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Environmental enrichment attenuates hippocampal neuroinflammation and improves cognitive function during influenza infection

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ABSTRACT

Recent findings from our lab have shown that peripheral infection of adult mice with influenza A/PR/8/34 (H1N1) virus induces a neuroinflammatory response that is paralleled by loss of neurotrophic and glial regulatory factors in the hippocampus, and deficits in cognitive function. Environmental enrichment has been shown to exert beneficial effects on the brain and behavior in many central nervous system (CNS) disorders, but its therapeutic potential during peripheral viral infection remains unknown. Therefore, the objective of the present study was to determine if long-term continuous exposure to environmental enrichment could prevent and/or attenuate the negative effects of influenza infection on the hippocampus and spatial cognition. Mice were housed in enriched or standard conditions for 4 months, and continued to live in their respective environments throughout influenza infection. Cognitive function was assessed in a reversal learning version of the Morris water maze, and changes in hippocampal expression of proinflammatory cytokines (IL-1β, IL-6, TNF-α, IFN-α), neurotrophic (BDNF, NGF), and immunomodulatory (CD200, CX3CL1) factors were determined. We found that environmental enrichment reduced neuroinflammation and helped prevent the influenza-induced reduction in hippocampal CD200. These changes were paralleled by improved cognitive performance of enriched mice infected with influenza when compared to infected mice in standard housing conditions. Collectively, these data are the first to demonstrate the positive impact of environmental enrichment on the brain and cognition during peripheral viral infection, and suggest that enhanced modulation of the neuroimmune response may underlie these beneficial effects.

1. Introduction

Influenza is a common and highly contagious viral pathogen that remains a significant health concern worldwide. Neurological symptoms have been reported following respiratory infection with pandemic strains of influenza A (CDC, 2009; Ravenholt and Foege, 1982; Studahl, 2003; Wang et al., 2010), suggesting that peripheral infection can impact central nervous system (CNS) function. While the mechanisms underlying the effects of non-neurotropic viral strains on the brain remain unclear (Schlesinger et al., 1998; Studahl, 2003), it has been hypothesized that glia-mediated neuroinflammation in association with common respiratory viruses may lead to cumulative neuronal damage across the lifespan (Majde, 2010).

Using a mouse model of influenza, we have recently shown that peripheral infection with influenza A/PR8/34 (H1N1) induces a neuroimmune response, and impacts hippocampal structure and function (Jurgens et al., 2012). Infected mice demonstrated cognitive deficits in hippocampal-dependent tasks that were paralleled by significant alterations in hippocampal neuron morphology in the CA1 region and dentate gyrus. Concurrent with cognitive impairment, influenza-infected mice had increased hippocampal expression of inflammatory cytokines (interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and interferon (IFN)-α), and decreased expression of brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Previous work has shown that elevated levels of proinflammatory cytokines in the hippocampus can inhibit neurotrophic factors (Barrientos et al., 2004; Tong et al., 2008) and impair hippocampal synaptic plasticity (Lynch, 2002; Mendoza-Fernandez et al., 2000; Pickering and O'Connor, 2007), providing a potential mechanism by which hippocampal neuroinflammation can induce cognitive dysfunction. In addition, influenza significantly reduced the levels of immunomodulatory factors CD200 and CX3CL1 (fractalkine), which play an important role in the neuronal control of microglia activation (Cardona et al., 2006; Hoek et al., 2000), and have recently been found to impact synaptic development and integrity (Costello et al., 2011; Paolicelli et al., 2011; Ransohoff and Stevens, 2011). Overall, the loss of neurotrophic

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support along with dysregulated neuron-microglia crosstalk, could negatively impact cognitive function and leave the brain vulnerable to inflammatory damage during influenza infection.

A potential strategy for increasing brain plasticity and resiliency is environmental enrichment. Environmental enrichment (EE) is classically defined as a "complex combination of social and inanimate stimulation" (Rosenzweig and Bennett, 1996), in comparison to standard housing conditions. While there is variation in experimental protocols (Simpson and Kelly, 2011), in general EE refers to conditions that provide increased social, cognitive, and physical stimulation. An enriched environment for rodents typically includes group housing in a large cage with nesting materials, various toys, tunnels and ladders for exploration, and running wheels for voluntary exercise (Nithianantharajah and Hannan, 2006; van Praag et al., 2000). Items of enrichment are routinely changed during the experimental period to maintain novelty and provide continuous opportunity for exploration and learning.

Environmental enrichment has been shown to exert positive effects on cognitive and emotional behaviors including enhanced learning and memory (Bruel-Jungerman et al., 2005; Leggio et al., 2005; Nilsson et al., 1999) and improved ability to cope with fear, anxiety and stress (Fox et al., 2006; Larsson et al., 2002; Schloesser et al., 2010). Early work demonstrated that exposure to a complex and stimulating environment could produce changes in neuron morphology, myelination and synaptogenesis during development, adulthood and aging (Bennett et al., 1969; Diamond et al., 1976; Green et al., 1983; Greenough et al., 1986; Juraska and Kopcik, 1988). This suggests that neural plasticity in response to environmental experience lasts throughout the lifespan. In addition to morphological changes, EE has also been shown to elevate levels of neurotrophic factors (Ickes et al., 2000; Pham et al., 1999) and synaptic proteins (Nithianantharajah et al., 2004; Rampon et al., 2000), as well as enhance hippocampal synaptic plasticity (Artola et al., 2006; Green and Greenough, 1986) and increase neurogenesis (Kempermann et al., 1997; Rossi et al., 2006).

