Glial progenitor cells in the adult brain reveal their alternate fate

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Glial cells that express NG2 and platelet-derived growth factor receptor α are found throughout the mature CNS. These cells are mitotically active, but their functions remain enigmatic. A genetic fate-mapping study in this issue shows that these abundant glial cells can generate both oligodendrocytes and some cortical projection neurons in the adult brain.

Like a ship preparing for a long voyage, the brain forms its neurons and glia during early development and must survive life's tempests without the benefit of extensive cell replacement. Nevertheless, dividing cells have been observed throughout the mature CNS, suggesting that neural circuits are not formed exclusively from a fixed complement of cells. Progenitors located in the subventricular zone (SVZ) continually supply the rodent olfactory bulb with new interneurons and new hippocampal granule neurons are constantly formed from dividing cells in the subgranular zone (SGZ) of the dentate gyrus[1] (**Fig. 1a**,**b**). Proliferating cells in the adult CNS are not, however, restricted to these small niches, as the proliferation marker bromodeoxyuridine (BrdU) is also readily incorporated by small, highly ramified non-neuronal cells that are scattered throughout the adult white and gray matter[2]. In this issue of *Nature Neuroscience*, Rivers *et al.*[3] track the fate of these cycling glial cells in the adult brain using genetically modified mice and find that they can develop into both oligodendrocytes and excitatory projection neurons.

Most oligodendrocytes in rodents are formed during the first few weeks of life from oligodendrocyte precursors (OLPs), which are generated near the lateral ventricles, migrate throughout the brain, proliferate and eventually differentiate into myelinating oligodendrocytes. During this period, OLPs express the transcription factor Olig2, the chondroitin sulfate proteoglycan NG2 (*Cspg4*) and the alpha receptor for platelet-derived growth factor (PDGFRA). In the adult brain, most proliferating cells outside of the SVZ and SGZ also express these proteins[4], suggesting that OLPs may persist beyond the early postnatal period. However, this conclusion has remained controversial, as there is limited evidence for widespread oligodendrogenesis in the adult CNS and these NG2-immunoreactive ($NG2^+$) cells are abundant in the gray matter, where myelination is sparse. Further doubts about the function of $NG2^+$ cells have been raised by the discovery that they express ionotropic receptors for neurotransmitters, form synapses with glutamatergic and GABAergic neurons in gray matter[5,6] and

