ELSEVIER

Neurobiology of Aging 27 (2006) 723–732

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Peripheral infection and aging interact to impair hippocampal memory consolidation

Ruth M. Barrientos *, Emily A. Higgins, Joseph C. Biedenkapp, David B. Sprunger, Karli J. Wright-Hardesty, Linda R. Watkins, Jerry W. Rudy, Steven F. Maier

Department of Psychology and Center for Neuroscience, University of Colorado at Boulder, Campus Box 345, Boulder, CO 80309-0345, USA

Received 31 August 2004; received in revised form 7 January 2005; accepted 9 March 2005
Available online 11 May 2005

Abstract

We report that a peripheral injection of *Escherichia coli* produces both anterograde and retrograde amnesia in 24 month old, but not 3 month old rats for memories that depend on the hippocampus, that is, memory of context, contextual fear, and place learning. The anterograde effect was restricted to measures of long-term memory. Short-term memory was not affected, nor did *E. coli* produce amnesia for auditory-cue fear conditioning. There were no age related effects on memory in vehicle-treated rats. In addition to these age-related cognitive effects of *E.coli*, we report that it produced a marked increased in IL-1β levels in the hippocampus, but not in parietal cortex or serum. These findings support the hypothesis that age is a vulnerability factor that increases the likelihood that an immune challenge will produce a cognitive impairment. It is possible that this cognitive vulnerability is mediated by age-related changes in the glial environment that results in an exaggerated brain pro-inflammatory response to infection.

Keywords: Normal aging; Learning; memory; Glial priming; Hippocampus; *E. coli*; Intraperitoneal administration; Interleukin-1

1. Introduction

It has been well documented that the significantly elevated expression of pro-inflammatory cytokines above basal levels, particularly in the hippocampus, impairs both synaptic plasticity, as assessed by long-term potentiation (LTP) [46,47], and hippocampal-dependent memory [1,3,4,14,16–18,32,36,37]. Peripheral cytokines, which are induced by immune activating stimuli such as infectious agents are capable of signaling the brain via both neural and blood-borne routes [23]. Importantly, this immune-to-brain signaling results in the de novo production of pro-inflammatory cytokines within the brain [24,30,45], largely by glial cells [19]. Thus, populations or individuals that have either increased peripheral inflammatory responses to immune activating agents such as bacteria or viruses, or exaggerated brain pro-inflammatory responses to signaling events within the brain, are likely to be more susceptible to infection-induced memory impairments.

Peripheral inflammatory responses to immune activating agents, as well as brain cytokine responses to stimulation are altered with normal aging. For example, Saito et al. [40] reported that the levels of interleukin-6 (IL-6) in blood produced by cecal ligation and puncture, as well as by lipolysaccharide (LPS), were elevated more in aged than in young mice. Within the brain, IL-1β and tumor necrosis factor-α responses to peripheral LPS administration appear to increase with aging [42,50]. Moreover, in the brain, healthy elderly subjects exhibit microglia with many phenotypic features consistent with a state of activation compared to younger control subjects. For example, aged subjects exhibit increases in the expression of complement type 3 receptor (CD11b), major histocompatibility complex II (MHC II), and CD4 at the protein level [29,33], and MHC II, MHC II transcriptional activator (CIITA), interferon (IFN)-γ, and CD86 at the transcriptional level [13]. Astrocytic glial fibrillary acidic protein (GFAP) protein and mRNA have

* Corresponding author. Tel.: +1 303 492 0777; fax: +1 303 492 2967.
E-mail address: rbarr@psych.colorado.edu (R.M. Barrientos).

0197-4580/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2005.03.010

also been reported to be elevated in the hippocampus of aged subjects [41,48]. As already noted, glial cells are important in the central nervous system with regard to inflammatory processes, as they are part of the neural cascade that is initiated by immune-to-brain signaling that involves the production of pro-inflammatory cytokines within the brain [27]. Because in the healthy, but aging organism both peripheral and central inflammatory functions are increased, it may be that a peripheral immune challenge in older subjects will produce an exaggerated cytokine response in the brain, and thereby produce greater impairments of memory. Moreover, since the immune-induced induction of cytokines such as IL-1β is especially prominent in the hippocampus [24,30,45], any interference with memory that is produced could be selective for forms of memory that require the hippocampus.

