A *Fgfr3- CreER^{T2}*

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Fgfr3; Cre-lox; transgenic mice; astrocytes; radial glia; neural stem cells; spinal cord; SVZ; olfactory bulb; interneurons

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The lack of markers for astrocytes, particularly gr1.1128p/41.5(ft)]TT*[NICOocytes,)-344.4(partsignificant349.1(8nithind-341.5(st8(rrch,

expressed at a low level in early oligodendrocyte precursors (OLPs) and is upregulated only transiently as they start to differentiate into OLs (Bansal et al., 1996), so it

and transferred to glass slides for mounting and microscopy. Embryonic brain and spinal cord sections were collected directly onto coated glass slides.

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Sections (20 μm) for hybridization were collected on the surface of DEPC-treated PBS, transferred onto

anywhere in the CNS, either because recombination had not yet occurred or because the level of YFP protein was still below detectable levels. However, at P50 + 7 many YFP$^+$ cells were found throughout the gray matter of the brain (Fig. 2) and spinal cord (Supp. Info. Fig. 2). These cells were close-packed and filled almost the entire gray matter volume with YFP fluorescence. Within this sea of fluorescence were scattered "holes," corresponding to unlabeled cells (e.g. Fig. 2a–c, arrowheads). This pattern was consistent with the idea that the YFP-labeled cells were astrocytes and indicated that the efficiency of *26* - reporter gene activation was high, though less than 100%. We never observed any YFP$^+$ cells in *3-* ^{2}: *26* - mice that had not received tamoxifen.

The great majority of YFP$^+$ cells in *26* - reporters were S100β$^+$ (94% ± 4% of YFP$^+$ cells) od

Fig. 3. Fgfr3-CreER^{T2} marks astrocytes in the adult mouse fore-

the forebrain. Many (around half) of the OLIG2$^+$, YFP$^+$ cells expressed a low level of OLIG2 relative to the OLIG2$^+$, YFP-negative cells (Fig. 3g). Although S100β is widely used to detect protoplasmic astrocytes, it is also expressed by some OL lineage cells (Hachem et al., 2005; Vives etal., 2003). We therefore triple-labeled for YFP, S100β, and OLIG2. The great majority (92% ± 5%) of YFP$^+$ cells co-expressed S100β but not OLIG2 (Fig. 3r), confirming them as astrocytes.

In coronal sections of P50 + 7 corpus callosum, a major white matter tract, the vast majority (95% ± 3%) of YFP$^+$ cells were GFAP$^+$ fibrous astrocytes (Fig. 3k–m,o). A very small proportion (1.0% ± 0.8%) of YFP$^+$ cells co-labeled for OLIG2 (Fig. 3j,m) but none co-labeled for NeuN (Fig. 3m). A tiny minority of all SOX10$^+$ OL lineage cells (0.07% ± 0.01%) co-expressed YFP$^+$. Similarly, a tiny fraction of NG2$^+$ OL precursors (0.3% ± 0.1%) were YFP$^+$. Taken together, these data demonstrate that recombination in the *26* - reporter background is both efficient and astrocyte-specific.

To examine the detailed morphology of labeled cells we used the / reporter, in which eGFP is expressed in a Cre-inducible manner from a synthetic promoter composed of CMV and β-actin promoter elements (Novak

