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Effects of protein restriction during gestation and lactation on cell proliferation in the hippocampus and subventricular zone: Functional implications. Protein restriction alters hippocampal/SVZ cell proliferation

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ABSTRACT

There is no consensus about the effects of protein restriction on neurogenesis and behavior. Here, for the first time, we evaluated the effects of protein restriction during gestation and lactation, on the two major neurogenic regions of the adult brain, the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ), simultaneously. We also assessed different types of behavior relevant to each region. After mating, pregnant Wistar rats were divided into a control group (CG) that received a normal diet (20% protein); and a protein-restriction group (PRG) that received a low-protein diet (8% protein). After birth, the same diets were provided to the mother and pups until weaning, when some rats were analyzed and others received a normal-protein diet until adulthood. Different sets of rats were used for cellular and behavioral studies in juvenile or adult age. Brains were processed for immunohistochemistry anti-BrdU, anti-Ki67, or anti-pHisH3. Juvenile and adult rats from distinct litters also underwent several behavioral tests. Our data show that early protein restriction results in a reduction of hippocampal progenitors and deficits in object recognition during adult life. Moreover, longer periods of immobility in the tail suspension and in the forced swimming tests revealed that PRG rats show a depressive behavior at 21 days of age (P21) and in adulthood. Furthermore, we suggest that despite the reduced number/proliferation of neural stem cells (B and/or E cells) in SVZ there is a compensatory mechanism in which the progenitors (types C and A cells) proliferate in a higher rate, without affecting olfactory ability in adulthood.

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1. Introduction

Neurogenesis persists throughout adulthood in mammals, mainly in two regions: in the SGZ of the hippocampal dentate gyrus (Eriksson et al.,1998; Gage, 2000) and in the SVZ (Conover and Allen, 2002).

In the hippocampal dentate gyrus, a population of neural stem cells/progenitors divides continuously along the border between the hilus and the granular layer, giving rise to cells that migrate to the granular layer, where they differentiate into granular neurons (Kuhn et al., 1996; Seri et al., 2001).

The SVZ contains four major cell types: ependymal cells (E cells) that are in direct contact with the ventricular lumen (Bruni, 1998), representing a putative neural stem cell population that is mostly quiescent (Coskun et al., 2008; Johansson et al., 1999; Nakafuku et al., 2008); neural stem cells (type B cells) that are multipotent and self-renewing (Chiasson et al., 1999; Doetsch et al., 1999, 2003) and generate clusters of transitory amplifying progenitor cells (type C cells), which proliferate and give rise to migrating neuroblasts (type A cells). Type A cells migrate along the rostral migratory stream (RMS) into the olfactory bulb (OB) where they differentiate into granular and periglomerular neurons (Doetsch et al., 1999; Winner et al., 2002). It is known that the ratio of type A:B:C cells in the mouse brain is 3:2:1 (Lois et al., 1996; Doetsch et al., 1997). Since there is no consensus about the true identity of the neural stem cells in the SVZ and there is not a unique/specific marker for these cells (Coskun et al., 2008; Doetsch et al., 1999; Winner et al., 2002), here we decided to use B/E type cells to represent SVZ neural stem cells.

Several markers can be used to identify proliferating cells in the studies about adult neurogenesis, such as BrdU, Ki-67 and pHisH3. During the synthesis phase of the cell cycle, the BrdU intercalate into DNA of cell replacing the endogenous base thymine, and labeled cells can be identified by immunohistochemistry techniques, without requiring the use of autoradiography, unlike tritiated timidine (Nowakowski et al., 1989). Furthermore, BrdU can be a good tool to study the proliferation or differentiation of specific cell populations in the neurogenic niches, based on the differences in the length of the cell cycle among them (for example, stem cells versus progenitors), besides enabling the progeny tracing (Doetsch et al., 1999, 1997; Costa et al., 2011; Lennington et al., 2003; Alvarez-Buylla et al., 2001). However, some studies showed that in response to stress the cells can incorporate BrdU, without necessarily be proliferating, for example during DNA repair or abortive reentrance on the cell cycle (Kee et al., 2002; Taupin, 2007 for review).

