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Research report

Effects of age, experience and inter-alpha inhibitor proteins on working memory and neuronal plasticity after neonatal hypoxia-ischemia

Cynthia M. Gaudet[a], Yow-Pin Lim[b], Barbara S. Stonestreet[c], Steven W. Threlkeld[d,*]

[a] Department of Biology, Rhode Island College, 600 Mount Pleasant Ave., Providence, RI 02904, USA
[b] ProThera Biologics, Inc., 349 Eddy Street, Providence, RI 02903, USA
[c] Department of Pediatrics, The Alpert Medical School of Brown University, Women & Infants Hospital of Rhode Island, 101 Dudley Street, Providence, RI 02905, USA
[d] Department of Psychology, Rhode Island College, 600 Mount Pleasant Ave. Providence, RI 02904, USA

HIGHLIGHTS

- Early life working memory (WM) experience improves adult memory performance.
- IAIPs and early experience improve moderately demanding working memory performance in neonatal HI injured rats.
- Early WM experience led to a significant reduction in hippocampal CA1 basal dendrite length in control animals.
- IAIPs and early WM experience resulted in fewer basal hippocampal CA1 dendrites in HI injured rats.

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ABSTRACT

Neonatal cerebral hypoxia-ischemia (HI) commonly results in cognitive and sensory impairments. Early behavioral experience has been suggested to improve cognitive and sensory outcomes in children and animal models with perinatal neuropathology. In parallel, we previously showed that treatment with immunomodulator Inter-alpha Inhibitor Proteins (IAIPs) improves cellular and behavioral outcomes in neonatal HI injured rats. The purpose of the current study was to evaluate the influences of early experience and typical maturation in combination with IAIPs treatment on spatial working and reference memory after neonatal HI injury. A second aim was to determine the effects of these variables on hippocampal CA1 neuronal morphology. Subjects were divided into two groups that differed with respect to the time when exposed to eight arm radial water maze testing: Group one was tested as juveniles (early experience, Postnatal day (P) 36–61) and adults (P88–113), and Group two was tested in adulthood only (P88–113; without early experience). Three treatment conditions were included in each experience group (HI + Vehicle, HI + IAIPs, and Sham subjects). Incorrect arm entries (errors) were compared between treatment and experience groups across three error types (reference memory (RM), working memory incorrect (WMI), working memory correct (WMC)). Early experience led to improved working memory performance regardless of treatment. Combining IAIPs intervention with early experience provided a long-term behavioral advantage on the WMI component of the task in HI animals. Anatomically, early experience led to a decrease in the average number of basal dendrites per CA1 pyramidal neuron for IAIP treated subjects and a significant reduction in basal dendritic length in control subjects, highlighting the importance of pruning in typical early life learning. Our results support the hypothesis that early behavioral experience combined with IAIPs improve outcome on a relativity demanding cognitive task, beyond that of a single intervention strategy, and appears to facilitate neuronal plasticity following neonatal brain injury.

1. Introduction

Premature infants and high-risk full-term babies, exposed to abruptio placenta, prolonged labor, umbilical occlusion or prolapse, are at increased risk for cerebral oxygen deprivation (hypoxia)

* Corresponding author at: Rhode Island College, Horace Mann Hall 314,600, Mount Pleasant Ave., Providence, RI 02908, USA.
E-mail address: Sthrelkeld@ric.edu (S.W. Threlkeld).

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and insufficient blood flow (ischemia; [1–4]). Neonatal cerebral hypoxia-ischemia (HI) disrupts global physiological function often translating to long-term deficits in learning and memory both in humans [3–13] and in rodent models [12,14–20]. At this time, the only available treatment for humans following neonatal hypoxic-ischemic encephalopathy (HIE) is controlled hypothermia, which may reduce the body's inflammatory response to injury [21,22]. However, this treatment is only approved for use in full term neonates after HIE, and even after therapeutic hypothermia, over 40% of babies will suffer from death or moderate to severe physical or intellectual impairments [23–25]. Given limited treatment options, research has focused on developing alternative and/or complementary therapeutic strategies that can improve long-term cognitive function following neonatal HI injury.

Inter-alpha Inhibitor Proteins (IAIPs) are found in relatively high concentrations in human and rodent blood plasma and are thought to play an important role in inflammatory regulation [26–32]. Recent studies using rodent models have shown that IAIPs play a significant role in regulating the inflammatory response and increase cell survival in both the central nervous system and somatic cells following infection and brain injury [20,33,34]. Our group has shown that administration of human plasma derived IAIPs following neonatal HI brain injury in rats leads to neuronal and gross anatomical sparing across distinct developmental time windows (72 h post-injury (Postnatal day (P) 10) and adult (P80+)) and improves spatial and non-spatial learning outcomes in an age dependent manner in juvenile and adult rats [20].

