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The Olfactory Pathway of Decapod Crustaceans—An Invertebrate Model for Life-Long Neurogenesis

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Abstract

The first part of this review includes a short description of the cellular and morphological organization of the olfactory pathway of decapod crustaceans, followed by an overview of adult neurogenesis in this pathway focusing on the olfactory lobe (OL), the first synaptic relay in the brain. Adult neurogenesis in the central olfactory pathway has the following characteristics. 1) It is present in all the diverse species of decapod crustaceans so far studied. 2) In all these species, projection neurons (PNs), which have multiglomerular dendritic arborizations, are generated. 3) Neurons are generated by one round of symmetrical cell divisions of a small population of immediate precursor cells that are located in small proliferation zones at the inner margin of the respective soma clusters. 4) The immediate precursor cells in each soma cluster appear to be generated by repeated cell divisions of one or few neuronal stem cells that are located outside of the proliferation zone. 5) These neuronal stem cells are enclosed in a highly structured clump of small glial-like cells, which likely establishes a specific microenvironment and thus can be regarded as a stem cell niche. 6) Diverse internal and external factors, such as presence of olfactory afferents, age, season of the year, and living under constant and deprived conditions modulate the generation and/or survival of new neurons. In the second part of this review, I address the question why in decapod crustaceans adult neurogenesis persists in the visual and olfactory pathways of the brain but is lacking in all other mechanosensory–chemosensory pathways. Due to the indeterminate growth of most adult decapod crustaceans, new sensory neurons of all modalities (olfaction and chemo-, mechano-, and photoreception) are continuously added during adulthood and provide an ever-increasing sensory input to all primary sensory neuropils of the central nervous system. From these facts, I conclude that adult neurogenesis in the brain cannot simply be a mechanism to accommodate increasing sensory input and propose instead that it is causally linked to the specific "topographic logic" of information processing implemented in the sensory neuropils serving different modalities. For the presumptive odotopic type of information processing in the OL, new multiglomerular PNs allow interconnection of novel combinations of spatially unrelated input channels (glomeruli), whose simultaneous activation by specific odorants is the basis of odor coding. Thus, adult neurogenesis could provide a unique way to increase the resolution of odorant quality coding and allow adaptation of the olfactory system of these long-lived animals to ever-changing odor environments.

Key words: arthropod, decapod crustacean, local interneurons, proliferation, projection neurons

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Introduction

Contrary to beliefs held throughout most of the last century, neurogenesis persists in the brain of adult animals from various taxa. Although widespread in the animal kingdom, adult neurogenesis in each species only occurs in one or very few, specific brain areas, and only one or very few neuronal types in each of these areas are subject to it. In this sense, neurogenesis is the rare exception and not the rule among the numerous neuronal populations of the adult brain, or even the central nervous system (CNS). In this review, I offer an explanation for the sparse occurrence of adult neurogenesis in the brain of decapod crustaceans. Hopefully, this hypothesis can also shed some light on other systems in which adult neurogenesis exists among certain neuronal populations of higher brain regions—the olfactory bulb and hippocampus of the mammalian brain, and the mushroom bodies of the insect brain.

Neurogenesis in the adult brain occurs in most of the main classes of vertebrates, including elasmobranchs (Leonard et al. 1978), teleost fishes (Raymond and Easter 1983; Zupanc 1999, 2001; Byrd and Brunjes 2002; Zupanc et al. 2005), amphibians (Polenov and Chetverukhin 1993), reptiles (Pérez-Cañellas and García-Verdugo 1996; Font et al. 2002), songbirds (Goldman 1998), and mammals (reviews: Gross 2000; Ming and Song 2005; Christie and Cameron 2006; Gheusi and Lledo 2007). Among these, mammals are by far the best

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analyzed group. Across various rodent and primate species including humans, adult neurogenesis was consistently demonstrated in only 2 brain areas, the subventricular zone/olfactory bulb and the dentate gyrus of the hippocampus (Eriksson et al. 1998; Kornack and Rakic 2001; Ming and Song 2005; Christie and Cameron 2006). Adult neurogenesis in the mammalian olfactory bulb is reviewed in another paper in this issue (Gheusi and Lledo 2007). Among invertebrates, mainly arthropods have been analyzed with respect to the occurrence of adult neurogenesis, and within arthropods the analysis is restricted to only several species of insects and decapod crustaceans, leaving numerous taxa and countless species untested. Although restricted to only relatively few species in each case, these analyses reveal a consistent picture of adult neurogenesis in the brain of both insects and decapod crustaceans. In insect brains, adult neurogenesis only occurs among the Kenyon cells of the mushroom bodies, which are higher order multimodal integration centers receiving their most prominent input from the antennal lobes, the primary olfactory neuropils (Cayre et al. 1994, 1996; Gu et al. 1999; Dufour and Gadenne 2006). Adult neurogenesis in the mushroom bodies is lacking in several insect species (Fahrbach et al. 1995; Cayre et al. 1996) and lasts only several days into adulthood (Gu et al. 1999). Adult neurogenesis in insects is reviewed in detail in another paper contained in this issue (Cayre et al. 2007).

In decapod crustaceans, adult neurogenesis occurs only in 2 brain areas: the optic lobes (Schmidt 1997; Sullivan and Beltz 2005b) and the central olfactory pathway (Schmidt 2002a; Beltz and Sandeman 2003). Although evidence for adult neurogenesis in the optic lobes so far has been obtained for only 2 species (*Carcinus maenas*, *Cherax destructor*) and has not been analyzed in detail (Schmidt 1997; Sullivan and Beltz 2005b), evidence for adult neurogenesis in the central olfactory pathway has been obtained for all 9 species that have been analyzed, which represent the major infraorders of decapod crustaceans: Caridea, shrimp: *Sicyonia brevirostris*; Achelata, spiny lobster: *Panulirus argus*; Homarida, clawed lobster: *Homarus americanus*; Astacida, crayfish: *C. destructor*, *Procambarus clarkii*; Anomala, hermit crab: *Pagurus bernhardus*; and Brachyura, true crabs: *C. maenas*, *Cancer pagurus*, *Libinia emarginata* (Schmidt 1997, 2001, 2007; Sandeman et al. 1998; Harzsch, Miller et al. 1999; Schmidt and Harzsch 1999; Beltz et al. 2001; Hansen and Schmidt 2001, 2004; Song et al. 2005; Sullivan and Beltz 2005a, 2005b; Sullivan et al. 2007). Several aspects of adult neurogenesis in the central olfactory pathway of decapod crustaceans have been studied in more detail in some of the above-mentioned species. These include the fate (neuronal differentiation, apoptosis) of the newly generated cells; the regulation of adult neurogenesis by internal and external factors, such as age, afferent input, captivity, and yearly seasons; and the identification of putative neuronal stem cells at the basis of proliferation. In this review, I provide a short overview on each of these themes, and based on this I propose a hypothesis to explain why adult neurogenesis occurs in the olfactory pathway but not in the many other mechanosensory–chemosensory pathways of the decapod crustacean CNS.

The olfactory pathway of decapod crustaceans

The general neuroanatomical organization of the olfactory pathway of decapod crustaceans is very similar to that of insects (Schachtner et al. 2005) (Figure 1A). Sensory input to the olfactory pathway is provided by an array of specialized sensilla, called aesthetascs, which exclusively occur on the lateral flagellum of the antennules (first antennae) (Marcus 1911; Helm 1928; Laverack and Ardill 1965; Grünert and Ache 1988). The aesthetascs have ultrastructural features identifying them as olfactory sensilla (Laverack and Ardill 1965; Grünert and Ache 1988; Gleeson et al. 1996), they respond to a range of physiologically relevant odorants (Schmiedel-Jakob et al. 1989; Michel et al. 1993; Steullet, Cate, Michel, et al. 2000), and they drive behavioral responses to pheromones (Gleeson 1982; Horner and Derby 2005; Johnson and Atema 2005; Shabani et al. 2006). Aesthetascs are unique among arthropod sensilla in having a large number (40–500) of primary sensory neurons packaged into each sensillum (Marcus 1911; Laverack and Ardill 1965; Ghiradella et al. 1968; Snow 1973; Grünert and Ache 1988; Gleeson et al. 1996; Steullet, Cate, Michel, et al. 2000). This unusually high number of sensory neurons (olfactory receptor neurons or ORNs) combined in one sensillum suggests that the aesthetascs are specialized for providing a multitude of information-coding channels as required for the representation of the highly complex, multidimensional odor environment (Laurent 2002; Wilson and Mainen 2006).

Aesthetascs increase in number during adulthood and also undergo a slow "longitudinal" turnover (Sandeman et al. 1998; Steullet, Cate, and Derby 2000; Harrison, Cate, Swanson, et al. 2001; Derby et al. 2003). Both phenomena are tightly associated with molting, the process whereby these animals grow through shedding the exoskeleton. With each molt, new aesthetascs are born at the proximal edge of the aesthetasc array, and in consecutive molts they are slowly and discontinuously translocated distally, and finally they reach the very tip of the flagellum and are lost with the following molt. Functional maturation of newly generated ORNs in *P. argus* lasts several months, and the life span of ORNs is probably more than a year (Steullet, Cate, and Derby 2000; Harrison, Cate, Swanson, et al. 2001).

