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Research Report

Slow age-dependent decline of doublecortin expression and BrdU labeling in the forebrain from lesser hedgehog tenrecs

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ABSTRACT

In addition to synaptic remodeling, formation of new neurons is increasingly acknowledged as an important cue for plastic changes in the central nervous system. Whereas all vertebrates retain a moderate neuroproliferative capacity, phylogenetically younger mammals become dramatically impaired in this potential during aging. The present study shows that the lesser hedgehog tenrec, an insectivore with a low encephalization index, preserves its neurogenic potential surprisingly well during aging. This was shown by quantitative analysis of 5-bromo-2′-deoxyuridine (BrdU) immunolabeling in the olfactory bulb, paleo-, archi-, and neocortices from 2- to 7-year-old animals. In addition to these newly born cells, a large number of previously formed immature neurons are present throughout adulthood as shown by doublecortin (DCX) immunostaining in various forebrain regions including archicortex, paleocortex, nucleus accumbens, and amygdala. Several ventricle-associated cells in olfactory bulb and hippocampus were double-labeled by BrdU and DCX immunoreactivity. However, most DCX cells in the paleocortex can be considered as persisting immature neurons that obviously do not enter a differentiation program since double fluorescence labeling does not reveal their co-occurrence with numerous neuronal markers, whereas only a small portion coexpresses the pan-neuronal marker HuC/D. Finally, the present study reveals tenrecs as suitable laboratory animals to study age-dependent brain alterations (e.g., of neurogenesis) or slow degenerative processes, particularly due to the at least doubled longevity of tenrecs in comparison to mice and rats.

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Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; Calb, calbindin; CR, calretinin; Cy, carbocyanine; DCX, doublecortin; GAD, glutamate decarboxylase; -ir, -immunoreactivity; NDS–TBS-T, 5% normal donkey serum in TBS, containing 0.3% Triton X-100; Parv, parvalbumin; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; TBS–BSA, TBS, containing 2% bovine serum albumin; βIIITub, βIII-tubulin

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1. Introduction

Neuronal proliferation in the developing central nervous system (CNS) is known to rapidly decline in the postnatal period. However, neurogenesis persists in the adulthood (for review, see Rakic, 2002; Taupin and Gage, 2002; Kempermann, 2006; Taupin, 2006). Neurogenesis was primarily shown for the granule cells of rodent dentate gyrus (Altman and Das, 1965; Gage et al., 1998; Ehninger and Kempermann, 2008) and olfactory bulb (Altman 1969; Graziadei and Graziadei, 1979; Gritti et al., 2002) that contains numerous neurons originating in the subventricular zone and migrating rostralwards along the rostral migratory stream (Lois and Alvarez-Buylla, 1994; Doetsch et al. 1997).

All major vertebrate taxa display neurogenesis in adulthood (García-Verdugo et al., 2002; Gould, 2007; Ihunwo and Pillay, 2007), which had been additionally shown for reptiles and in classical studies for birds, e.g., canaries repeatedly learning new songs (reviewed by Nottebohm, 2008). Remarkably, adult neurogenesis was also reported for nonhuman primates (Kornack and Rakic, 1999, 2001; Pencea et al., 2001, Bernier et al., 2002; Rakic, 2002) and man (Eriksson et al., 1998; Johansson et al. 1999).

Currently, the preferred method for the detection of neurogenesis is the incorporation of the exogenously applied thymidine analogue 5-bromo-2′-deoxyuridine (BrdU) during the S-phase of the cell cycle into newly synthesized DNA and its subsequent immunohistochemical detection. The microtubule-associated protein doublecortin (DCX; Gleeson et al., 1999) was reported to indicate neurogenesis (Brown et al., 2003; Couillard-Després et al. 2005), and it is a widely accepted marker for the detection of immature neurons (Ehninger and Kempermann, 2008; von Bohlen und Halbach, 2007). Such DCX-immunopositive, immature neurons were described for several brain regions from various mammalian species (see, e.g., Gómez-Climent et al., 2008; Luzzati et al., 2009; Zhang et al., 2009).

