Mosaic Organization of Neural Stem Cells in the Adult Brain
Florian T. Merkle, et al.
Science 317, 381 (2007);
DOI: 10.1126/science.1144914

The following resources related to this article are available online at www.sciencemag.org (this information is current as of June 19, 2008):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/317/5836/381

Supporting Online Material can be found at:
http://www.sciencemag.org/cgi/content/full/1144914/DC1

This article cites 27 articles, 14 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/317/5836/381#otherarticles

This article has been cited by 23 article(s) on the ISI Web of Science.

This article has been cited by 8 articles hosted by HighWire Press; see:
http://www.sciencemag.org/cgi/content/full/317/5836/381#otherarticles

This article appears in the following subject collections:
Neuroscience
http://www.sciencemag.org/cgi/collection/neuroscience

Information about obtaining reprints of this article or about obtaining permission to reproduce this article in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl
Mosaic Organization of Neural Stem Cells in the Adult Brain

Florian T. Merkle, Zaman Mirzadeh, Arturo Alvarez-Buylla*

The in vivo potential of neural stem cells in the postnatal mouse brain is not known, but because they produce many different types of neurons, they must be either very versatile or very diverse. By specifically targeting stem cells and following their progeny in vivo, we showed that postnatal stem cells in different regions produce different types of neurons, even when heterotopically grafted or grown in culture. This suggests that rather than being plastic and homogeneous, neural stem cells are a restricted and diverse population of progenitors.

Every day, thousands of new neurons are generated by astrocyte-like stem cells residing in the subventricular zone (SVZ), a thin but extensive layer of cells lining the lateral wall of the lateral ventricle in the adult mouse brain (1). These newly born neurons (neuroblasts) migrate to the olfactory bulb in a complex network of chains that eventually merge to form the rostral migratory stream (RMS). On reaching the olfactory bulb, neuroblasts integrate and mature into several distinct cell types (2). It is not known how this diversity of neurons is generated, largely because it is difficult to study individual stem cells in vivo. Indeed, our understanding of stem cells is strongly influenced by the in vitro techniques with which they were first isolated and later defined by their ability to be passed (demonstrating self-renewal) and differentiated into astrocytes, neurons, and oligodendrocytes (demonstrating multipotency) (3, 4). These results led to the widely held assumption that neural stem cells are a homogeneous population of multipotent, plastic progenitors (Fig. 1A). Similarly, it was thought that neuroblasts born in the SVZ might be equivalent until they reach the olfactory bulb and begin to differentiate. However, recent evidence suggests that neuroblasts are heterogeneous before reaching the olfactory bulb (5, 6). We therefore hypothesized that stem cells are not equivalent and that they specify the fate of the neurons they produce (Fig. 1B). To test this hypothesis, we labeled stem cells in different regions and followed their progeny in vivo.

We have previously shown that adult neural stem cells are derived from radial glia present in the neonatal (P0) mouse brain (7). Radial glia, which are now recognized as the principal stem cell of the embryonic and early postnatal mouse brain (8, 9), have a unique morphology that allows them to be targeted specifically. Their cell bodies line the ventricles and they send a long, radial process to the brain surface. Adenoviruses readily infect these processes and are transported to the cell body. When an adenovirus expressing Cre recombinase (Ad:Cre) is injected into green fluorescent protein (GFP) reporter (Z/EG) mice (10), infected radial glia and their progeny become permanently labeled with GFP (7). Because adenoviruses do not diffuse readily in the brain, the localized injection of a small volume (20 nl) of Ad:Cre labels a spatially restricted patch of neural stem cells.

