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Adult Neurogenesis: Lessons from Crayfish and the Elephant in the Room

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Key Words

Adult neural stem cells · Immune system · Self-renewal · Hemocytes · Bone marrow · Blood-brain barrier

Abstract

The 1st-generation neural precursors in the crustacean brain are functionally analogous to neural stem cells in mammals. Their slow cycling, migration of their progeny, and differentiation of their descendants into neurons over several weeks are features of the neural precursor lineage in crayfish that also characterize adult neurogenesis in mammals. However, the 1st-generation precursors in crayfish do not self-renew, contrasting with conventional wisdom that proposes the long-term self-renewal of adult neural stem cells. Nevertheless, the crayfish neurogenic niche, which contains a total of 200–300 cells, is never exhausted and neurons continue to be produced in the brain throughout the animal's life. The pool of neural precursors in the niche therefore cannot be a closed system, and must be replenished from an extrinsic source. Our in vitro and in vivo data show that cells originating in the innate immune system (but not other cell types) are attracted to and incorporated into the neurogenic niche, and that they express a niche-specific marker, glutamine synthetase. Further, labeled hemocytes that undergo adoptive transfer to recipient crayfish generate cells in neuronal clusters in the olfactory pathway of the adult brain. These hemocyte descendants express appropriate neurotransmitters and project to target areas typical of neurons in these regions. These studies indicate that under natural conditions, the immune system provides neural precursors supporting adult neurogenesis in the crayfish brain, challenging the canonical view that ectodermal tissues generating the embryonic nervous system are the sole source of neurons in the adult brain. However, these are not the first studies that directly implicate the immune system as a source of neural precursor cells. Several types of data in mammals, including adoptive transfers of bone marrow or stem cells as well as the presence of fetal microchimerism, suggest that there must be a population of cells that are able to access the brain and generate new neurons in these species.

Introduction

Adult neurogenesis – the production and integration of new neurons into circuits in the nervous system of mature organisms – is a phenomenon found in both vertebrate and invertebrate species. In mammals, the hippocampus and olfactory bulb are the best-known regions that incorporate new neurons throughout life. Many types of evidence indicate that adult neurogenesis is important in learning and memory mechanisms [Lemaire et

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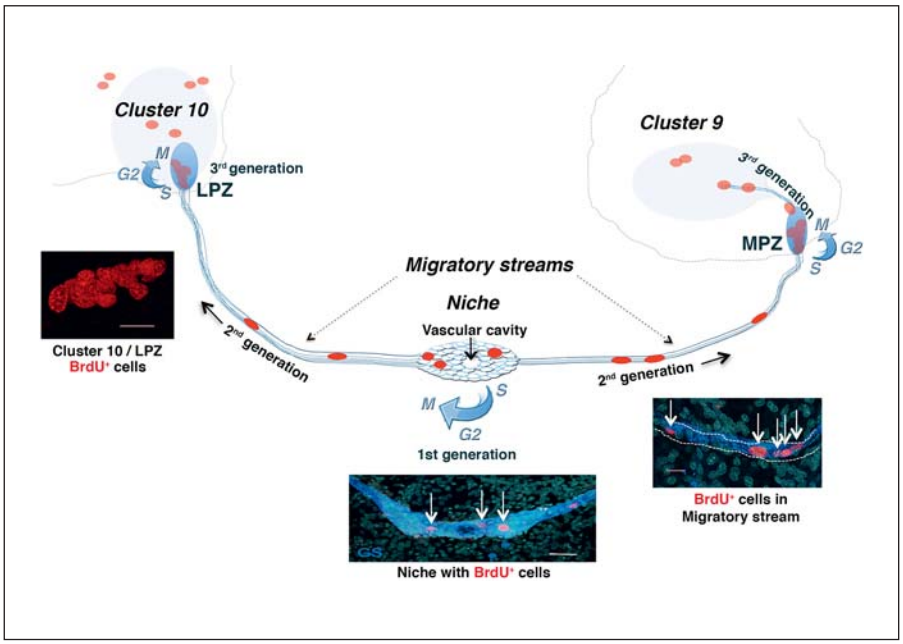

