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SUSTAINED EXPRESSION OF INTERLEUKIN-1β IN MOUSE HIPPOCAMPUS IMPAIRS SPATIAL MEMORY

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Abstract—Glial activation and neuroinflammation occur in neurodegenerative disease and brain injury, however their presence in normal brain aging suggests that chronic neuroinflammation may be a factor in age-related dementia. Few studies have investigated the impact of sustained elevation of hippocampal interleukin-1β, a pro-inflammatory cytokine upregulated during aging and Alzheimer's disease, on cognition in mice. We utilized the IL-1β^{XAT} transgenic mouse to initiate bilateral hippocampal overexpression of interleukin-1β to determine the influence of sustained neuroinflammation independent of disease pathology. Fourteen days following transgene induction, adult male and female IL-1β^{XAT} mice were tested on non-spatial and spatial versions of the Morris water maze. For the spatial component, one retention trial was conducted 48 h after completion of a 3 day acquisition protocol (eight trials per day). Induction of IL-1β did not impact non-spatial learning, but was associated with delayed acquisition and decreased retention of the spatial task. These behavioral impairments were accompanied by robust reactive gliosis and elevated mRNA expression of inflammatory genes in the hippocampus. Our results suggest that prolonged neuroinflammation response *per se* may impact mnemonic processes and support the future application of IL-1β^{XAT} transgenic mice to investigate chronic neuroinflammation in age- and pathology-related cognitive dysfunction.

Key words: neuroinflammation, water maze, IL-1β^{XAT} transgenic mouse, hippocampus.

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Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; CCR2, chemokine (C–C motif) receptor 2; COX, cyclooxygenase; CXCR2, chemokine (C-X-C motif) receptor 2; FIV-Cre, feline immunodeficiency virus-Cre recombinase protein; FIV-GFP, feline immunodeficiency virus-green fluorescent protein; GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium binding adaptor molecule-1; IL-1α, interleukin-1α; IL-1β, interleukin-1β; IL-1ra, interleukin-1 receptor antagonist; KC, keratinocyte chemoattractant; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MHC-II, major histocompatibility complex II; MIP-2, macrophage-inflammatory protein-2; MWM, Morris water maze; PG, prostaglandin; qRT-PCR, quantitative real-time RT-PCR; SEM, standard error of the mean; tOD, threshold optical density; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick end labeling.

Chronic neuroinflammation is a prominent feature of Alzheimer's disease (AD) and is believed to contribute to the molecular cascade that ultimately manifests as cognitive dysfunction. It is well known that the single most important risk factor for AD is age. One reason for this association is that the progression from initial pathophysiological event to clinical detection is likely to be on the order of decades. Although glial activation is influenced by neuronal plaques and tangles, its presence in the aged brain (Nichols et al., 1993; Perry et al., 1993; Conde and Streit, 2006; Beach et al., 2007; Gavilan et al., 2007), independent of AD-like neuropathology, suggests that chronic neuroinflammation may be an initial component of and factor in age-related dementia (Cagnin et al., 2001; Weaver et al., 2002).

Reactive glia produce a variety of molecules that trigger and contribute to chronic neuroinflammation. Termed "the cytokine cycle" (Griffin et al., 1998), pro-inflammatory cytokines participate in a spectrum of signaling events that continuously feedback and influence each other. Microglial-derived IL-1β appears to be a driving force in this process. IL-1β has been previously shown to be a potent immunomodulating cytokine that induces multiple inflammatory mediators in astrocytes and neurons (Mrak et al., 1995). IL-1β overexpression is a consistent feature of postmortem AD brain, with double-labeling immunohistochemical studies localizing IL-1 to plaque-associated microglia (Griffin et al., 1995, 2000; Shaftel et al., 2008). In addition to initiating and sustaining inflammation-related events and modulating neurons, IL-1β appears to have direct relation to pathophysiological alterations in AD (for review, refer to; Moore and O'Banion, 2002; Shaftel et al., 2008). Its regional expression around plaques and its temporal profile of immunoreactivity relative to pathology implicates IL-1β as a mediator of plaque and tangle formation. Not restricted to AD pathology, increased IL-1β is found in the hippocampus of aged rats (Murray and Lynch, 1998; Griffin et al., 2006), reinforcing the hypothesis that chronic neuroinflammation may be initiated by the normal process of aging. However, the role of chronic neuroinflammation in cognitive dysfunction has yet to be clearly determined.

Understanding the role of neural expression of pro-inflammatory molecules in memory processes has been addressed in animal models. Numerous investigators have described learning and memory impairments associated with acute central IL-1β induction ($\leq$5 days) following peripheral lipopolysaccharide (LPS) stimulus (Shaw et al., 2001; Yirmiya et al., 2002; Sparkman et al., 2005a,b; Wu et al., 2007) and direct central administration of IL-1β (Yirmiya et al., 2002; Goshen et al., 2007; Hein et al., 2007). Impaired hippocampal-dependent performance has

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also been reported at 24 h following intracerebroventricular infusion of IL-1 receptor antagonist (IL-1ra; Yirmiya et al., 2002) and in transgenic mice lacking expression of IL-1ra (Avital et al., 2003), further suggesting a role of constitutive IL-1 signaling in cognition. However, only a limited number of studies address the adult onset and ongoing presence of neuroinflammation that is representative of aging and AD. Substantial research has been conducted in a rat model of chronic (>7 days) neuroinflammation (Hauss-Wegrzyniak et al., 1998a,b, 2000a,b) and transgenic mouse models of AD neuropathology. Yet, considering the prevalence of mice as the standard research model given the potential and ease of genetic manipulation, data are sparse on the effect of prolonged region-specific glial activation and elevated IL-1β brain concentrations *per se* on behavior in adult mice. Therefore, we utilized a recently established transgenic mouse model (IL-1β^{XAT}; Shaftel et al., 2007a,b) to determine the effect of induced human IL-1β in the hippocampus on spatial memory in adult C57BL/6 mice. Our results suggest that sustained (14 days) elevation of hippocampal hIL-1β and the accompanying increase in pro-inflammatory molecules drive mnemonic deficits, establishing the IL-1β^{XAT} mouse as a valid model to investigate the consequence of chronic hIL-1β expression.