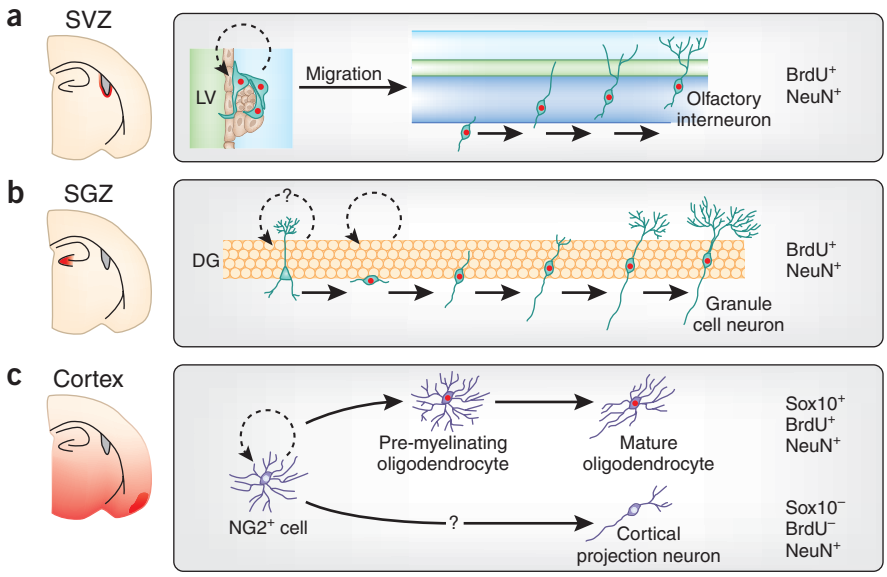

Figure 1 Sites of neurogenesis in the adult brain. (**a**,**b**) The SVZ adjacent to the lateral ventricles (LV) (**a**) and the SGZ of hippocampal dentate gyrus (DG) (**b**) are sites that are known to generate new neurons in the adult brain. Stem cells and dividing progenitors in the SVZ (type B, C and A cells) and SGZ (type 1 and type 2 cells, see ref. 1) give rise to precursors that can be detected by BrdU incorporation (red nucleus), which then migrate and/or develop into mature neurons that express NeuN. Adapted from ref. 1. (**c**) In ventral forebrain regions such as piriform cortex, resident glial cells that express the alpha receptor for PDGF and NG2, termed $NG2^+$ cells or oligodendrocyte precursors (OLPs), can generate both neurons and oligodendrocytes ($Sox10^+$). However, unlike neurogenesis in the SVZ and SGZ, generation of these glutamatergic projection neurons may occur through transdifferentiation of postmitotic $NG2^+$ cells.

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axons of passage in white matter[7,8], and may be capable of firing action potentials in some regions[9,10], properties that seem unusual for progenitors that are merely destined to become oligodendrocytes.

Interest in the fate of these cells has also been fueled by a report that OLPs that were isolated from the developing optic nerve differentiated into astrocytes, oligodendrocytes or neurons *in vitro* on exposure to different combinations of growth factors[11]. If NG2$^+$ cells could develop into these major cell types of the nervous system *in vivo*, it would have profound implications for mechanisms of information storage in the brain and could present new opportunities for cell replacement following injury or disease.

To study the fate of OLPs in the adult CNS, Rivers *et al.*[3] developed transgenic mice that express a tamoxifen-inducible form of Cre recombinase (*creER^{T2}*) under the control of the *Pdgfra* promoter using a phage artificial chromosome carrying large segments of regulatory sequence to help ensure that Cre expression was restricted to OLPs. The resulting *Pdgfra-creER^{T2}* mice were crossed with *Rosa26-yfp* mice, which carry a *loxP*-flanked stop sequence in front of the yellow fluorescent protein sequence (YFP); when Cre activity is induced, the stop sequence is excised and YFP is expressed under the control of the ubiquitously active Rosa26 promoter. The excision event is irreversible, and Cre-expressing OLPs and all of their progeny are expected to express YFP.

Despite the presence of a large portion of regulatory sequence, however, transgenes often do not exactly mimic the expression pattern of the endogenous gene, presumably because they lack key regulatory elements or are subject to abnormal regulation at the site(s) of integration. For accurate fate mapping, it is therefore essential that tamoxifen induces Cre activity only in the progenitor pool of interest. By *in situ* hybridization, the authors found that *Pdgfra* mRNA was detected in nearly all *cre* mRNA–expressing cells in both gray and white matter. A tradeoff with inducible control is that the efficiency of recombination is typically lower than can be achieved with constitutive Cre expression. Indeed, with a maximal tamoxifen administration protocol only about half of the PDGFRA$^+$ population was found to express YFP, although it is likely that these cells comprise a representative sample of the population.

The authors used these *Pdgfra-creER^{T2}/Rosa26-yfp* mice to determine the fate of resident PDGFRA$^+$ cells by examining the phenotype of YFP$^+$ cells days to months after a brief administration of tamoxifen. As the length of time post–tamoxifen induction increased, the number of YFP$^+$ cells in the brains of these mice also increased, indicating that OLPs continued to proliferate, in accordance with previous reports of BrdU incorporation by PDGFRA$^+$ cells in adults. However, the rate of YFP-positive cell accumulation was slower at 3 months than at 1 month of age, and was slower in gray matter (cortex) than in white matter (corpus callosum), suggesting that the behavior of these progenitors is influenced by their local environment. With increasing delay, more YFP$^+$ cells became PDGFRA negative; however, they retained Sox10 expression, indicating that they remained in the oligodendrocyte lineage. In the corpus callosum, these YFP$^+$ PDGFRA$^-$ cells extended processes that ran parallel to axons and expressed both CNPase and myelin basic protein (MBP), properties that are consistent with myelinating oligodendrocytes. Far from providing a minor contribution to adult myelin, the authors estimate that nearly a third of oligodendrocytes in this region were generated in a 6-month period after postnatal day 45 (P45). The great majority of these YFP$^+$ oligodendrocytes were BrdU$^+$ when OLPs were preloaded with BrdU, indicating that most oligodendrocytes in the adult brain are generated from proliferating progenitors rather than from differentiation of postmitotic OLPs.