The ORNs innervating the aesthetascs project their axons into the brain where they terminate in the elongated, wedge-shaped glomeruli of the olfactory lobe (OL), the most prominent neuropil of the deutocerebrum (Sandeman and Denburg 1976; Mellon et al. 1989; Mellon and Munger 1990; Schmidt and Ache 1992; Mellon and Alones 1993; Sandeman DC and Sandeman RE 1994) (Figures 1A and 3). Most ORNs innervate only one glomerulus of the OL

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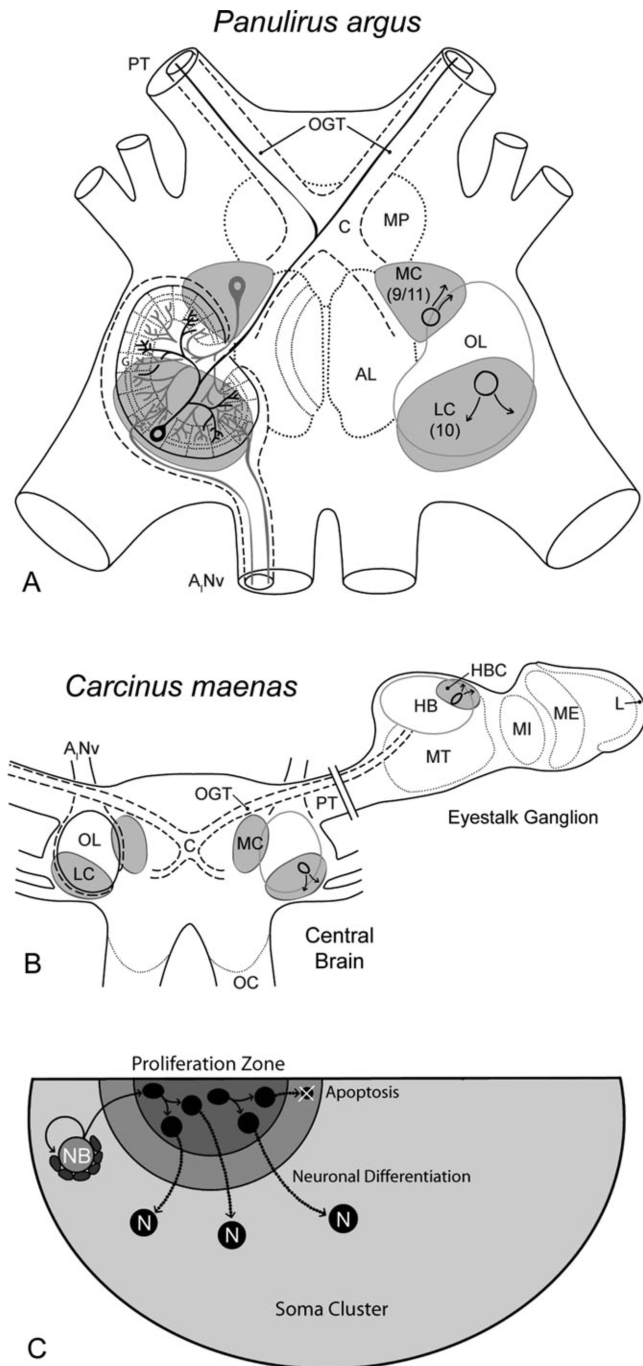

Figure 1 Adult neurogenesis in the central olfactory pathway of decapod crustaceans. **(A)** Overview of the organization of the olfactory deutocerebrum in *Panulirus argus* (modified after Schachtner et al. 2005). On the left: schematic drawing of main neuronal types—ascending PNs in black, local interneurons and ORNs in gray; on the right: location of proliferation zones in soma clusters indicated by black circles. **(B)** Overview of the organization of the central olfactory pathway in *Carcinus maenas* (modified after Schmidt 1997). On the right: location of proliferation zones in soma clusters indicated by black circles. **(C)** Schematic representation of the cellular basis of adult neurogenesis in the central olfactory pathway of decapod crustaceans. Large putative adult NBs in the vicinity of proliferation zones act as primary neuronal stem cells. By asymmetric cell divisions, they self-renew and generate daughter cells migrating into the proliferation zone. Here they divide once symmetrically, and both daughter cells are pushed out of the proliferation zone and either differentiate into neurons (N) or die by apoptosis (very few). In essence, this process resembles closely neurogenesis in the nervous system of embryonic and larval crustaceans and insects. One important difference is that the putative adult NBs are associated with specialized clumps of small cells that may represent stem cell niches critically important for the unusual, life-long self-renewal and proliferative capacity of the putative adult NBs. Abbreviations: antennular nerve (A_INv), hemiellipsoid body (HB), hemiellipsoid body soma cluster (HBC), commissure of olfactory globular tract (C), glomeruli of OL (G), lamina (L), lateral soma cluster (LC, cluster 10), medial protocerebrum (MP), medial soma cluster (MC, cluster 9/11), medulla externa (ME), medulla interna (MI), medulla terminalis (MT), esophageal connectives (OC), olfactory globular tract (OGT), olfactory lobe (OL), and protocerebral tract (PT).

(Schmidt and Ache 1992; Mellon and Alones 1993), and the topographical arrangement of the aesthetascs on the lateral flagellum is not preserved in the projections of their ORNs to the OL glomeruli (Mellon and Munger 1990), suggesting that the OL has an odotopic rather than somatotopic representation. The number of glomeruli per OL varies substantially between different species, ranging from approximately 150 to 1300, with marine species generally having a higher number of glomeruli than freshwater or semiterrestrial species (Blaustein et al. 1988; Schmidt and Ache 1992; Beltz et al. 2003; Schachtner et al. 2005). The OL is closely associated with some other neuropils, of which the accessory lobe (AL) is the largest in some infraorders (Achelata, Homarida, Astacida, Thalassinida) (Sandeman and Scholtz 1995; Sullivan and Beltz 2004) (Figure 1A). The AL is also organized into glomeruli but does not receive any direct sensory input. Together, these neuropils form the olfactory deutocerebrum representing the first stage of the central olfactory pathway (Sandeman et al. 1992; Schachtner et al. 2005). Two types of neurons constitute the olfactory deutocerebrum, and the somata of these neuronal types are segregated neatly into 2 distinct soma clusters. One type is local interneurons with arbors restricted to deutocerebral neuropils and somata forming the medial soma cluster (MC; cluster 9/11 according to the terminology of Sandeman et al. 1992) (Figures 1A,B and 2B,J). The second type is projection neurons (PNs) with arborizations either in the OL (OL-PNs) or AL (AL-PNs), an axon projecting to the lateral protocerebrum, and somata forming the lateral soma cluster (LC; cluster 10 according to the terminology of Sandeman et al. 1992) (Figures 1A,B and 2A). LNs that mainly arborize in the OL come in 2 morphological types (core neurons and rim neurons), both of which innervate most if not all glomeruli (Schachtner et al. 2005). The OL-PNs have multiglomerular arborization patterns with dense, bush-like branching in only some (3–10) OL glomeruli and very sparse branching in many others (from 10% to 80% of all glomeruli) (Wachowiak and Ache 1994; Schmidt and Ache 1996b).

The second level of the central olfactory pathway is constituted by neuropils of the lateral protocerebrum located in the eyestalk ganglia, which are the target of the PNs: the hemiellipsoid body and the medulla terminalis (Mellon et al. 1992;

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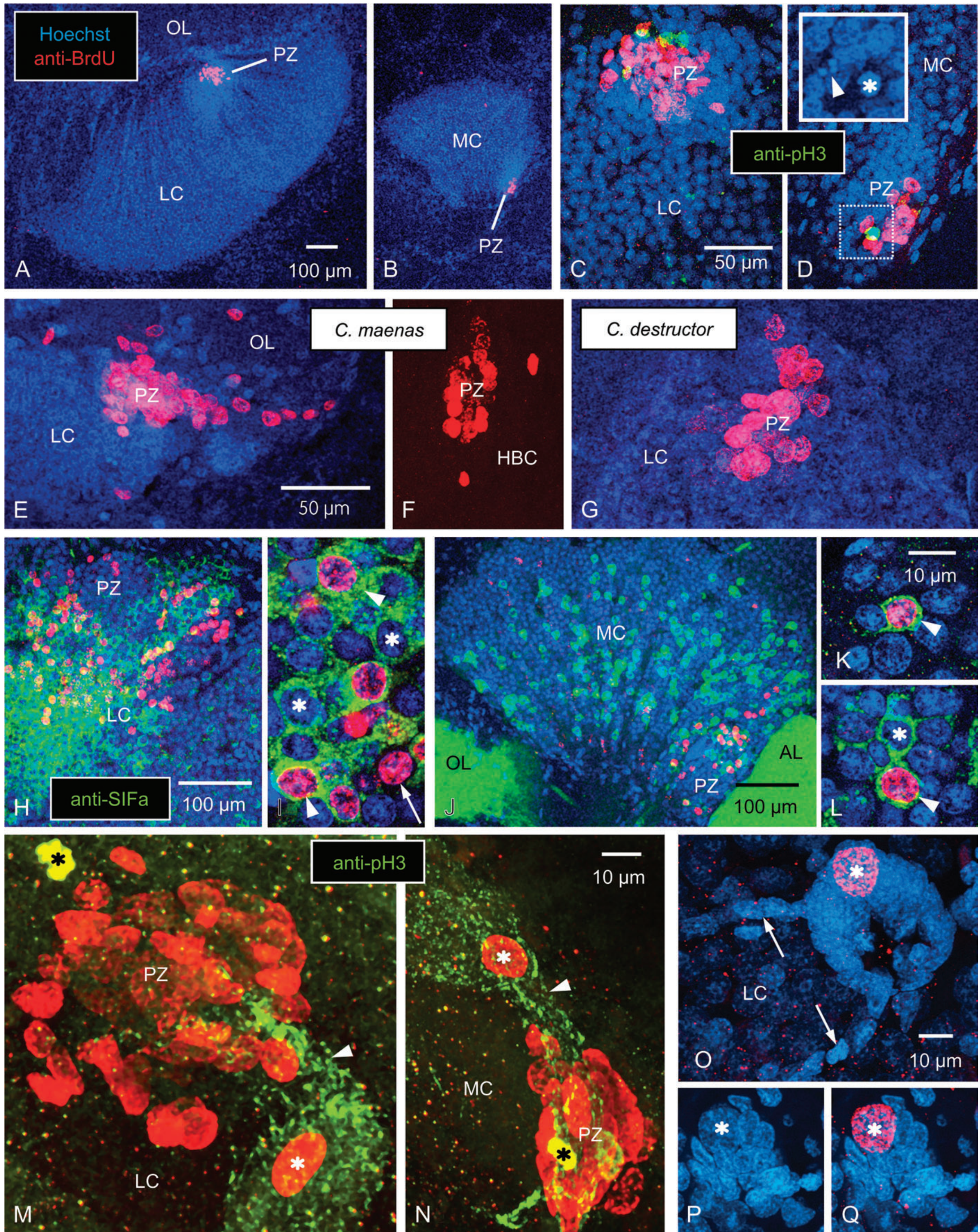