To test for stem cell labeling, we generated neurosphere cultures from the SVZ and spinal cords of *3-2*: *26* - mice at increasing times (7–80 days) post-tamoxifen (Fig. 5b,c) and determined the proportion of neurospheres that was YFP$^+$ in each culture (Fig. 5d). At all times post-tamoxifen, 100% of spinal cord-derived neurospheres were uniformly YFP$^+$ (Fig. 5d). EZ stem cells are the only cells capable of generating neurospheres in the normal healthy spinal cord, so all adult EZ stem cells must be targeted by the *3-2* transgene. In SVZ cultures, both stem cells (type-B) and progenitor cells (type-C) have the capacity to generate neurospheres (Young et al., 2007). If only the stem cell population expresses *3-2*, then only a fraction of neurospheres should be YFP$^+$. However, this fraction would be expected to increase, the longer the delay between tamoxifen administration and establishment of the SVZ cell cultures. This is because YFP$^+$ stem cells give rise to YFP$^+$ intermediate progenitor cells, while preexisting (YFP-negative) progenitors generate migratory neuroblasts that leave the SVZ to join the rostral migratory stream (RMS) and move towards the OB. This is what we found experimentally. Only 31% ± 11% of neurospheres were YFP$^+$ in SVZ-derived cultures that were established at the shortest time post-tamoxifen (P70 + 7) (Fig. 5d). However, the proportion of neurospheres that was YFP$^+$ increased with time post-tamoxifen, to 89% ± 4% at P70 + 56 and 90% ± 6% at P70 + 80 (Fig. 5d). These data indicate that ~90% of SVZ stem cells recombine following tamoxifen administration (i.e. recombination efficiency ≅ 90%), similar to the recombination rate in fibrous and protoplasmic astrocytes. Consistent with this, BrdU labeling experiments showed that 91% ± 2% of BrdU$^+$ cells in the SVZ of adult P50 + 80 *3-2*: *26* - mice were YFP$^+$ (four BrdU injections in 24 h; Fig. 5e).

If *3-2* is expressed in migratory neuroblasts (type-A cells), one would expect to find YFP-labeling of PSA-NCAM$^+$ neuroblasts in the SVZ, RMS, and

