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Cellular Composition and Organization of the Subventricular Zone and Rostral Migratory Stream in the Adult and Neonatal Common Marmoset Brain

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Abstract

The adult subventricular zone (SVZ) of the lateral ventricle contains neural stem cells. In rodents, these cells generate neuroblasts that migrate as chains toward the olfactory bulb along the rostral migratory stream (RMS). The neural-stem-cell niche at the ventricular wall is conserved in various animal species, including primates. However, it is unclear how the SVZ and RMS organization in nonhuman primates relates to that of rodents and humans. Here we studied the SVZ and RMS of the adult and neonatal common marmoset (*Callithrix jacchus*), a New World primate used widely in neuroscience, by electron microscopy, and immunohistochemical detection of cell-type-specific markers. The marmoset SVZ contained cells similar to type B, C, and A cells of the rodent SVZ in their marker expression and morphology. The adult marmoset SVZ had a three-layer organization,

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as in the human brain, with ependymal, hypocellular, and astro-cyte-ribbon layers. However, the hypocellular layer was very thin or absent in the adult-anterior and neonatal SVZ. Anti-PSA-NCAM staining of the anterior SVZ in whole-mount ventricular wall preparations of adult marmosets revealed an extensive network of elongated cell aggregates similar to the neuroblast chains in rodents. Time-lapse recordings of marmoset SVZ explants cultured in Matrigel showed the neuroblasts migrating in chains, like rodent type A cells. These results suggest that some features of neurogenesis and neuronal migration in the SVZ are common to marmosets, humans, and rodents. This basic description of the adult and neonatal marmoset SVZ will be useful for future studies on adult neurogenesis in primates.

INDEXING TERMS

common marmoset; subventricular zone; rostral migratory stream

Neurogenesis mostly occurs during development. However, neurons are also generated in the mammalian brain throughout life, as demonstrated in rodents to humans (Altman, 1969; Eriksson et al., 1998; Kornack and Rakic, 2001; reviewed by Okano and Sawamoto, 2008). In adult rodents, neurogenesis has been described in two fore-brain regions: the subventricular zone (SVZ) of the lateral ventricles (Altman and Das, 1965; Bayer, 1983) and the subgranular layer of the dentate gyrus (DG) of the hippocampus (Altman and Das, 1965; Bayer et al., 1982).

The largest neurogenic region in the adult mammalian brain is the SVZ, where astrocytes or type B cells are the stem cells that give rise to neuroblasts (type A cells) through a transit amplifying cell type, the type C cells (Doetsch et al., 1999a). These newly generated cells migrate toward the olfactory bulb (OB) in chains ensheathed by astrocytes, forming the rostral migratory stream (RMS). After reaching the OB, the neuroblasts differentiate into local interneurons (Lois and Alvarez-Buylla, 1994; Doetsch et al., 1997). The cytoarchitecture and composition of the adult rodent SVZ and RMS have been well characterized at the ultrastructural level (Doetsch et al., 1997). The SVZ is largely conserved in various mammalian species, including the cow (Perez-Martin et al., 2003) and rabbit (Luzzati et al., 2003), albeit with significant differences from the rodent SVZ.

The human SVZ has been studied at the ultrastructural level, and its organization differs from that of rodents. In the human SVZ, a population of proliferating multipotent astrocytes forms a ribbon that is separated from the ependyma by a gap or hypocellular layer (Sanai et al., 2004). This composition of layers is similar to that found in *Macaca fascicularis* (Gil-Perotin et al., 2009). A region corresponding to the RMS has been identified in the adult human brain (Sanai et al., 2004; Curtis et al., 2007), but whether it is a conduit for neuronal migration in adults remains controversial (Sanai et al., 2007).

