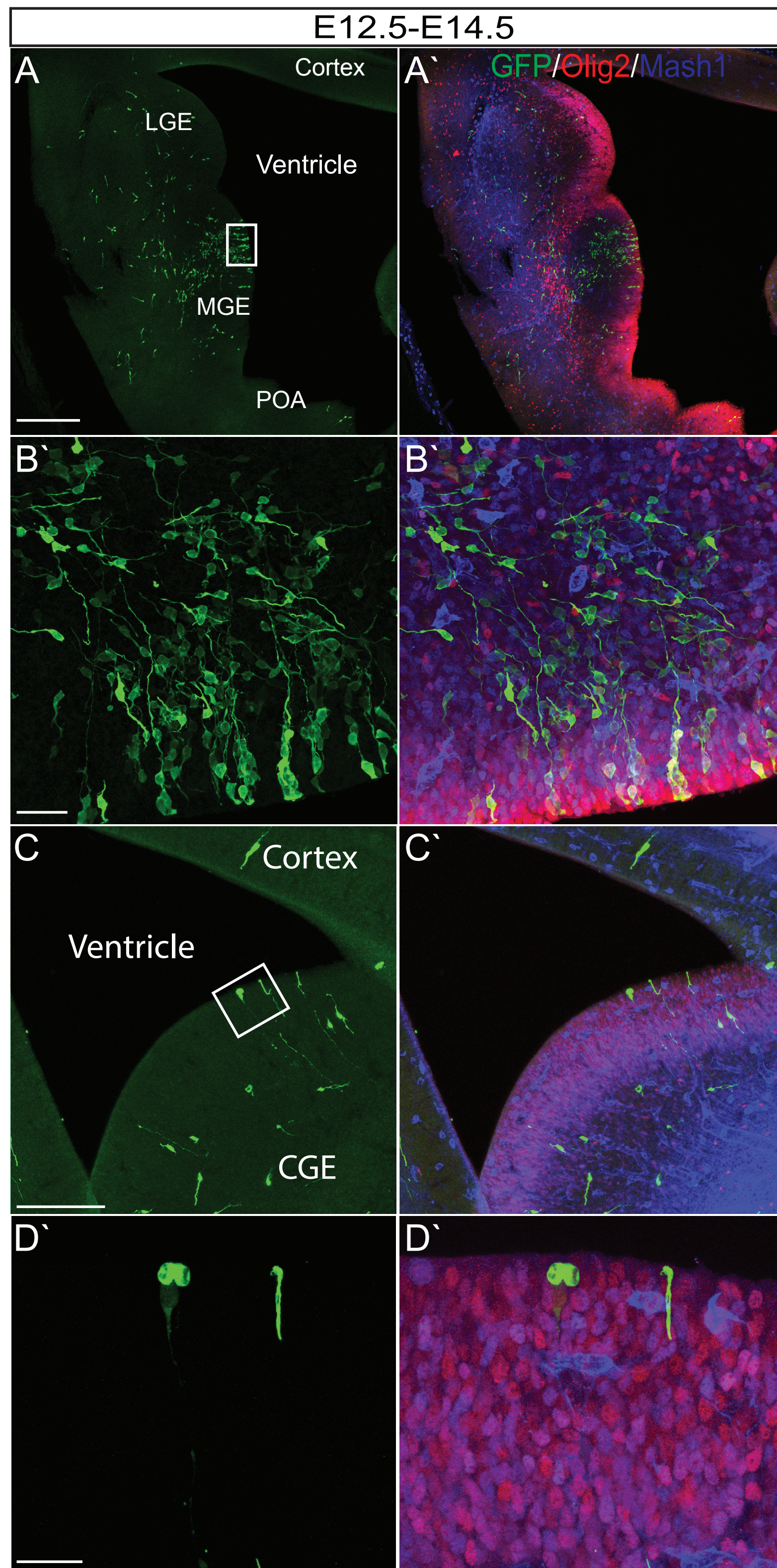
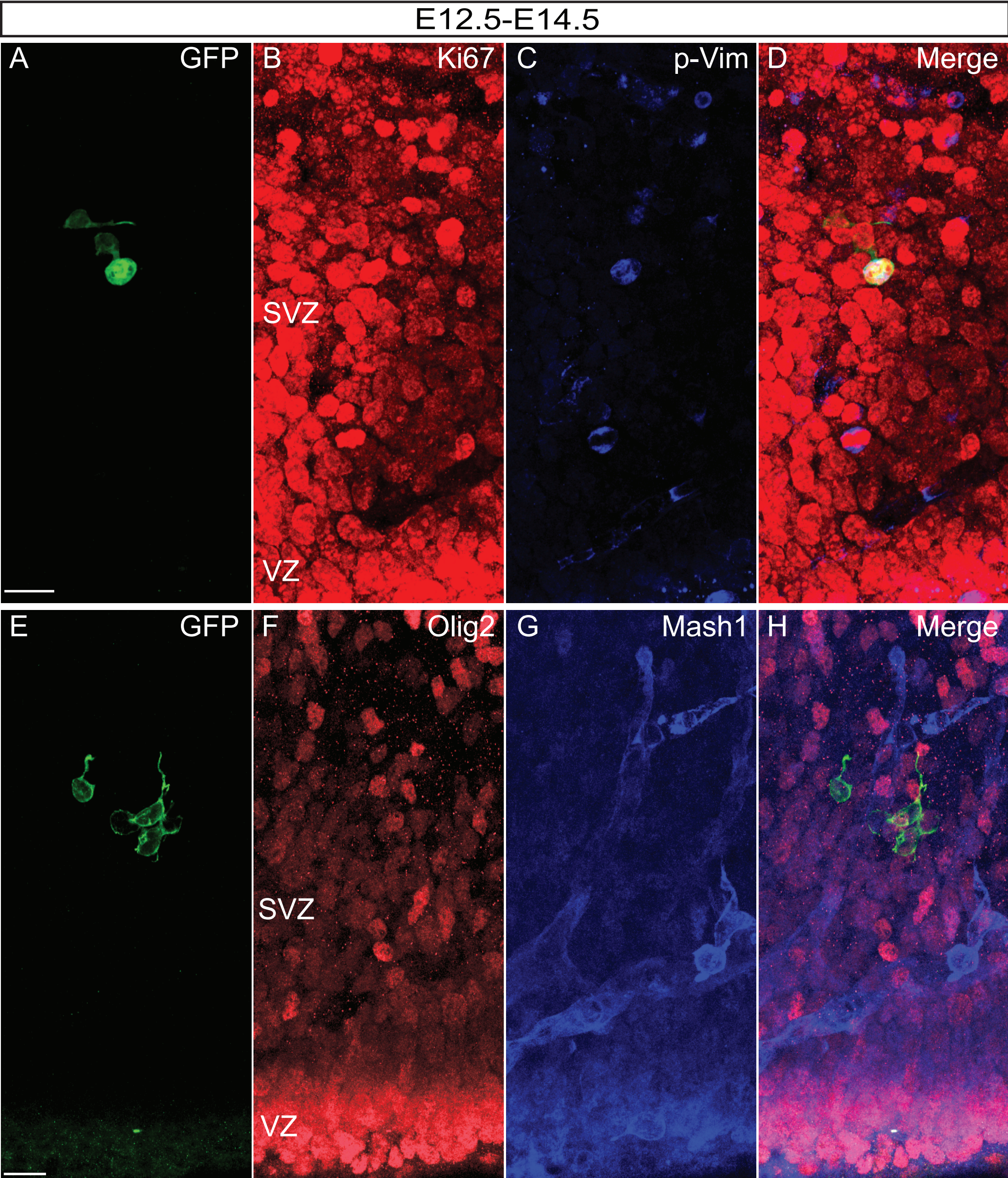
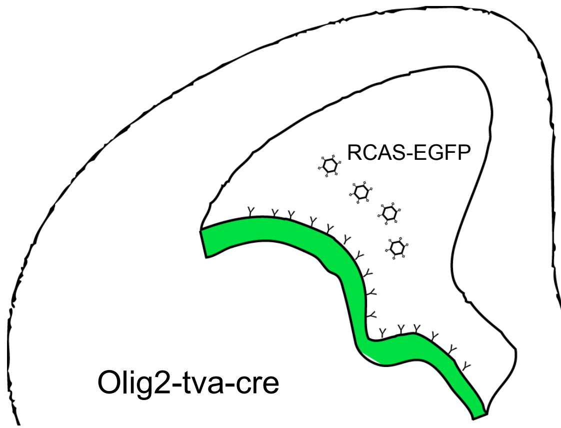
Harwell et al. Figure S1