Fig. 1. The cellular machinery producing adult-born neurons in the crayfish brain consists of a neurogenic niche containing 1st-generation precursors, migratory streams containing 2nd-generation precursors, and two clusters of olfactory interneurons (clusters 9 and 10). The spatial relationships between these regions are indicated in the schematic drawing. First-generation precursors in a neurogenic niche divide symmetrically, both daughters migrating to proliferation zones (MPZ, LPZ) in clusters 9 and 10, where they divide at least once more before progeny differentiate into neurons. Solid black arrows next to the streams indicate the direction of migration; curved, thick blue arrows indicate locations of cell divisions. The niche is connected to the blood system via a central 'vascular cavity' in the niche, illustrated in the diagram and appearing as a black region in the center of the niche image; propidium iodide (cyan) labeling of niche cell nuclei is also shown. Glutamine synthetase immunoreactivity (blue) highlights the niche and streams in these images. Examples of BrdU labeling (red) are shown in each region of the niche-stream-cluster system. Scale bars: niche, 50 μm; cluster, 10 μm; streams, 20 μm. Adapted from Benton et al. [2014].

al., 2000; Shors, 2004; Kee et al., 2007] as well as in neuropsychiatric disorders [Eisch et al., 2008]. However, there are still considerable gaps in our knowledge. One of these concerns the functional relationships between adult neurogenesis and the immune system. The immune system plays critical roles in combating disease, in neural physiology and development, and in regulating neural stem cell (NSC) niches in both vertebrate and invertebrate species [Perry et al., 1993, 2010; Gonzalez-Perez et al., 2010, 2012; Paolicelli et al., 2011; Benton et al., 2014]. However, while immunological mechanisms involved in adult neurogenesis are of potential broad importance to our understanding of how the normal brain is maintained throughout life, the contributions of the immune system to adult neurogenesis remain largely unknown.

In spite of much progress in understanding the cell biology of adult neurogenesis in mammals, particularly in rodents, the locations and complexity of the neurogenic regions that generate neurons in the hippocampus and olfactory bulb have posed challenges in unraveling basic mechanisms and the interrelationships between the different precursor cell generations. However, it is well established that many cellular, molecular and regulatory aspects of neurogenesis – from embryonic development to adult life – are highly conserved even in distantly related animal groups [e.g. Sullivan et al., 2007; Beltz et al., 2011; Brand and Livesey, 2011; Hartenstein and Stollewerk, 2015]. Taking advantage of this relationship, invertebrates with their comparably less complex nervous systems can often provide experimental opportunities not afforded by vertebrate organisms. The relative simplicity of the neuron-producing system in the brains of adult crayfish, for instance, makes this group of crustaceans a favorable model for examining mechanisms underlying adult neurogenesis and contributions from the immune system. Thus, lessons from the crustaceans may well lead the way to insights in mammals, including humans.

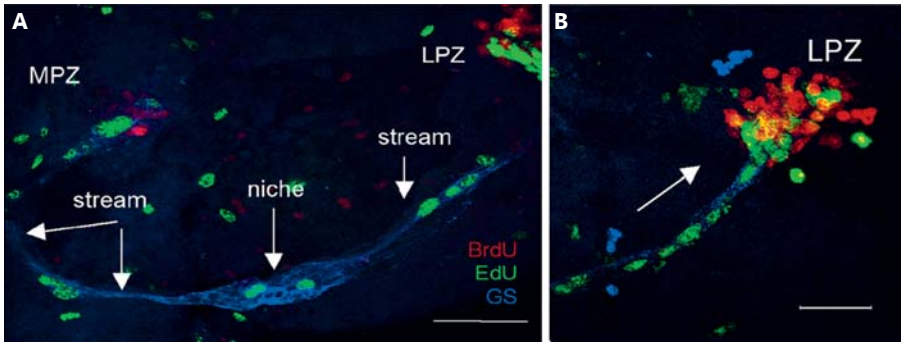

Fig. 2. Double-nucleoside labeling demonstrates that 1st-generation neural precursors are not self-renewing. Crayfish were incubated for 24 h in BrdU (red), then maintained in pond water for 7 days. Six hours before sacrifice, they were treated with EdU (green). Brains also were labeled immunocytochemically for glutamine synthetase (blue) to reveal the niche and streams. **A** $BrdU^+$ cells were found only in the proliferation zones (MPZ and LPZ), not in the niche. Only EdU labels S phase cells in the niche, showing that niche cell divisions are not self-renewing and that migration is unidirectional, away from the niche. **B** Higher magnification image of the LPZ. Arrow: direction of migration. Scale bars: 100 μm (**A**); 50 μm (**B**). Adapted from Benton et al. [2011].