In adult nonhuman primates, proliferating cells are present in the SVZ and hippocampus, but this proliferation declines with age (Leuner et al., 2007). Neurogenesis has also been shown in the DG of the adult macaque (Gould et al., 1999; Kornack and Rakic, 1999). The adult nonhuman primate SVZ also contains neuronal progenitors whose progeny migrate toward the OB (Kornack and Rakic, 1999). In the macaque monkey, new cells migrate more than 2

cm by forming chains along the olfactory peduncle, and they differentiate into OB granule inter-neurons. The RMS organization in the primate forebrain appears similar to that in rodents (Kornack and Rakic, 2001; Pencea et al., 2001; Gil-Perotin et al., 2009). However, there are technical limitations associated with the use of macaque monkeys for studying adult neurogenesis, which include their restricted availability, slow sexual maturation, and large body size.

The New World primate *Callithrix jacchus* (common marmoset) is small (300–500 g at maturity) and easy to maintain and breed in an animal facility. More important, because common marmosets can be bred in experimental colonies their supply is stable and reliable, with adequate genetic and microbiological control to minimize biases. Several neurological disease models have been created in the marmoset monkey (Mansfield, 2003). Moreover, we recently reported the successful creation of transgenic marmosets (Sasaki et al., 2009), indicating that this primate model will be valuable to many fields of biomedical research, including that of adult neurogenesis.

The aims of this study were to describe the organization of the SVZ and RMS in the marmoset brain and to analyze how it relates to that of rodents and humans. For this purpose we studied the forebrain of adult marmosets using electron microscopy (EM) and the immunohisto-chemical detection of cell-type-specific markers. We found the marmoset SVZ to have a very similar organization to that of humans, and identified a sparse migration of neuroblasts toward the OB. To investigate the migration of newborn neurons we studied the neonatal marmoset, which exhibited greater cell proliferation.

MATERIALS AND METHODS

Animals

Common marmosets reach sexual maturity at 12–18 months after birth. Adult (250–450 g; older than 31 months) and neonatal (0–1-day-old) marmosets obtained from Clea (Japan) were used in this study. Animal care procedures were performed in accordance with the Laboratory Animal Welfare Act, the *Guidelines for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD), and the Guidelines and Policies for Animal Surgery provided by the Animal Study Committee of the Central Institute for Experimental Animals, Keio University and Nagoya City University.

Tissue processing

Adult and neonatal marmosets were deeply anesthetized with Nembutal and perfused transcardially with 0.9% saline, followed by 100 mL of Karnovsky's fixative (2% paraformaldehyde [PFA] and 2.5% glutaraldehyde) for conventional EM, 100 mL of 4% PFA and 0.1% glutaraldehyde for immunoelectron microscopy, or 100 mL of 4% PFA for immunohistochemistry. The heads were removed and postfixed in the same fixative overnight. The brain was removed from the skull and washed in 0.1 M phosphate buffer (PB, pH = 7.4) for 2 hours.

We subdivided the lateral ventricle in both neonates and adults into three rostrocaudal levels (Fig. 1F): anterior, medial, and posterior, which correspond to 12.5 mm, 11.5 mm, and 10

mm, respectively, anterior to the line passing through the bilateral center of the external auditory meati (Stephan, 1980). We studied the SVZ cell organization in each level along its dorsoventral extent. We used four adults and two neonates for EM, and at least three animals for each immunohistochemical staining experiment.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) experiments were performed using a 7 T MRI unit (PharmaScan 70/16; Bruker Biospin, Ettlingen, Germany), in a horizontal bore magnet equipped with 300 mT/m actively shielded gradient coils. Two adult marmosets were used for brain imaging. One was a sacrificed animal used to depict the lateral ventricles in fine detail by long-term acquisition, and the other was an anesthetized animal used to depict the lateral ventricles in the living state. The head of the anesthetized marmoset was firmly fixed in a specially designed acrylic head positioner and placed over the center of the integrated radiofrequency (RF) transmitting and receiving coil (62-mm inner diameter, Bruker Biospin) in the magnet bore. For the postmortem MRI, one monkey under deep anesthesia (pentobarbital sodium, 100 mg/kg, intravenous [iv]) was transcardially perfused with phosphate-buffered saline (PBS) and then with 4% PFA. The brain was carefully removed and postfixed in 4% formalin. After sufficient time for external fixation the brain was placed into a 34-mm inner diameter plastic tube filled with formalin and the tube was placed over the center of the RF coil (38-mm inner diameter, Bruker Biospin).