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Sullivan and Beltz 2001, 2004; McKinzie et al. 2003). In species lacking an AL, the OL-PNs primarily target the hemiellipsoid body (Sullivan and Beltz 2004), whereas in species that have a prominent AL, the OL-PNs instead terminate in the medulla terminalis and the hemiellipsoid body receives innervation by AL-PNs (Sullivan and Beltz 2001; McKinzie et al. 2003). The neuroanatomy of the eyestalk ganglia is very complex and only partially analyzed (Blaustein et al. 1988). Both medulla terminalis and hemiellipsoid body appear to be mainly comprising different types of local interneurons, some of which have their somata in a cluster that is located in the vicinity of the hemiellipsoid body and is named HBC (Schmidt 1997; Schmidt and Harzsch 1999; Hansen and Schmidt 2001, 2004) (Figure 1B) or soma cluster A (Sullivan and Beltz 2005a).

It is noteworthy that the aesthetascs occur in a stereotypical arrangement with various other sensilla types (guard setae, companion setae, and asymmetric setae), all of which appear to be bimodal containing mechano- and chemosensory receptor neurons (Laverack 1964; Derby 1982; Spencer and Linberg 1986; Cate and Derby 2001; Schmidt and Derby 2005). The afferents of these ''nonaesthetasc'' sensilla target another neuropil of the deutocerebrum, the lateral antennular neuropil (LAN), which also contains major arborizations of antennular motoneurons and thus represents the antennular sensory-motor center (Schmidt et al. 1992; Schmidt and Ache 1993, 1996a; Roye 1994). Due to the strict coexistence of particular nonaesthetasc sensilla with the aesthetascs, the continuous increase in the number of sensilla and receptor neurons during adulthood and the slow longitudinal turnover along the lateral flagellum not only applies to the aesthetascs and the ORNs but also to these accompanying sensilla and their chemo- and mechanoreceptor neurons.

Neurogenesis in the brain throughout postlarval life

Decapod crustaceans, after completion of embryonic development in the egg, transform into larvae that usually are free-swimming planktonic organisms (Williamson 1982; Scholtz 2000) and only in few taxa, such as crayfish, remain in the egg (Sandeman R and Sandeman D 1991). After going through several, sometimes as many as 17 (Palinuridae: Kittaka et al. 2005) morphologically distinct larval stages, the animals molt into first juveniles, which closely resemble adults in morphology (Felder et al. 1985; Sandeman R and Sandeman D 1991). Through successive molts with ever-increasing intervals, these juvenile animals incrementally grow in size until they reach adulthood, defined as the state of sexual maturity (Hartnoll 1982; Conan et al. 2001). Afterward, most species continue to grow by further molts throughout their entire adult life (indeterminate growth);

Figure 2 Immunocytochemical characterization of adult neurogenesis in the central olfactory pathway of decapod crustaceans. Micrographs represent collapsed stacks (in A–H, J, M, N) or substacks (in I, K, L, O–Q) of optical sections (thickness 0.3–1 μm) taken with a confocal microscope from horizontal, 80-μm thick vibrating microtome sections at different excitation wavelengths. Red: BrdU-like immunoreactivity; blue: labeling with the nuclear marker Hoechst 33258; green: phosphorylated histone 3-like (pH3) immunoreactivity (in C, D, M, N) or SIFamide-like immunoreactivity (in H–L). (A–D) Identification of proliferation zones in the LC and the MC of the spiny lobster, *Panulirus argus*, by in vivo labeling with a single injection of BrdU followed by a short survival time (6 h). **(A, B)** Overview; scale bar in (A) also applies to (B). In both soma clusters, a small region at the inner margin contains BrdU-positive nuclei and is characterized by a very high nuclear density. This region is the proliferation zone (PZ). **(C, D)** Higher magnification reveals that in both soma clusters the BrdU-positive nuclei form a compact group within the proliferation zone. The proliferation zone also contains some nuclei in mitosis, as indicated by the labeling with anti-pH3. Inset in (D) shows highlighted area in higher magnification. Note presence of an apoptotic nucleus (arrowhead) in close proximity to a mitotic nucleus (asterisk). Scale bar in (C) also applies to (D). **(E–G)** Identification of proliferation zones in the brain of other decapod crustaceans, the shore crab, *Carcinus maenas* (E, F), and the crayfish, *Cherax destructor* (G), by in vivo labeling with a single injection of BrdU followed by a short survival time (ca. 12 h). Scale bar in (E) also applies to (F) and (G). (E) In the LC of *C. maenas*, the BrdU-positive nuclei form a compact group and a strand attached to it. (F) The soma cluster of the hemiellipsoid body (HBC) of *C. maenas* contains a compact group of BrdU-positive nuclei. (G) The LC of *C. destructor* contains a compact group of BrdU-positive nuclei. Note that these are much larger than in the other species. **(H–L)** Demonstration of neuronal maturation of newly generated cells in the lateral (LC) and the MC of *P. argus*. In vivo labeling with a single injection of BrdU followed by a long survival time of 7 months was combined with immunostaining for the neuropeptide SIFamide expressed in many somata of LC and MC. Scale bar in (H) also applies to (J), and scale bar in (K) also applies to (I) and (L). (H, I) In the LC, numerous somata of PNs express SIFamide-like immunoreactivity (green), and many cells outside of the proliferation zone (PZ) are BrdU-positive, indicating that they were born 7 months earlier and have moved toward the periphery of the soma cluster since then. (H) Lower magnification reveals largely overlapping populations of SIFamide- and BrdU-positive cells. (I) Higher magnification reveals that numerous cells (arrowheads) are double labeled by BrdU (red nucleus) and anti-SIFamide (green cytoplasm) demonstrating that these cells born 7 months earlier have undergone neuronal maturation. Note that other cells in the same group are SIFamide positive but BrdU negative (asterisks) or BrdU positive but SIFamide negative (arrow). **(J–L)** In the MC, several somata of local interneurons express SIFamide-like immunoreactivity (green), and some cells outside of the proliferation zone (PZ) are BrdU positive, indicating that they were born 7 months earlier and have moved toward the periphery of the soma cluster since then. (J) Lower magnification reveals partial overlap between the populations of SIFamide- and BrdU-positive cells. (K, L) Higher magnification reveals that some cells (arrowheads) are double labeled by BrdU (red nucleus) and anti-SIFamide (green cytoplasm), demonstrating that these cells born 7 months earlier have undergone neuronal maturation. Note that most SIFamide-positive cells are BrdU-negative (asterisk) and that most BrdU-positive cells are SIFamide negative. **(M–Q)** Identification of putative NBs at the basis of adult neurogenesis in the LC and the MC of *P. argus*. In vivo labeling with 3 injections of BrdU for approximately 2 days was combined with immunostaining for pH3 and nuclear labeling. (M, N) In the LC and the MC, the multiple injections of BrdU caused labeling of one extra nucleus (white asterisks) that was not detectable after single BrdU injections. This nucleus is located outside of the proliferation zone (PZ), is larger than the BrdU-positive nuclei in the PZ, and has hence been identified as putative adult NB. Anti-pH3 not only labels mitotic nuclei in the PZ (black asterisks) but also fibrous material surrounding the putative adult NB and forming a tube-like connection to the neighboring PZ (arrowheads). (O–Q) Nuclear labeling in the LC reveals that the structure surrounding the adult NB (asterisks) is a dense accumulation of small cells that is connected to some arterioles (arrows). Likely this clump of specialized cells represents a stem cell niche for the adult NB.

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only some species, such as the brachyuran crab *L. emarginata*, stop molting and growing after a terminal molt (Sullivan and Beltz 2005a). As the other sensory pathways, the visual and the central olfactory pathways, usually develop and differentiate in the embryo; in crayfish, the central olfactory pathway differentiates in postembryonic stages (Helluy et al. 1993; Harzsch et al. 1998; Harzsch, Benton, et al. 1999; Harzsch, Miller, et al. 1999; Benton and Beltz 2002). In embryonic development, neurogenesis is widespread in the brain and in other parts of the CNS and, as in insects, is based on the activity of typical neuroblasts (NBs) (Hartenstein et al. 1987; Truman and Bate 1988; Scholtz 1992; Zacharias et al. 1993; Harzsch and Dawirs 1994; Doe and Skeath 1996; Harzsch et al. 1998; Harzsch, Benton, et al. 1999; Harzsch 2001, 2003). NBs are large precursor cells that in a series of asymmetric cell divisions renew themselves and give rise to midsized ganglion mother cells (GMCs). These in turn divide once symmetrically and give rise to 2 primordial neurons (a possible exception to this has been reported for the embryonic lobster brain by Benton and Beltz 2002). Throughout larval development, neurogenesis in the brain and in other parts of the CNS continues in a similar way (Harzsch and Dawirs 1994, 1996a, 1996b; Harzsch et al. 1998). But with the metamorphosis to the first juvenile stage, neurogenesis ceases in most brain areas except the central olfactory pathway (Harzsch and Dawirs 1996b) and the optic lobes (Harzsch and Dawirs 1996a). Thus, from the first juveniles throughout the juvenile and the entire adult life, neurogenesis continues in these 2 areas of the brain. However, neurogenesis in the central olfactory pathway differs between early juveniles and adults in the number of proliferating cells, the temporal dynamics of cell divisions, and its regulation by intrinsic factors (Schmidt 2001; Goergen et al. 2002; Song et al. 2005; Song C-K, Johnstone LM, Schmidt M, Derby CD, Edwards DH, unpublished results), indicating that the neurogenic processes in these stages need to be analyzed separately before general conclusions can be derived. A further confounding effect is that in many species, the transition between juvenile and the sexually mature adult stage is not accompanied by distinct morphological changes (Conan et al. 2001). Thus, in many studies on adult neurogenesis, late juvenile animals have also been included, under the premise that they do not differ from adults in gross morphology or the specific and quantifiable aspects of neurogenesis. This review will focus on neurogenesis in the central olfactory pathway of adult (including late juvenile) decapod crustaceans.

