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# Early life inflammatory pain induces long-lasting deficits in hippocampal-dependent spatial memory in male and female rats

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ABSTRACT

The present experiment tested the hypothesis that neonatal injury disrupts adult hippocampal functioning and that normal aging or chronic stress during adulthood, which are known to have a negative impact on hippocampal function, exacerbate these effects. Male and female Sprague–Dawley rats were given an intraplantar injection of the inflammatory agent carrageenan (1%) on the day of birth and their memory was tested in the hippocampal-dependent spatial water maze in adulthood and again in middle age. We found that neonatal injury impaired hippocampal-dependent memory in adulthood, that the effects of injury on memory were more pronounced in middle-aged male rats, and that chronic stress accelerated the onset of these memory deficits. Neonatal injury also decreased glucocorticoid receptor mRNA in the dorsal CA1 area of middle-aged rats, a brain region critical for spatial memory. Morphine administration at the time of injury completely reversed injury-induced memory deficits, but neonatal morphine treatments in the absence of injury produced significant memory impairments in adulthood. Collectively, these findings are consistent with our hypothesis that neonatal injury produces long-lasting disruption in adult hippocampal functioning.



## 1. Introduction

In the United States alone, approximately 500,000 babies are born prior to 37 weeks gestation and are considered preterm (Martin, Hamilton, Osterman, Curtin, & Mathews, 2013; National Center for Health Statistics., 2014). Premature infants spend an average of 25 days in the neonatal intensive care unit (NICU), where they undergo 10–18 invasive and painful procedures each day, including endotracheal intubation, surgery, catheterization, and mechanical ventilation (Barker & Rutter, 1995; Carbajal et al., 2008; National Perinatal Information System/Quality Analytic Services., 2011; Simon, Lazareff, Diament, & Kennedy, 2003). Although preterm infants can respond to painful stimuli (Anand & Hickey, 1987; Bartocci, Bergqvist, Lagercrantz, & Anand, 2006; Grunau et al., 2005; Slater et al., 2006), approximately 65% of these procedures are performed in the absence of analgesia (Bouza, 2009; Carbajal et al., 2008; Rodkey & Pillai Riddell, 2013; Simon et al., 2003; Walter-Nicolet, Annequin, Biran, Mitanchez, & Tourniaire, 2010).

Evidence suggests that neonatal pain activates the hypothalamic pituitary adrenal (HPA) axis. In preterm infants undergoing surgery, the opioid analgesic fentanyl significantly decreases plasma levels of stress hormones, including cortisol (Anand & Hickey, 1987), and the number of skin-breaking procedures preterm infants experience in the NICU is associated with increased cortisol levels in later development (8–18 months; Grunau, Weinberg, & Whitfield, 2004; Grunau et al., 2007). A preclinical study on the effects of early life pain in rodents also found significantly elevated corticosterone (CORT) levels 24 h following inflammatory pain, with CORT levels remaining elevated above

*Abbreviations:* ANOVA, analysis of variance; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BDNF, brain derived neurotrophic factor; CGN, carageenan; CeA, central amygdala; CORT, corticosterone; dCA1, dorsal CA1; FST, forced swim test; GR, glucocorticoid receptor; HPA, hypothalamic pituitary adrenal; IL-1β, interleukin 1β; LTP, long-term potentiation; mCVS, mild chronic variable stress; NICU, neonatal intensive care unit; P, postnatal day; ROI, regions of interest; TNFα, tumor necrosis factor alpha.

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handled controls at 7 days post-injury (Victoria, Karom, Eichenbaum, & Murphy, 2014b).

Stress and associated high levels of CORT negatively impact the hippocampus, a brain area critical for episodic (autobiographical) memories (Eichenbaum, 2004; Shapiro, Kennedy, & Ferbinteanu, 2006; Tulving, 1972). Increased CORT down-regulates hippocampal glucocorticoid receptor (GR) expression (Kitraki, Kremmyda, Youlatos, Alexis, & Kittas, 2004), decreases dendrite number (Conrad & Bimonte-Nelson, 2010; McLaughlin, Gomez, Baran, & Conrad, 2007) and synapses (Tata, Marciano, & Anderson, 2006), and impairs hippocampal-dependent memory (Conrad, Lupien, & McEwen, 1999; Conrad, Lupien, Thanasoulis, & McEwen, 1997; McLaughlin et al., 2007; Wright & Conrad, 2005). Similarly, early life pain in rodents decreases GR mRNA and protein site-specifically in the dorsal CA1 (dCA1) region of the adult hippocampus (Victoria, Inoue, Young, & Murphy, 2013; Victoria et al., 2014b). As increased CORT and decreased hippocampal GR expression are associated with significant memory deficits, these data suggest collectively that the stress associated with unresolved early life pain may produce long-lasting deficits in hippocampal-dependent function.