Adult Neurogenesis in Crayfish

New neurons in the adult crayfish brain are incorporated into two cell clusters (9 and 10; terminology according to [Sandeman et al., 1992]) that contain local and projection interneurons in the olfactory pathway [Schmidt and Harzch, 1999]. The precursor cell lineage producing these neurons has been identified (fig. 1). The 1st-generation precursors are located in bilaterally paired niches that are found on the ventral surface of the brain, in close association with the vasculature [Sullivan et al., 2007]. Niche cells, including the 1st-generation neural precursors, are distinctive because they label for glutamine synthetase. The 1st-generation precursors divide in the niche, producing 2nd-generation precursors that migrate along fibrous streams formed by the processes of bipolar niche cells, until they reach proliferation zones in cell cluster 9 or 10 [Benton et al., 2011, 2013, 2014] (fig. 1). There, they divide at least once more before differentiating into neurons [Sullivan and Beltz, 2005; Kim et al., 2014]. Because of the spatial separation of the generations in the precursor lineage, each generation can be examined independently, allowing quantitative assessments of individual precursor cell types [e.g. Zhang et al., 2011].

First-Generation Neural Precursors Are Not Self-Renewing

Unlike embryonic NSCs in crustaceans, the 1st-generation precursors residing in the niche of the adult brain divide morphologically symmetrically [Zhang et al., 2009]. Pulse-chase experiments using the nucleoside markers 5-bromo-2′-deoxyuridine (BrdU) and 5-ethynyl-2′-deoxyuridine (EdU), which are incorporated into newly replicated DNA during the S phase, have demonstrated that the 1st-generation neural precursors in the crayfish niche are not self-renewing [Benton et al., 2011]. BrdU (presented first) is not retained in the 1st-generation precursors in the niche, and is instead replaced with EdU, which was presented 3–7 days later (fig. 2). Rapid division and dilution of the BrdU label cannot account for these findings because the cell cycle of the 1st-generation precursors in the niche is long (~48 h) [Benton et al., 2011]. In the absence of cell death, both daughters of a division must therefore exit the niche, a conclusion that is supported by the observation of pairs of cells migrating together along the initial segments of the streams. In spite of this lack of self-renewal, the 1st-generation precursors are never depleted and neurons continue to be generated throughout the animals' long lives. However, the total cell population of the niche is only 200–300 cells, depending on animal size [Zhang et al., 2009]. We have therefore proposed that the pool of neural precursors must be replenished from an extrinsic source, i.e. a cell-producing region outside of the niche.

Mind the Gap

The existence of such an extrinsic source was confirmed by single-pulse experiments in which adult crayfish were injected with BrdU, and labeling in niche cells was documented daily for 1 week and then at intervals until 21 days after injection. We hypothesized that 1st-generation precursors in the niche that are in the S phase

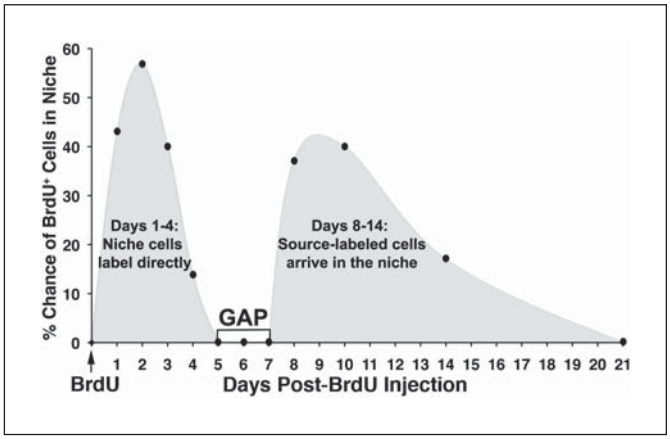

Fig. 3. Actively proliferating ($BrdU^+$) cells in the neurogenic niche have a bimodal temporal distribution. $BrdU^+$ cells were quantified in the niches of crayfish that were sacrificed daily for 1 week after BrdU injection, and at intervals thereafter for 21 days. The probability of observing $BrdU^+$ cells in the niche was then plotted for each of the sampling days. $BrdU^+$ cells were observed in the niche on days 1–4 following injection. On days 5–7, the niches contained no $BrdU^+$ cells. However, between days 8 and 14 after injection, $BrdU^+$ cells were once again observed in the niche. Adapted from Benton et al. [2014].

during BrdU exposure would be labeled, as would cells in potential non-niche tissues that provide additional neural precursors. However, it was anticipated that cells labeled in extrinsic sources that are destined to become neural precursors will arrive at the niche after a delay (due to the time required to complete their lineages, be released, and travel to the niche). Indeed, 1st-generation precursors in the niche label reliably with BrdU on days 1–4 following exposure (fig. 3), as shown previously [Sullivan et al., 2007]. On days 5–7 ('the gap'), no $BrdU^+$ cells are observed in the niche because the ~2-day BrdU clearing time [Benton et al., 2011] is over and the originally labeled cells have divided and exited the niche via the streams. However, on days 8–14 following injection, intensely labeled $BrdU^+$ cells are once again observed in the niche. As BrdU is no longer available for renewed labeling of neural precursors in the niche, our interpretation is that the 'second wave' of labeled cells incorporated BrdU in their source tissue and then traveled to the niche.