Occurrence and localization of adult neurogenesis in the central olfactory pathway

Adult neurogenesis in the central olfactory pathway of decapod crustaceans was demonstrated by counting neuronal somata or axons (Schmidt 1997; Sandeman et al. 1998) and by *in vivo* labeling with the S-phase marker 5-bromo-2′-deoxyuridine (BrdU) (Schmidt 1997, 2001, 2007; Sandeman et al. 1998; Harzsch, Miller, et al. 1999; Schmidt and Harzsch 1999; Beltz et al. 2001; Hansen and Schmidt 2001, 2004; Sullivan and Beltz 2005a, 2005b; Sullivan et al. 2007). Counting neurons showed in the shore crab *C. maenas* and in the Australian crayfish *C. destructor* that the number of PNs increases substantially (by more than 100%) and in a linear fashion throughout the life span of the animals (Schmidt 1997; Sandeman et al. 1998). In *C. destructor*, LNs also increase in number, although less substantially (Sandeman et al. 1998). By in vivo labeling with BrdU in these and other species (as detailed above), the presence of small groups of proliferating cells in soma clusters of the central olfactory pathway was consistently demonstrated (Figure 1A,B). Constant across all tested species is the presence of groups of proliferating cells in the LCs comprised by the somata of deutocerebral PNs (Figure 2A,C,E,G) (Schmidt 1997, 2001; Sandeman et al. 1998; Harzsch, Miller, et al. 1999; Schmidt and Harzsch 1999; Beltz et al. 2001; Hansen and Schmidt 2001, 2004; Sullivan and Beltz 2005a, 2005b). In all species of Reptantia (spiny and clawed lobsters, crayfish, hermit crabs, and true crabs), which are evolutionary more advanced than the Natantia (shrimps) (Sandeman et al. 1993), groups of proliferating cells are also present in other soma clusters of the central olfactory pathway: in the MCs comprised by somata of deutocerebral LNs (Figure 2B), in the HBCs (or cluster As) comprised by protocerebral LNs (Figure 2F), or in both (Schmidt 1997, 2001, 2007; Sandeman et al. 1998; Harzsch, Miller, et al. 1999; Schmidt and Harzsch 1999; Beltz et al. 2001; Hansen and Schmidt 2001, 2004; Sullivan and Beltz 2005a, 2005b; Sullivan et al. 2007). A hypothesis linking the occurrence of proliferation in these soma clusters to the presence or absence of a well-developed AL (Schmidt and Harzsch 1999) has recently been invalidated by the discovery of proliferation in the MC and the HBC of a brachyuran crab species (*L. emarginata*) (Sullivan and Beltz 2005a).

Except in protocerebral cluster A of *L. emarginata* (Sullivan and Beltz 2005a), only one group of proliferating cells is present in a soma cluster, its position within the soma cluster is invariant, and the number of cells it comprises is very small (ca. 20–100) (Schmidt 1997, 2001, 2007; Harzsch, Miller, et al. 1999; Schmidt and Harzsch 1999; Hansen and Schmidt 2001, 2004; Sullivan and Beltz 2005a, 2005b) in comparison to the total number of neuronal somata (10 000–200 000) in that cluster (Schmidt and Ache 1996b; Schmidt 1997; Sandeman et al. 1998). Furthermore, this small area, which is always located at the inner surface of the respective soma cluster close to the neuropil innervated by the neurons contained in the cluster, can also be identified in tissue sections stained with cytological or immunocytochemical techniques (Figure 2A–D). Cells in this area differ in several characteristic ways (i.e., smaller size and less spherical shape, darker staining with methylene blue, and lack of neuropeptide-like immunoreactivity) from the neuronal somata in the surround (Schmidt 1997, 2001, 2007; Sullivan and Beltz 2005a, 2005b; Sullivan et al. 2007), and at each time point some of them are in mitosis

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(Schmidt 1997, 2001, 2007). Based on these findings, the area containing the group of BrdU-positive cells in each of the soma clusters showing proliferation has been identified as a morphologically specialized proliferation zone. In this organizational aspect, adult neurogenesis in the central olfactory pathway of decapod crustaceans parallels adult neurogenesis in the mammalian brain, which is also based on proliferation taking place in morphologically specialized germinal areas, the subventricular zone (generation of new local interneurons of the olfactory bulb: Luskin 1993; Doetsch et al. 1999), and the subgranular zone (generation of new granule cells of the hippocampus: Seri et al. 2001, 2004; Christie and Cameron 2006). However, in contrast to decapod crustaceans where proliferation is concentrated in very small areas, the germinal areas in the mammalian brain are extended layers in which proliferation is dispersed.

Fate of proliferating cells: long-term survival, apoptosis, and neuronal maturation

BrdU pulse-chase experiments with short (days) and long (months) survival times were used to study the fate of the proliferating cells in the soma clusters of the central olfactory pathway (Schmidt 1997, 2001; Harzsch, Miller, et al. 1999; Beltz et al. 2001; Sullivan and Beltz 2005a, 2005b). Counting BrdU-positive cells after different survival times showed that their number doubles within about 2 weeks and then remains constant, indicating that all cells that are labeled (and thus are in the S-phase of their cell cycle at the time of BrdU injection) divide once but do not undergo further rounds of cell divisions (Schmidt 1997, 2001). Even after many months (up to 14), numerous BrdU-positive cells remain detectable in the respective soma clusters, suggesting that most if not all the daughter cells arising in this round of cell divisions survive for a very long time, possibly the entire life span of the animal (Figure 2H,J) (Schmidt 2001; Sullivan and Beltz 2005a, 2005b).

Evidence for apoptotic cell death has been obtained in some studies of the fate of the proliferating cells (Sandeman et al. 1998; Harzsch, Miller, et al. 1999; Schmidt 2001). Two conflicting distribution patterns of apoptotic cells have been reported, with the results dependent on the method of identification: TUNEL labeling versus observation of condensed, pyknotic nuclei in tissue sections stained with methylene blue or labeled with the nuclear marker Hoechst 33258. TUNEL-positive cells were found in moderate numbers and were scattered throughout the entire soma cluster without any preferred location (Sandeman et al. 1998; Harzsch, Miller, et al. 1999). In contrast, condensed, pyknotic nuclei were found in only very small numbers (1–6) and were restricted in their distribution to the immediate vicinity of the proliferation zone of the respective soma cluster (Figure 2D, inset) (Schmidt 2001). In the LC of *H. americanus*, nuclei darkly stained by methylene blue were equated with nuclei of TUNEL-positive cells (Harzsch, Miller, et al. 1999), but in a recent morphological study, nuclei with similar appearance in the LC of *P. argus* have been identified as nuclei of a novel type of glial cell (Schmidt 2007). It thus seems likely that as in many other systems (Charriaut-Marlangue and Ben-Ari 1995; Stähelin et al. 1998; Tateyama et al. 1998), TUNEL labeling performed in the central olfactory pathway of decapod crustaceans produced false-positive results that need to be critically reevaluated. The occurrence of some condensed, pyknotic nuclei in the immediate vicinity of proliferation zones suggests that only very few of the newly generated cells undergo programed cell death shortly after their birth. A similar mode of interplay between proliferation and programed cell death appears to occur in both areas of adult neurogenesis in the mammalian CNS (Morshead and Van der Kooy 1992; Gage et al. 1998).

BrdU pulse-chase experiments with longer survival times have revealed that newly generated cells leave the proliferation zones within about 4 weeks and that thereafter they slowly and continuously move further toward the periphery of the respective soma cluster (Schmidt 1997, 2001; Harzsch, Miller, et al. 1999; Beltz et al. 2001; Sullivan and Beltz 2005a, 2005b). After about 3 months, they reach areas of the respective soma clusters, which almost exclusively comprising somata of morphologically mature neurons (Figure 2H,J) (Schmidt 2001; Sullivan and Beltz 2005a, 2005b). Maturity of the cells in these regions is indicated by the expression of specific neuropeptides (MC: FMRFamide, substance P, orcokinin, allatostatin; LC: SIFamide) and in the case of the LC of *P. argus* also by receiving innervation from descending giant neurons with FMRFamide- and substance P-like immunoreactivity (Sandeman et al. 1990; Schmidt and Ache 1994, 1997; Yasuda et al. 2004; Yasuda-Kamatani and Yasuda 2006; Sullivan et al. 2007). Neuronal differentiation and maturation of cells born in the MC and the LC has been demonstrated directly by combining BrdU pulse-chase experiments with neuron-specific labeling (Schmidt 2001; Sullivan and Beltz 2005a, 2005b; Sullivan et al. 2007). In one approach, expression of the above mentioned neuropeptides was examined in double-labeling experiments. These experiments revealed that newly generated cells in the MC of *P. argus* and in the LC of *C. destructor* and *L. emarginata* mature into neurons within 3–6 months and that populations of neurons in the MC expressing different neuropeptides mature at different times (Figure 2I,K,L) (Schmidt 2001; Sullivan and Beltz 2005a, 2005b; Sullivan et al. 2007). In another approach, backfills of PNs with biocytin or fluorescent dextrans were performed in double-labeling experiments (Schmidt and Demuth 1998; Sullivan and Beltz 2005b). These experiments confirmed neuronal maturation in the time frame of several months and demonstrated that OL-PNs and AL-PNs are generated in the LC of adult *C. destructor* (Sullivan and Beltz 2005b). No evidence currently exists for the differentiation of other cell types, in particular glial cells, from BrdU-positive cells in the proliferation zones, although glial markers have been used in double-labeling

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experiments (Sullivan and Beltz 2005b; Schmidt 2007; Sullivan et al. 2007).