EXPERIMENTAL PROCEDURES

Subjects

IL-1β^{XAT} transgenic mice. Twenty-two IL-1β^{XAT} mice (13 male and nine female) were used in this study. Creation and genotyping of the IL-1β^{XAT} mice on a C57BL/6 background have been described previously (Shaftel et al., 2007a,b). Briefly, the IL-1β^{XAT} mice harbor a transgene construct consisting of a murine glial fibrillary acidic protein (GFAP) promoter (Stalder et al., 1998), loxP flanked transcriptional stop, and downstream ssIL-1β transgene coding for the signal sequence from the human IL-1ra (75 bp) fused to the cDNA sequence of human mature IL-1β (464 bp) (Wingren et al., 1996). Transgene activation occurs upon feline immunodeficiency virus (FIV)-Cre recombinase protein (Cre) mediated excision of a transcriptional stop. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at University of Rochester and Santa Clara University for compliance with federal regulations before the initiation of the study.

Feline immunodeficiency virus. The construction and packaging of FIV-Cre has been described previously (Lai et al., 2006b). Briefly, the FIV-Cre virus encodes the nuclear localization sequence (nls), Cre, and V5 epitope tag under the control of a cytomegalovirus promoter. FIV-Cre and FIV-green fluorescent protein (GFP; System Biosciences, Mountain View, CA, USA) were packaged to a final titer of 1×10^7 infectious viral particles (IVP) per ml. *In vivo* stereotactic injections were performed at 12 weeks of age and used 1.5 μl of virus to deliver 1.5×10^4 IVP to the mouse hippocampus. Viral titers were established in the 293^{FT} cell line using an anti-V5 antibody (Invitrogen, Carlsbad, CA, USA) or GFP fluorescence.

Stereotactic injections. Intrahippocampal injections were performed as described previously (Shaftel et al., 2007a,b). Briefly, mice were anesthetized with 1.75% isoflurane in 30/70% oxygen/nitrogen gas. While secured to a Kopf stereotaxic apparatus in a biosafety level two approved facility, two 0.5 mm burr holes were drilled in the skull at −1.8 mm caudal and 1.8 mm horizontal on each side of bregma. A pre-loaded 33 gauge needle was lowered first into the right hippocampus, 1.75 mm from the brain surface over 2 min after which 1.5 μl of virus was injected at a constant rate over 10 min. After allowing 5 min for diffusion of the virus, the needle was raised over 2 min. A second injection was performed in an identical manner on the contralateral side. The burr holes were sealed with bone wax and the scalp incision was closed with 6–0 nylon suture (Ethicon, Somerville, NJ, USA). Control animals received bilateral intrahippocampal injections of FIV-GFP using the same procedures.

Behavioral apparatus and procedures

Fourteen days after bilateral hippocampal injections, mice were tested on non-spatial and spatial learning using the Morris water maze (MWM) adapted for mice (Vorhees and Williams, 2006). The MWM consisted of a black, plastic circular tub (88 cm diameter, 12 cm deep) filled with water (25±1 °C) made opaque using non-toxic white tempera paint. A removable 10 cm^2 plexiglas platform with weighted base was placed in the water approximately 1 cm below the surface of the water. Visual cues were placed in various locations of the testing room. A ceiling mounted digital camera and ANY-Maze® software (Stoelting Co., Chicago, IL, USA) was used to automatically collect data during behavioral testing. Both non-spatial (visible platform) and spatial (hidden platform) versions of the test were conducted for each mouse.

Shaping. One day prior to training, mice were introduced (shaped) to the water maze by placing the animal on the visible escape platform for 10 seconds (s) and then placing the animal in the water at successively greater distances from the platform over three 45 s trials. The mouse was guided to the escape platform if the trial ended without the mouse finding the platform. General motor ability was observed and swim speed was measured.

Non-spatial task. One day following shaping, animals were trained to escape to a visible platform in the water maze during two sets of four trials with inter-trial and inter-set intervals of 5 and 120 min, respectively. In this non-spatial task, the platform was made visible by raising it 1 cm above the surface of the water and marking it with patterned tape and a perpendicular cue. For each trial, the mouse was placed in the maze (facing tub wall) at the same drop point with the position of the visible platform alternating between quadrants.

Spatial task. One day following acquisition of the non-spatial task, each mouse experienced 3 days of spatial training in locating a hidden escape platform. Each training day consisted of two sets of four trials with inter-trial and inter-set intervals of 5 min and 120 min, respectively. The starting point of each trial varied between four designated drop points. After completion of the 3 day training protocol, one final trial was performed 48 h later to test spatial memory retention.

For all trials (non-spatial and spatial), a maximum of 45 s was allowed for the mouse to find the hidden platform. The trial automatically ended when the mouse remained on the hidden platform for 3 s. If the trial ended without the mouse finding the escape platform, the investigator guided the mouse to the platform where it remained for 10 s. For all testing phases, mice were placed in a heated holding chamber for inter-trial intervals and, upon demonstration of normal behavior (e.g. grooming, rearing, exploring), returned to their home cages for inter-set intervals.

Latency (duration of trial), path length (distance traveled), swim speed, proportion of time and distance spent in the periphery and average distance from the platform were recorded per trial.