The 3D shape of the lateral ventricle in the postmortem marmoset brain was verified on volume-rendering images that were analyzed and reconstructed using Amira software (v. 5.2, Mercury Computer Systems, Houston, TX) from a high-resolution 3D T₂-weighted volumetric dataset obtained with the following acquisition parameters: 3D fast spin-echo MR sequence, TR/TE = 1000/16 ms, field of view of 40 × 40 × 20 mm³, matrix = 512 × 512 × 256, producing an isotropic resolution of 78 μm.

To analyze the dorsolateral extension of the lateral ventricle, we performed high-resolution 2D T₂-weighted coronal imaging in which the first slice was positioned at the anterior horn of the lateral ventricle of the living marmoset brain, with a 2D-fast spin-echo MR sequence. (TR/TE = 10,000/100 ms, field of view of 44 × 44 mm², matrix = 320 × 320, producing an in-plane resolution of 137 μm, slice thickness = 1.2 mm).

BrdU administration

Monkeys received an intraperitoneal (i.p.) injection of 4-bromo-2'-deoxyuridine (BrdU) (50 μg/g body weight). Two hours or 1 month after the injection the monkeys were perfused transcardially with saline followed by 4% PFA. The heads were removed and postfixed in the same fixative overnight. The 2-hour timepoint was used to investigate the location of proliferating cells in the brain, since 2 hours is sufficient for BrdU uptake into cells in S phase but not for the completion of mitosis or migration (Gould et al., 1999). The 1-month timepoint was used to investigate the migration and differentiation of the newly born cells. In the squirrel monkey, a New World primate, newly born cells can differentiate into mature neurons within a 5-week period (Bedard et al., 2002).

Immunohistochemistry

Sections (50- μ m thick) were cut on a vibratome and incubated for 1 hour in blocking solution (10% donkey serum and 0.5% Triton X-100 in PBS) and then overnight with the primary antibodies. After being washed the sections were incubated with a biotinylated secondary antibody (1:500) or an Alexa Fluor-conjugated secondary antibody (1:300; Invitrogen, La Jolla, CA). The biotinylated antibodies were visualized using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). The nuclei were stained with Hoechst. Images were obtained using an Axioplan2 and a confocal laser microscope LSM510 (Zeiss, Germany). We adjusted the contrast and brightness of our images using Photoshop (Adobe Systems, San Jose, CA) imaging software.

Primary antibody characterization

Table 1 lists all the primary antibodies used.

The anti-PSA-NCAM antibody recognizes the PSA portion of the high-molecular-weight neural cell adhesion molecule on western blots of the P6 rat cerebral cortex (Seki and Arai, 1991). It stains immature neurons in embryonic and postnatal rat brains (Seki and Arai, 1991). Endo N, a PSA-specific endoneuraminidase, abolishes the immunostaining by this antibody in the hippocampus (Seki and Rutishauser, 1998). This antibody stained cells with the morphology of immature neurons in our study. We obtained a similar staining pattern with antibodies against Doublecortin (Dcx) and β III-tubulin.

The anti-Dcx antibody detects a single band at 40 kDa on western blots of the adult rat OB (Brown et al., 2003) and is a marker of migrating neurons. It stains young neurons in the SVZ and RMS (Nacher et al., 2001). We obtained a similar staining pattern with antibodies against PSA-NCAM and β III-tubulin in the SVZ and RMS of marmoset.