The present study assessed the impact of early life pain on adult hippocampal-dependent spatial water maze memory and GR expression in male and female rats. We further determined whether normal aging and chronic stress, life events previously shown to have a negative impact on hippocampal function, exacerbated the effects of early life pain on memory (Bizon et al., 2009; Driscoll et al., 2006; Gazova et al., 2013; Golomb et al., 1993). The ability of morphine to attenuate the long-term consequences of early life pain on memory was also examined.

## 2. Materials and methods

Experiment 1 tested the effects of neonatal inflammatory pain on memory in the spatial water maze in adult and middle-aged male and female rats and on hippocampal GR mRNA expression (Fig. 1). Experiment 2 determined if chronic stress accelerated the onset of the early life pain-induced memory deficits and if analgesia given at the time of injury mitigated the impact of early life pain.

### 2.1. Animals

Pregnant Sprague–Dawley rats were received on gestational day 14 (Charles River, Wilmington, MA) and housed individually under a 12:12-h light:dark cycle with *ad libitum* access to food and water. On the day of birth (postnatal day 0; P0), pups were subjected to treatments described below. Previous studies have established that the P0 rat pup is comparable to a third trimester human infant in terms of neurodevelopment (Workman, Charvet, Clancy, Darlington, & Finlay, 2013). All experiments adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Georgia State University Animal Care and Use Committee.

### 2.2. Neonatal injury

In Experiment 1, pups were separated from their dam on P0, maintained on a warm surface, and sexed by examination of anogenital distance. Neonatal injury was induced by intraplantar administration of the inflammatory agent carageenan (CGN; 5 μL, 1% dissolved in saline; Sigma–Aldrich, St. Louis, MO). Intraplantar CGN causes paw edema lasting approximately 48–72 h (LaPrairie & Murphy, 2007) and does not produce permanent skin, nerve or other damage in the inflamed area (Lidow, Song, & Ren, 2001; Ren, Hylden, Williams, Ruda, & Dubner, 1992; Traub, 1996). Control litters were handled in an identical manner but skin was not broken. Intraplantar saline, which has been previously shown to induce an inflammatory response (<24 h; LaPrairie & Murphy, 2007), was not administered. Pups were separated from their dam for less than 20 min and litters were returned to their home cage as a group. Maternal behavior directed toward the injured and handled control pups is not significantly different (LaPrairie & Murphy, 2007). All pups within a litter received the same neonatal treatment and were undisturbed except for cage changes and weaning (P21).

In Experiment 2, on P0 male rat pups were given morphine sulfate (2 mg/kg, i.p.) or equivolume saline (0.9%, i.p.) 15 min prior to intraplantar CGN or handling ($n = 9$ handled-saline, $n = 5$ injured-saline, $n = 5$ handled-morphine, and $n = 6$ injured-morphine). At peak paw inflammation (5-h post-CGN), a second dose of morphine or saline was administered (LaPrairie & Murphy, 2009). Only male rats were used in Experiment 2 given our finding in Experiment 1 that there was no significant effect of injury in female middle-aged rats.

### 2.3. Estrous cycle

Starting on P144, vaginal lavage was performed once per day in female rats for 2 weeks in the morning between 8:00 am and 10:00 am. Vaginal secretions were collected using a plastic pipette filled with deionized water. Estrus stage was defined by the presence of stage-specific epithelial cells (Becker et al., 2005) in ⩾90% of the cell population under a light microscope (Nikon Instruments Inc., Melville, NY) as described previously (Victoria et al., 2013). Male rats were removed from their cage by the base of the tail, placed

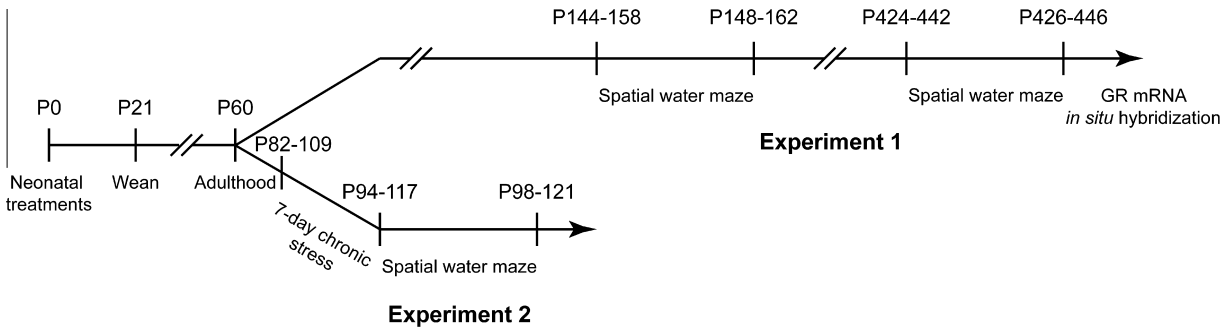


**Fig. 1.** Experimental timeline.