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Research Report

Reproductive experience facilitates recovery from kainic acid-induced neural insult in female Long–Evans rats

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ABSTRACT

The hormones of pregnancy and lactation (e.g., estrogen, progesterone, and oxytocin) have been shown to modulate learning, memory, and the restructuring of brain areas not traditionally associated with maternal behavior. Given the impact of reproductive experience on plasticity of brain areas such as the hippocampus, kainic acid (KA) was used in the current study to induce hippocampal-specific neurotoxic insult in adult multiparous and virgin Long–Evans rats. In Experiment I, Fluoro-Jade B, an indicant of degenerating cells, revealed significant neuronal damage in KA-treated hippocampi at 16 h post-injection in both maternal and virgin rats. In Experiment II, maternal and virgin rats were assessed in spatial and novel object preference tasks to determine the effects of KA on subsequent behavioral and cognitive responses. Twenty-four hours post injection, saline maternal animals exhibited superior memory in a spatial task. Further, maternal saline-injected rats were more similar to maternal KA-injected rats than both the virgin groups. Forty-eight hours following the KA or saline injection, compared to virgins, maternal animals demonstrated enhanced memory in the novel object memory test, regardless of type of injection. Further, neurobiological assessments in Experiment II indicated that virgin KA exposed rats had significantly more glial fibrillary acidic protein (GFAP)-immunoreactivity in the hippocampus, suggesting that they were in an earlier stage of neural recovery compared to maternal animals or, alternatively, may have exhibited more trauma than maternal animals. Together, these data suggest that the previously reported plasticity of the maternal brain may facilitate neural and behavioral recovery from neural insults.

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Abbreviations: ABC, avidin–biotin complex; ANOVA, analysis of variance; CA1, cornu ammonis region 1; CA3, cornu ammonis region 3; DAB, 3-3′ diaminobenzidine tetrahydrochloride; DG, dentate gyrus; FITC, fluorescein isothiocyanate; FJB, Fluoro-Jade B; GFAP, glial fibrillary acidic protein; HFm, hippocampal fissure medial; HFl, hippocampal fissure lateral; i.p., intraperitoneal; IR, immunoreactivity; KA, kainic acid; NOP, novel object preference task; PBS, phosphate-buffered saline; RT, room temperature; SEM, standard error of the mean

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1. Introduction

Maternal experience, and its accompanying exposure to various reproductive hormones such as prolactin, progesterone and estrogen for extended durations, has dramatic effects on the brains of mammals, effects which may extend beyond stimulating the onset of maternal behavior (Kinsley and Lambert, 2006, 2008; Lambert and Kinsley, 2008). Past research, for example, indicates that maternal experience alters hippocampal plasticity, which may contribute to brain regions and behaviors that support care of young. Compared to virgins, late pregnant and lactating rats have denser populations of hippocampal dendritic spines (Kinsley et al., 2006). Further, reproductive status affects morphological changes in hippocampal astrocytes (Salmaso et al., 2005). Lower levels of amyloid precursor protein (associated with cognitive decline) have also been observed in the hippocampi of senescent rats with prior maternal experience, indicating that certain aspects of reproduction-induced modifications persist throughout the animal's life (Gatewood et al., 2005). Together, these results confirm that maternal experience alters hippocampal plasticity, an effect that, in some cases, leads to the expression of neuroplasticity and complementary neuroprotective effects across the lifespan.

Hippocampus-dependent behavioral change has also been assessed in maternal rats. Multiparous rats perform better than virgins in the radial-arm maze; further, both maternal and pup-sensitized rats exhibited a stronger spatial memory than virgins in a version of the Morris water maze known as the dry land maze (Kinsley et al., 1999). In a competitive spatial foraging task, multiparous rats exhibited shorter latencies to reach food rewards than age-matched primiparous and virgin rats (Love et al., 2005). In corroboration with the long-lasting effects observed in relevant neuroanatomy studies, as animals age, maternal rats have been shown to continue to outperform their virgin counterparts in spatial tasks (Gatewood et al., 2005; Love et al., 2005).