The anti-glial fibrillary acidic protein (GFAP) antibody recognizes the glial fibrillary acidic protein of 50 kDa expressed in astrocytes (Debus et al., 1983). In this study, this antibody stained cells with the morphology and distribution expected for astrocytes (Yang et al., 2007).

Immunohistochemistry using the Pan-Dlx (Distal-less) antibody in invertebrate tissues shows expression patterns that are indistinguishable from the RNA expression patterns (Panganiban et al., 1995). Immunohistochemistry using vertebrate tissues shows expression patterns that are indistinguishable from the sum of the Dlx1, 2, 5, and 6 mRNA expression patterns (Stuhmer et al., 2002a,b). Colabeling experiments using anti-Dlx2 and anti-pan-Dlx antibodies in the mouse brain showed that all the Dlx2+ cells are also pan-Dlx+ (Zhao et al., 2008). The pan-Dlx-positive cells observed in this study were similar to those reported previously (Sakaguchi et al., 2006).

The anti- β III-tubulin antibody detects a single band at 46 kDa on western blots (manufacturer's technical information) and is a marker for immature neurons (Lee et al., 1990). This antibody stained cells with the morphology of immature neurons in our study.

The anti-Mash1 antibody detects a specific 34-kDa band on western blots of lysates from rat embryonic brain (manufacturer's technical information). Mash1 is expressed in neuronal progenitor cells (Parras et al., 2004). The Mash1-positive cells observed in this study were similar to those reported previously in the mouse SVZ (Kohwi et al., 2005; Sakaguchi et al., 2006).

The anti-BrdU antibody reacts with free BrdU, BrdU in single-stranded DNA, or BrdU attached to a carrier protein (manufacturer's technical information). No staining was observed when we used the antibody to stain tissue from non-BrdU-injected specimens (data not shown).

The anti-NeuN antibody recognizes two or three bands in the 46–48-kD range and another band at 66 kD in western blots of the rat brain (manufacturer's technical information). This antibody reacts with the RNA-binding, neuron-specific protein NeuN (Kim et al., 2009), which is present in most central nervous system (CNS) and peripheral nervous system (PNS) neuronal cells. This antibody shows broad reactivity across species, including primates (manufacturer's data sheet). In this study, this antibody labeled cells with the same morphology and distribution in the marmoset DG as reported previously (Leuner et al., 2007).

Electron microscopy

Transverse 200- μ m brain sections were cut on a vibratome, postfixed in 2% osmium for 2 hours, rinsed, dehydrated, and embedded in araldite (Durcupan; Fluka, Buchs, Switzerland). The organization of the different regions (SVZ, RMS, olfactory tract [OT], and OB) was studied on 1.5- μ m semithin sections, which were cut with a diamond knife and stained with 1% toluidine blue. We analyzed 10 consecutive semithin sections obtained from at least one tissue block of each animal and region of interest. To identify individual cell types, ultrathin (70 nm) sections were cut with a diamond knife, stained with lead citrate, and examined under a transmission electron microscope (Tecnai Spirit G2, FEI, OR) using a digital camera (Morada, Soft Imaging System, Olympus, Japan). At least three ultrathin sections cut from each of these blocks were studied. Some SVZ levels were larger than the grid, so separate ultrathin sections were cut for the dorsal and ventral areas. For 3D reconstructions, consecutive ultrathin sections from the intermediate area from the anterior SVZ region were analyzed. Usually 10 grids, containing an average of 30 ultrathin sections each were analyzed. For immunoelectron microscopy, 50- μ m-thick sections were incubated for 1 hour in blocking solution (10% donkey serum and 0.5% Triton X-100 in PBS) and overnight with the primary antibodies. The sections were washed and antibody staining was revealed with 0.02% diaminobenzidine (DAB) and 0.01% H₂O₂. Immunostained sections were postfixed in 1% osmium in 0.1 M PB, dehydrated, and embedded in araldite. Semithin sections (1.5 μ m) were cut with a diamond knife and stained with 1% toluidine blue, reembedded for ultrathin sectioning, and examined under the electron microscope.