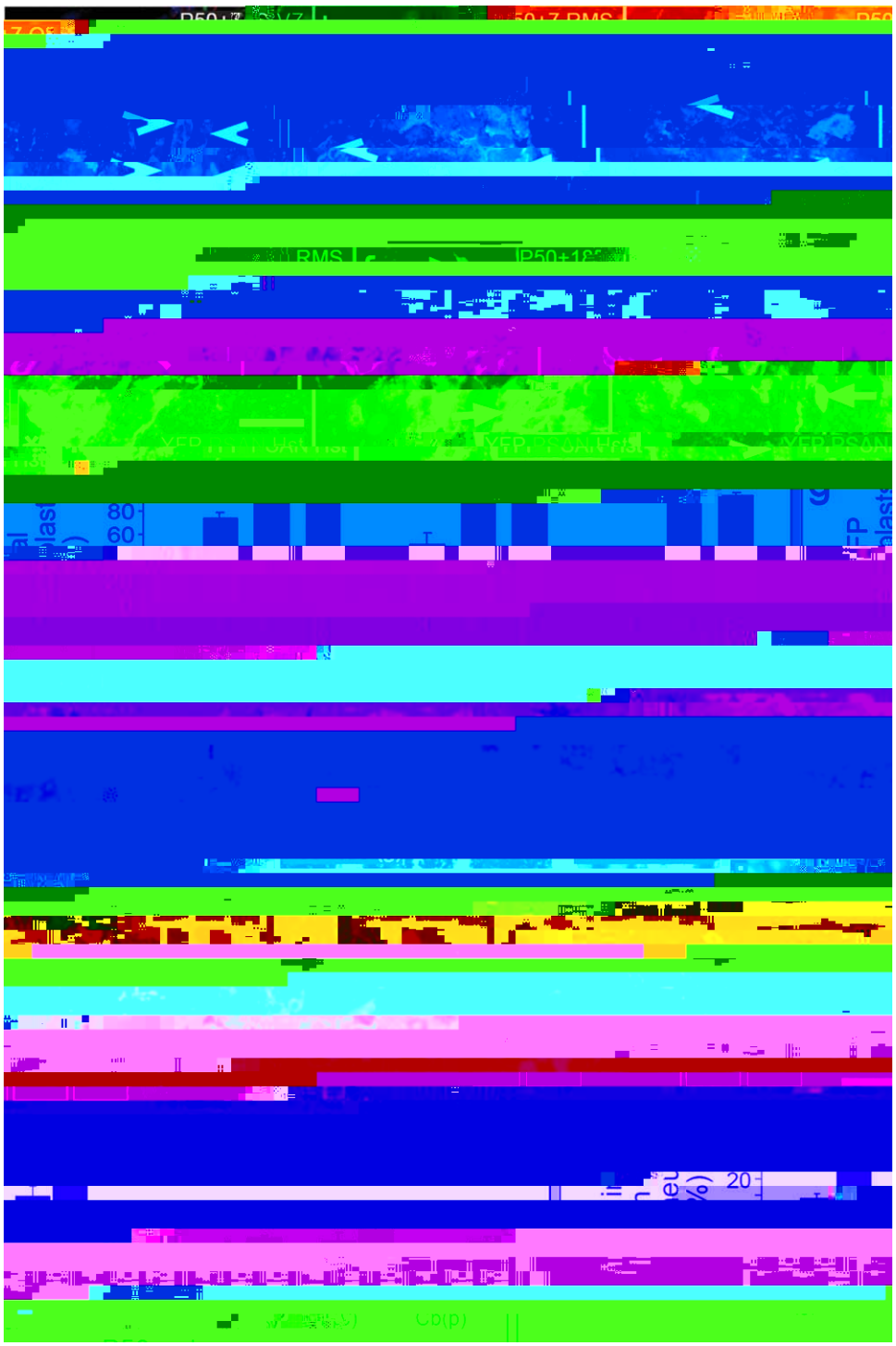

Fig. 6. OB interneurons inherit a recombined *26* - from SVZ stem cells via intermediate PSA-NCAM^{+} progenitors. Coronal forebrain sections through the SVZ, RMS and OB of P50 + 7 (–), P50 + 14, P50 + 80 and P50 + 180 (–) *3-* *2*: *26* - mice were immunolabeled for YFP (green) and PSA-NCAM (red). The proportions of PSA-NCAM^{+} cells that were also YFP^{+} are shown in ( ). At each

, ‘, ^{2}: 26 - , mice, demonstrating that post-mitotic OB neurons are themselves , 3-negative (Fig. 6h). We took advantage of this to investigate the rate of arrival and accumulation of SVZ-derived interneurons in the adult OB. We administered tamoxifen to , 3-, ‘, ^{2}: 26 - , mice starting on P50 and analyzed OB sections by immunolabeling for YFP and NeuN 7, 14, 80, or 180 days later (Fig. 6h–j). In addition, we identified interneuron subtypes by immunolabeling for Calretinin, Calbindin, or Tyrosine Hydroxylase (Fig. 6k–m). The proportion of each interneuron subtype that was YFP-labeled increased between P50 + 14 and P50 + 80 and again between P50 + 80 and P50 + 180

mice, we found few if any YFP-labeled reactive astrocytes. These data suggest that reactive astrocytes are formed from preexisting parenchymal astrocytes and/or EZ stem cells but not from -expressing OL precursors/NG2cells.

We used the fact that our 3- 2 transgene is expressed in SVZ stem cells to assess their contribution to adult neurogenesis in the OB. There is a variety of interneuron subtypes in the OB. These include the three major populations of GABAergic interneurons in the periglomerular layer, which can be distinguished by immunolabeling for the calcium binding proteins Calbindin and Calretinin and the dopamine synthesizing enzyme tyrosine hydroxylase. The lifespans and functions of these new interneurons has been a matter of great interest in recent years. We report that newly born neurons (YFP^+, $NeuN^+$) accumulate in the periglomerular and granule neuron layers of the OB for at least 6 months after tamoxifen-induced labeling of the SVZ stem cells. By this time, the adult-born interneurons comprise ~15% of all periglomerular interneurons and ~35% of all granule neurons. These data are con-

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