Previous studies have suggested that OLPs in the adult brain can also be generated in the SVZ from a subset of GFAP$^+$ neurogenic stem cells (also called type B cells) that express PDGFRA[12]. Their contribution to oligodendrogenesis could not be assessed with *Pdgfra-creER^{T2}* mice, as Cre was not expressed in SVZ cells. Rivers *et al.*[3] therefore carried out similar fate-tracing experiments using another line of mice (*Fgfr3-icreER*) in which Cre activity can be induced in nearly all GFAP$^+$ B cells. Few, if any, OLPs or oligodendrocytes were generated from SVZ B cells over a 3-month period starting on P45, indicating that oligodendrocytes in the adult corpus callosum arise from local PDGFRA$^+$ precursors rather than from subventricular stem cells.

The authors did not observe YFP$^+$ astrocytes in *Pdgfra-creER^{T2}/Rosa26-yfp* mice at any time after tamoxifen administration. Although this finding is seemingly at odds with recent fate-mapping studies performed with *NG2-cre* mice, where astrocytes (along with OLPs and oligodendrocytes) were observed in the ventral brain[13], it is possible that the NG2 promoter is active in less-committed progenitors during prenatal or early postnatal periods.

One of the most controversial issues regarding NG2$^+$ cells is whether they have the capacity to differentiate into neurons *in vivo*. Rivers *et al.*[3] discovered that YFP$^+$ neurons appeared in several regions of the ventral forebrain in *Pdgfra-creER^{T2}/Rosa26-yfp* mice within a few weeks of tamoxifen administration at P45. These cells were NeuN$^+$ (but negative for Sox10, Olig2, NG2 and PDGFRA), extended TAU1$^+$ processes to toward the pial surface and did not express markers that are associated with interneurons, indicating that they were glutamatergic projection neurons. This remarkable finding suggests that neurons in the adult brain can be generated from resident glial cells and supports the contention that neurogenesis in the cortex is widespread and not merely restricted to the germinal SVZ and SGZ areas[14] (**Fig. 1c**). Notably, the approach taken by Rivers *et al.*[3] does not suffer from many of the caveats associated with earlier reports of cortical neurogenesis, as it did not require *in vitro* analysis, cell transplantation or identification of newly generated cells on the basis of immunohistochemical labeling of proliferation markers (such as BrdU or PSA-NCAM). Although neurogenesis in the SVZ and SGZ originates from proliferating stem/progenitor cells, the neurons here appeared to arise from transdifferentiation of postmitotic OLPs, as YFP$^+$ neurons did not stain for BrdU when OLPs were preloaded with this indicator of mitosis. Furthermore, the YFP$^+$ neurons that appeared in these mice were projection neurons, rather than SVZ-derived interneurons (olfactory bulb) or SGZ-derived granule cells (dentate gyrus), which integrate into local circuits. It will thus be important to determine how the OLP-derived projection neurons find their distant targets and to understand why this neurogenesis occurs preferentially in ventral areas of the brain.

Evidence for neurogenesis from OLPs *in vivo* is particularly surprising given that neurons were not observed in recent fate-mapping experiments performed with either *NG2-cre*[13] or *Olig2-creER^{TM}* mice[15], both of which should sample PDGFRA$^+$ cells. One possibility is that recombination was induced in mature neurons in *Pdgfra-creER^{T2}* mice through ectopic or 'leaky' PDGFRA promoter activity. However, significant numbers of YFP$^+$ neurons were not observed until a month after tamoxifen administration and they became more abundant with increasing time post-tamoxifen, observations that are most consistent with ongoing neurogenesis from precursors. A more detailed kinetic analysis of the appearance of YFP$^+$ neurons

and evidence of transitional phenotypes could have strengthened this exciting conclusion.