Cell counts

The number of cells corresponding to the different cell types along the ventricular wall of the anterior horn of the adult monkey ($n = 2$) was quantified in specific regions of ultrathin

sections under the electron microscope. Anterior, medial, and posterior regions of the SVZ of the lateral ventricle were included in the analysis. Cells with only small fragments of cytoplasm or nucleus in a given section were classified as unidentified. For all quantifications, the experiments were performed blind as to the location of the specimen in the SVZ. For cell counts, type A cells were identified by their small size, scanty and electron-dense cytoplasm, and fusiform appearance; type B cells were identified as large, less electron-dense cells, rich in intermediate filaments; and putative precursors were characterized by the presence of a large, irregular nucleus and a lack of intermediate filaments. The different cell types in the adult SVZ were scored as the percentage of each cell type over the total number of cells counted in each SVZ region, as described previously (Doetsch et al., 1997). Cells were counted on one ultrathin section selected from the 2–3 contained in a grid. We performed the cell counting in the intermediate part of the SVZ. We counted at least three ultrathin sections for each region in two different animals.

Matrigel culture

The SVZ was dissected from the neonatal brain in L-15 medium (Invitrogen), cut into small pieces (300–500 μm diameter), and embedded in a 3:1 Matrigel (BD Biosciences, San Jose, CA)/L-15 mixture. The SVZ explants were cultured in Neurobasal medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 2% B-27 (Invitrogen), and 50 U/mL penicillin–streptomycin, at 37°C in a 5% CO₂ incubator. Forty-eight hours later the explants were fixed and processed for immuno-staining and nuclear staining by the same protocol used for the sections, except that the explants were fixed for 30 minutes at room temperature. Images were obtained using an Axiovert100 (Zeiss). Live imaging of migrating cells was performed using an IX-70 inverted microscope (Olympus) equipped with a CZI-3 incubation chamber (Zeiss) and an ORCA-ER digital camera (Hamamatsu). Phase-contrast images were obtained every 20 seconds and were analyzed with Metamorph software (Micro-BrightField, Colchester, VT). To determine the speed of individual cells in the migrating chain, the position of the cell body of 11 cells was recorded at 1-minute intervals using a Multi Track J plug-in bundled with the ImageJ shareware program (NIH). The average speed of the cells was determined. Cells engaged in chain migration show moving and resting phases (Wichterle et al., 1997). To determine the average speed in the active moving phase we only calculated speeds from the change in cell position for the 1-minute intervals in which the cell position was significantly altered with respect to a stationary point, as shown in the movie (average + 2 $\times$ SD = 85.7 $\mu\text{m}/\text{h}$).

RESULTS

We studied the cytoarchitecture and cellular characteristics of the SVZ and RMS in the marmoset at two developmental stages: adult (older than 31 months) and neonate (0–1-day-old). We found that the marmoset SVZ was organized into three defined layers: the ependymal, hypocellular gap, and astrocyte ribbon layers (see Fig. 6A), as described for the human brain (Sanai et al., 2004). This organization into three well-defined layers was clearly observed in the posterior SVZ. However, the gap layer was very thin or absent in the neonatal SVZ and in the adult anterior SVZ, which showed instead groups of migrating neuroblasts under the ependymal lining. The medial wall of the lateral ventricles facing the

septum did not contain an SVZ; instead, it was lined by a single layer of cubical ependymal cells next to a layer of astrocytes.

Adult

Marmoset ventricular system—To visualize the morphology of the lateral ventricles in the marmoset brain, dorsal (Fig. 1A) and lateral (Fig. 1B, F) views of the 3D shape of the lateral ventricle (blue) were reconstructed from serial MRI images of an adult postmortem marmoset brain. C-shaped cavities located in both hemispheres were clearly observed. To understand the organization of the ventricular system, high-resolution 2D T2-weighted coronal imaging, with the first slice positioned at the anterior horn of the lateral ventricle in the adult living marmoset brain, was performed using a 2D-fast spin echo MR sequence. At the posterior levels, a small space between the corpus callosum and the striatum at the lateral telencephalon contained cerebrospinal fluid and was continuous with the lateral ventricles (Fig. 1C–E). We did not observe an open olfactory ventricle.

