cerebrovascular diseases (Mookadam and Arthur, 2004; Brainin and Dachenhausen, 2013; Holt-Lunstad and Smith, 2016), and is associated with increased all-cause mortality (House et al., 1988; Rebelin and Uchino, 2008). In contrast, social support is associated with a positive impact on health and disease outcome (DeVries et al., 2007; Karelina and DeVries, 2011). Although loneliness cannot be assessed in non-human animals, the physiological changes triggered by isolation in social species mirror those experienced by humans encountering loneliness (Hawkley and Capitanio, 2014). At times, the health benefits of social interaction are attributed to improved health behaviors, but the ability to recapitulate the effects of social behavior on health outcomes in non-human animals suggests that this explanation is not sufficient (Karelina and DeVries, 2011). Indeed, there is growing evidence that social modulation of the immune system may explain social influences on a wide range of experimental and clinical settings.

Studies from a range of species, including humans, indicate that social environment can modify risk and outcome to cerebral ischemia (Glass et al., 1993; Reeves et al., 2008; Karelina and DeVries, 2011; Brainin and Dachenhausen, 2013). Specifically,

Abbreviations: CA/CPR, cardiac arrest/cardiopulmonary resuscitation; OGD, oxygen-glucose deprivation; aCSF, artificial cerebrospinal fluid; rtPCR, real-time polymerase chain reaction; MHC II, major histocompatibility complex II; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin 1 beta; IL-6, interleukin 6; Nfκb, nuclear factor kappa B; Iba-1, ionized calcium-binding adaptor 1.

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rodent experiments have demonstrated that the negative effects of social isolation on cerebral ischemia include an increased neuroinflammatory response, greater neuronal death, and impaired functional recovery (Craft et al., 2005; Weil et al. 2008; Norman et al., 2010a; Venna et al., 2012; Karelina et al., 2011). These detrimental effects are likely linked to increased ischemia-induced activation of microglia among socially isolated mice relative to socially integrated mice (Weil et al. 2008; Karelina et al., 2011). There is ample evidence that a wide range of stressors, including social stress, can prime microglia to respond in an exaggerated and prolonged manner to a subsequent inflammatory stimulus (Wohleb et al., 2011; Frank et al., 2012). Thus, microglial sensitization may underlie the exacerbating effects of social isolation of cerebral ischemia. Microglia perform important homeostatic functions in the brain (Davalos et al., 2005; Kim and deVilles, 2005), but rapidly become activated in response to neuronal injury, including cerebral ischemia (Danon and Dietrich, 2003; Iadecola and Anrather, 2011). Activated microglia can secrete pro-inflammatory cytokines and produce reactive oxygen species (Hanisch, 2002; Block et al., 2007; Iadecola and Anrather, 2011). Specifically, following cerebral ischemia, gene expression of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and nuclear factor kappa b (Nfκb) are significantly upregulated, indicating a pro-inflammatory response (Schneider et al., 1999; Stephenson et al., 2000; Norman et al., 2010a; Doll et al., 2014). Generally, increased post-ischemic neuroinflammation is associated with increased cell death and worse neurological outcome (Wang and Shuaib, 2002; Weil et al. 2008; Neigh et al., 2009; Norman et al., 2010a; Stuller et al., 2012; Doll et al., 2014; Wang et al., 2015).

The present work utilizes both *in vivo* and *ex vivo* models of global cerebral ischemia, to examine social modulation of the neuroinflammatory response to ischemia. Cardiac arrest is an *in vivo* model of global cerebral ischemia, in which heart stoppage eliminates blood flow throughout the body, leading to global ischemia (Kofler et al., 2004; Neigh et al., 2004; Noppens et al., 2005; Hutchens et al., 2012; Khodanovich and Kisel, 2015). In this model, body temperature is lowered to protect the peripheral tissues while the brain is maintained at typical body temperature, thus limiting the direct effects of ischemia cell death to the brain (Kofler et al., 2004; Neigh et al., 2004). The neurological damage that evolves following cardiopulmonary resuscitation (CPR) results from both the direct effects of cerebral ischemia and reperfusion, as well as secondary effects of the associated physiological alterations, such as disruption of the blood brain barrier. On the other hand, oxygen-glucose deprivation (OGD) is an *ex vivo* model of global cerebral ischemia (Whittingham et al., 1984; Tasca et al., 2015). Following dissection and sectioning of the brain, control samples are incubated in artificial cerebrospinal fluid (aCSF) while the ischemic samples are incubated in similar conditions minus the glucose and oxygen (Xie et al., 2008; Tasca et al., 2015). An advantage of the *ex vivo* models of cerebral ischemia is the availability of tissue from the same animal for both the control and ischemic group, minimizing individual variability and animal usage. Nonetheless, it limits the investigation to that occurring in the isolated brain, eliminating the possible influence of other physiological systems. Thus, any differences in OGD outcome in the current studies are attributed to the pre-ischemic state of the brain tissue.