To label radial glia in a regionally specific manner, we developed a method to stereotaxically inject Ad:Cre in P0 mice (Fig. 1E) (11). Injections resulted in the reproducible labeling of spatially segregated patches of radial glia (Fig. 1, F and G). In contrast, cells labeled locally at the injection site do not give rise to olfactory bulb neurons or neural stem cells (7). By systematically varying the injection location (Fig. 1H), we targeted 15 different populations of radial glial cells at six different rostrocaudal levels (i to vi) (11). We targeted the dorsal (D) or ventral (V) lateral wall of the lateral ventricle at four different rostrocaudal levels (ii to v) and the entire dorsoventral extent of the lateral wall at level vi. We also targeted the RMS (i), the medial (septal) wall (iiM), and the cortical wall of the lateral ventricle (iic to vc) because these regions have been suggested to contain neural progenitors (1, 4, 12, 13). When we analyzed the brains of mice 4 weeks after Ad:Cre injection, we observed a patch of labeled cells in the same anatomical location as the targeted radial glial cell bodies, indicating that neural stem cells do not disperse tangentially (Fig. S1). Figure 1, F and G, show examples of SVZ labeling after neonatal targeting of dorsal and ventral SVZ radial glia, respectively.

We then examined the mature GFP-labeled neurons in the olfactory bulb. The two principal types of adult-born olfactory neurons, periglomerular cells and granule cells, are interneurons that modulate the activity of neurons that project to olfactory cortex. Periglomerular cells can be subdivided into three nonoverlapping populations of cells: calretinin- (CalR+) and calbindin-expressing (CalB+) cells, and tyrosine hydroxylase-expressing (TH+) dopaminergic cells (14). Granule cells include deep, superficial, and CalR+ cells (Fig. 2A) (15, 16). These subtypes are thought to be distinct functional elements of the olfactory bulb circuitry (14, 15, 17).

Olfactory bulb interneurons were produced from all labeled regions including regions i, iiM, and iiiC to vC, which extend beyond the lateral wall of the lateral ventricle, the accepted boundary of the neurogenic adult SVZ. Notably, each region gave rise to only a very specific subset of interneuron subtypes. Anterior and dorsal regions produced periglomerular cells (Fig. 2B and fig. S2, A to D). Interestingly, the highest percentage was produced in region iiM (Fig. 2B and fig. S2, A and B). Periglomerular cell subtypes were also produced in a region-specific manner. Dorsal regions (iiC to vC) produced the highest percentage of TH+ cells (Fig. 2C and fig. S3, A to C), whereas CalB+ cells were produced mainly ventrally, in regions iiV and iiiV (Fig. 2D and fig. S3, D to F). CalR+ periglomerular cells were less frequently observed when these regions were targeted, but were frequently labeled with targeting of regions i and iiiC (Fig. 2E and fig. S3, G to I). Each targeted region produced granule cells (Fig. 2F), though region iiiC produced relatively few (Fig. 2B and fig. S2, A and B). Dorsal regions (iiC to vC, iiiD, iiiIiD) tended to produce superficial granule cells, whereas ventral regions (iiV to viV) produced mostly deep granule cells (Fig. 2F and fig. S2, E and H). CalR+ granule cells were produced mostly from the anterior regions (i and iiiC) that also produced many CalR+ periglomerular cells (Fig. 2G and fig. S3, J to L). This is consistent with a recent study showing that CalR+ olfactory bulb neurons are derived from SP8+ cells, which are found embryonically in regions i and iiiC (18).

Each neonatally targeted region continued to produce neuroblasts in the adult brain, suggesting the continued presence of a neural stem cell (fig. S2, B, D, F, and H). Neonatal radial glia convert into SVZ astrocytes that express glial fibrillary acid protein (GFAP) and pericyte-like cells (fig. S2, E and F).

References


Department of Neurosurgery and Developmental and Stem Cell Biology Program, University of California, San Francisco, San Francisco, CA 94143-0525, USA.

