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Systemic inflammation induces acute working memory deficits in the primed brain: relevance for delirium

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Abstract

Delirium is an acute, severe neuropsychiatric syndrome, characterized by cognitive deficits, that is highly prevalent in aging and dementia and is frequently precipitated by peripheral infections. Delirium is poorly understood and the lack of biologically relevant animal models has limited basic research. Here we hypothesized that synaptic loss and accompanying microglial priming during chronic neurodegeneration in the ME7 mouse model of prion disease predisposes these animals to acute dysfunction in the region of prior pathology upon systemic inflammatory activation. Lipopolysaccharide (LPS; 100 μg/kg) induced acute and transient working memory deficits in ME7 animals on a novel T-maze task, but did not do so in normal animals. LPS-treated ME7 animals showed heightened and prolonged transcription of inflammatory mediators in the central nervous system (CNS), compared with LPS-treated normal animals, despite having equivalent levels of circulating cytokines. The demonstration that prior synaptic loss and microglial priming are predisposing factors for acute cognitive impairments induced by systemic inflammation suggests an important animal model with which to study aspects of delirium during dementia.

Keywords: Microglia; Delirium; Dementia; Priming; Synaptic; Predisposition; Infection; Inflammation; Animal model

1. Introduction

Delirium is an acute and transient impairment of global cognitive function, causing marked impairments in consciousness, attention, immediate recall, orientation (temporal and spatial), and perception (Burns et al., 2004; Meagher, 2009). It is highly prevalent in the aged and demented population and it is now well accepted that episodes of delirium hasten cognitive and functional decline and increase mortality (Fick et al., 2002; MacLullich et al., 2009; McCusker et al., 2001; Murray et al., 1993; Pitkala et al., 2005; Rahkonen et al., 2000; Rockwood et al., 1999). Recent high profile studies have reiterated this with a specific focus on patients with Alzheimer's disease (Fong et al., 2009b). Despite these important economic and medical imperatives, delirium is understudied and poorly understood (Fong et al., 2009a). One reason for this is the lack of biologically relevant animal models.

It is well accepted that systemic inflammation, caused by infection, surgery, or injury can induce episodes of delirium in elderly or demented patients (Belloosesky et al., 2007; Elie et al., 1998; Lemstra et al., 2008; Lerner et al., 1997; van Munster et al., 2008) while much less commonly causing similar dysfunction in younger or nondemented patients. The mechanisms of this dysfunction remain unclear. We have previously shown that microglia, the major macrophage population of the brain, are primed by prior neurodegenerative pathology to respond more robustly to systemic inflammatory signals (Cunningham et al., 2005). Here, we hypothesized that systemic inflammation, induced in animals with early stage neurodegeneration (ME7 prion disease), characterized by synaptic loss and primed micro-

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glia in the hippocampus, would induce acute onset and transient working memory dysfunction that is not induced by similar challenges in normal animals. In previous studies we reported a systemic inflammation-induced learning deficit in a reference memory task (Cunningham et al., 2009a) but novel approaches were necessary to investigate whether prior hippocampal pathology would be sufficient to predispose animals to systemic inflammation-induced working memory deficits that were acute onset and transient. These are key criteria in the clinical diagnosis of delirium.

ME7 is a prion disease strain that has been mouse-adapted and constitutes a real progressing neurodegenerative disease, characterized by extracellular amyloidosis, synaptic loss, robust neurodegeneration, and progressive cognitive decline (Betmouni et al., 1999; Cunningham et al., 2003). Because the early pathology is chiefly hippocampal, disease-associated cognitive impairments consist of failure on spatial working and reference memory tasks that, in the mouse, are hippocampal-dependent. We predicted that acute systemic inflammation would induce hippocampal-dependent working memory impairments at a time when such impairments were not yet present in disease per se. Because systemic inflammation causes changes in appetite, locomotor activity, motivation, and stress, which can confound many commonly used tests of hippocampal function in mice (Cunningham and Sanderson, 2008), we have designed and validated a novel shallow water T-maze alternation task that is less sensitive to these confounding factors. We challenged prion-diseased (ME7) and normal mice, at 12 weeks postinoculation, with bacterial endotoxin at 100 μg/kg and analyzed the acute effects (3–8 hours) on working memory. We also assessed learning in an egocentric Y-maze task to assess the generality of performance deficits. In order to assess the degree of, and possible mechanisms underpinning, the primed brain's heightened responses to systemic inflammation, we examined a number of inflammatory receptors and assessed the time course of expression of systemic and central nervous system (CNS) cytokines and effector molecules. Our findings suggest a model system that will be important in delineating the systemic and central inflammatory contributions to acute cognitive dysfunction, as seen in delirium.