In order to understand mechanisms underlying the production of adult-born neurons in crustaceans, identifying the source(s) of the 1st-generation neural precursors is obviously of prime interest. In vitro and in vivo studies demonstrate that cells extracted from the hemolymph, but not cells from other tested tissues, are attracted to and integrated into the niche [Benton et al., 2011, 2014]. Further, by 7–10 days after adoptive transfer of EdU^+ hemocytes from EdU-injected donor crayfish into nontreated recipient crayfish, EdU^+ cells are found in the migratory streams, and by 7 weeks they are found in brain cell clusters 9 and 10 of the recipients. Here, the EdU^+ cells express neurotransmitters appropriate for olfactory neurons found in those clusters (fig. 4) [Benton et al., 2014], and their processes project to appropriate target regions [Platto, 2015]. Adoptive transfers of specific hemocyte types (separated with Percoll gradients) have shown that cells resembling semigranular hemocytes, but no other circulating cells, integrate into the niche and generate cells in clusters 9 and 10 [Cockey et al., 2015].

The Innate Immune System of Crayfish

Unlike mammals, invertebrates lack an adaptive immune system and oxygen-carrying erythrocytes, but do have a well-developed innate immune system. In crayfish, oxygen is transported in the hemolymph by direct binding to the molecule hemocyanin. Thus, while bone marrow is a source of both erythrocytes and immune cells in mammals, the primary function of the hematopoietic system in crayfish is innate immunity.

Freshwater crayfish (e.g. *Pacifasticus leniusculus* and *Procambarus clarkii*) live for up to 20 years and possess distinct hematopoietic tissues that produce hemocytes throughout the animal's lifetime. Innate immunity has been intensively studied in *P. leniusculus*, including one of the most important mechanisms, the prophenoloxidase-activating (proPO) system, which leads to the activation of the Toll pathway and production of antimicrobial peptides [Noonin et al., 2012; Jearaphunt et al., 2014]. Astakines, crustacean cytokines belonging to the prokineticin family, are synthesized in hematopoietic tissues and are required for the proliferation and release of hemocytes into the circulation [Söderhäll et al., 2005; Lin et al., 2010; Lin and Söderhäll, 2011]. By stimulating the expression of crustacean hematopoietic factor, astakine 1 (AST1) specifically promotes the release of semigranular cells from hematopoietic tissues [Lin et al., 2011]. Interestingly, serotonin appears to regulate the expression of AST1 (Söderhäll and Benton, unpublished results), potentially explaining the increased rate of adult neurogenesis following exposure of crayfish to serotonin [Sandeman et al., 2009; Zhang et al., 2011]. In vertebrates, prokineticins are involved in diverse processes, including angiogenesis and neurogenesis; adult neurogenesis in the olfactory bulb is dependent on prokineticin 2 sig-