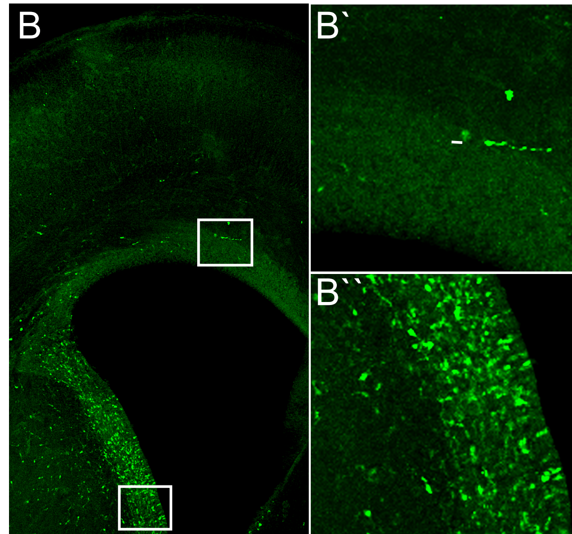


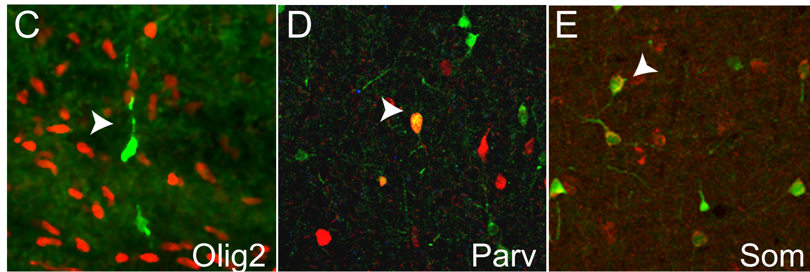
Harwell et al. Figure S2

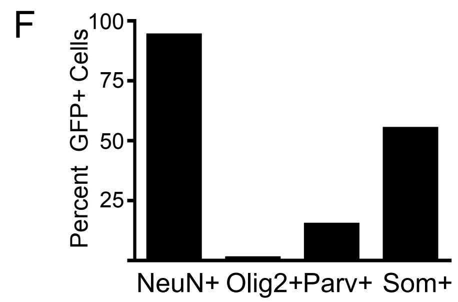


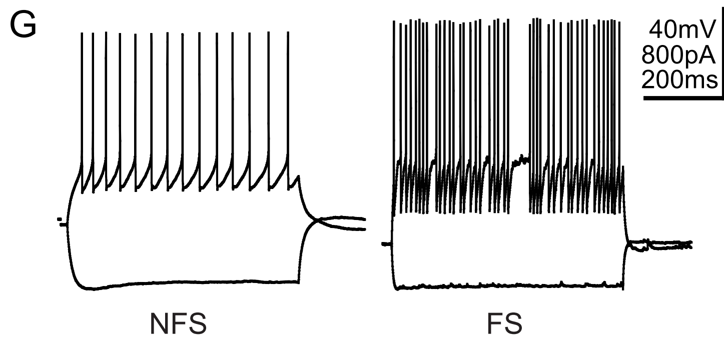
Harwell et al. Figure S3

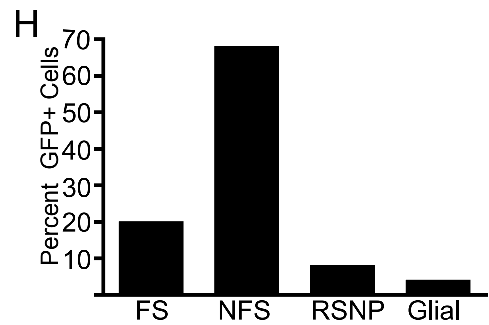




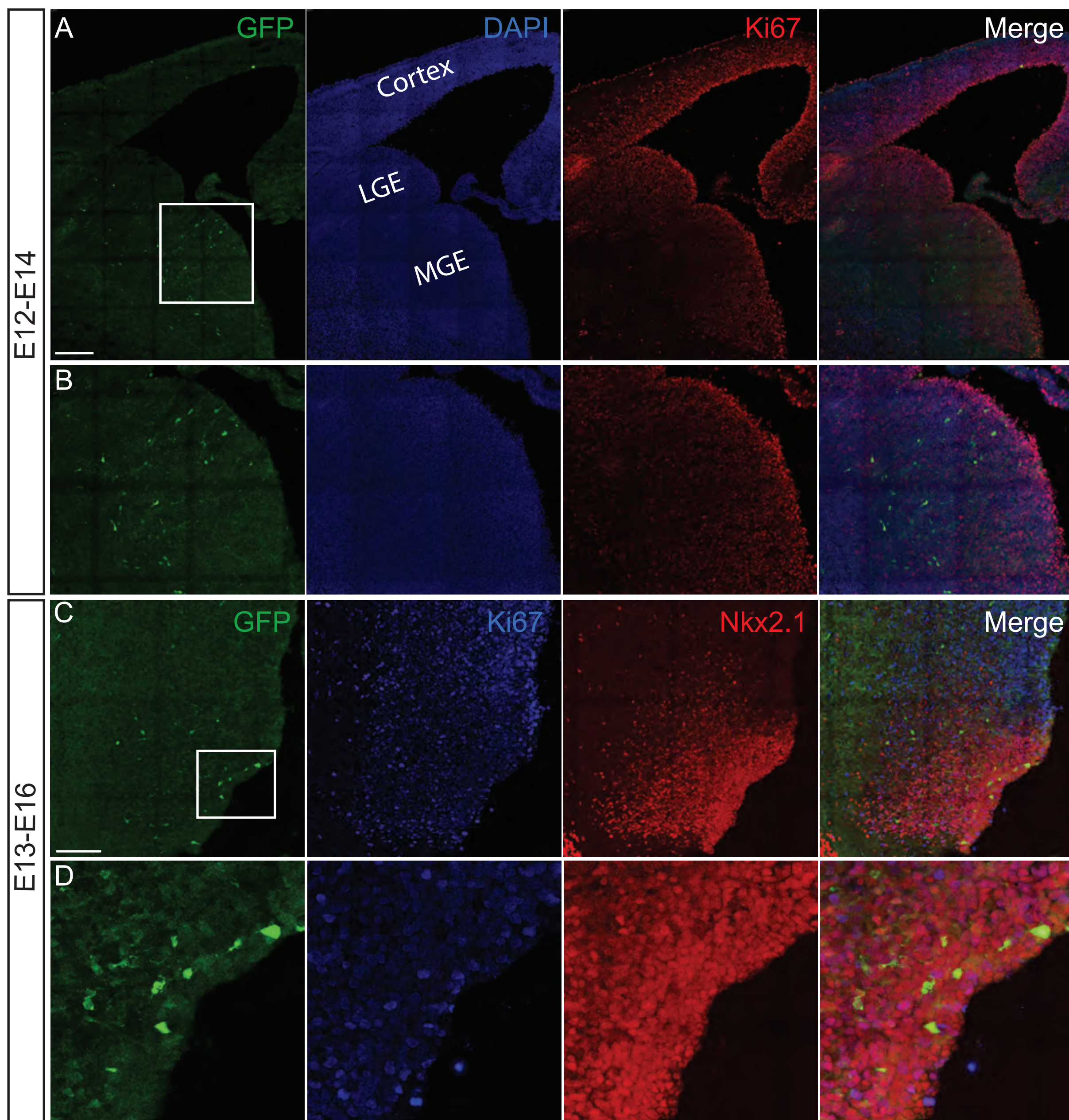








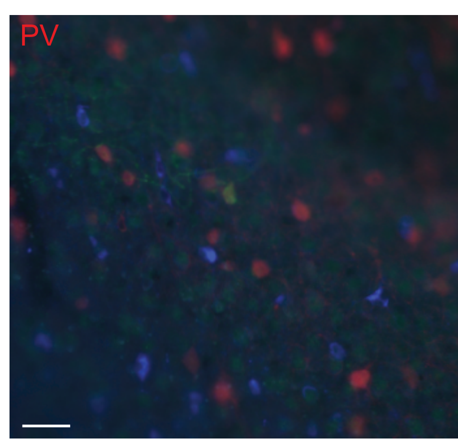
Harwell et al. Figure S4

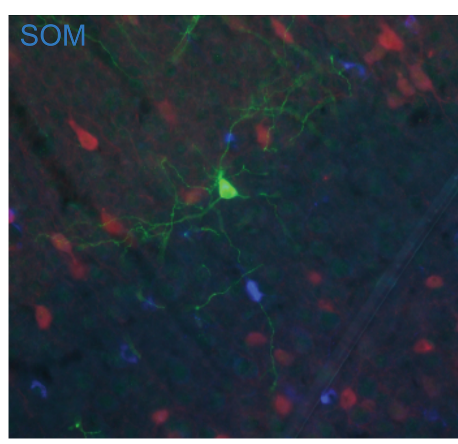


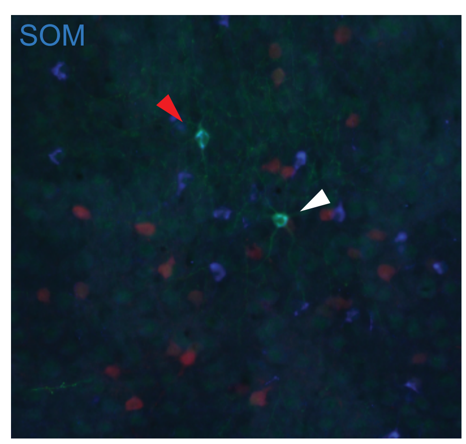
Harwell et al. Figure S5

A

B







Supplementary Figure Legends

Figure S1

Related to Figure 1. Retroviral Infection of Ventral Telencephalic Progenitors

(A) Radial arrays of MGE progenitors 48h after injection of EGFP retrovirus. The majority of EGFP labeled progenitors co-label with antibodies against bHLH transcription factors Olig2 (red) and Mash1 (blue). (B) Zoomed image of progenitors located inside white boxes from panel (C) GFP labeled clones in both dorsal (cortex) and ventral (CGE) parts of the developing telencephalon. Ventral progenitors can be distinguished by the boundaries of labeling of Olig2 (red) and Mash1 (blue). (D) Olig2 positive Mash1 negative mitotic progenitor at the floor of the ventricle. CGE: Caudal Ganglionic Eminence, MGE: Medial Ganglionic Eminence, POA: Preoptic Area. Scale bars: 150μm (A-E), 50μm (F)

Figure S2

Related to Figure 1. Retroviral Infection of Intermediate Progenitors in the SVZ of the Ganglionic Eminence

(A) In the background a pair of EGFP labeled newborn neurons, and in the foreground is an intermediate progenitor cell in M-phase, evidenced by morphology and positive labeling for proliferative cell markers (B) Ki67 (red) and (C) phospho-vimentin (blue), (D) merged image. The neuron pairs are the likely progeny of a symmetric proliferative division of intermediate progenitor cells. Scale bars: 50μm