Ki-67 is a nuclear protein expressed in all phases of the cell cycle (except in G0 and in a short period in early G1), which has a very short half life and is expressed exclusively during the cell cycle (Scholzen and Gerdes, 2000). Many studies used only this marker to assess cell proliferation in rodents (Moraes et al., 2012) and also in humans (Boekhoorn et al., 2006; Boldrini et al., 2009; Coras et al., 2010). On the other hand, pHisH3 is a protein expressed only during the G2/M phase, what makes it a very reliable marker of cell proliferation (Mandyam et al., 2007). Using Ki67 and pHisH3 in cell proliferation studies present advantages, since to be endogenous markers their expression in tissues are not dependent on dose, bioavailability or diffusion, as in the case of BrdU.

Postnatal neurogenesis can be modulated by diverse stimuli such as: ischemia and trauma (Bengzon et al., 1997; Gould and Tanapat, 1997; Taupin, 2006; Tureyen et al., 2004), infusion of growth factors (Craig et al., 1996), exposure to an enriched environment (Barnea and Nottebohm, 1994; Kempermann et al., 1998, 1997; Nilsson et al., 1999), voluntary physical activity (van Praag et al., 1999) and also dietary restriction (King et al., 2004; Lee et al., 2000). It was previously reported that after caloric restriction, hippocampal neurogenesis can be either stimulated (Lee et al., 2002, 2000; Mattson, 2005) or inhibited (Akman et al., 2004). In addition, it has been reported that protein restriction limited to the gestational period alters postnatal hippocampal neurogenesis, and that this effect persists even after nutritional rehabilitation (Debassio et al., 1996). However, studies of the effects of protein restriction during gestation and lactation on SVZ and hippocampal neurogenesis have not been reported so far.

The functional roles for adult neurogenesis are still being debated (Bardy and Pallotto, 2010; Deng et al., 2010; Eisch et al., 2008; Gheusi et al., 2009; Lacefield et al., 2012; Ma et al., 2009; Yau et al., 2011). The idea that hippocampal neurogenesis may play an important role in memory is due largely to the observation that several environmental and genetic factors affecting hippocampal neurogenesis result in changes in cognitive performance, such as physical exercise, exposure to an enriched environment, stress, and aging (Drapeau et al., 2003; Kempermann et al., 1997; Ohl and Fuchs, 1999; van Praag et al., 1999). For example, voluntary exercise increases cell proliferation in SGZ (Kee et al., 2007), while exposure to an enriched environment promotes the survival of immature neurons (Tashiro et al., 2007), and both factors improve performance in the Morris water maze (Nilsson et al., 1999; van Praag et al., 1999). Environmental enrichment also improves recognition memory (Bruel-Jungerman et al., 2005). However, more studies are necessary to clarify the physiological role of neurogenesis in the adult hippocampus.

On the other hand, neurogenesis in the SVZ has been associated with olfaction, due to evidence that it can be modulated by the olfactory experience of animals. The neuronal activity and sensory experience are critical to the survival of young neurons in the olfactory bulb (Lledo and Saghatelyan, 2005). Moreover, enrichment of odors increases the survival of newly generated neurons and also olfactory memory, suggesting a role of neurogenesis in the memory process (Lledo et al., 2006; Magavi et al., 2005). Alonso and collaborators (Alonso et al., 2006) observed that more of the generated neurons survived in the olfactory bulb in mice that learned an olfactory discrimination task.

Here, we investigated whether protein restriction during gestation and lactation modulates cell proliferation in the rat hippocampal dentate gyrus and SVZ. Behavioral parameters were also assessed. We found that early protein malnutrition results in a reduction of hippocampal neural progenitors and in object-recognition deficits during adulthood, as well as a depressive behavior already present at P21 that persists into adult life. In the SVZ we found alterations in the duration of