In parallel, studies from our group and others have shown that early experience (enriched housing or more domain specific experience (e.g., auditory processing)) can lead to improved adult behavioral outcomes in rats with various forms of developmental brain injury [16,20,35]. Animals with early life exposure to domain-specific tasks show robust improvements in adult performance relative to inexperienced animals allowed to mature typically [35]. This is significant because, in humans with neurodevelopmental disorders, behavioral training/intervention has been shown to improve long-term cognitive and linguistic outcomes and appears to shift brain activation (using fMRI) patterns closer to that of typical individuals [36–41]. Furthermore, early life enriched housing or behavioral training has been shown to modify dendritic branching across divergent brain regions in rats (e.g., hippocampus, frontal cortex), relating to improved behavioral performance later in life [42,43]. Interestingly, the age at which behavioral experience takes place and the length of experience may influence whether a reduction/refinement of branching or dendritic expansion occurs following brain injury [44]. Taken together, the current literature suggests that early behavioral and/or sensory intervention is critical for improving long-term functional recovery in at risk developmental populations and is likely to facilitate neuronal plasticity.

Regardless of how effective new pharmacological treatments may be at improving neurological outcome, many infants will not meet criteria for treatment or will miss optimal timing windows required for treatment effectiveness. In addition, optimal drug dosage may be compromised due to difficulty predicting injury severity in human populations. Therefore, behavioral interventions will continue to be a valuable tool to improve long-term outcome, even in cases of severe birth trauma. However, given the central role of inflammation in neonatal HI injury and evidence for the benefits of early behavioral training, combining pharmacological and behavioral interventions will likely provide greater improvement in functional recovery than a single intervention alone. Thus, the purpose of the current study was to explore the efficacy of a multi-factorial intervention strategy to modulate inflammatory mediated brain injury with the use of Inter-alpha Inhibitor Proteins (IAIPs) in conjunction with early-life spatial working and reference memory experience in an effort to maximize cognitive recovery and facilitate neuronal plasticity. We hypothesized that administration of IAIPs in combination with early task-specific experience would significantly improve spatial working and reference learning in rats with neonatal HI injury beyond improvements from a single treatment. We also predicted that early experience would result in morphological changes in basal dendrites of hippocampal CA1 neurons, which are central to spatial processes important for navigation in the eight-arm radial water maze.

2. Methods

2.1. Subjects

Subjects were 57 male Wistar rats born from time-mated dams shipped on embryonic day five of pregnancy and (Charles River Laboratories, Wilmington, MA) housed in the Fogarty Life Sciences Vivarium at Rhode Island College. On post-natal day 1 (P1), pups were separated into litters of eight males and two females to control for sex ratio and litter size. This study was limited to the assessment of male subjects due to prior research showing that rodent males exhibit more prominent deficits following neonatal HI brain injury as compared to females [45,46], findings that parallel observations in humans [12,47–53]. Subsets of subjects were randomly assigned to receive either hypoxic-ischemic (HI) insult or Sham surgical procedure on P7. Subjects were weaned on P21, right or left ear marked, and pair housed using a 12:12 light/dark cycle with food and water available *ad libitum*. Prior to weaning, approximately half of the subjects from each litter were randomly assigned into two groups for behavioral testing on the eight-arm-radial water maze. To minimize between-litter effects, subjects from all litters were represented in each treatment and experience group. Group 1 (G1; Sham $n = 8$, HI + Vehicle $n = 13$, HI + IAIPs $n = 9$) received behavioral testing as juveniles (P36–P61; G1A) and also as adults (P113–138; G1B). Group 2 (G2; Sham $n = 7$, HI + Vehicle $n = 12$, HI + IAIPs $n = 8$) was tested only as adults (P113–138) to assess maturational effects (Fig. 1). All procedures were conducted in compliance with the National Institutes of Health guidelines for care and use of laboratory animals and all protocols were approved by the Rhode Island College Institutional Animal Care and Use Committee (IACUC).

2.2. Surgical procedure

Surgery was performed in the Fogarty Life Science Vivarium at Rhode Island College (Providence, RI) using aseptic techniques. On postnatal day 7 (P7), subjects were randomly assigned to treatment (HI + Vehicle, HI + IAIPs, or Sham) and experience groups (Group 1: juvenile and adult testing or Group 2: adult testing only). All treatment conditions were balanced across litters. A modified version of the Rice–Vannucci method was followed to induce the hypoxic-ischemic injury [54–56]. In summary, each animal was stabilized on a surgical surface and anesthetized using 3–4% isoflurane administered through a nose cone. Total absence of leg withdrawal and tail-pinch reflexes were verified prior to advancing with 1–2% isoflurane for maintenance. A midline ventral incision was made in the neck. The right common carotid artery (RCCA) was located and completely cauterized. Sham subjects followed the same procedure except for an absence of RCCA cauterization. The neck incision was stitched using vicryl sutures, and each pup was labeled with a footpad ink injection (~10 μL). Body temperature was maintained throughout the procedure at 37 °C, using an isothermal heating pad (Braintree Scientific, Braintree, MA), as reduced temperature has been shown to provide a degree of neuroprotection in humans and animal models [22,57–59].