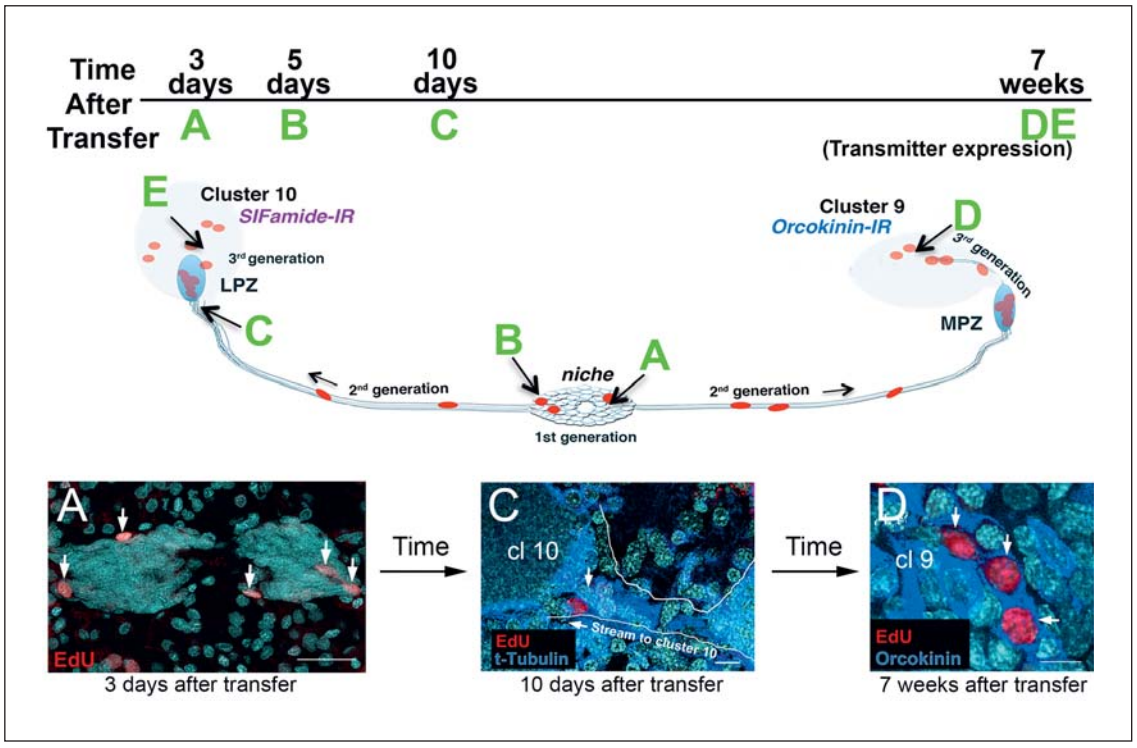

Fig. 4. EdU$^+$ cells were observed in the niche, streams, and in clusters 9 and 10 of recipient crayfish after adoptive transfer of EdU$^+$ hemocytes from donor animals. **A**, **D** *P. clarkii.* **C** *P. leniusculus.* The schematic diagrams illustrate (top) the experimental timeline for each sample (time after transfer, **A–E**), and (bottom) the locations of the EdU$^+$ cells that were observed. **A** Three days after adoptive transfer of hemocytes, EdU$^+$ cells (red) were observed in the niche. Hoechst 33342 (cyan) labeled nucleic acids in all niche cells as well as nearby cells. **C** Ten days after hemocyte transfer, cells were observed in the distal ends of the streams, near clusters 9 and 10 (arrow). Immunoreactivity for tyrosinated tubulin (blue) highlights the niche and streams, which are also outlined. **D** Seven weeks after adoptive transfer of hemocytes from donor to recipient crayfish, EdU$^+$ cells (red) were observed in cell clusters 9 and 10. Some of these cells in cluster 9 express orcokinin, a peptide transmitter used by many cluster 9 cells. Examples of cell labeling at **B** (5 days, in the niche) and at **E** (7 weeks, in cluster 10) are not shown. Scale bars: 40 μm (**A**); 10 μm (**C**); 10 μm (**D**). Adapted from Benton et al. [2014].

naling [Ng et al., 2005]. Together, these studies point to an evolutionary ancient role for the prokineticin superfamily as one link between the immune and nervous systems.

The Immune System: A Source of Neural Precursors

We have further tested the relationship between the immune/hematopoietic system and the neurogenic niche by manipulating the number of circulating hemocytes and subsequently assessing changes in the neural precursor cell lineage of the brain. When the total hemocyte count was either increased by injection of recombinant AST1 into crayfish or decreased by partial ablation of hematopoietic tissues, total hemocyte count and cell number in the neurogenic niche were tightly correlated (R^2 = 0.91). The reduction in niche cell number resulting from hematopoietic tissue ablation can be 'rescued' by injection of recombinant AST1 prior to sacrifice, also suggesting that semigranular hemocytes are the neural precursors. Further, when recombinant AST1 is injected into crayfish at the same time as BrdU and the BrdU single-pulse experiment (fig. 3) repeated as described above, the shape of the resulting peaks is altered in two ways: (1) the peak on days 1–4 has two maxima (presumably because astakine promotes the proliferation of 1st-generation precursors in the niche [Benton et al., 2014], leading to a delayed second maximum on day 4), and (2) a new peak of cells fills the gap, demonstrating that the premature cytokine-stimulated release of semigranular cells from hematopoietic tissues is correlated with an earlier appearance of source-labeled cells in the niche [Cockey et al., 2015]. Our data therefore indicate that 1st-generation

neural precursors in crayfish are derived from semigranular hemocytes, and that the innate immune system controls the production of adult-born neurons in the crayfish brain.

