Supp. Fig.1



Supplementary Figure 1. Neuronal and glial markers expressed in SVZ GAD65-GFP⁺ cells of adult GAD65-GFP mice. (a) Merged confocal images of the anterior subventricular zone (ASVZ) show GAD65-GFP⁺ cells co-labeled with antibodies against Ki67, Mash1, NG2, Nkx2.2, Olig2, Pax6, and Dcx. Dotted lines bound the ASVZ. Scale bar = 500 μ m. (b) The majority of ASVZ GAD65-GFP⁺ cells expressed neuronal markers (Pax6, Dcx). Bar graphs represent mean ± s.e.m. (n = 4 brains per marker). (c) RT-PCR from FACS-purified GAD65-GFP⁺ and GAD65-GFP⁻ cells from ASVZ shows GAD65-GFP⁺ cells expressing neuronal and oligodendrocytic genes. (d) Images of cultured (5 days) FACSsorted GAD65-GFP⁺ cells from ASVZ in bright field (left) and fluorescence (right) microscopy show neuronal morphology of GAD65-GFP⁺ cells. Scale bar = 50 μm. (e) Percentages of different cell types in FACS-purified SVZ GAD65-GFP⁺ cells cultured for 5 days. All GAD65-GFP⁺ cells displayed a neuronal phenotype, as demonstrated by MAP2 immunoreactivity; no Olig2, GalC or GFAP expression was detected (data from 2 independent experiments). ND = not detectable. (f) GAD65-GFP⁺ cells FACS-purified from the adult SVZ and cultured for 5 days were immunostained with anti-MAP2 (red). Scale bar = $20 \mu m$.



Supp. Fig. 2

Supplementary Figure 2. Characterization of GAD65-GFP⁺ neuroblasts in the SVZ. (a–d) Tri-colored images (5dpl) of GAD65-GFP⁺ cells expressing Dcx (in red) together with Pax6 or Dlx2 (in blue) after NaCl (a,c) and LPC injections (b,d) into the corpus callosum. (e) Percentages of GAD65-GFP⁺Dcx⁺ cells co-expressing Pax6 and Dlx2 at 5 dpl. No differences were found in percentages of GAD65-GFP⁺Dcx⁺ neuroblasts that express Pax6 and Dlx2 in demyelinated corpus callosum, compared to NaCl-injected brains. Bar graphs represent mean \pm s.e.m. (n = 4 brains, P < 0.5, *t*-test).





Supplementary Figure 3. GAD65-GFP⁺ cell proliferation increases in corpus callosum after demyelination. Tri-colored confocal images from NaCl- (a,c,e) and LPC-injected (b,d,f) corpus callosum (10 dpl) after intraperitoneal injection of BrdU (every 24 hrs for 5 consecutive days) and double immunostaining with antibodies against BrdU together with anti-Olig2 (a,b), anti-CC1 (c,d), or anti-GFAP (e,f) antibodies. Dotted lines bound corpus callosum. Cells pointed by arrows are magnified in insets. Scale bar = 50 µm.

Supp. Fig.4



Supplementary Figure 4. GAD65-GFP⁺ cells found in demyelinated corpus callosum migrate from the SVZ. Tri-colored images from LPC-injected corpus callosum (a–c). GAD65-GFP⁺ cells were infected with dsRed virus and labeled with anti-Olig2 (a), anti-CC1 (b), or anti-S100 β (c) antibodies at 14 dpl. Inserts show higher magnification of retrovirally labeled GAD65-GFP⁺dsRed⁺ cells expressing Olig2, CC1, and S100 β in demyelinated corpus callosum. Arrows point to GAD65-GFP⁺dsRed⁺Olig2⁺, GAD65-GFP⁺dsRed⁺CC1⁺, or GAD65-GFP⁺dsRed⁺S100 β ⁺ cells in corpus callosum. Scale bar = 50 µm.

Supp. Fig.5



Supplementary Figure 5. Microarray analysis of SVZ tissue from NaCI- and LPC-injected brains. (a1, a2) Gene expression analysis shows upregulation of Crhr2, Cx3cr1, II10r, Stat3, and Tgfb1 after demyelination (4dpl). Corticotropin releasing hormone receptor 2 (Crhr2), Chemokine receptor 1 (Cx3cr1), Growth hormone releasing hormone (Ghrh), Glia maturation factor gamma (Gmfg), Hypocretin (orexin) receptor 2 (Hcrtr2), Interleukin 10 (110), Interleukin 10 receptor beta (*II10r*), Interleukin 1 beta (*II1b*), Interleukin 1 receptor, type I (*II1r1*), Nuclear receptor coactivator 1 (*Ncoa1*), Neuropeptide Y receptor Y1 (*Npy1r*), Neuregulin 4 (*Nrg4*), Signal transducer and activator of transcription 1 (*STAT1*), Signal transducer and activator of transcription 3 (STAT3), Transforming growth factor beta 1 induced transcript 1 (*Tgfb1*). (b) Western blot analysis showed upregulation of chordin after demyelination (LPC), compared to NaCl-injected tissue (NaCl; representative samples - 4 independent corpus callosum samples were analyzed in each group). (c) RT-PCR revealed decreased BMP4, and higher expression of chordin, noggin, and ChorR in SVZ after LPC injection into corpus callosum (4 independent corpus callosum samples analyzed per group).







Supplementary Figure 6. Culture homogeneity of Dcx-GFP cells. Images of FACS-purified Dcx-GFP⁺ cells immunostained with anti-MAP2 (**a**–**d**) and anti-Olig2 (**e**–**h**) antibodies at 24hr in culture. (**i**) Bar graph represents percentages of Dcx-GFP⁺ cells expressing MAP2 and Olig2. The Dcx-GFP⁺ cell population expresses the neuronal marker MAP2 (99.5%). Bar graph represents means \pm s.e.m. (n = 3 independent cultures, ****P* < 0.001, *t*-test)