In addition to this new information about the developmental potential of adult OLPs and the rates of oligodendrogenesis in the mature brain, Rivers *et al.*[3] have developed a line of mice that can be used to selectively delete genes from OLPs. These *Pdgfra-creER*^{T2} mice will provide new opportunities for studying the signaling pathways that guide OLP differentiation. Such work could help us to understand why OLPs eventually fail to remyelinate axons in chronic stages of multiple sclerosis and to identify new therapeutic targets for enhancing cell replacement in the CNS after injury or disease.

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Switching gears in the spinal cord

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A study in this issue shows that zebrafish larvae deploy different groups of excitatory spinal interneurons to drive slow, fast and top speed swimming. As one set is gradually activated, the others are partially or fully inhibited.

Small fish such as zebrafish spend most of their time moving slowly, searching for food. Slow swimming involves paddling with the pectoral fins and weak undulatory body movements at a comparatively low frequency. When a predator appears, however, they must accelerate and swim quickly to escape danger. An escaping zebrafish folds the fins tightly to the body wall and relies entirely on rapid body undulations to provide a forceful thrust. In this issue, McLean *et al.*[1] asked whether slow and fast swimming are generated by the same or by different interneuronal networks. They found that different sets of excitatory interneurons produce these two distinct patterns of motor behavior. When the interneuronal network for the fast pattern was recruited, the slow motor pattern was turned off by active inhibition. With increasing speed, interneurons were recruited following an orderly arrangement, from the most ventral to the most dorsal aspect of the spinal cord. As new dorsal interneurons were recruited, the most ventral interneurons, active at lower speeds, were inhibited. This pattern is very different from the well-known pattern of motoneuron recruitment. There, the ventral motoneurons that are active at low speeds remain active as speed increases and progressively more dorsal motoneurons are activated[2].

The interneurons that drive slow swimming are the excitatory, glutamatergic so-called 'multipolar commissural descending' interneurons (MCoDs). They are located in the most ventral aspect of the spinal cord, with axons crossing the midline and providing a phasic drive in each swim cycle to a subset of ventral contralateral motoneurons. The trunk movements that occur during slow swimming are entirely dependent on the activation of these interneurons. When caudal MCoDs are inactivated, slow trunk-swimming movements cannot be generated. Under these conditions, however, the pectoral fins in the rostral part of the spinal cord can still produce rhythmic locomotor movements[2].

When the swimming speed was gradually increased, the MCoDs received a profound inhibition through glycinergic synapses, completely blocking their activity, while the 'circumferential ipsilateral descending' interneurons (CiDs) became activated. The CiDs are an ontogenetically separate group of excitatory interneurons with ipsilateral axons belonging to the specific developmental class V2a, which is present in species ranging from fish to mammals[3,4]. They provide direct excitation to the ipsilateral motoneurons controlling the segmental trunk muscles[3]. As swimming speed increased further from intermediate to top speed, the authors observed another switching of 'gears'. With trunk locomotion at moderate speed, the most ventral CiDs were active, but as speed increased further, progressively more dorsal interneurons were recruited. During very fast escape swimming, the most dorsal CiDs were active, whereas it appeared as if the ventral CiDs became inhibited to some degree, though much less so than the MCoDs (**Fig. 1**).

The zebrafish develops rapidly from the egg through larval and juvenile stages. Around 30 d after birth, young fish have all of the adult anatomical features and they become sexually mature at 3 months. Very young fish swim with a very high frequency, but as physical dimensions grow with age, the frequency of swimming undulations decreases progressively in most species, including the zebrafish. McLean *et al.*[1] carried out their study on 4-d-old zebrafish larvae, when basic features of slow and fast swimming are already manifest. The frequency of swimming is, however, still quite high, ranging from 15 to 75 Hz, whereas in the adult[5], the frequency span is down to around 2 to 40 Hz. There are a number of technical advantages to using this early larval stage, such as transparency of the body allowing easy imaging, but it also introduces some possible limitations to the conclusions, as the authors are looking at a still-developing nervous system.

At earlier stages, the frequency range is even higher than it is at the 4-d stage. As the motor system develops, the highest swimming frequencies are mediated by the large primary motoneurons that are the first

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