Neonatal infection modulates behavioral flexibility and hippocampal activation on a Morris Water Maze task

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HIGHLIGHTS

• Neonatal infection increases memory accuracy on a challenging water maze task.
• Memory is impaired in neonatally infected rats on a reversal water maze task.
• Arc expression in the dentate gyrus correlates with memory during the challenging task.

ARTICLE INFO

ABSTRACT

Neonatal infection has enduring effects on the brain, both at the cellular and behavioral levels. We determined the effects of peripheral infection with *Escherichia coli* at postnatal day (P) 4 in rats on a water maze task in adulthood, and assessed neuronal activation in the dentate gyrus (DG) following the memory test. Rats were trained and tested on one of 3 distinct water maze task paradigms: 1) minimal training (18 trials/3 days), 2) extended training (50 trials/10 days) or 3) reversal training (extended training followed by 30 trials/3 days with a new platform location). Following a 48 h memory test, brains were harvested to assess neuronal activation using activity-regulated cytoskeleton-associated (Arc) protein in the DG. Following minimal training, rats treated neonatally with *E. coli* had improved performance and paradoxically reduced Arc expression during the memory test compared to control rats treated with PBS early in life. However, neonatally-infected rats did not differ from control rats in behavior or neuronal activation during the memory test following extended training. Furthermore, rats treated neonatally with *E. coli* were significantly impaired during the 48 h memory test for a reversal platform location, unlike controls. Specifically, whereas neonatally-infected rats were able to acquire the new location at the same rate as controls, they spent significantly less time in the target quadrant for the reversal platform during a memory test. However, neonatally-infected and control rats had similar levels of Arc expression following the 48 h memory test for reversal. Together, these data indicate that neonatal infection may improve the rate of acquisition on hippocampal-dependent tasks while impairing flexibility on the same tasks; in addition, network activation in the DG during learning may be predictive of future cognitive flexibility on a hippocampal-dependent task.

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1. Introduction

Early-life experiences have significant and enduring effects on the health and normal functioning of many organisms. The central nervous system (CNS) develops rapidly during the perinatal period and, thus, is especially vulnerable to disruption. A growing body of research suggests that immune activation during early life has lasting consequences for the immune system, the CNS, and the communication between these two systems [1]. Notably, adult hippocampal function is often especially vulnerable to disruption following immune challenges during critical periods of development [2–6]. For instance, maternal immune activation or neonatal immune challenge with a diverse number of pathogens and immune activators (*e.g.*, polyriboinosinic-polyribocytidylic acid (poly(I:C)), *Escherichia coli*, lipopolysaccharide (LPS), human immunodeficiency virus (HIV)-1 and interleukin (IL)-6) similarly disrupts spatial learning in adult male rats, suggesting convergence of immune activation onto common plasticity mechanisms [7–12]. Our laboratory has extensively characterized the lifelong effects of early-life infection with *E. coli* on hippocampal function throughout the lifespan [13–17]. Previously, we have shown that in young adulthood neonatally-infected male rats acquire a platform location more quickly than controls on a Morris Water Maze task. Aged male rats that were treated with *E. coli* on P4, however, have impaired memory

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for the platform location 24 h after testing [18]. We have also demonstrated alterations at the cellular level in neonatally-infected rats. *E. coli* infection on P4 significantly reduced proliferation of neurons in the CA1 and CA3 sub-regions of P6 pups and reduced the maturation and integration of neurons in the CA1, CA3 and dentate gyrus (DG) regions of P33 rats [19]. Nevertheless, our previous work has not examined the effects of *E. coli* infection on hippocampal neuronal networks. The persistent changes in neurogenesis following early-life infection may be indirect evidence that hippocampal circuitry is enduringly altered in these rats.

Neuronal activation during behavioral tasks can be measured in a variety of ways. Immediate early gene (IEG; *e.g. Arc/Arg 3.1, Zif268, cFos*) expression is a non-invasive technique for measuring cellular activation in the brain. Protein expression of IEGs has a well-defined time course; thus, assessing protein expression at specific times following behavior reveals the populations of neurons that were activated during a given behavioral task [20–25]. The intracellular kinetics of activity-regulated cytoskeleton-associated (*Arc*) mRNA and protein are well-characterized [21,26,27] and extensive work on Arc at both the mRNA and protein levels demonstrates a significant role for its activity-dependent transcription and translation as a mechanism for synapse-specific plasticity [for reviews, see 28, and 29]. Inhibiting Arc protein expression impairs long-term potentiation (LTP) and memory consolidation [30]. Thus, we characterized Arc protein in this study as both a time-sensitive readout of neuronal activation and a representation of possible plasticity during a learning and memory task.