The lateral ventricle was studied at three rostrocaudal levels, anterior, medial, and posterior (Fig. 1F), and the organization of the SVZ was examined along its dorsoventral extent at each level.

Light microscopy: histology and immunohistochemistry—The rostral ventricular surface was smooth, and the ventral and posterior ventricular surfaces were more irregular and sporadically invaginated. We frequently observed displaced ependymal cells, far away from the ventricle. Displaced ependymal cells were identified as cell groups surrounding a toluidine blue-stained diffuse material. After examining ultrathin sections made by reembedding and resectioning these samples, we identified this diffuse material as microvilli and cilia from ependymal cells. The ventricular cavity was lined by a monolayer of ependymal cells. However, occasional clusters of small cells were observed that were irregularly dispersed and predominantly anterior; they tended to disappear in the posterior interdigitated regions.

To identify neuroblasts in the adult SVZ, we stained coronal brain sections with an anti-PSA-NCAM antibody (Fig. 2A–F). In the anterior SVZ, PSA-NCAM-positive neuroblasts were found all along the lateral ventricular wall (Fig. 2A, C, D). In the posterior SVZ of the adult marmoset, neuroblasts were found only in the small segment of the lateral ventricle between the corpus callosum and the striatum, in the lateral telencephalon (Fig. 2E, F).

We also stained the lateral wall of the lateral ventricle as a whole mount, with the anti-PSA-NCAM antibody (Fig. 2G, H). This experiment revealed an extensive network of neuroblast chains in the adult marmoset SVZ, similar to those seen in mice.

PSA-NCAM labeling of sagittal sections through the adult OB and OT showed individually migrating cells with an elongated shape in the core of the OB and in the granule cell layer (Fig. 3A–C). We did not find chains of neuroblasts in these regions. In coronal sections we found neuroblasts in the core of the OB (Fig. 3D, E) and in the granule cell layer (Fig. 3F).

Proliferation and neurogenesis—To label proliferating cells in the adult brain we injected BrdU into adult marmosets. The brains were perfused and analyzed 2 hours or 1 month after the injection (Fig. 4, Table 2). BrdU-labeled cells were sparse in the SVZ, RMS, OT, and OB at 2 hours (Fig. 4 A–D) as well as at 1 month (Fig. 4 E–H) after the injection. All of the BrdU+ cells observed 2 hours after the injection were negative for Dcx. On the other hand, some of the BrdU+ cells were positive for Dcx at the 1-month timepoint (Fig. 4 I–N), indicating that BrdU+ Dcx+ neuroblasts were generated in the adult brain. We did not detect BrdU+ mature OB neurons in our samples, whereas some BrdU+NeuN+ cells were observed in the DG of the hippocampus (data not shown), as previously reported (Leuner et al., 2007).

Electron microscopy

General organization: We next examined the organization of the adult marmoset SVZ at the ultrastructural level. The three-layer organization previously described for humans and primates (Sanai et al., 2004; Gil-Perotin et al., 2009), was also well defined in the adult marmoset (Fig. 5A). Layer I was formed by a continuous ependymal cell lining, next to a hypocellular gap (layer II). This gap layer was composed of cytoplasmic expansions of astrocytes and ependymal cells and contained microtubules and intermediate filaments. The astrocytic expansions were light and contained many intermediate filaments and microtubules close to the membrane, while the ependymal expansions were more electron-dense and contained only a few intermediate filaments. Between the gap layer expansions, myelinated axons, oligodendrocytes, microglia, and migrating neuroblasts were also observed. Subjacent to the hypocellular layer, there was a thick astrocyte ribbon (layer III), which was still thinner than in the adult human.