*To whom correspondence should be addressed. E-mail: abuylla@stemcell.ucsf.edu
acidic protein (GFAP) and function as the adult neural stem cells (1, 7, 19). To test whether adult neural stem cells were also regionally specified, we constructed an adenovirus that expresses Cre under the control of the murine GFAP promoter (Ad:GFAP-Cre). This virus induces recombination in GFAP-expressing cells including adult neural stem cells, but not the more differentiated cells they give rise to (fig. S4, A to C) (11). We injected P60 Z/EG mice with Ad:GFAP-Cre in regions i, iiD, iiiV, and vC (fig. S4, D to G) and killed the animals 28 days later to examine the olfactory bulb cell types produced. Each targeted region produced olfactory bulb neurons and neuroblasts (Fig. 3, A to D), suggesting that they contain long-lived GFAP+ neurogenic progenitors. This finding agrees with previous work suggesting the presence of a neurogenic progenitor in the adult RMS (5, 20, 21) and subcallosal zone (regions ivC and vC) (12). Furthermore, these labeled cells produced superficial and deep granule cells (Fig. 3, A to E), as well as TH+, CalB+, and CalR+ cells (Fig. 3F) in virtually the same region-specific pattern obtained from neonatal labeling. A recent study suggests that the potential of progenitors to produce different types of periglomerular cells changes over development (22). This study might have inadvertently examined progenitors and tangentially migrating neuroblasts from different regions at different ages, whereas our technique targets only primary progenitors. The apparent maintenance of stem cell potential over postnatal development suggests that the factors specifying neural progenitors in the SVZ are maintained throughout development in a regionally specific manner.

These factors could be either environmental or intrinsic to stem cells. To distinguish between these two possibilities, we challenged neonatal stem cells by heterotopic transplantation (11). If environmental factors specify the fate of newborn neurons, grafted (donor) stem cells and their progeny should respond to these factors and make cell types produced in the host region. To obtain labeled neural stem cells, neonatal radial glia were infected with Ad:Cre as described above, microdissected after 2 hours, dissociated to a single-cell suspension, and homotopically or heterotopically grafted into the littermates of donor animals (fig. S5A). We then analyzed the olfactory bulb cell types produced 40 days after grafting and found that labeled radial glia continued to produce the cell types appropriate to their region of origin (fig. S5B, C and D). To determine if progenitors would maintain their region-specific potential in the absence of any environmental cues, we cultured anterior, dorsal, or ventral progenitors under adherent conditions that recapitulate postnatal SVZ neurogenesis (23). Cultures were expanded, differentiated (fig. SSD), and immunostained for cell type--specific markers (fig. S6). Despite being removed from their complex environment and exposed to a cocktail of growth factors, neural stem cells maintained their region-specific potential (fig. S5E). We confirmed this result by targeting dorsal or ventral radial glia, culturing them for 2 weeks, and grafting them heterotopically into wild-type neonatal mice (fig. S5F). Again, grafted cells produced cell types appropriate to their region of origin, not their grafted location (fig. S5G).

The above experiments suggest that neural stem cells are not readily respecified by environmental factors present in the postnatal brain, but we cannot eliminate the possibility that unlabeled cells grafted alongside labeled stem cells could be carrying factors from the donor environment into the host graft site. Therefore, we microdissected progenitors from different regions of neonatal wild-type or ActB-GFP mice (24) and cultured them for three passages over 2 weeks. To ensure that grafted cells were surrounded by foreign environmental cues, we mixed GFP+ cells from the donor region with a 10:1 excess of unlabeled wild-type cells from the host region before grafting them into wild-type neonatal mice (Fig. 4A). Four weeks later, grafted brains contained labeled astrocyte-like cells at the graft site (fig. S7, A, C, E, and G) and mature neurons in the olfactory bulbs (fig. S7, B, D, F, and H). Because grafted cells continued to produce neuroblasts as efficiently as directly targeted primary progenitors (fig. S8), grafted cells were most likely stem cells and not intermediate progenitors. Again, heterotopically grafted stem cells produced neuronal subtypes.