However, there are only restricted data on the time course of DCX-immunoreactivity (ir) in forebrain neurons during aging (Siwak-Tapp et al., 2007). In addition, to the best of our knowledge, comparable data on DCX-immunolabeling of immature neurons and neurogenesis are still lacking for mammals with poorly differentiated brains, particularly tenrecs (see also Lindsey and Tropepe, 2006). The lesser hedgehog tenrec (*Echinops telfairi*) classically considered an insectivore, but more recently grouped among the superorder Afrotheria (Murphy et al., 2007; Poux et al., 2008) shows one of the lowest encephalization indices among mammals (Stephan et al., 1991) and a poorly differentiated cerebral cortex (Rehkämper, 1981; Künzle, 1995; Morawski et al., 2010). Such features make this species a good choice (i) to investigate how far the preservation of neurogenic potential depends on the phylogenetic stage of the animal, (ii) to show differences in the age-dependent decline of neuronal proliferation among differently encephalized mammals, and (iii) to investigate whether the continuously generated new neuronal pool enters differentiation.

Therefore, the present study was primarily focused on age-dependent BrdU labeling in the forebrain, which was quantified in groups of 2-, 3-, 4, 5-, and 7-year-old tenrecs. In parallel, the distribution pattern of DCX in these animals was analyzed including the quantification of immunosignals in selected regions of interest. Finally, the characterization of DCX-immunopositive cells was assessed by combined labeling of DCX and BrdU or numerous neuronal markers.

2. Results

2.1. *Qualitative findings of BrdU and DCX immunoperoxidase labeling*

BrdU labeling of cell nuclei occurred throughout all layers and regions of the selected brain fields, i.e., olfactory bulb, paleo-, archi-, and neocortices, and was observed in animals from all age groups investigated. Labeled profiles lined the ventricular wall both in the olfactory bulb (Fig. 1A) and, more caudally, of the lateral ventricle (Fig. 1B). In the hippocampus, immunoreactive cells were typically found in the subgranular zone of the dentate gyrus in younger animals (Fig. 1C) as well as in old ones (Fig. 1D).

DCX immunostaining also revealed periventricular (and other) cells in the selected brain areas; this label was cytoplasmic and, thus, provided an impression of the morphology of the cells as exemplified for 2- and 7-year-old tenrecs (Figs. 2 and 3). While the distribution patterns of BrdU- and DCX-immunoreactive cells appeared similar, e.g., in the dentate gyrus, it differed in the paleocortex and neocortex.

In the olfactory bulb (for morphological details, see Radtke-Schuller and Künzle, 2000), DCX-immunoreactive cells at the ventricular wall were densely arranged, showing a darkly stained cell body but scarcely visible processes (Figs. 2A and B, 3A and B). More distant from the ventricular wall, cells were less densely arranged, but displayed numerous long, mostly radially aligned, processes. No labeled cells were found in the outer fourth of the olfactory bulb. In the hippocampus (Künzle and Radtke-Schuller, 2001), DCX-immunoreactive cells were detectable only in the granular layer of the dentate gyrus, particularly in its subgranular zone, whereas none was found in the indusium griseum and the neocortex (Figs. 2C and 3C).

Independent of their age, all tenrecs contained DCX-immunopositive cells and fibers in the nucleus accumbens (Figs. 2D and 3D) and in the amygdala (Figs. 2E, F, and 3F). In the paleocortex (Künzle and Radtke-Schuller, 2000), immunoreactive cells typically accumulated in layer II, the densocellular layer (Figs. 2F and 3E). In addition to the transversally oriented dendrites, their radially directed long processes could well be followed deep into layer III. Whereas DCX-immunoreactive somata and processes were sporadically detected in layer III, none was found in the molecular layer (layer I) of the paleocortex. In parallel, the whole neocortex was also devoid of labeled cells.

2.2. *Age-dependent decrease but persisting BrdU-labeled profiles*

In all four fields investigated, the number of BrdU-labeled profiles (as calculated for 1 mm^3) was significantly higher in younger animals, i.e., at 2 or 3 years of age, than in older animals (Figs. 4A–F). No statistically significant differences in BrdU cell densities were found between animals older than 3 years of age, although a gradual decrease with the aging of the animals was evident. Similarly, the ratio of immunoreactive cells detected at the ventricular wall to all labeled cells in the selected area was