Cell types in the adult SVZ: Ependymal cells showed interdigitating cytoplasmic expansions and sometimes a thin radial process (Fig. 5B). These cells contained abundant intermediate filaments and occasional electron-dense crystals in their cytoplasm (Fig. 5C). Mitochondria were located close to the basal bodies in the apical compartment of these cells. Abundant microvilli and cilia covered the apical cell surface.

We observed displaced ependymal cells throughout the SVZ, a phenomenon that was greater in the posterior region (Fig. 5D). These cells formed rosette-like structures containing cilia, basal bodies, and microvilli.

The astrocytes had an electron-translucent cytoplasm that was rich in intermediate filaments and an irregularly shaped, invaginated nucleus. We identified different types of astrocytes (type B). The first type was located in the ribbon layer, and had cytoplasmic extensions that projected into the gap layer. These astrocytes were rich in intermediate filaments and had large nucleoli that contained electron-translucent areas and were less compact than those of other astrocytes (Fig. 6A). Above the gap layer and the astrocytic ribbon was a population of structural astrocytes that contained fewer intermediate filaments (Fig. 6B). Finally, a subset of astrocytes (type B1) were in contact with the ventricle and displayed a single, short cilium with a perpendicularly orientated centriole (Fig. 6C, D). All of the astrocytes contained

GFAP+ intermediate filaments, as confirmed by preembedding immunocytochemistry (Fig. 6E, F).

Migrating neuroblasts were abundant in the anterior and medial regions and formed clusters of densely packed cells (Fig. 7A). They formed chains with intercellular spaces that were surrounded by cytoplasmic expansions from ependymal cells and astrocytes. The neuroblasts were elongated and showed a sparse dark cytoplasm and microtubule-rich processes (Fig. 7B).

We also identified a cell type with a morphology intermediate between that of astrocytes and neuroblasts, possibly equivalent to rodent type C cells or intermediate precursors (Fig. 7C). These cells were large and displayed long cytoplasmic extensions. Their cytoplasm was dark, rich in microtubules, and contained mostly mitochondria, rough endoplasmic reticulum (RER), and free ribosomes. Their nucleus was large, with deep invaginations, and lax chromatin.

To further characterize the cellular composition of the marmoset SVZ, we counted the number of different cell types in ultrathin sections in the anterior, medial, and posterior levels using EM images and show the proportion of each cell type as a percentage of the total cells counted (Tables 3 and 4). As described above, neuro-blasts were frequent in the anterior regions (3.9%) and tended to disappear caudally (0.2% to 0%). A similar distribution was observed for putative precursors, which were, however, less abundant (1.1% in the anterior regions). In contrast, astrocytes were more frequent in the anterior and medial regions (19.9% and 20.4%, respectively). Interestingly, although displaced ependymal cells were present in the anterior SVZ (3.4%), their number greatly increased in the medial and posterior regions (13.1% and 16.3%, respectively). Figure 8 shows the cell-type composition of the SVZ at the anterior and posterior levels. The anterior levels contained migrating cells and some putative precursors. In contrast, the posterior levels lacked neuroblasts and putative precursors but contained abundant displaced ependymal cells.

Adult RMS: Neuroblasts that formed the RMS were first identifiable at the anteroventral SVZ, where we had seen clusters of PSA-NCAM+ cells (Fig. 2B). EM observation revealed that these cells were densely packed and surrounded by astrocytic processes and cell bodies. The migrating neuroblasts were smooth, elongated, and rich in microtubules, while the astrocytes were irregularly shaped and contained intermediate filaments (Fig. 9A, B). The RMS became thinner as it approached the OB, and did not appear as a continuous chain within the OT; only individual migrating neuroblasts were visible at some points.