Future Directions

Transgenic Crayfish

In order to further probe adult neurogenesis in the crustacean brain, we are generating transgenic crayfish that will provide a source of green fluorescent protein (GFP)-expressing hemocytes and other cell types for adoptive transfer. Our previous work using donor EdU^+ hemocytes [Benton et al., 2014] demonstrated the attraction of hemocytes to the niche in vivo, and their progression through the neural precursor lineage to produce cells in clusters 9 and 10. In contrast to BrdU- and EdU-labeling methods, GFP expression can be observed without the use of fixatives, allowing electrophysiological and other approaches in live preparations. In addition, GFP fills the entire cell, and thus GFP^+ cells in clusters 9 and 10 can be visualized in whole mount brains to confirm the projections of these cells to the olfactory and accessory lobes [Platto, 2015]. Therefore, this transgenic approach will allow further characterization of the cells in clusters 9 and 10 that are derived from adoptive transfer of hemocytes.

Next-Generation Sequencing

One challenge of studying adult neurogenesis in a nonmodel invertebrate is the relatively small number of readily available markers for cells and tissues of interest. We have therefore started transcriptome studies and set out to identify key genes known to be involved in (adult) neurogenesis or expressed in the immune cells of other arthropod models (often with homologues serving similar functions in mammals [Brand and Livesay, 2011]). Among the molecules of prime interest are factors that have been shown to play important roles in the adult hematopoietic-neural lineage of crayfish (e.g. astakines and their receptors, crustacean hematopoietic factor, or serotonin receptors) [Lin et al., 2010, 2011; Lin and Söderhäll, 2011], as well as genes acting early during neurogenesis and remaining in part active during neural differentiation (e.g. homologues of the *Achaete-Scute* Complex or the *SoxB* transcription factors). NSC factors involved in cell maintenance or promoting self-renewal, such as *SoxB*, *Asense*, *Deadpan* (*Hes*-related), or *Snail* family transcription factors [Doe, 2008; Southall and Brand, 2009; Luo et al., 2015] are also of interest, as are genes encoding proteins that have been shown to counteract self-renewal in NSCs, and instead promote neural differentiation in their progeny, such as *Prospero* [Stergiopoulos et al., 2015] or *Brain tumor* and *Numb* [Doe, 2008; Southall and Brand, 2009].

Beyond this candidate gene approach, however, comparative transcriptome analyses will provide a considerably broader perspective. They will allow identification of unique and shared transcripts in the relevant cell types/tissues, and assessment of significant differences in relative gene expression levels. Based on this approach, we expect to gain considerable new insights into the molecular fabric of the different generations of cell types within the hematopoietic-neural cell lineage of crayfish. This will help us to identify and eventually unravel the genetic mechanisms governing the transition of cells originating in the immune system to neural precursors and neurons.

The Elephant in the Room

The still widely accepted model of adult neurogenesis in mammals suggests that neurons are generated by NSCs that undergo long-term self-renewal, and that a lifetime supply of NSCs resides in the brain. This is in sharp contrast to our findings in crayfish, in which the neural precursor cells do not self-renew and are replenished by cells originating from the immune system. However, the proposal that cells from the immune system play a central role in adult neurogenesis is not a novel idea. There is substantial data suggesting that cells from outside the nervous system contribute to the neuronal population in the adult brain, and that stem cells from bone marrow and blood cells have an intriguing ability to generate cells with neural properties [e.g. Sanchez-Ramos et al., 2000; Mezey et al., 2003; Munoz-Elias et al., 2004; Chan et al., 2012; Lee et al., 2015]. This has been a controversial topic due to the belief in the long-term self-renewal of stem cells and the rarity of transdetermination/transdifferentiation events.

The existing literature includes many studies suggesting that the immune system in mammals may be capable of providing neural precursors [reviewed in Beltz et al., 2015]. Among these are reports of adoptive transfer experiments in rodents and humans, which have shown that cells extracted from bone marrow and mesenchymal stem cells are capable of differentiating not only into a variety of mesodermal cell types, but also into astrocytes and neurons in the adult brain [Brazelton et al., 2000; Mezey et al., 2000, 2003]. Thus it was concluded that

'… bone marrow can make brain …' [Cogle et al., 2004]. However, this interpretation was tempered by later work suggesting that the neuronal phenotype may have been induced by secreted factors or resulted from cell fusion, rather than from natural physiological mechanisms [Maltman et al., 2011; Mezey, 2011]. Further, unlike adoptive transfers in crayfish, these types of studies in mammals require immune suppression so that the adoptively transferred cells are not attacked by the recipient's immune system; irradiation and other forms of immune suppression also are thought to make the blood-brain barrier (BBB) less restrictive. Thus, in the existing studies, when adoptively transferred cells reached the brain, did they arrive there by natural mechanisms, or because the integrity of the BBB had been compromised?