In the present experiments we assessed the impact of peripheral immune challenge/infection with live, replicating *E. coli* on memory formation in aging and young rats. To evaluate the effect of the *E. coli* challenge we used three memory paradigms that are known to depend on the hippocampus. Two versions of contextual fear conditioning were used. In the standard version, rats were simply placed into a conditioning chamber and presented with a tone-shock pairing. They were later tested for fear of the context (in the absence of the tone), and again for fear of the tone (in an altered context). It is important to note that the context test is a measure of hippocampal-dependent memory, whereas the tone test is not [22,35]. In the other version, we used a paradigm that produces what is called the context pre-exposure facilitation effect (CPFE). It allows the study of the rat's memory of an explored context. The CPFE derives from Fanselow's [10] analysis of the failure of shock delivered immediately after the subject is placed into an experimental apparatus to produce fear conditioning to the experimental context. However, if the subject is pre-exposed to the experimental context the day before fear conditioning, then immediate shock produces substantial fear conditioning [49]. According to Fanselow, immediate shock fails to support conditioning because the rat does not have time to encode a representation of the context prior to immediate shock. Pre-exposure thus facilitates the amount of conditioning produced by immediate shock because it allows the rat to establish a memory representation of the context before the immediate shock. This memory is then retrieved prior to the immediate shock by a subset of features that make up the context or by cues associated with transporting the rat to the conditioning chamber [39]. It is this retrieved memory representation of the context that is then associated with the immediate shock, and this facilitation effect depends on the hippocampus [2,28,38]. The third paradigm was the Morris place learning task. It requires rats to learn and retain the location of a platform that is submerged in a circular pool of water.

We evaluated the ability of the *E. coli* infection to produce both anterograde and retrograde amnesia for these tasks. Retrograde amnesia was studied by injecting the *E. coli* immediately following training, and anterograde amnesia was studied by injecting *E. coli* several days prior to training on the task. These experiments compared 3 and 24 month old male F344XBN F1 rats. We purposely chose the older group to be at an age below senescence (perhaps 32 months in this strain). This was done to minimize the possibility that there would be large memory impairments in the absence of challenge. Thus, the present experiments were designed to examine the effects of aging rather than old age.

2. Materials and methods

2.1. Subjects

Subjects were male F344XBN F1 rats obtained from the National Institute on Aging (Bethesda, MD). Upon arrival at our facility, older rats were 24 months old and weighed approximately 575 g. Younger rats were 3 months old and weighed approximately 280 g. Older and younger rats were housed 2 or 4 to a cage (52 cm × 30 cm × 21 cm; $L \times W \times H$), respectively. The animal colony was maintained at 22 °C on a 12-h light/dark cycle (lights on at 07:00 h). All rats were allowed free access to food and water and were given 1 week to acclimate to colony conditions before experimentation began. All experiments were conducted in accordance with protocols approved by the University of Colorado Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

2.2. Immune challenge

In all experiments, animals received an intraperitoneal (i.p.) injection of either Escherichia coli (a ubiquitous bacterial strain), or vehicle. One day prior to experimentation, stock *E. coli* cultures (ATCC 15746; American Type Culture Collection, Manassas, VA) were thawed and cultured overnight (15–20 h) in 40 mL of brain-heart infusion (BHI; DIFCO Laboratories, Detroit, MI) in an incubator (37 °C, 95% air + 5% CO_2). The number of bacteria in cultures was quantified by extrapolating from previously determined growth curves. Cultures were then centrifuged for 15 min at 4 °C, 3000 rpm, supernatants discarded, and bacteria resuspended in sterile phosphate buffered saline (PBS). Bacteria were resuspended with a volume of PBS to achieve a concentration of 1.0×10^{10} CFU/mL. A volume of 250 μL was injected i.p. regardless of body weight for a final dose of 2.5×10^9 CFU. Thus, the older rats received a lower "dose" than did the younger subjects relative to body weight, a procedure that was adopted as a conservative measure. Vehicle-treated rats received an injection of sterile PBS of an equal volume (250 μL).

2.3. Apparatus

2.3.1. Context pre-exposure and contextual fear conditioning

For the context pre-exposure experiment, rats were pre-exposed to either a control or conditioning context.