Figure S3

Related to Figure 2. Olig2-tva-cre Allows for Selective Infection of Progenitors of the Ganglionic Eminences

(A) Diagram of the region of Olig2-tva-cre expression (green) and *in utero* intraventricular injection of RCAS-EGFP viral particles infecting progenitors of the ventral telencephalon. (B) Coronal brain section of Olig2-tva-cre mouse 72 h

after intraventricular injection with RCAS-EGFP. The upper box shows an absence of GFP-labeled progenitors, with only a small number of migratory cells in the dorsal telencephalic progenitor zones (magnified in B`), while the lower box shows an abundance of GFP-labeled cells in the ventral telencephalon (magnified in B``). (C) Migratory neurons entering the cortex no longer express Olig2 (red). (D and E) Examination of P28 neurons shows that the majority of GFP-labeled neurons co-label with the cortical interneuron subtype markers parvalbumin (Parv) or somatostatin (Som). (F) Quantification of the percentage of GFP-positive cells in P28 Olig2-tva-cre mice that co-label with the neuronal marker NeuN, Olig2, parvalbumin or somatostatin. (G) Example electrophysiology traces from recorded GFP-positive neurons, representing the two major subtypes (FS and NFS) of cortical interneurons. (H) Quantification of the major electrophysiological subtypes that were recorded from GFP-labeled cells in P28 Olig2-tva-cre mice that were injected with RCAS-EGFP at E12.

Figure S4

Related to Figure 2. Nkx2.1cre;LSL-tva Allows for Selective Infection of MGE Progenitors

(A) Coronal section of E14 Nkx2.1;LSL-tva mouse brain 48h after infection with pseudotyped GFP retrovirus. All GFP positive cells are located in the progenitor zones (Ki67 positive), or mantle zone of the MGE. (B) Zoomed in region of the MGE showing GFP positive cells exclusively in the MGE. (C) Ventral telencephalic progenitor zone of E16 Nkx2.1;LSL-tva mouse brain 72h after infection where GFP cells positive for Ki67 (blue) are located in the Nkx2.1 (red) positive region of the ventral telencephalon. Scale bars: 150μm.

Figure S5

Related to Figure 4. Wide Dispersion and Diversity of Cortical Clones

(A and B)Representative schematic of a widely dispersed three-cell cortical clone containing two somatostatin (SOM) (blue) positive neurons and a single parvalbumin (PV) (red) positive cell. (B) Note in the third panel an unrelated SOM cell (red arrowhead) located next to the cell that is a part of the multi-cell clone (white arrowhead).

Supplemental Movie

Symmetric Divisions of IPCs Followed by Rapid Migration of Progeny. Related to Figure 1.

This movie shows and example of subventricular zone intermediate progenitor first undergoing symmetric proliferative division and then each cell dividing a second time to produce two pairs of neurons which then migrate rapidly out of the field of view. Images were acquired every 20min. White and red arrowheads denote the two daughters of the first IPC division, and additional arrowheads mark the progeny of the second set of divisions. White and red bars appear in frames where IPC divisions are taking place.

Distribution of Clonal Siblings and Unrelated Cells. Related to Figure 3.

Summary table showing the clonal lineage, forebrain location, cell type identity and Euclidean coordinates of cells collected from brain QCOL4, as described in Figure 4D.

Retrovirus Production and In utero Injections

Replication-incompetent EGFP-expressing retrovirus was produced from a stably transfected packaging cell line (293gp NIT-GFP). RCAS-EGFP plasmid, and DF-1 chicken cell line were were utilized to produce RCAS-EGFP virus. Uterine horns of E11.5 - E13.5 gestation stage pregnant swiss webster mice (Charles River Laboratories) were exposed in a clean environment. Retrovirus (~1.0 µl) with Fast green (2.5 mg ml^{-1}, Sigma) was injected into the embryonic cerebral ventricle through a bevelled, calibrated glass micropipette (Drummond Scientific). After injection, the uterine horns were replaced, and the wound was closed.