It may also be significant that male microchimerism of fetal origin has been documented in the healthy mouse and human brain, in both neuron- and astrocyte-like cell types [Tan et al., 2005; Zeng et al., 2010; Chan et al., 2012]. In mice, fetal cells (also called pregnancy-associated progenitor cells) are integrated into the adult maternal brain and persist for up to 7 months postpartum, and cell fusion with maternal neurons has been ruled out. Further, data indicate that these cells '… undergo a molecular and morphological maturation program similar to that observed during adult neurogenesis …' [Zeng et al., 2010]. These findings suggest that a population of neural precursor cells is indeed able to cross the BBB under natural conditions.

While there are many unanswered questions about the relationship between bone marrow and brain, as well as the role of NSCs and adult neurogenesis in disease mechanisms, many beneficial clinical therapies are based on an infusion of bone marrow, without a complete understanding of why these treatments are helpful. For example, following adoptive transfer of human mesenchymal stem cells, rats show remarkable recovery from stroke [Li et al., 2002]. In addition, for boys with cerebral X-linked adrenoleukodystrophy, a bone marrow or cord blood transplant early in the course of the disease can stop the progression of the disease [Shapiro et al., 2000]. Likewise, bone marrow-derived mesenchymal stem cell therapies hold promise for treatment of Parkinson's disease and multiple system atrophy, both of which are neurodegenerative disorders [Lee and Park, 2009]. It is generally thought that the trophic influences of bone marrow cells account for many of the clinical benefits of these transplants [Li et al., 2002; Kan et al., 2011], but a direct contribution of NSCs derived from bone marrow has not been ruled out.

Long-Term Self-Renewal of Adult NSCs?

The long-term self-renewal of NSCs, a cornerstone of the 'stem cell' definition that has been unchallenged until recently, precludes the necessity for an ongoing source of neural precursors. However, several lines of recent evidence now suggest that the concept of long-term self-renewal – while a clear characteristic of NSCs in vitro and of hematopoietic stem cells in vivo [Kiel et al., 2007] – may not apply to NSCs in vivo. Direct tests of the self-renewal capacity of NSCs in the subgranular and subventricular zones generating hippocampal and olfactory bulb neurons, respectively, suggest that these cells are relatively short-lived once activated, and undergo a limited number of divisions [Encinas et al., 2011; Calzolari et al., 2015; Fuentealba et al., 2015].

Using BrdU pulse-labeling in a reporter mouse line, Encinas and colleagues [2011] followed the proliferation and fate of neural precursors in the dentate gyrus. The first-generation NSCs [quiescent neural progenitors (QNPs)] and their offspring [amplifying neural progenitors (ANPs)] were identified, in addition to neuroblasts and immature neurons. A dramatic depletion especially of QNPs but also of ANPs in the hippocampus between 3 weeks and 2 years of life was documented, suggesting that the loss of NSCs may explain the age-related decline in adult neurogenesis that has been observed in mice. If putative NSCs might themselves be replenished from the immune system as we hypothesize, then the age-related decline would presumably correspond to a reduction in the production or availability of the immune precursors. In addition, a pulse-chase double-nucleoside labeling approach similar to that used in crayfish (fig. 2) showed that QNPs undergo approximately three rapid divisions to generate ANPs, and then withdraw from the NSC pool by differentiating into astrocytes, rather than resuming a quiescent phase. Based on these data, Encinas and colleagues [2011] propose a 'disposable stem cell' (or single-use) model in the hippocampus. It should be noted, however, that one clonal analysis study indicated the capacity for some adult stem cell self-renewal in the subgranular zone [Bonaguidi et al., 2011].

In the subventricular zone, clonal analysis indicates that the adult NSCs may be exhausted after division [Fuentealba et al., 2015] as proposed for the hippocampus [Encinas et al., 2011]. This also is in agreement with the work of Calzolari and colleagues [2015], which tracked the progeny of single NSCs in the subventricular zone in vivo and found limited self-renewal and exhaustion of clones within a few weeks. Therefore, the weight of current data does not appear to support the long-term self-

renewal of adult NSCs in the subgranular and subventricular zones. The debate continues.

The final answer to the self-renewal question may have critical implications. Even if limited self-renewal of NSCs is ultimately discovered, the numbers of neurons generated per day in the hippocampus and olfactory bulb may pose a quandary in terms of the supply of NSCs, particularly in long-lived animals. In humans, for example, roughly 700 neurons are added to each hippocampus every day [Spalding et al., 2013], a number that does not take into account the likely death of a high proportion of the cells that are initially produced. It is interesting to ask whether the human brain can physically store an adequate supply of NSCs – in the absence of long-term self-renewal – to provide adult-born neurons over our long lifespan.

