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BRAIN RESEARCH

Research Report

A morphologically distinct granule cell type in the dentate gyrus of the red fox correlates with adult hippocampal neurogenesis

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ARTICLE INFO

Article history:
Accepted 25 February 2010
Available online 4 March 2010

Keywords:
Carnivore
Cytoarchitecture
Immunohistochemistry
Stereology
Proliferation

ABSTRACT

Wild red foxes, proverbially cunning carnivores, are investigated for adult hippocampal neurogenesis and morphological characteristics of the dentate gyrus. Adult red foxes harbor almost 15-times more young, doublecortin-positive neurons in their dentate gyrus than domesticated dogs. The number of doublecortin-positive cells corresponds to 4.4% of the total granule cell number, whereas dividing cells amount to only 0.06%. Compared to laboratory mice, proliferating (Ki67-positive) and dying cells are rare, but the percentage of new neurons is quite similar. The numbers of proliferating cells, young cells of neuronal lineage and dying cells correlate. Resident granule cells can be divided into two types with strikingly different morphologies, staining patterns and distinct septotemporal distributions. Small sized granule cells with a nuclear diameter of 7.3 μm account for ~83% of all granule cells. The remaining granule cells are significantly larger with a nuclear diameter of 9.4 μm diameter and stain heavily for NeuN. Septally and mid-septotemporally, densely packed small cells dominate. Here, only few large granule cells are scattered throughout the layer. Temporally, granule cells become more loosely packed and most of the cells are of the large type. High rates of neurogenesis are observed in foxes with high numbers of large granule cells, whereas the number of small granule cells does not correlate with any of the neurogenesis-related cell counts. Staining for parvalbumin, glutamate receptor 2/3, GAP-43 and dynorphin shows an anatomical context that is a composite of features common also to other mammalian species. In summary, we report a morphologically distinct granule cell type which correlates with adult hippocampal neurogenesis in the fox. Furthermore, the maturation phase of the young neurons may be prolonged as in other long living species such as primates.

1. Introduction

Red foxes (*Vulpes vulpes*) are the most widespread carnivore species of the world. Even though humans have hunted larger carnivores for centuries, foxes survived in their ancestral habitats. Foxes are proverbially cunning and show exceptional adaptability to natural and manmade environmental changes. This flexibility has been explained by non-specialized food and habitat preferences of this species, as '… the most important requirement within any

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doi:10.1016/j.brainres.2010.02.075

habitat to a fox, is another fox' (Lloyd, 1980). Group-living foxes form highly sophisticated hierarchies with one male, one breeding female and female helpers (Macdonald, 1979). The suppression of reproduction in subordinate females is associated with socially-mediated stress, population density and food availability (Macdonald, 1979). In rodents, social stress, mating and maternal-related factors can influence adult hippocampal neurogenesis (for review see Gheusi et al., 2009). Foxes display additional behavioral traits which have been associated with adult hippocampal neurogenesis in rodents. Foxes show highly developed spatial memory skills, and successfully retrieve cached food (Macdonald, 1976; MacDonald et al., 1994). In rodents, successful retrieval of food in baited radial mazes has been positively correlated with neurogenesis (Veena et al., 2009). Red foxes have also been shown to be more curious and exploratory than other carnivores kept in zoos (Kusak and Huber, 1991), however, when living within cities, they remain shy and alert towards humans. In rodents, explorative behavior in elevated plus maze does not correlate with neurogenesis (Shors et al., 2002), but the level of neurogenesis correlates with the behavioral pattern in an open field test (Naylor et al., 2008), and object recognition (Bruel-Jungerman et al., 2005; Jessberger et al., 2009).

In short-living rodents, high neurogenic activity in young animals declines rapidly while aging, both in wild and laboratory mice (Amrein et al., 2004a; Ben Abdallah et al., 2010). In a comparative study (Amrein et al., 2004a) we could show that basal proliferation rates differ between rodent taxa. Increased species-specific complexity of the habitat can be compensated by increased survival of the newly born cells or by increasing total granule cell number. Long life expectancy and late development of the hippocampal formation however might require other strategies. We have shown that in long living bats hippocampal neurogenesis in adulthood is the exception (Amrein et al., 2007). In other long living species such as marmoset monkeys, proliferation occurs on a comparatively low level and also decreases with age (Leuner et al., 2007). However, marmosets might compensate for a low rate of cell division by an extended maturation of the adult born neurons, as has been shown for rhesus monkeys (Ngwenya et al., 2006).

