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Review

First-generation neuronal precursors in the crayfish brain are not self-renewing

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ABSTRACT

Adult-born neurons in crayfish (*Procambarus clarkii*) are the progeny of 1st-generation precursor cells (functionally analogous to neuronal stem cells in vertebrates) that are located in a neurogenic niche on the ventral surface of the brain. The daughters of these precursor cells migrate along the processes of bipolar niche cells to proliferation zones in the cell clusters where the somata of the olfactory interneurons reside. Here they divide again, producing offspring that differentiate into olfactory local and projection neurons. The features of this neuronal assembly line, and the fact that it continues to function when the brain is isolated and perfused or maintained in organotypic culture, provide opportunities unavailable in other organisms to explore the sequence of cellular and molecular events leading to the production of new neurons in adult brains. Further, we have determined that the 1st-generation precursor cells are not a self-renewing population, and that the niche is, nevertheless, not depleted as the animals grow and age. We conclude, therefore, that the niche is not a closed system and that there must be an extrinsic source of neuronal stem cells. Based on *in vitro* studies demonstrating that cells extracted from the hemolymph are attracted to the niche, as well as the intimate relationship between the niche and vasculature, we hypothesize that the hematopoietic system is a likely source of these cells.

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1. Introduction

According to current understanding, stem cells by definition are "*capable of dividing and renewing themselves for long periods*" *in vivo*, although adult stem cells are not capable of long-term self-renewal *in vitro*, as are embryonic stem cells (National Institutes of Health, http://stemcells.nih.gov/info/basics/). A second fundamental tenet is that adult stem cells *in vivo* "*generate the cell types of the tissue in which they reside. For example, a blood-forming adult stem cell in the bone marrow normally gives rise to . . . blood cells. . . . a hematopoietic stem cell . . . cannot give rise to the cells of a very different tissue, such as nerve cells in the brain.*" Our studies concerning the lineage of precursor cells that generates neurons in the adult crayfish brain challenge both of these principles. In this paper, we review what is currently known about the precursor cells underlying adult neurogenesis in the crayfish brain. These findings are discussed in relation to studies of bone marrow stromal (*i.e.*, mesenchymal) stem cells and the generation of new neurons in adult mammalian brains.

Abbreviations: 5-HT, serotonin; AL, accessory lobe; APC, anterior proliferation center; BrdU, 5-bromo-2′-deoxyuridine; CTG, CellTracker™ Green CMFDA; EdU, 5-ethynyl-2′deoxyuridine; GS, glutamine synthetase; HPT, hematopoietic tissue; LPS, lipopolysaccharide; LPZ, lateral proliferation zone; MPZ, medial proliferation zone; MMS, methiothepin mesylate salt; MSC, mesenchymal stem cell; OGT, olfactory globular tract; OL, olfactory lobe; ROS, reactive oxygen species.

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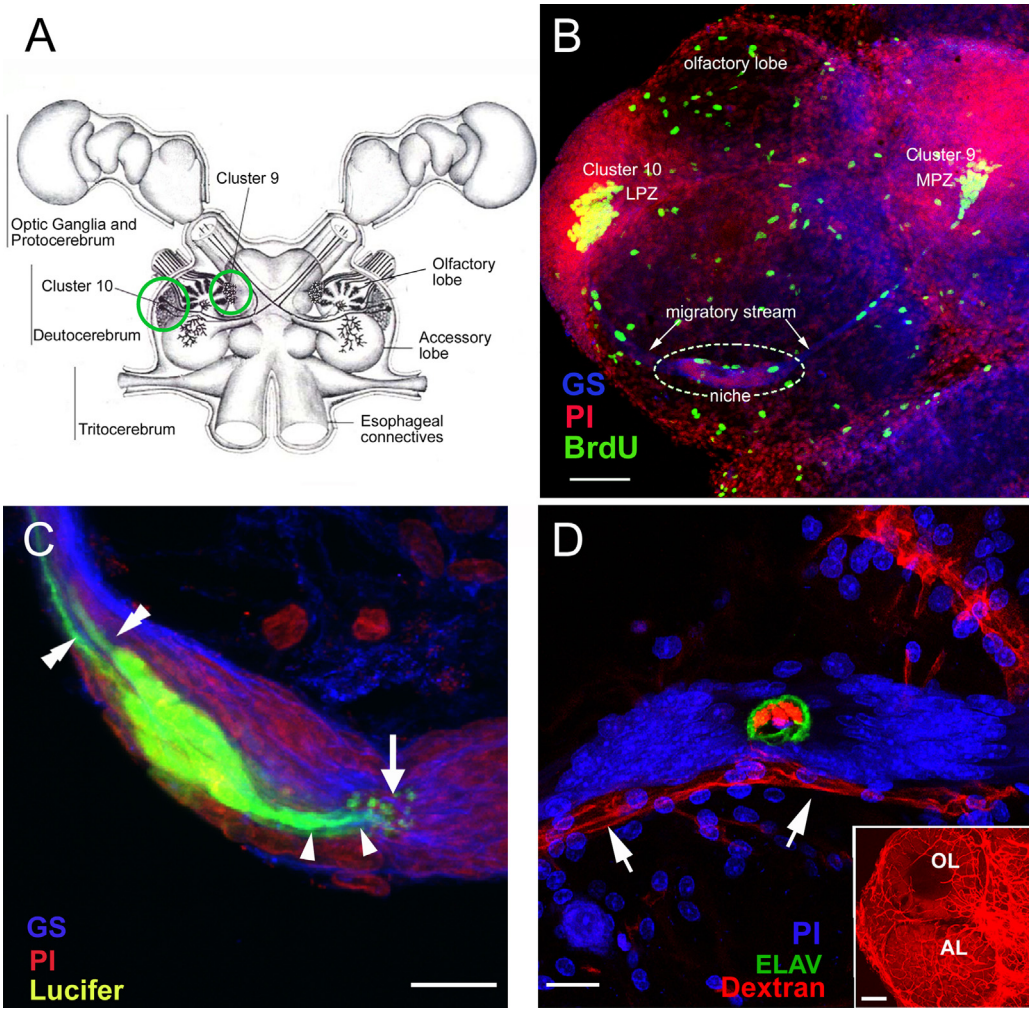