Origin of proliferating cells: identification of putative neuronal stem cells

Neurogenesis in the brain of adult decapod crustaceans differs substantially from neurogenesis in the CNS of embryos and larvae with respect to the size and distribution of nuclei labeled after a single application of BrdU, suggesting that adult neurogenesis might be based on other neuronal stem cells than embryonic and larval neurogenesis. As detailed above, neurogenesis in the embryonic and larval CNS of decapod crustaceans is based on the proliferative activity of typical NBs. Single applications of BrdU have consistently labeled small groups of closely associated nuclei with substantially different sizes, which comprise one large nucleus of a NB, several smaller nuclei of GMCs, and some even smaller nuclei of primordial neurons (Scholtz 1992; Harzsch and Dawirs 1994, 1996a; Harzsch et al. 1998; Harzsch, Benton, et al. 1999; Harzsch 2001, 2003).

In contrast to the situation in embryos and larvae, a single injection of BrdU in adults usually labels one compact group of nuclei within the proliferation zones (as detailed above), and these nuclei do not substantially vary in size (Figure 2C–G) (Schmidt 2001, 2007). Based on the fact that the BrdU-positive cells in the proliferation zone are the immediate precursors of neurons and give rise to them by one round of symmetrical cell divisions, they have been interpreted as equivalent to GMCs in the CNS of embryonic insects and decapod crustaceans (Schmidt 2001). This leaves open the question how these cells are generated or replenished, made even more pressing by the fact that after survival times of more than 4 weeks no BrdU-positive cells are retained in the proliferation zones (Figure 2H,J) (Schmidt 2001; Sullivan and Beltz 2005b). Recently, the problem of identifying the neuronal stem cells at the basis of adult neurogenesis in the olfactory midbrain has been tackled in *P. argus* and *P. clarkii* with 2 different approaches. These yielded different results and have led to contrasting conclusions about the nature of the putative neuronal stem cells and their cell cycle properties.

In *P. argus*, multiple BrdU injection over a time period of about 2 days were used in an attempt to label putative neuronal stem cells that are not labeled by single injections of BrdU because their mitoses may be timed differently (Schmidt 2002b, 2003, 2007). In these experiments, single or very few additional nuclei in an invariant position close to the proliferation zones of LC and MC were consistently labeled by BrdU (Figure 2M,N). These ''extra'' BrdU-positive nuclei were significantly larger than nuclei of neurons or BrdU-positive cells in the proliferation zones. Because the defining morphological feature of NBs in embryonic neurogenesis in the Tetraconata (insects and crustaceans) is being larger than their progeny, it has been proposed that these large extra BrdU-positive nuclei represent ''putative adult NBs.'' Only one putative adult NB is present in each soma cluster. It is enclosed in a highly organized clump of small cells (Figure 2O–Q), which connects to the proliferation zone by a tube-like structure expressing phosphorylated histone 3-like immunoreactivity (Figure 2M,N). Cells in the clumps share morphological and immunocytochemical characteristics with newly identified putative glial cells in the soma clusters but clearly differ from another type of glial cells that surround the neuropils of the olfactory midbrain and were previously identified based on the expression of histamine- and glutamine synthetase–like (GS-like) immunoreactivity (Orona et al. 1990; Linser et al. 1997).

In adult *P. clarkii*, a single 6-h long exposure to BrdU labeled not only groups of nuclei in the proliferations zones of the LC and MC but also a string of isolated nuclei extending out from each of the soma clusters toward the ventral surface of the AL (Sullivan et al. 2005, 2007). The strings of nuclei are embedded in strands of material expressing GS-like immunoreactivity, and the strands extending from the LC and MC originate in a clump of small somata that also express GS-like immunoreactivity and hence have been identified as glial cells. Only prolonged exposure to BrdU (10–14 days) labeled few nuclei located within the clumps of small cells, and based on these results these cells have been interpreted as very slowly dividing neuronal stem cells of glial identity (Sullivan et al. 2005, 2007). In juvenile *P. clarkii*, a single 24-h long exposure to BrdU yielded very similar results with respect to the identification of strings of BrdU-positive nuclei extending from the proliferations zones of the LC and MC, and labeling with anti-GS and anti-tubulin also led to the identification of a clump of small cells at the juncture of the strings originating in the LC and MC (Song et al. 2005; Song C-K, Johnstone LM, Schmidt M, Derby CD, Edwards DH, unpublished results).

The results obtained in adult *P. argus* have been interpreted as evidence that single adult NBs are the neuronal stem cells at the basis of adult neurogenesis in the olfactory midbrain. Based on the available evidence, it has been proposed that these putative adult NBs undergo rapid asymmetric divisions in which they self-renew and generate one daughter cell—a GMC—that translocates to the adjacent proliferation zone where it undergoes one symmetrical cell division that lasts much longer (in the range of several days) and generates 2 primordial neurons. Thus, adult neurogenesis in the brain of *P. argus* appears to have a very similar cellular basis as neurogenesis in the CNS of embryos and larvae and might therefore be viewed as a continuation of these early neurogenic events. Similarly, neurogenesis in the mushroom bodies of adult insects also appears to be a scaled down direct continuation of neurogenesis in larval and pupal development of the mushroom bodies. Two or more large NBs located in different aspects of the Kenyon cell soma cluster (depending on the species) give rise to smaller GMCs by asymmetrical divisions, and these in turn divide once symmetrically to generate 2 neurons (Cayre et al. 1994, 1996; Gu et al. 1999; Dufour

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and Gadenne 2006). In contrast, the results obtained in adult *P. clarkii* have been interpreted as evidence that specialized cells of glial identity are the neuronal stem cells. These cells supposedly divide extremely slowly, and their progeny migrate along a strand of glial fibers to the respective proliferation zones of the LC and MC where they divide further. This scenario is highly reminiscent of the cellular processes underlying adult neurogenesis in the subventricular zone/olfactory bulb of mammals, where the neuronal stem cells are of glial identity, divide very slowly, and produce progeny that migrate along a path established by glial cells (the rostral migratory stream) to their final destination, the olfactory bulb (Doetsch et al. 1999; Doetsch 2003; Garcia et al. 2004). Obviously, the data sets obtained for *P. argus* and *P. clarkii* are incongruent as they lead to mutually exclusive conclusions about the cellular identity of the neuronal stem cells and their cell cycle properties. Because both species are closely related (both belong to the order Decapoda within the class Malacostraca) and share many features of adult neurogenesis (occurrence of proliferation zones in similar positions in the same soma clusters, generation of the same types of neurons with similar time frames for neuronal maturation), it seems highly unlikely that adult neurogenesis in these species is indeed based on different types of neuronal stem cells. This strongly suggests that the current data sets are incomplete and that more experimental work is needed to resolve the current controversy.

In spite of these differences, the results of studies on *P. argus* and *P. clarkii* conform in one important aspect, the close association of the presumptive neuronal stem cells with a clump of small cells of glial identity (Schmidt 2002b, 2003, 2007; Sullivan et al. 2005, 2007; Song et al. 2005; Song C-K, Johnstone LM, Schmidt M, Derby CD, Edwards DH, unpublished results). The clumps of cells can be interpreted as structures that provide a specific microenvironment for maintaining the proliferative and self-renewal capacity of the putative neuronal stem cells. Thus, they seem to constitute ''stem cell niches'' as they have recently also been identified in the neurogenic regions of the adult mammalian brain, the subventricular and the subgranular zone (Palmer et al. 2000; Alvarez-Buylla and Lim 2004; Seri et al. 2004; Ma et al. 2005). It seems likely that in the context of adult neurogenesis in the decapod crustacean brain, such stem cell niches are required to maintain the proliferative and self-renewal potential of the putative adult neuronal stem cells for the entire life time of the animals, which is on the scale of years to decades (Farmer 1973; Belchier et al. 1998; Sheehy et al. 1999; Bluhm and Brey 2001). This is in stark contrast to the situation in the embryonic and larval CNS of insects and crustaceans, where NBs are active for only some days or weeks and die by apoptosis after a predetermined number of cell divisions (about 50) (Bate 1976; Hartenstein et al. 1987; Bello et al. 2003) and in the mushroom bodies of adult insects where the NBs remain active for only several days into adulthood (Gu et al. 1999). In both cases, similar stem cell niches for the NBs have not been reported to date.

Regulation of adult neurogenesis by internal and external factors

Adult neurogenesis in the mammalian brain is modulated by diverse internal factors such as hormonal status, age, physical activity, and presence and activity of ORNs, but also by a host of external influences such as stress, dominance hierarchy formation, learning situations, and environmental richness (Ming and Song 2005). Adult neurogenesis in the mushroom bodies of insects is controlled by hormonal status (Cayre et al. 1994, 1997) and by environmental richness (Lomassese et al. 2000).