Mouse Lines

Timed pregnant (E1 day of plug) Swiss Webster mice were obtained from Charles River Laboratories. Animals were maintained according to protocols approved by the Institutional Animal Care and Use Committee at the University of California at San Francisco.

Brain sectioning, cortical slice culture, viral infection on slice and time-lapse imaging

Brains were dissected out into ice-cold artificial cerebro-spinal fluid (ACSF) containing (in mM): 125 NaCl, 5 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 2 $CaCl_2$, 25 $NaHCO_3$ and 20 glucose; pH 7.4, 310 mOsm/L. Brains were embedded in 4% low-melting agarose in ACSF and sectioned at 300 µm using a vibratome (Leica microsystems). Brain slices that were transferred onto a slice culture insert (Millicell) in a glass-bottom petri dish (MatTek Corporation) with culture medium containing (by volume): 66% BME, 25% Hanks, 5% FBS, 1% N-2, 1% Penicillin/Streptomycin/Glutamine (all from Gibco) and 0.66% D-(+)-glucose (Sigma). Cultures were maintained in a humidified incubator at 37°C with constant 5% CO_2 supply. All the time-lapse images were acquired using an inverted Leica TCS SP5 with an on-stage incubator (while streaming 5% CO_2, 95% O_2) and a 40X objective lens.

Immunohistochemistry and confocal imaging

Mouse brain slices were fixed in 4% PFA in PBS at 4°C overnight. Incubated for one hour at room temperature in a blocking solution (10% normal goat or donkey serum as appropriate, 0.1% Triton X-100, and 0.2% gelatin in PBS), followed by incubation with the primary antibodies 3 days at 4°C. Sections were then washed in 0.1% Triton X-100 in PBS and incubated with the appropriate secondary antibody for one to two hours at room temperature.

The primary antibodies used were: rabbit anti-olig2 (Millipore, 1:500) mouse anti-phospho-vimentin (MBL International D076-3s (Ser55), or D095-s (Ser82), 1:500); chicken anti-GFP (Aves Labs 1:500); Rabbit anti-Calretinin (Millipore, 1:1000); Rabbit anti-somatostatin (Millipore, 1:750); Mouse anti-parvalbumin (Millipore 1:1000). Secondary antibodies used were: Alexafluor 488 (1:1000), 546 (1:500), or 647 (1:500) conjugated donkey anti-mouse, anti-rabbit or goat (Invitrogen). DNA was stained with 4',6-diamidino-2-phenylindole (DAPI) (Molecular Probes). Images were acquired with a Leica TCS SP5 broadband laser confocal microscope, and analyzed with LCS Leica confocal software (Leica), Imaris imaging software (Bitplane) and Photoshop (Adobe Systems).

Slice preparation and recording

Coronal brain slices containing somatosensory cortex were cut 300μm thick from P21 to P32 mice of the C57/BL6 strain. Slices were incubated at 32°C for 30 min after slicing, then held at room temperature until being moved to a 32°C submersion-type chamber for recordings. The bathing solution contained (in mM) 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 10 dextrose, and 2 $CaCl_2$, saturated with 95% O_2/5% CO_2. Whole cell current clamp recordings were obtained from pairs of GFP+ or mCherry+ neurons in layers II-IV of somatosensory cortex. Cells were visualized with infrared-differential interference contrast microscopy using an Olympus BX51WI microscope and a CCD camera (Retiga Exi). Patch pipettes were made from 1.5 mm OD/0.86 mm ID glass (Sutter) and filled with (in mM) 130 potassium gluconate, 4 KCl, 2 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, 14 phosphocreatine-Tris (pH 7.25,

280–290 mOsm). Recordings were performed with a MultiClamp 700B amplifier (Molecular Devices). Series resistances were typically between 15 and 30 MΩ and were continually monitored and compensated throughout the recording sessions. Both series resistance and electrode capacitance were corrected conventionally using the built-in circuitry of the amplifiers. Recordings were acquired and analyzed using pClamp version 10 (Molecular Devices) and data were further analyzed in Excel (Microsoft). All averages reported appear as mean ± SE.