Reports about adult neurogenesis in other carnivores such as cats and dogs suggest that we can expect newborn neurons in the adult fox hippocampus as well (Altman, 1963; Siwak-Tapp et al., 2007). Since we argue that foxes show exceptional high behavioral plasticity, and adult hippocampal neurogenesis adds to the structural and functional plasticity of the brain, we expected neurogenesis in the fox hippocampus to be higher than in other long living species. In order to compare our findings with other species all measurements are normalized to total granule cell number. To provide an anatomical context for these findings, we briefly describe the basic morphology of the fox dentate gyrus and the distributions of some antigens used in studies of the functional anatomy of this part of the brain. The extent of newly born cells in the adult fox dentate gyrus is compared to data in other carnivores and rodents, and the findings are discussed in a comparative context.

2. Results

2.1. *Morphology*

The morphology of the fox dentate gyrus (Fig. 1) closely resembles that of the dog (Hof et al., 1996a). The granule cell layer (Fig. 2a) is typically 6 to 8 granule cell bodies deep and well-delimited from the molecular layer and polymorphic layer of the hilus. There is no clear cell sparse zone between the granule cell layer and the polymorphic cell layer of the hilus (Fig. 1c), which is seen in rabbits or primates (Geneser, 1987; Rosene and van Hoesen, 1987). A narrow continuation of the stratum radiatum separates the polymorphic cell layer of the hilus from CA4 (as used by Rosene and van Hoesen, 1987; the reflected blade of the CA3 pyramidal cell layer), which is continuous with the polymorphic cell layer close to its infrapyramidal tip (Fig. 1c). In contrast to the dog, the cells of CA4 do not form a compact layer but a very loosely organized band (Fig. 1c) similar to that in pigs or primates (Holm and Geneser, 1991; Rosene and van Hoesen, 1987).

2.1.1. *Two distinct granule cell types in the fox dentate gyrus*

The number of total granule cells in the fox hippocampi varies by a factor of two, with the animal identified as being the oldest (approximately four years) having the highest score (Table 1).

Within the population of granule cells, two clearly distinct cell types were found and analyzed separately (Figs. 2a,b). The prevalent cell type is round, with a mean nuclear diameter of 7.3 μm, evenly distributed fine heterochromatin granules and a narrow rim of cytoplasm. This cell type accounts for most cells in the septal and mid-septotemporal part of the dentate gyrus and represents 83% (min 71%; max 89%) of the total granule cell population. The other type consists of larger, ovoid-shaped granule cells with a mean nuclear size of 9.4 μm, scarce heterochromatin and one distinct nucleolus. They are found scattered in the septal and mid-septotemporal granule cell layer and prevalent in the temporal part. The cytoplasm of these large cells is wider and extends into a prominent axon hillock. Cells are oriented perpendicular to the polymorphic layer in the septal and medial part of the dentate gyrus, while in the temporal part the orientation of the axonal hillock is more variable. Nuclear diameters of the two cell type are significantly different ($p<0.0001$, Fig. 3).

Total granule cell number is largely determined by the number of small granule cells ($r_{\text{Total GC - small GC}}=0.998$, $p<0.0001$), whereas the number of large granule cells does not correlate with the number of granule cells ($r_{\text{Total GC-large GC}}=-0.58$, ns., Fig. 4).

2.2. *Neurogenesis*

2.2.1. *Ki67-, DCX-, PSA-NCAM- and NeuroD-immunoreactivity*

The morphological appearance of the Ki67-positive cells is equal to that seen in rodents. Positive cells often appear in clusters in the subgranular layer, while single positive cells are comparatively rare (Figs. 2c,d). Most DCX-stained cells are located in the subgranular layer. In the granule cell layer, they