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Research Report

Age-related declines in exploratory behavior and markers of hippocampal plasticity are attenuated by prenatal choline supplementation in rats

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ABSTRACT

Supplemental choline in the maternal diet produces a lasting enhancement in memory in offspring that resists age-related decline and is accompanied by neuroanatomical, neurophysiological and neurochemical changes in the hippocampus. The present study was designed to examine: 1) if prenatal choline supplementation alters behaviors that contribute to risk or resilience in cognitive aging, and 2) whether, at old age (25 months), prenatally choline-supplemented rats show evidence of preserved hippocampal plasticity. A longitudinal design was used to look at exploration of an open field, with and without objects, at 1 and 24 months of age in male and female rats whose mothers were fed a diet supplemented with choline (SUP; 5 mg/kg choline chloride) or not supplemented (CON; 1.1 mg/kg choline chloride) on embryonic days 12–17. Aging caused a significant decline in open field exploration that was more pronounced in males but interest in novel objects was maintained in both sexes. Prenatal choline supplementation attenuated, but did not prevent age-related decline in exploration in males and increased object exploration in young females. Following behavioral assessment, rats were euthanized to assess markers of hippocampal plasticity. Aged SUP males and females had more newly proliferated cells in the hippocampal dentate gyrus and protein levels of vascular endothelial growth factor (VEGF) and neurotrophin-3 (NT-3) were significantly elevated in female SUP rats in comparison to all other groups. Taken together, these findings provide the first evidence that prenatal choline supplementation causes changes in exploratory behaviors over the lifespan and preserves some features of hippocampal plasticity that can be seen even at 2 years of age.

1. Introduction

Choline is an essential nutrient important for a variety of biological processes in humans and other mammals (for reviews see Blusztajn, 1998; Zeisel, 2004; 2006). In addition to its importance as the precursor to the neurotransmitter, acetylcholine, choline also contributes to the construction of cellular membranes and lipid transport as a constituent of

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phosphatidylcholine. Via its conversion to betaine it becomes a methyl group donor altering gene and histone methylation processes, and as a component of the platelet aggregating factors, lysosphosphatidylcholine and sphingosylphosphorylcholine, it participates in cell signaling. An abundant literature clearly reveals that the choline content during early development has a profound impact on the developing organism with measurable, often marked, effects on behavior and neural function that persist well into adulthood (see Hohmann and Berger-Sweeney, 1998; Meck and Williams, 2003; Meck et al., 2008).

In rodents, supplementation of the maternal diet with choline on embryonic days (ED) 11–17, significantly enhances spatial memory of the adult offspring (e.g., Meck and Williams, 1997; Meck et al., 1988; 1989; also see Schenk and Brandner, 1995; Tees and Mohammadi, 1999) and prevents the memory decline that normally occurs with advanced age (Meck et al., 2008; also see Meck and Williams, 2003). This long-term enhancement in cognitive function is accompanied by facilitation of hippocampal long-term potentiation (LTP) in young adult and middle-aged rats (Jones et al., 1999; Pyapali et al., 1998) and is positively correlated with the acetylcholine (ACh) content of hippocampal slices following stimulated release in aged rodents (Meck et al., 2008). To date, the precise behavioral and neural mechanisms mediating the life-long persistence of prenatal choline supplementation effects on memory and on hippocampal function are not known. The present study was designed to examine: 1) if prenatal choline supplementation alters behaviors that contribute to risk or resilience in cognitive aging, and 2) whether, at 25 months of age, prenatally choline-supplemented rats show evidence of preserved hippocampal plasticity. A longitudinal design was used to examine the activity, exploration and plasma corticosterone responses to acute stress in prepubertal male and female rats. At 24 months of age, these rats were retested for their tendencies to exploration and their general activity levels. Markers of hippocampal plasticity (i.e. cell proliferation and levels of several growth factors) were determined in all rats once behavioral assessments were complete, at approximately 25 months of age.