The BBB and More Questions

An important feature of all NSC niches in both invertebrate and vertebrate species is a rich network of blood vessels in and around these sites [Tavazoie et al., 2008; Ming and Song, 2011]. In crayfish, the neurogenic niches are situated directly on a blood vessel on the ventral surface of the brain [Sullivan et al., 2007], and are isolated from all other tissues, with the exception of hemolymph [Chaves da Silva et al., 2012]. No neural innervation of the niche has thus far been discovered, but it is clear that there is an exchange with the hemolymph via a vascular cavity located centrally in the niche and the hemolymph that bathes the niche [Sullivan et al., 2007]. In vitro experiments suggest that hemocytes invading the niche that become the 1st-generation neural precursors gain access via this vascular connection or from the blood sinus around the brain in which the niche lies. While crayfish do have a BBB [Otopalik et al., 2012; Schmidt, 2016], the flow of at least some cell types between the vasculature and the niche does not appear to be restricted.

In mammals, the BBB is a dynamic multicellular structure that separates the central nervous system (CNS) from the vascular circulation. It is now recognized that the BBB is a highly selective filter and avenue of communication that is critical in regulating ionic fluxes, access to oxygen and nutrients, and protecting the CNS from pathogens. The endothelial cells that form the core of the BBB are unique from endothelial cells in other tissues by virtue of their tight junctions, which limit movement between cells, and a variety of receptor and transporter proteins involved in transcellular transport. While most cell types are excluded from passage across the BBB, both leukocytes and dendritic cells are known to traffic across this barrier, at least in certain inflammatory and disease-related situations. Experiments directly testing the interactions between mesenchymal stem cells and endothelial cells have yielded inconsistent results regarding the potential for transmigration of mesenchymal stem cells across the BBB under healthy circumstances [reviewed in Liu et al., 2013]. A 'leaky' BBB is characteristic of many neurological diseases such as multiple sclerosis [Greenwood et al., 2011; Sagar et al., 2012], Alzheimer's disease, and amyotrophic lateral sclerosis [Keaney and Campbell, 2015], but it is not clear whether these changes are a cause or consequence of the disease. However, it is known that the healthy BBB is 'tighter' and highly selective.

Yet, recent studies have discovered that even in the absence of disease, the restrictiveness of the BBB is subject to dynamic changes. Sleep deprivation, for example, also increases BBB permeability [Gomez-Gonzalez et al., 2013; He et al., 2014], but BBB integrity is restored with a normal sleep-wake cycle. Even microbiota in the gut are known to alter BBB permeability [Braniste et al., 2014]. It is also significant that the BBB associated with the subventricular zone lacks the normal glial end feet and endothelial cell tight junctions found in other brain regions, and as a result is relatively permeable, presumably supporting communication between NSCs and circulating regulatory factors [Tavazoie et al., 2008; Lin and Iacovitti, 2015]. Whether specific cells may have access to the brain at these sites is not known, although conventional wisdom suggests that invasion of cells across the BBB and into the brain is not characteristic of a healthy state. However, as we learn more about how normal fluctuations in gut microbiota, sleep cycles, nutrition, and other dynamics of living influence the permeability of this structure, there may well be 'normal' situations where the BBB is more (or less) permissive. Thus, there is still a great deal to learn about the dynamic nature of a 'healthy' BBB.

Can Blood Make Brain?

In crayfish, the conclusion from different lines of evidence is 'yes'. In mammalian species, the answer is not clear, although there are provocative indications in several studies that allude to this possibility. There is still a great deal to learn about adult neurogenesis in the many species that display this phenomenon, including to what degree the underlying mechanisms have been preserved by evolution. Is long-term self-renewal limited to NSCs in vitro, as some recent data suggest? If so, then does the vast supply of NSCs required for the life-long production

of neurons reside in the brain from birth? Or might there be a source of NSCs extrinsic to the CNS, from which the pool can be replenished? If so, does this replenishment occur on an ongoing basis, or might this be a selective response to inflammation or disease? And, if such a source of NSCs is required, what is that source? Eric Kandel has said, '… as Charles Darwin might have predicted, once nature finds a solution that works, it tends to hold on to it' [Bear et al., 2016]. In this spirit, it is intriguing to ask whether the production of adult-born neurons by precursors originating in the immune system is an anomaly that is confined to some crustaceans, or if these data provide a clue to our understanding of adult neurogenesis in mammals, including ourselves.

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