Adult neurogenesis in the central olfactory pathway of decapod crustaceans is also modulated by diverse factors. One factor is the presence of ORNs. Unilateral ablation of the olfactory sensilla (or the appendage bearing them, the lateral flagellum) in *C. destructor* caused a substantial decrease in the number of PNs and LNs on the affected side accompanied by a higher number of apoptotic cells (see critique of method above) than on the intact side (Sandeman et al. 1988). The effect of unilateral ablation of the lateral flagellum on the number of BrdU-positive cells in the proliferation zones of LC and HBC of *C. maenas* was also examined (Hansen and Schmidt 2001). By manipulating the relative times of ablation and BrdU injection, it was shown that unilateral ablation of the lateral flagellum negatively affects the generation of new cells from precursors (putative NBs) but also the survival of newly generated, postmitotic cells in both soma clusters. The effect of the unilateral ablations on the survival of postmitotic cells was lateralized, but the proliferation of neuronal precursors was impaired bilaterally. The latter finding and the influence of ORN ablation on proliferation in the HBC cannot be explained by a direct influence of the presence or activity of ORN afferents on proliferation among cells that eventually will integrate into the neuropil innervated by these afferents. Instead, these observations suggest that some effects of ORN ablation on neurogenesis in the central olfactory pathway are indirect and therefore likely mediated by hormones or other far ranging signals.

The effect of age, season, and captivity on adult neurogenesis in LC and HBC was examined in *C. maenas* (Hansen and Schmidt 2004). All these factors differentially modulated neurogenesis in both soma clusters. With increasing age (measured as carapace width), the number of proliferating cells significantly decreased in the LC but remained almost constant in the HBC. The number of proliferating cells in both soma clusters showed substantial seasonal variations with 2 peaks (spring and late summer) in the LC and one peak (early summer) in the HBC. The number of proliferating cells in both soma clusters was significantly lower during autumn and winter than during spring and summer. Keeping the animals in captivity for 3 months under constant and impoverished conditions caused an overall decrease in the number of proliferating cells in both soma clusters but did not eliminate the seasonal changes in either of them. These data suggest

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that proliferation in LC and HBC of *C. maenas* is regulated independently and that it is controlled by an endogenous circannual rhythm.

Social interactions, enriched or impoverished living conditions, and circadian rhythm affect neurogenesis in the central olfactory pathway of crayfish, by altering proliferation and/or survival of neuronal precursor cells (Sandeman R and Sandeman D 2000; Goergen et al. 2002; Song et al. 2005, Song C-K, Johnstone LM, Schmidt M, Derby CD, Edwards DH, unpublished results). However, these studies were exclusively focused on very young juveniles, in which neurogenesis in the central olfactory pathway appears to have very different temporal properties than in adults (see above), and therefore they are not further reviewed here.

Why have adult neurogenesis in olfactory and not in other sensory pathways of the decapod crustacean CNS?

The specific organization of the numerous peripheral and central sensory pathways and their unique mode of indeterminate growth throughout adulthood make decapod crustaceans an attractive animal model to address the question why adult neurogenesis in the CNS is restricted to certain areas—here the olfactory and visual pathways. This question addresses a basic issue of adult neurogenesis because it does not have the simple answer that these 2 are the only pathways in the decapod crustacean CNS that receive an ever-increasing sensory input during adult growth. To the contrary, input to all primary sensory neuropils of the decapod crustacean CNS appears to increase in much the same way during adult growth.

Sensory input to the CNS of decapod crustaceans, as in insects, is provided by compound eyes mediating vision (Bernhards 1921; Meinertzhagen 1991) and by small cuticular organs called sensilla mediating all other sensory modalities (Derby 1982; Schmidt and Gnatzy 1984; Hallberg and Hansson 1999). Compound eyes comprise numerous small units called ommatidia, each of which contains 8 photoreceptor neurons projecting their axons directly into the underlying lamina, the first neuropil of the optic lobe (Nässel 1976; Meinertzhagen 1991). Each sensillum houses from one to many primary sensory neurons projecting their axon directly into a target neuropil of the CNS (Derby 1982; Schmidt and Gnatzy 1984; Hallberg and Hansson 1999). Sensory neurons are unimodal and most of them are either mechano- or chemosensory (Derby 1982; Schmidt and Gnatzy 1989; Voigt and Atema 1992; Cate and Derby 2002), with only one reported exception (Hatt 1986). Sensilla are differentiated into many morphologically distinct types, each of which houses a specific complement of sensory neurons mediating one or several modalities. In decapod crustaceans, aesthetascs are the only sensillum type containing exclusively chemosensory neurons (Laverack and Ardill 1965, Spencer and Linberg 1986, Grünert and Ache 1988, Gleeson et al. 1996), few sensillum types exclusively contain mechanosensory neurons (Kouyama and Shimozawa 1982; Schmidt 1990), and most sensillum types are bimodal containing mechano- and chemosensory neurons (Altner et al. 1983; Schmidt and Gnatzy 1984; Cate and Derby 2001, 2002). Sensilla of various types occur in high numbers (in many cases thousands or tens of thousands) on almost all parts of the body and on all of its appendages, and their distribution is highly patterned. This is particularly obvious in the flagella (distal-most segments) of the 2 pairs of antennae of the decapod crustacean head, which consist of a series of pseudosegments called annuli each bearing a specific arrangement of various types of sensilla (Laverack 1964; Derby 1982; Sandeman 1989; Cate and Derby 2001).

From the first juvenile stage, in which a morphology closely resembling that of adults is obtained (Felder et al. 1985), decapod crustaceans grow in size by successive molts, and in most species this growth continues throughout the entire adult life (Hartnoll 1982). An enormous increase in body size can be obtained, often in the range of 1000-fold and in some species up to 10 000-fold (Helluy et al. 1995). The ever-increasing surface area accommodates more and more sensilla. Clearly, all types of sensilla increase in number with increasing body size (Thomas 1970; Letourneau 1976; Laverack 1987, 1988; Schmitz 1993), including the aesthetascs and other all sensilla on the lateral flagellum of the antennule (Tiews 1954; Sandeman et al. 1998; Steullet, Cate, and Derby 2000; Harrison, Cate, Steullet, et al. 2001). The compound eyes also increase in size with increasing body size due to both enlargement of the existing ommatidia and addition of new ommatidia (Bernhards 1921; Meyer-Rochow et al. 1990). Consequently, the number of sensory afferents projecting into the CNS increases substantially with body size, regardless of the sensory modality.

In light of this scenario, the answer to the initial question why adult neurogenesis is restricted to certain brain areas must lie in specific properties of these brain areas themselves which can only or at least better be achieved by adult neurogenesis and not by other forms of neuronal plasticity, such as synaptogenesis or sprouting of new arborizations. Thus, the initial question can be phrased more specifically as: Are there fundamental differences in information processing among the different sensory pathways of the decapod crustacean CNS that indicate a particular benefit of adult neurogenesis for the olfactory and the visual but not for the other sensory pathways? I believe that this is indeed the case and hypothesize that the fundamentally different ''topographic logic'' of information processing in the olfactory, visual, and other sensory pathways (which are primarily chemo- and mechanosensory pathways) is causal for the occurrence or the lack of adult neurogenesis. The topographic logic of information processing and its consequences for neurogenesis will be elaborated separately for 3 types of sensory pathways.

The visual pathway represents the most transparent case. The axons of the photoreceptor cells project in a topographically ordered way into vertical columns of the lamina

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constituting a correct 1:1 retinotopic map of the retinal ommatidia onto the lamina columns (Nässel 1976; Strausfeld and Nässel 1981; Meinertzhagen 1991). The neuropils of the optic lobes—the medulla externa and medulla interna—are also organized into vertical columns, and the retinotopic mapping seems to be preserved in their connections with each other and with the lamina. The columns in all 3 optic neuropils are mainly constituted by local columnar neurons, and these are horizontally connected by amacrine cells and centripetal large-field PNs (Strausfeld and Nässel 1981). When new ommatidia are added to the compound eyes during adult growth (Bernhards 1921; Meyer-Rochow et al. 1990), correct processing of their input requires the generation of new corresponding columns in at least the lamina and likely also in the medulla externa and medulla interna. Because these columns mainly consist of designated local neurons, these must be newly generated. The horizontally connecting neurons, on the other hand, could just grow new arborizations to adequately integrate novel columns into their information processing. Thus, this hypothesis gives rise to the testable prediction that the neurons formed by adult neurogenesis in the optic lobes of decapod crustaceans (Schmidt 1997; Sullivan and Beltz 2005b) likely are local columnar neurons and not amacrine cells and large-field PNs.