In light of the growing literature on enduring cognitive changes following perinatal infection or inflammation, this study examines the effects of bacterial infection on spatial learning and, indirectly, its underlying neural correlates. We assessed the impact of neonatal *E. coli* infection on water maze acquisition and memory in adulthood, and measured Arc expression following the memory probe to examine hippocampal activation patterns in rats exposed to bacterial infection early in life, along with age-matched controls. Based on the acquisition and memory behavior that we observed and the potential for neonatal infection to alter many brain regions and not solely the hippocampus, we then examined reversal learning acquisition and memory on the water maze task to assess cognitive flexibility in our neonatally-infected rats.

2. Material and methods

Specific animal, apparatus and procedural details appear below in the General methods section.

2.1. Experiment 1

The goal of this experiment was to test the effects of neonatal infection on a challenging paradigm of minimal training on the Morris Water maze (MWM) task in adulthood. We trained the rats with limited, minimal exposure to the apparatus and, thus, increased the difficulty of the task compared to training over a greater number of days or with more trials per day. We trained neonatally-infected and control rats for 3 days, 6 trials per day, to assess memory for a platform location after training. On the third day, half of the trained animals (n = 36) were tested on a memory probe trial 2 h following their final training trial. The other half of the group (n = 36) was tested on the probe trial 48 h following their last training trial. One hour after their respective probe trials, rats were taken for euthanasia. In addition to the trained rats, we assessed the effect of a single experience in the MWM on Arc expression in the DG, compared to expression after repeated experiences during training and testing. These rats were given a single 60 s trial in the pool without a submerged platform. One hour after their single trial, rats were taken for euthanasia.

2.2. Experiment 2

Based on our findings in Experiment 1, we assessed memory performance following extended training on the MWM task, increasing both trials per day and number of days of training. Rats received 5 days of 10 trials per day during training. One hour after a 48 h probe trial (60s), rats were euthanized and brains were collected. We assessed a separate group of rats that was "yoked" by latency to the trained rats to examine the importance of learning on neuronal activation in the DG during a hippocampal-dependent task. Yoked rats were treatment- and latency-matched to rats from the Extensive Training group. All yoked trials were conducted without a submerged platform, allowing for a similar experience in the environment without the act of learning. One hour after a memory probe trial (60 s trial 48 h after last training trial), rats were euthanized and brains were collected.

2.3. Experiment 3

We next assessed cognitive flexibility with a reversal task paradigm on the MWM. Once rats were trained to the first platform location (5 days × 10 trials per day, as in Experiment 2) and tested on memory probe trials, the platform was moved to another location in the opposite quadrant (SE) of the pool. Rats were trained for 3 days of 10 trials per day on the new platform location until they reached criterion (less than 10 s average escape latency). They were tested 48 h after the last trial for memory for the reversal platform location. Rats were taken 1 h after the 48 h probe trial for brain collection. The reversal task was assessed in 2 separate groups of rats (total n = 16) and their data for acquisition, reversal and the 48 h probe trial for the reversal platform location were pooled.

2.4. General methods

2.4.1. Animals

Adult male and female Sprague–Dawley rats were obtained from Harlan (Indianapolis, IN) and were pair housed for breeding after a week of acclimation to the facility. Female breeders were visually examined daily for confirmation of pregnancy, and male breeders were removed from cages prior to the birth of pups (P0). All rats were housed in ventilated polypropylene cages with *ad libitum* access to food and filtered water. The colony was maintained at 22 °C on a 12:12 h light:dark cycle (lights on at 0700 h). Sentinel animals were housed in the colony room and screened periodically for the presence of common rodent diseases; all screens were negative. All experiments were conducted with protocols approved by the Duke University Institutional Animal Care and Use Committee.

2.4.2. Neonatal manipulations and bacterial cultures

All litters were culled on P4 to a maximum of 10 pups/litter, retaining 2 female and as many male pups as possible. The females were retained to prevent single sex litters, but all female pups were euthanized at weaning (P21). All litters were born within 1 week of each other, and all studies were limited to males, limiting the conclusions about the data to males alone. *E. coli* culture (ATCC 15746; American Type Culture Collection, Manassas, VA) vial contents were hydrated and grown overnight in 30 ml of brain–heart infusion (BHI; Difco Labs, Detroit, MI) at 37 °C. Cultures were aliquoted into 1 ml stock vials supplemented with 10% glycerol and frozen at −20 °C. One day before injections, a stock culture was thawed and incubated overnight in 40 ml of BHI at 37 °C. The number of bacteria in cultures was read using a microplate reader (Bio-Tek Instruments Inc., Winooski, VT) and quantified by extrapolating from previously determined growth curves. Cultures were centrifuged for 15 min at 4000 rpm, the supernatants were discarded, and the bacteria were re-suspended in the dose-appropriate volume of sterile Dulbecco's PBS (Invitrogen Corp., Carlsbad, CA). Male pups were injected