While the beneficial effects of EE have been explored during aging, as well as in animal models of neurodegenerative disease, psychiatric disorders and brain injury (Laviola et al., 2008; Mora et al., 2007; Nithianantharajah and Hannan, 2006; Will et al., 2004), the impact of EE on the central immune response and cognitive behavior during peripheral viral infection remains unknown. Since influenza infection has been shown to negatively impact measures of hippocampal and cognitive function, which are influenced by EE, the present study sought to determine if the deleterious effects of influenza infection on the CNS could be prevented or attenuated by long-term continuous exposure to environmental enrichment. We examined the impact of environmental enrichment on influenza-induced sickness response and cognitive behavior, and assessed changes in inflammatory, neurotrophic, and immunomodulatory factors in the hippocampus. We focused our study on the hippocampus, as this is a brain region that demonstrates a high degree of plasticity, is vulnerable to inflammation, and plays an important role in cognitive function. We hypothesized that continuous exposure to an enriched environment throughout adulthood and during influenza infection would help modulate inflammation, preserve levels of neurotrophic and glial regulatory factors, and prevent or reduce cognitive deficits induced by peripheral infection with live influenza virus.

2. Materials and methods

2.1. Subjects

Young (6 weeks old) male BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). On arrival, mice were group housed and allowed at least 7 days to acclimate before experimental housing treatments were initiated. Following acclimation, animals were randomly assigned to the enriched environment (EE, $n = 5–8$/cage) or standard environment (SE, $n = 1$/cage) conditions. Briefly, the enriched environment consisted of a large cage ($100 \times 51 \times 37$ cm) equipped with toys, tunnels, ladders, housing chambers, nesting material and two running wheels (Fig. 1). Mice were housed continuously in the enriched environment with rearrangement of toys, tunnels, and wheels 3–4 times per week to maintain novelty. The standard environment consisted of a laboratory polypropylene cage with bedding. All mice were maintained under a reverse 12:12 h light:dark cycle at 24 °C and given *ad libitum* access to food and water. All animal care and experimental procedures are in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of Illinois Institutional Animal Care and Use Committee.

2.2. Mouse viral infection

Mouse-adapted human influenza A/Puerto Rico/8/34 (H1N1) virus was a generous gift from Dr. John Sheridan and Dr. David Padgett (The Ohio State University). Mice were lightly anesthetized with isoflurane and intranasally (i.n.) inoculated with 1 HAU (hemagglutinating unit) of influenza A/PR/8/34 virus in 50 μl sterile PBS. Control animals were inoculated (i.n.) with 50 μl of sterile PBS. Use of isoflurane anesthesia allowed for quick recovery (<2 min) and accurate delivery of viral dose. Following viral infection, mice were monitored daily for signs of morbidity, including response to handling, food intake, and physical appearance (i.e. ruffled fur, huddled or hunched behavior). In mouse models of influenza, weight loss is considered a reliable indicator of disease progress (Matsuoka et al., 2009), thus body weight was measured at the same time daily (between 09:00 and 10:00) and percent change from initial body weight (day 0) was calculated. Previous studies (Lowder et al., 2005) have shown that during influenza infection, mice can lose a substantial amount of body weight (30–35%) and still recover, however as an established humane endpoint, mice that reached a 30% loss of their initial body weight were euthanized (day 7 post-inoculation).

2.3. Morris water maze

The effects of environmental enrichment and influenza infection on spatial learning and memory were assessed in a reversal learning version of the Morris water maze. In this hippocampal-dependent task, the animal must learn to use distinctive distal visuo-spatial cues surrounding the pool to navigate a direct path to the hidden platform (D'Hooge and De Deyn, 2001; Morris, 1984). The Morris water maze (MWM) consisted of a circular pool (130 cm diameter, 23–25 °C) with a transparent round platform (10 cm diameter) hidden 0.5 cm below the surface of the water. Mice were inoculated (day 0) and 2 days later began a 5-day acquisition phase (days 2–6 post-inoculation). The platform remained in the same location during the acquisition training. Animals were placed on the platform for 30 s preceding the start of each training session. The trials were conducted using a pseudorandom protocol in which mice were placed in the water in one of three preset entry locations. Mice were allowed to swim freely for 60 s or until the platform was reached. If the platform was not located during the 60 s, mice were guided to the platform and allowed to remain for 30 s. After completion of three consecutive trials, mice were placed in their home cage under a heat lamp to dry for 10 min. On day 7 post-inoculation (24 h after the last day of acquisition training), the platform was removed and mice received a 30 s probe trial to assess spatial memory for the platform location. Following the