All primary sensory neuropils of the decapod crustacean CNS except the lamina of the optic lobe and the OL receive input from chemo- and mechanosensory sensilla on the body surface or the appendages. The tegumentary neuropils located in the tritocerebrum of the brain receive input from sensilla on the head (Tautz 1987a), the antenna II neuropils (AnN) receive input from sensilla on the second antennae (Tautz and Müller-Tautz 1983; Sandeman 1989), the LANs receive input from the nonaesthetasc sensilla on the first antennae (antennules) (Schmidt et al. 1992; Schmidt and Ache

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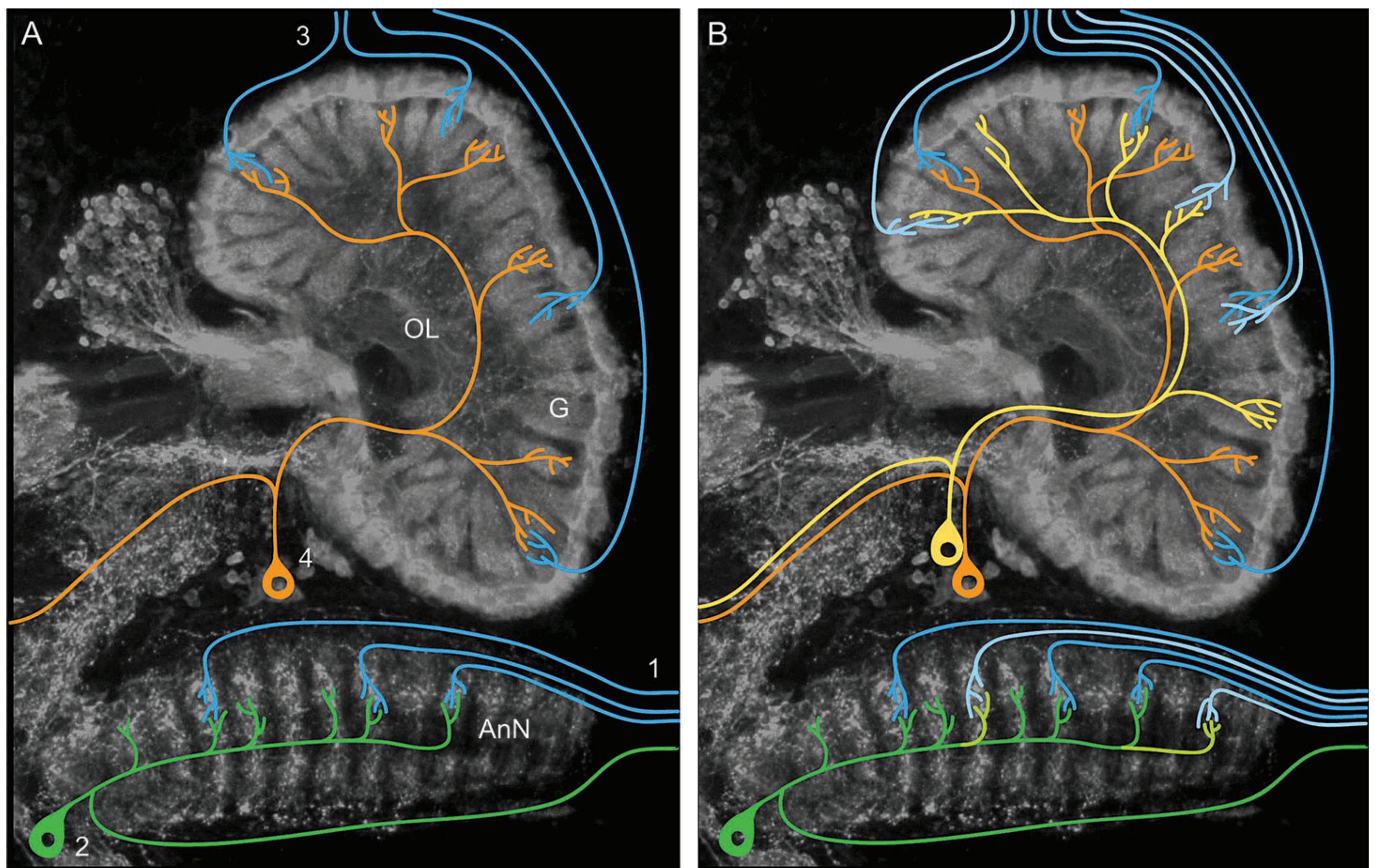

Figure 3 Half-schematic demonstration of the hypothesis that differences in the topographic logic of information processing are the root cause for the selective occurrence of adult neurogenesis in the OL and its lack in all chemo- and mechanosensory neuropils. Simplified neurons are drawn on a template representing a section through the brain of the hermit crab *Pagurus bernhardus* stained with an antibody against the neuropeptide FMRFamide. The FMRFamide-like immunoreactivity highlights the distinctly different spatial organization of the OL consisting of numerous columnar glomeruli (G) and the antennal neuropil (AnN) comprised of a stack of discs. The following neuronal types are depicted: chemo- and mechanosensory afferents from the antenna (1, blue), antennal motoneurons (2, green), olfactory afferents from the aesthetascs on the lateral flagellum of the antennule (3, blue), and ascending PNs of the OL (4, orange). **(A)** Basic layout of neuronal arborizations in both neuropils. **(B)** Putative changes in neuronal wiring during adult growth due to the addition of sensory neurons in the periphery (light blue), the extension of new arborizations from existing neurons in the brain (light green), and the addition of new brain neurons by adult neurogenesis (light orange). See Discussion for further details.

1996a), the neuromeres of the subesophageal ganglion receive input from sensilla on the corresponding mouthparts, the thoracic ganglia receive input from sensilla on the corresponding pereiopods (chelae or walking legs), the abdominal ganglia receive input from sensilla on the corresponding pleopods, and the terminal ganglion receives input from the tailfan (telson and uropods) (Sandeman 1982; Antonsen and Edwards 2003). The unpaired median antennular neuropil located in the deutocerebrum of the brain represents a special case in that it receives mainly mechanosensory input from the statocysts, sense organs dedicated to gravity perception (Sandeman and Okajima 1973; Schmidt et al. 1992). Especially, the neuropils that receive input from the long annulated antennae (AnN and LAN) have an elongated shape and are obviously structured by a striation perpendicular to their long axis, generating the appearance of a stack of discs (Tautz and Müller-Tautz 1983; Schmidt and Ache 1996a). This structure suggests a somatotopic representation of the afferent sensory input in the corresponding neuropils (Figure 3A). This assumption, although not supported by direct evidence from sensilla backfills (which to date have not been possible in decapod crustaceans), is strengthened by the observation that the length of these neuropils corresponds to the length of the represented appendages (Helm 1928; Sandeman et al. 1993) and by the structure of the afferent terminals, which are either restricted to certain discs or are elongated with regularly spaced perpendicular side branches into several adjacent discs (Tautz and Müller-Tautz 1983; Schmidt et al. 1992; Schmidt and Ache 1996a). Information processing in these neuropils relies heavily on ascending or descending PNs and on motoneurons, whereas local interneurons seem to be scarce or absent (Tautz and Müller-Tautz 1983; Tautz 1987a; Schmidt and Ache 1993, 1996a). PNs and motoneurons show branching patterns that correspond to the striated structure of the neuropils in having repetitive perpendicular side branches interdigitating with the afferent terminals. Together, these structural features strongly suggest that the chemo- and mechanosensory input in these neuropils is processed somatotopically, which would allow identification of the position of a stimulus on the elongated flagella of the antennae. Behavioral data support the notion that decapod crustaceans are able to retrieve positional information upon chemo- and mechanosensory stimulation of the antennae (Maynard and Dingle 1963; Zeil et al. 1985; Tautz 1987b; Wilkens et al. 1996). How exactly chemosensory input is integrated in these neuropils is currently unknown, but the low number of putative chemoreceptor neurons (5–10) in the sensilla providing input to these neuropils (Cate and Derby 2001, 2002) together with the stereotyped, reflexive behaviors elicited by chemosensory stimulation (Schmidt and Derby 2005) suggests that these pathways are hard-wired and work according to the labeled-lines model of chemosensory information processing (Erickson 2000). Further support for the somatotopic mode of information processing in primary chemo- and mechanosensory neuropils is provided by backfills of sensilla on the legs of insects, by which it was shown directly that chemo- and mechanosensory neurons form parallel and overlapping somatotopic maps in the corresponding leg ganglia (Newland et al. 2000). In addition, behavioral experiments show that insects retrieve positional information from chemo- and mechanosensory stimulation of diverse body parts (Newland and Burrows 1997; Rogers and Newland 2000; Gaaboub et al. 2005). In neuropils organized for this somatotopic mode of information processing, additional sensory input arising during adult (or juvenile) growth can correctly be integrated into the preexisting circuitry by extending new side branches from PNs and motoneurons at the appropriate locations (Figure 3B). The generation of new neurons of these types would not add novel capacities for extracting positional information, and I propose that this is the underlying reason for the lack of adult neurogenesis among them. Indeed, the central and peripheral arbors of motoneurons innervating leg muscles of *H. americanus* increase enormously in size and density during juvenile and adult growth, but their number remains constant (Govind 1984; Laverack 1987, 1988; Purves 1988; Govind and Pearce 1989).

How is the topographic logic of information processing different in the OL? The OL of decapod crustaceans is organized into glomeruli receiving the afferent axons of the ORNs (Sandeman and Luff 1973; Sandeman and Denburg 1976; Blaustein et al. 1988; Mellon and Munger 1990; Schmidt and Ache 1992; Sandeman DC and Sandeman RE 1994), as is the case for primary olfactory neuropils in other taxa, especially the antennal lobe of insects and the olfactory bulb of vertebrates (Ache and Young 2005). Further parallels are the prevalent uniglomerular arborizations of the afferents (Schmidt and Ache 1992; Mellon and Alones 1993), their nontopographical projections to the OL (Sandeman and Denburg 1976; Mellon and Munger 1990), the participation of large numbers of local interneurons in information processing (Schmidt and Ache 1996b; Sandeman et al. 1998), and the multi- to totiglomerular arborization patterns of the local interneurons (Mellon and Alones 1995; Schmidt and Ache 1996b; Wachowiak et al. 1997). A stark contrast between decapod crustaceans on the one hand and insects and mammals on the other is the different arborization patterns of the PNs. In decapod crustaceans, PNs are multiglomerular with dense arbors in 3–10 glomeruli in different parts of the OL and sparse branching in many other glomeruli (Wachowiak and Ache 1994; Schmidt and Ache 1996b). On the other hand, PNs of insects and mammals usually have uniglomerular arborizations (Dryer and Graziadei 1994; Schachtner et al. 2005). Generally, each ORN of mammals and insects expresses one odorant receptor type out of a much larger repertoire (ca. 100 in insects and up to 1000 in mammals), and the axons of ORNs expressing the same odorant receptor type converge in one or a pair of glomeruli with stereotypic positions (Mombaerts et al. 1996; Couto et al. 2005). Thus, the projections of the ORNs onto the glomeruli of the primary olfactory neuropil constitute an "odotopic" map in