In the current studies, male mice were housed in social isolation or with an ovariectomized female beginning one week prior to the induction of cerebral ischemia and then through the reperfusion period; similar housing manipulations have been used to recapitulate the effects of social environment on a wide array of health conditions (Weil et al. 2008; Norman et al., 2010b; Karelina et al., 2011). To date, all studies identifying social interaction as neuroprotective against ischemic damage were completed using *in vivo* models (Craft et al., 2005; Weil et al. 2008; Norman et al., 2010a; Venna et al., 2012; Venna et al., 2014; Verma et al., 2014). With the *ex vivo* OGD model one can determine whether the social environment prior to an ischemic event is sufficient to influence the post-ischemic inflammatory response. If so, this will further support the hypothesis that upon social isolation animals undergo physiological changes that can prime their immune responses, and that a poor social environment may convey disease vulnerability.

2. Results

2.1. Experiment 1

Adult male mice were socially isolated or pair-housed with an ovariectomized female for a week. Following tissue collection, the hippocampus and pre-frontal cortex were dissected and assayed to examine gene expression.

2.1.1. Gene expression in the hippocampus and cortex following isolation

Analysis of the hippocampal and cortical mRNA expression of MHC II and Nfκb1 suggests that seven days of social isolation sensitizes but does not activate the immune system. There is a statistically significant increase in the hippocampal (Fig. 1A; $t_{19} = 3.163$, $p < 0.05$) and cortical (Fig. 1B; $t_{19} = 3.960$, $p < 0.05$) mRNA expression of MHC II among isolated mice relative to paired mice. In contrast, gene expression of Nfκb1 in the hippocampus (Fig. 1C; $t_{19} = 0.2838$, $p > 0.05$) and cortex (Fig. 1D; $t_{21} = 0.2592$, $p > 0.05$) does not vary in response to the social environment.

2.2. Experiment 2

Adult male mice were socially isolated or pair-housed for a week, and then received a sham control or cardiac arrest/cardiopulmonary resuscitation (CA/CPR) procedure. Twenty-four hours later, tissue was collected for either gene expression analysis or histological analysis of microglia.

2.2.1. Hippocampal gene expression of pro-inflammatory cytokines after CA/CPR

Analysis of the hippocampal mRNA expression of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, indicates that CA/CPR elicits neuroinflammation, some components of which are modified among pair-housed mice. There is a statistically significant difference among the treatment groups in hippocampal mRNA expression of TNF-α (Fig. 2A; $F(2,33) = 15.83$, $p < 0.05$). Specifically, the sham group expresses significantly lower TNF-α mRNA, than both the paired CA/CPR and the isolated CA/CPR groups ($p < 0.05$ for both). Hippocampal mRNA expression of IL-1β also differs significantly among experimental groups (Fig. 2B; $F(2,33) = 6.723$, $p < 0.05$). Specifically, the sham group has significantly lower IL-1β mRNA expression within the hippocampus relative to the isolated CA/CPR group ($p < 0.05$), whereas IL-1β mRNA expression does not differ between the sham group and the paired CA/CPR group ($p > 0.05$). The pattern of hippocampal IL-6 mRNA expression is similar to TNF-α; there is a statistically significant difference among the experimental groups (Fig. 2C; $F(2,32) = 11.24$, $p<0.05$), with the sham group expressing significantly lower IL-6 mRNA than both the paired CA/CPR ($p < 0.05$) and the isolated CA/CPR ($p < 0.05$) groups.

2.2.2. Cortical gene expression of pro-inflammatory cytokines after CA/CPR

In the cortex, increased expression of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, indicates that CA/CPR elicits