Fig. 1. Specific regional targeting of neural stem cells. (A and B) In the traditional model of SVZ stem cell potential (A), equivalent stem cells (black dots) generate multiple neuron types, which are produced by a diverse stem cell population in the proposed model (B). (C and D) Diagram of a neonatal mouse brain showing targeting of dorsal (C) or ventral (D) radial glia (green) with virus deposited at the injection site and along the needle tract (gray circle and bar). (E) Stereotactic setup showing an acrylic model of a neonatal pup positioned in a customized head mold for viral injection parallel to radial glial processes. (F and G) Photomicrograph of a P28 Z/EG brain injected at P0 to target dorsal (F) or ventral (G) radial glia, visualized with immunoperoxidase staining for GFP. (H) Diagram of brain regions targeted. Representative frontal sections of the right hemisphere traced from the adult brain are shown relative to a photomicrograph of an adult lateral ventricular wall whole mount outlined in blue (30). Targeted regions, indicated by green dots, are named for their anterior-posterior level (i to vi) followed by the location within that level, where C is cortical, M is medial, D is dorsal, and V is ventral.
with the same regional specificity observed by in vivo lineage tracing (Fig. 4, B to E). The lack of evidence for even partial respecification indicates that grafted cells behaved like a single population that was resistant to respecification. However, we cannot discard the possibility that some environmental factors were not totally eliminated in our experiments. Neural stem cell potential could also be altered by factors not included in our culture conditions or after extended periods of time ex vivo. Previous studies have suggested that adult hippocampal or spinal cord progenitors might be respecified when heterotopically grafted (25, 26). Our findings suggest that although SVZ neural stem cells retain the potential to produce astrocytes, oligodendrocytes, and neurons, they are restricted in the types of neurons they can generate. We conclude that postnatal neural stem cells are diverse and are organized in an intricate mosaic in the postnatal germinal zone.

These findings suggest that, as during embryonic brain development (27–29), the potential of postnatal neural stem cells is determined by a spatial code, supporting the model shown in Fig. 1B. Stem cells do not appear to migrate tangentially as they mature from radial glia into astrocyte-like adult cells and must have integrated positional information at some point in development. We suggest that this positional information becomes encoded in the progenitors, perhaps by expression of a transcription factor code, and maintained into adulthood. This insight is a key step toward understanding the molecular mechanisms of neural stem cell potential and for future efforts to use these cells for brain repair. The mosaic distribution of progenitors also raises the possibility that the activity of stem cells is regionally modulated in order to regulate the production of different types of neurons. This may provide a mechanism for the brain to dynamically fine tune the olfactory bulb circuitry.

References and Notes

Queen Pheromone Blocks Aversive Learning in Young Worker Bees

Vanina Vergoz, Haley A. Schreurs, Alison R. Mercer*

Queen mandibular pheromone (QMP) has profound effects on dopamine signaling in the brain of young worker honey bees. As dopamine in insects has been strongly implicated in aversive learning, we examined QMP’s impact on associative olfactory learning in bees. We found that QMP blocks aversive learning in young workers, but leaves appetitive learning intact. We postulate that QMP’s effects on aversive learning enhance the likelihood that young workers remain in close contact with their queen by preventing them from forming an aversion to their mother’s pheromone bouquet. The results provide an interesting twist to a story of success and survival.

To advertise her presence in the colony and to exert influence over its members, a honey bee queen produces a complex blend of substances known as queen mandibular pheromone (QMP) (1). Young workers, attracted to the queen by QMP are enticed not only to feed her, but also to lick and to antennate her body. As they do so, they gather samples of QMP, which they distribute to other members of the colony (2, 3). At the colony level, QMP inhibits the rearing of new queens (4), influences comb-building activities (5), helps prevent the development of worker ovaries (6), and modulates the behavioral development of workers (7, 8).

We recently showed that brain dopamine levels, levels of dopamine receptor gene expression, and brain tissue responses to this amine are altered significantly in young workers exposed to QMP (9). Among its many functions, dopamine contributes to cellular events underlying learning and memory. In insects, dopamine signaling is essential for aversion learning (10–12), which raises an interesting question: Does QMP, through its actions on brain dopamine function in young workers, alter their ability to establish aversive olfactory memories?

We examined QMP’s impact on associative olfactory learning in bees exposed to QMP from the time of adult emergence. Bees of the same age maintained under identical conditions but without exposure to QMP were used as controls. To begin, we examined QMP’s effects on aversive learning in young (6-day-old) bees. A differential conditioning paradigm was used to train bees to extend their sting to an odorant paired...