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which neither the position of the ORNs in the periphery nor their physical correlation to each other (by being packaged in the same sensilla in insects) is preserved (Couto et al. 2005). Stimulation with different odorants causes distinct spatial and temporal patterns of glomerular activation (Wang et al. 2003; Wachowiak et al. 2004), and the activity of local interneurons mediating lateral interactions between glomeruli seems to be critical for encoding these patterns and thus odor quality (Stopfer et al. 1997). Although odorant receptor genes have not yet been identified in decapod crustaceans and the projections of afferents of different classes of ORNs are currently unknown, the high structural similarity of their ORNs and primary olfactory neuropils with those of insects and mammals strongly suggests that also in the OL of decapod crustaceans the topographical logic of information processing is based on an odotopic map (Figure 3A). Consequently, odorant quality most likely is encoded in glomerular activation patterns. In contrast to the situation in the antennal lobe of insects and the olfactory bulb of mammals, the PNs of the OL of decapod crustaceans are multiglomerular and thus could themselves be able to integrate particular patterns of glomerular activation and hence encode odor quality. Because exposure to novel olfactory stimuli will generate novel glomerular activation patterns, new PNs with dense arborizations in novel combinations of spatially unrelated glomeruli would be uniquely suitable to encode such novel olfactory stimuli (Figure 3B). In contrast to the situation in the somatotopic mode of processing mechano- and chemosensory information, the sprouting of new branches to other glomeruli by preexisting PNs would severely alter the logic of information coding in the odotopic mode because different glomerular activation patterns code for distinctly different odor qualities (Wang et al. 2003; Wachowiak et al. 2004). Thus, it seems that adult decapod crustaceans generate new PNs as a means to continuously adapt to changing and novel odorants. The very long life expectancy of many adult decapod crustaceans in the range of years or even decades (Farmer 1973; Belchier et al. 1998; Sheehy et al. 1999; Bluhm and Brey 2001), and a lifestyle that often includes extended migrations and change of habitats (Herrnkind and McLean 1971; Boles and Lohmann 2003), would make such adaptability to changing odorant environments particularly advantageous. The very long life expectancy is also prerequisite for the newly generated neurons to become physiologically relevant because their maturation lasts several weeks to months (Schmidt 2001; Sullivan and Beltz 2005b).

The hypothesis that new PNs of adult decapod crustaceans are generated to allow adaptation to novel odor stimuli has some interesting collaterals. 1) Most importantly, it implies that the increase in the number of ORN afferents during adult growth is not the root cause for the generation of new PNs. This notion is strongly supported by several observations. Firstly, the generation of new ORNs is discontinuous because it is coupled to molting (Sandeman et al. 1998; Steullet, Cate, and Derby 2000; Harrison, Cate, and Swanson, et al. 2001), whereas the generation of new PNs is a continuous process and therefore mainly occurring during the long intermolt phase (Schmidt 1997, 2001). Secondly, in *L. emarginata*, a decapod crustacean species that stops molting and growing as an adult after which it does not produce any new ORNs, the generation of new PNs continues in the remaining adult life (Sullivan and Beltz 2005a). Thirdly, the number of OL glomeruli does not change during adult life (Helluy et al. 1996), indicating that all newly generated ORN afferents project into these preexisting glomeruli all which are already innervated by numerous PNs. The increase in the number of afferents could adequately be accommodated simply by enlarging the glomeruli and the arbors of the preexisting PNs that they contain. Glomerular growth has indeed been reported for the OL of adult decapod crustaceans (Helluy et al. 1996). Very likely it provides a mechanism to increase odor sensitivity; however, a more differentiated coding of odor quality cannot be achieved in this way. 2) In the OL, not only PNs but also small LNs have multiglomerular arborizations, whereas large LNs have totiglomerular arborizations (Schachtner et al. 2005). Therefore, according to the scheme developed above, also new small LNs could provide novel connectivity between glomeruli and thus improve odor quality coding. This might be the reason that in some species of decapod crustaceans also new LNs are continuously generated during adult life albeit in much lower numbers than PNs (Sandeman et al. 1998; Schmidt and Harzsch 1999; Schmidt 2001; Sullivan and Beltz 2005a, Sullivan et al. 2007). Why neurogenesis of LNs, if it is beneficial for odor quality coding, occurs only in some but not in all species is currently unclear. An answer possibly lies in the multitude of morphologically and immunocytochemically distinct types of small LNs (Schachtner et al. 2005), as it is currently unknown which of these types occur in different species of decapod crustaceans and more importantly which types are newly generated by adult neurogenesis, if it occurs. 3) Why does adult neurogenesis not also occur in the antennal lobe of insects—especially because it implements odotopic information processing? The answer may lie in the comparatively short life expectancy of adult insects, which is in the range of weeks to some months, and in their generally specialized lifestyle which is associated with a more constant odor environment. The short life expectancy makes it unlikely that new neurons generated during adulthood could actually take effect, given that in decapod crustaceans and mammals neurons generated by adult neurogenesis need several weeks to months to mature (Schmidt 2001; Ming and Song 2005; Sullivan and Beltz 2005b). The more constant odor environment would mean that changes in odor coding achieved by adult neurogenesis during the adult phase would have little or no adaptive advantage. In this context, it would be of great interest to study adult neurogenesis in the brain of the queens of social insects (e.g., honey bees), as individuals of this cast live much longer than their worker siblings but spend most of their

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adult life in the extremely constant odor environment of the hive or nest. 4) A striking difference between adult neurogenesis in the OL of decapod crustaceans and the olfactory bulb of mammals is the types of neurons generated. In decapod crustaceans, primarily PNs are generated; LNs are generated only in some of the tested species, and in all these species they are always generated in much lower number than PNs (see above). In contrast, in mammals, exclusively LNs (mostly periglomerular neurons, but also some granule cells) are generated (Luskin 1993; Hack et al. 2005; Kohwi et al. 2005). This difference may be due to the fundamentally different dendritic branching pattern of the PNs of both taxa. While the dendritic arbors of PNs are multiglomerular in decapod crustaceans (see above), they are uniglomerular in PNs of mammals (mitral cells and tufted cells) (Dryer and Graziadei 1994). Thus, according to the arguments developed above, generation of new PNs in the olfactory bulb of mammals would not generate novel connectivity between olfactory glomeruli, whereas generation of new LNs, which form lateral connections between glomeruli (Aungst et al. 2003), would. An interesting group of vertebrates to test the validity of this conclusion are teleost fish because most or at least some of their PNs (mitral and tufted cells) have multiglomerular dendritic arbors (Kosaka and Hama 1982; Oka 1983; Fuller et al. 2006). Occurrence of adult neurogenesis in the olfactory bulb of teleost fish has been documented repeatedly (Byrd and Brunjes 2002; Zupanc et al. 2005; Adolf et al. 2006; Grandel et al. 2006), but the types of neurons that are generated have not been identified conclusively. Recent immunocytochemical evidence indicates that mainly GABAergic and dopaminergic local interneurons are generated but the production of new PNs has not been excluded (Adolf et al. 2006).

Concluding remarks

Neurogenesis persists in the adult brains of diverse animals, making it a common phenomenon that is not correlated with phylogeny or overall complexity of their brains. However, adult neurogenesis is restricted to only one or very few brain regions, making it a rare event with respect to the numerous neuronal populations comprising the brain. Decapod crustaceans, which show adult neurogenesis in 2 brain areas—the optic lobes and the central olfactory pathway—are useful animal models because they provide insight into why adult neurogenesis is restricted to certain brain regions. Their usefulness arises from the fact that most of them show indeterminate growth as adults, which results in a continuous increase in the number of sensory afferents of all modalities providing input to numerous primary sensory neuropils of the brain and other parts of the CNS. Obviously, the increase in the number of sensory afferents per se cannot be the root cause for adult neurogenesis, as this is lacking in most of the primary sensory neuropils, the ones that process mechano- and chemosensory information from all parts of the body and its appendages. I hypothesize that instead fundamental differences in the topographic logic of information processing in the 3 types of primary sensory neuropils of decapod crustaceans (visual, olfactory, and mechano- and chemosensory) account for the occurrence or the lack of adult neurogenesis in them. In the primary visual neuropil, which has a retinotopic representation of the periphery, adult neurogenesis is required to produce more local interneurons forming new cartridges accommodating an ever-increasing number of ommatidia (=pixels). Thus, it is essential for an increase in light sensitivity and, more importantly, for an increase in the spatial resolution of vision. The numerous primary mechano- and chemosensory neuropils, which have a somatotopic representation of the periphery, can have their ever-increasing sensory input adequately integrated by simply increasing the size and density of the dendritic arbors of preexisting PNs and motoneurons, which seem to carry out most of the information processing. Thus, an increase in sensitivity and spatial resolution can be achieved without the generation of new neurons. In the primary olfactory neuropil, which very likely has an odotopic representation of the periphery, new neurons provide a means to interconnect novel combinations of spatially unrelated input channels (glomeruli), whose simultaneous activation by specific odorants is at the root of odor coding. Thus, adult neurogenesis would allow encoding of the quality of novel odor stimuli without losing the resolution for other odor qualities, whereas an increase in the arborization of preexisting neurons would be sufficient to increase sensitivity. Animals with a long life expectancy and an active lifestyle are likely to encounter novel odor stimuli throughout their life, and this could explain why adult neurogenesis of olfactory interneurons is widespread among vertebrates and decapod crustaceans but not insects.

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