Explorations of responses to acute brain injuries have also indicated the importance of reproductive hormones in recovery from neural insults. When male and female rats, for example, were exposed to traumatic brain injury, females displayed less cerebral edema than males; additionally, females with the highest levels of progesterone exhibited the lowest incidence of secondary edema from the neural insult (Roof et al., 1993). These findings suggest potential neuroprotective and neuroregenerative effects of progesterone, a steroid hormone with largely estrogenic interactions (e.g., Carroll et al., 2007; He et al., 2004; Nilsen and Brinton, 2002, 2003). Furthermore, contact between astrocytes and neurons represent another essential component of the neuroprotective role of estrogens. For instance, astrocytes express estrogen receptors and participate in the regulation of synaptic plasticity, and estrogens modulate the release of neurotrophic factors and inflammatory molecules by astrocytes (Azcoitia et al., 2010; Barreto et al., 2009). Progesterone's effects include the facilitation of myelination (e.g., Baulieu and Schumacher, 2000; Schumacher et al., 2007), increased mitochondrial activity (e.g., Irwin et al., 2008), suppressed inflammation (Gibson et al., 2005) and protection against the breakdown of cell membranes, perhaps due to anti-oxidant properties (Stein, 2008). Progesterone receptors are found in many areas throughout the brain including the hippocampus, a particularly sensitive region for recovery of normal function in individuals suffering from acute brain injuries (Guerra-Araiza et al., 2000, 2001, 2003). Increased hippocampal cell death and hippocampal-dependent neural activity, for example, have been observed in animal models of traumatic brain injury (Barha et al., 2011). Recently, it was reported that male rats with bilateral frontal cortex contusions exhibited a more normalized pattern of cell proliferation and degeneration following progesterone treatment (Barha et al., 2011). Alternatively, though, recent studies have reported that progesterone may interfere with the neuroprotective action of other steroids, such as estradiol (Azcoitia et al., 2011).

In addition to reports indicating that the reproductive hormone progesterone facilitates recovery from neural insults, research suggests that lactating animals exhibit protection against neural insult, as they show less excitotoxicity-induced cell damage in the dorsal hippocampus than nulliparous rats following an acute neural insult (Morales, 2011).

Considering the accumulating evidence that reproductive experience influences the brain's ability to recover from various insults, the purpose of the current set of experiments was to determine the effects of maternal experience on both neural and behavioral recovery from neural insult in Long–Evans rats caused by kainic acid (KA), a naturally-occurring analog of the excitatory amino acid neurotransmitter glutamate—known to induce seizures and subsequent damage to the CA3 area of the hippocampus (Benkovic et al., 2004; Kesslak and Gage, 1986; Krajewska et al., 2011). Thus, KA was used to generate an acute brain injury; that is, a neurotoxic lesion (Hilton et al., 2006; Nadler et al., 1978; Olney et al., 1979; Sperk et al., 1983). The sustained excitatory amino acid exposure and associated hippocampal neurodegeneration precede a reactive gliosis which parallels the secondary effects observed in other models of acute brain injuries such as traumatic brain injury (Genarelli and Graham, 2005). Secondary injuries occur during the brain's recovery attempts following a brain injury and include such compensatory events as edema, apoptosis/degeneration of nerve cells, decreased cranial blood pressure, generation of free radicals, and the release of cytokines (Bouma and Muizelaar, 1992; McIntosh, 1994; Panter and Faden, 1992).

Here, the neurotoxic effect of KA was initially confirmed using Fluoro-Jade B (FJB), a fluorescent marker with a high affinity for degenerating cells (Schmued and Hopkins, 2000). Glial fibrillary acidic protein (GFAP) immunohistochemistry was also employed in maternal and virgin animals to determine the extent of kainate-induced reactive gliosis. In a second experiment, both spatial and non-spatial memory in maternal and virgin rats was assessed pre- and post-insult to determine the regulatory impact of maternal experience and neurotoxin-induced neural insult on recovery of function following KA exposure and the subsequent brain injury.

2. Results

2.1. *Seizure scoring*

In Experiments I and II, maternal experience failed to affect the mean seizure intensity (Experiment I: $F_{1,15}=0.68$, $p=0.42$