2. Methods

2.1. Animals and stereotaxic surgery

Female C57BL/6 mice at 8–10 weeks of age (Harlan Olac Ltd, Bicester, United Kingdom) were housed in cages of 5 at 21 °C with a 12 : 12 hour light-dark cycle with food and water ad libitum. They were anesthetized with intraperitoneal Avertin (Sigma, Poole, UK) and stereotactically injected with 1 μl of a 10% w/v scrapie (ME7 strain)-infected C57BL/6 brain homogenate (or 10% w/v normal brain homogenate (NBH): at bregma: anterior-posterior −2.5 mm, lateral −1.7 mm, depth −1.6 mm) using a Hamilton microsyringe (Sigma, Poole, UK). Additional animals (n = 8) were injected intracerebrally (4 injections bilaterally) with N-methyl-D-aspartic acid (NMDA; 10 mg/mL) to ablate the hippocampus under isoflurane anesthesia (approximately 2%) with perisurgical analgesia (carprophen 5 mg/kg). Chlordiazepoxide (CDZP; 10 mg/kg) and atropine (0.075 mg/kg) were given to minimize seizure activity and bronchial secretions respectively. In all other respects the hippocampal lesions were performed as previously described (Deacon et al., 2002). Sham-operated animals (n = 12) had 8 holes drilled in the skull, but no intracerebral injections were made. All animal procedures were done in strict accordance with UK Home Office license and Irish Department of Health regulations.

2.2. Intraperitoneal challenges

Experimental groups with ME7 or NBH at 12 weeks postinoculation were injected intraperitoneally (i.p.) with 100 μg/kg (or 200 μg/kg to age-matched naive mice) of lipopolysaccharide (LPS; equine abortus, Sigma L5886, Poole, UK) in a volume of 200 μl saline. This dose mimics a mild infection, producing small changes ($<$ 1 °C) in core body temperature. Controls were administered 200 μl non-pyrogenic saline in each case.

2.3. T-maze alternation: working memory

We assessed hippocampal-dependent working memory (3–8 hours post-LPS) using alternation behavior in a novel "escape from shallow water" T-maze task to allow assessment of performance in animals experiencing sickness behavior. The T-maze was constructed of black Perspex with dimensions (cm): long axis 67, short axis 38, depth 20, and arm width 7. There was a single 40 mm diameter hole at the end of each choice arm, 2 cm from the floor. Black exit tubes were inserted into these holes (these may also be blocked to prevent exit). A "guillotine" door was inserted to prevent access to 1 or other choice arm. This maze was filled with water at 20 °C to a depth of 2 cm to motivate mice to leave the maze by "paddling" or walking "on tip-toe" to an exit tube. Animals were taken with their cage mates to a holding cage. Each mouse was placed in the start arm of the maze with 1 arm blocked such that they were forced to make a left (or right) turn, selected in a pseudorandom sequence (equal numbers of left and right turns, no more than 2 consecutive runs to the same arm). On making this turn the mouse could escape from the water by entering the small tube, and then a transit tube, in which it was carried to another holding cage. The mouse was held here for 25 seconds (intratrial interval) during which time the guillotine door was removed and the exit tube was switched to the alternate arm. The mouse was then replaced in the start arm and could choose either arm. The mouse must alternate from its original turn to escape. On choosing correctly mice escape to the transit tube as before and are returned to their home cage. On choosing incorrectly the mice were allowed to self-correct to find the