Fig. 1. (A) Diagram of the eureptantian (crayfish, lobster) brain including the optic ganglia, and showing the locations of the proto-, trito- and deutocerebral neuropils. Cell Clusters 9 and 10 (circles), locations of neurogenesis in the adult brain, flank two prominent neuropil regions of the deutocerebrum, the olfactory and accessory lobes. (Names of brain areas are according to Sandeman et al., 1992.) (B) Left side of the brain of *Procambarus clarkii* labeled immunocytochemically for the S-phase marker BrdU (green). Labeled cells are found in the lateral proliferation zone (LPZ) contiguous with Cluster 10 and in the medial proliferation zone (MPZ) near Cluster 9. The two zones are linked by a chain of cells in the migratory stream, labeled immunocytochemically for glutamine synthetase (GS; blue). These streams originate in the oval region 'niche' (dotted circle) containing cells labeled with the nuclear marker propidium iodide (PI, red). (C) Several niche cells are labeled by intracellular injection of Lucifer yellow. Each of these has a short process (arrowheads) projecting to the vascular cavity (arrow) and longer fibers (double arrowheads) that fasciculate to form the tracts projecting to the LPZ and MPZ, along which the daughters of the niche cells (2nd-generation neuronal precursors) migrate (the 'streams'). Blue, GS; red, propidium iodide (PI). (D) The vascular connection of the cavity in the center of the glial soma cluster was demonstrated by injecting a dextran dye into the dorsal artery. The cavity, outlined in green by its reactivity to an antibody to ELAV, contains the dextran dye (red), which is also contained within a larger blood vessel that runs along beneath the niche. PI (blue) labeling of nuclei in the niche cells is also shown. Inset: dextran-filled vasculature in the olfactory (OL) and accessory (AL) lobes on the left side of the brain. Scale bars: B, 100 μm; C and D, 20 μm; inset in D, 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
C and D from Sullivan et al. (2007a).

1.1. Adult neurogenesis in the crayfish brain

Our studies focus on life-long neurogenesis among interneuronal populations in the olfactory pathway of the crustacean brain (Fig. 1A; Schmidt, 1997; Harzsch et al., 1999; Schmidt and Harzsch, 1999). The sensory, local and projection neurons of the crustacean midbrain are functionally analogous to groups of neurons in the vertebrate olfactory system that have a similar capacity for life-long neurogenesis (Lois and Alvarez-Buylla, 1994; Hildebrand and Shepherd, 1997).

The crustacean olfactory system consists of sensory neurons that synapse on local and projection interneurons within the glomeruli of the olfactory lobes (OL), which are involved in the primary processing of olfactory information. The cell bodies of olfactory interneurons are clustered in functional groups: the local interneurons located medial to the OL in Clusters 9 and 11, and the projection neurons lateral to the OL in Cluster 10 (Fig. 1A; terminology of Sandeman et al., 1992). Cluster 9 interneurons innervate both the OL and accessory lobe (AL); Cluster 10 projection neurons innervate *either* the OL or AL (Sullivan et al., 2000), and their axons project *via* the olfactory globular tract (OGT) to neuropil regions in the lateral protocerebrum (Sullivan and Beltz, 2001). The AL is involved in higher-order integration of olfactory, visual and mechanosensory information (Sandeman et al., 1995; Sullivan and Beltz, 2005).

Neuronal proliferation in most regions of the decapod brain ceases in the period around hatching when the embryonic precursor cells (neuroblasts) disappear (Beltz and Sandeman, 2003). The exception to this is in the central olfactory pathway where mitotic activity continues throughout life (Harzsch and Dawirs, 1996; Schmidt, 1997; Schmidt and Harzsch, 1999; Harzsch et al., 1999). Adult neurogenesis also occurs in the visual pathway (Sullivan and Beltz, 2005), but has been studied in much less detail. In the