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Signals Regulating Neurogenesis in the Adult Olfactory Bulb

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Introduction

Most mammalian brain cells develop from neural progenitor or stem cells that reside in the ventricular and subventricular zone. Interestingly, in rodents and primates, neurogenesis does not end when the olfactory bulb reaches adult size but rather continues throughout life (Kaplan *et al.*, 1985; Kuhn *et al.*, 1996; Pencea *et al.*, 2001). For neurons at two locations in the olfactory bulb, the granule cell layer and glomerular layer, a persistent proliferative activity of progenitor cells can be observed in the subventricular zone (SVZ) of the lateral ventricles. The committed neuronal progenitor cells migrate rostrally through the remnant of the embryonic olfactory ventricle wall, the so-called rostral migratory stream (RMS), towards the olfactory bulb. Once the olfactory bulb is reached, the majority of cells disperse throughout the granule cell layer to develop into GABAergic granule cells. A small percentage, however, moves into the periglomerular region to develop into interneurons of mostly GABAergic and dopaminergic phenotype.

Elimination as well as long-term survival of young neurons

The continuous addition of new neurons to the olfactory bulb leads to substantial growth of the structure over the adult life of a rodent, which is achieved mostly through an increase in neuronal density (Kaplan *et al.*, 1985). Yet, the amount of proliferating and migrating cells appears to outnumber the growth rate of the olfactory bulb, and therefore, we investigated cell death within the neurogenic regions of the adult brain and found an up to 100-fold higher incidence of apoptotic cell death within the SVZ, RMS and olfactory bulb compared with non-neurogenic regions (Biebl *et al.*, 2000).

The coexistence of neurogenesis and cell death in the olfactory bulb leads to the question, whether old cells are replaced or whether the number of developing neurons is adjusted to the necessary amount. After injecting rats at 2 months of age with bromodeoxyuridine (BrdU), we quantified the newly generated cells over a period of 19 months (Winner *et al.*, 2002). A peak of new neurons is reached in the olfactory bulb 1 month after BrdU injection, when the labeled cells have finished migration from the ventricle wall. At this point the majority of new cells (>90%) express the mature neuronal marker NeuN, although the first cells begin expressing NeuN already as early as 7–10 days after birth. Thereafter, we observed a reduction of BrdU-positive cells to ~50%. We confirm by dUTP-nick end labeling (TUNEL) that progenitors and young neurons undergo programmed cell death. Nevertheless, cells that survived the first 3 months after BrdU injection were detectable as granule cells for up to 19 months. A similar elimination of newly generated neurons was observed for the periglomerular interneurons (Winner *et al.*, 2002). Rather than replacing old neurons, these results suggest that new neurons are added to the adult olfactory bulb and that apoptotic elimination of young neurons is used to control the growth of these neuronal populations in the olfactory bulb.

Sensory stimulation of olfactory neurogenesis

It is well established that during embryonic brain development a large proportion of neural progenitors and young neurons are eliminated by programmed cell death unless the cells receive synaptic input or trophic support (for a review, see Oppenheim, 1991). Newly generated neurons in the adult brain depend on sensory stimulation as demonstrated by decreased neurogenesis due to increased cell death after naris closure (Corotto *et al.*, 1994), whereas sensory stimulation through exposure to novel odorants had the opposite effect (Rochefort *et al.*, 2002). On the other hand, exposure to an enriched environment and physical activity, such as voluntary wheel running, increase neurogenesis in the dentate gyrus of adult mice and rats (Kempermann *et al.*, 1997; Nilsson *et al.*, 1999); but no difference in SVZ progenitor proliferation or neurogenesis in the olfactory bulb was detectable under these conditions (Brown *et al.*, 2003). Conversely, odorant enrichment was ineffective in raising the hippocampal neurogenesis level (Rochefort *et al.*, 2002), thus arguing for local, yet unidentified mechanisms that specify neurogenic signals in the adult brain. Using anosmic mice, Alvarez-Buylla and colleagues found that sensory input was critical for the survival of young granule cells during maturation, and once synaptically connected, their survival depended on the level of activity that they received (Petreanu and Alvarez-Buylla, 2002).

Molecular regulators of olfactory neurogenesis

Direct mitogenic stimulation of progenitor cells in the adult brain appears to be mediated via growth factors and trophic factors. It is still unclear to what extend endogenous production of several candidate growth factors, such as FGF-2 and BDNF, play a role in the ongoing spontaneous olfactory bulb neurogenesis. Possible mechanisms include local expression or direct action of factors passing through the blood-brain barrier. But other mechanisms, such as angiogenesis, could also be triggered, which have a secondary positive effect on neurogenesis (Palmer *et al.*, 2000; Louissaint *et al.*, 2002; Shen *et al.*, 2004). In this context it is important to note, that several blood-derived growth factors such as erythropoietin and vascular endothelial growth factor (VEGF) are potent stimulators of neurogenesis, when applied directly into the ventricular system (Shingo *et al.*, 2001; Jin *et al.*, 2002; Schänzer *et al.*, 2004).

The effect of neurotransmitter systems on adult neurogenesis has been extensively studied in the hippocampus. Glutamatergic input from the entorhinal cortex has a negative impact on granule cell production (Cameron *et al.*, 1995; Bernabeu and Sharp, 2000; Kitamura *et al.*, 2003; Nacher *et al.*, 2003), whereas the serotonergic input from the raphé nuclei is an activator of hippocampal neurogenesis. Treatments with the antidepressants, which act as stimulators of the serotonergic system, have demonstrated a positive influence neurogenesis (Malberg *et al.*, 2000; Czeh *et al.*, 2001; Santarelli *et al.*, 2003). In a recent study we were able to demonstrate that neurogenesis in the olfactory bulb as well as in the dentate gyrus is reduced after lesion of the cholinergic basal forebrain system using immunotoxin lesions (Cooper-Kuhn *et al.*, 2004). These studies

indicate that the extracellular milieu in the neurogenic regions can be substantially influenced by the release of neurotransmitters. However, it remains to be shown, whether and at what stage the immature cells express neurotransmitters receptors in order become directly responsive to these signals.

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