There is considerable evidence that enriched environments, activity/exercise, and stress modulate hippocampal plasticity and cognitive aging. Enriched environments increase rats' exploratory behavior as measured in an open field (Fernández et al., 2004), and improve cognitive function in aged rodents (Lores-Arnaiz et al., 2006). Exercise has dramatic effects on hippocampal plasticity and improves memory function (see Cotman and Berchtold, 2002). And, it is well known that stress-induced activation of the hypothalamic–pituitary–adrenal axis causes loss of hippocampal spines, inhibition of hippocampal cell proliferation, and cognitive impairment (Lupien et al., 1998; McEwen, 1999), while reduction of corticosterone in old age reverses these effects (see Cameron and McKay, 1999). Therefore, we reasoned that prenatal choline supplementation might modify cognitive aging by altering rats' activity, exploratory behavior and/or stress reactivity. If this hypothesis is correct, then prenatally choline-supplemented rats may have life-long changes in the way in which they interact with their environment compared to control rats and this behavioral change over the lifespan may contribute to the maintenance of neural plasticity and might help to explain why they show attenuated cognitive decline during aging (Meck et al., 2008; Meck and Williams, 2003).

One age-related change in hippocampal plasticity is greatly diminished neurogenesis in the dentate gyrus (DG), which begins during middle age in the rodent (Kuhn et al., 1996; Nacher et al., 2003; Rao et al., 2005). Moreover, it has been suggested that this loss in plasticity may contribute to age-related learning and memory impairments (Drapeau et al., 2003). While the causal factor or factors underlying decreased hippocampal neurogenesis beginning in middle age are not known, a number of proliferation/growth factors also show reduced concentrations in the hippocampus as rats age, including fibroblast growth factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF) (Shetty et al. 2005), and brain-derived neurotrophic factor (BDNF) (Bimonte-Nelson et al., 2008, Hattiangady et al., 2005, but see also Katoh-Semba et al., 1998). On the other hand, nerve growth factor (NGF) and neurotrophin 3 (NT-3) show varied patterns with aging, rising or staying constant at least through 24 months of age in the rat (see Bimonte-Nelson et al., 2008; Katoh-Semba et al., 1998). All these factors have been shown to modulate the proliferation of stem/progenitor cells in the subgranual zone of the dentate gyrus and/or the survival of new neurons (Lichtenwalner et al., 2001; Jin et al., 2002; Lee et al., 2002; Cao et al., 2004; Shimazu et al., 2006; Frielingsdorf et al., 2007).

These data are of particular interest because prenatal choline supplementation to rat mothers leads to enhanced basal levels of hippocampal neurogenesis in adult offspring that is accompanied by increased BDNF (Glenn et al., 2007; Wong-Goodrich et al., 2008a), NGF (Sandstrom et al., 2002; Wong-Goodrich et al., 2008a), and IGF-1 (Wong-Goodrich et al., 2008a, 2008b) and -2 (Napoli et al., 2008) levels. These data are strongly suggestive of a plasticity mechanism underlying the cognitive effects of prenatal choline supplementation. As has been suggested by Kempermann (2008), organisms with an increased capacity for hippocampal plasticity may have a "neurogenic reserve" that may make them more resilient to age-related cognitive decline. This provocative idea was tested in the present study by examining whether markers of plasticity, hippocampal cell proliferation and growth factor content (e.g., BDNF, NGF, VEGF, NT-3, and IGF-1), are elevated in rats aged to 25 months that were treated *in utero* with choline supplementation compared to rats fed a control diet. We hypothesized that the aged brains, and specifically the hippocampi, of prenatal choline-supplemented rats would show more features consistent with enhanced plasticity when compared to control rats.

2. Results

2.1. *Behavioral measures in young and old rats*

2.1.1. *Open field exploration and activity*

The behavior of female and male rats in a large open field was assessed at 1 and again at 24 months of age. Latency to enter the center area of the field and the total amount of time rats