Neonate

Our results from the adult marmoset SVZ and RMS (Figs. 2–9) revealed that the migrating neuroblasts are generated in the SVZ, but they do not travel efficiently to the OB, much as in humans. Therefore, we next examined whether the SVZ and RMS of neonatal marmosets have a more evident RMS, like that of rodents.

Light microscopy: histology and immunohistochemistry—We studied the neonatal SVZ at the equivalent rostro-caudal levels as used for the adult brain.

The ventricular surface was flat in the anterior and medial levels, and it became irregular along its dorsoventral expanse in the posterior region, but it showed deeper invaginations than the adult in the ventral horn (Fig. 10A, B). Ependymal cells formed a monolayer epithelium lining the ventricular cavity.

In the anterior region, the ependymal layer was lined by a dense band of small cells that formed compact groups surrounded by more lightly stained areas. These groups of cells were distributed irregularly along the dorsoventral axis. They formed a layer up to 10 cells thick in the ventral parts of the anterior SVZ (Fig. 10C). The thickness of this layer decreased posteriorly, to 2–3 cells, and sometimes none at all were seen (Fig. 10D). Besides these groups of cells, we found dispersed cells with lightly stained cytoplasm. The SVZ was richly vascularized, with blood vessels extending close to the ventricle between the SVZ cells.

We stained coronal sections for GFAP, which labels astrocytes, including neural stem cells in the SVZ (Doetsch et al., 1999a) (Fig. 10E), and for markers of neuronal progenitors, including *Dlx* (Panganiban and Rubenstein, 2002) (Fig. 10F), β III-tubulin (Doetsch et al., 1997) (Fig. 10G), and *Dcx* (Brown et al., 2003) (Fig. 10H–J). In addition to GFAP+ cells, many cells expressing *Dcx*, *Dlx*, and β III tubulin were found in the SVZ, suggesting active neurogenesis. To study the distribution of these neuroblasts, we stained coronal (Fig. 10H) and sagittal (Fig. 10I, J) brain sections for *Dcx*, and found a number of them in the dorsolateral corner of the lateral ventricles.

As previously described in other nonhuman primates (Kornack and Rakic, 2001; Pencea et al., 2001; Gil-Perotin et al., 2009), the RMS began at the anterior ventral SVZ. Neuroblasts, similar to those described above in the SVZ, formed irregular groups that invaded the subjacent parenchyma. Figure 11A–E shows the distribution of *Dcx*+ neuroblasts in the RMS of neonatal animals. As in rodents, in the newborn marmoset we found a large number of *Dcx*+ neuroblasts that formed an RMS, which ran initially ventrally and then turned rostrally to join the OT.

We followed the trajectory of the migratory pathway by examining the OT and OB under a light microscope. The OT started as an individual elongated tract that remained in close contact with the brain surface. *Dcx*+ neuroblasts were found in the core of the OT (Fig. 11F, G) and the OB (Fig. 11H, I). They formed a compact group concentrated in the center of the OT, where we found a large accumulation of dark cells (Fig. 11J).

Proliferation—To study cell proliferation, neonatal marmosets were injected with BrdU 2 hours before perfusion. Many BrdU-labeled proliferating cells were distributed along the SVZ-RMS-OB pathway, including the anterior SVZ (Fig. 12A, D), posterior SVZ (Fig. 12B, E), and the core of the OB (Fig. 12C, F). Most BrdU-labeled cells appeared in clusters separated from the ependymal layer. We also performed double immunohistochemistry for Mash1, a marker for transit-amplifying cells (Parras et al., 2004; Sakaguchi et al., 2006),

together with BrdU (Fig. 12G–I). We found that 88% ($n = 51$ cells) of the BrdU+ cells were Mash1+ cells.

Electron microscopy

General organization: The boundaries between cell layers that we identified in the adult SVZ were not as clearly defined in the neonate (Fig. 13A), and the layered organization was well defined only in the posterior SVZ of the neonate. The ependymal cell layer formed a pseudo-stratified epithelium, which showed less apparent stratification at the posterior levels. Next to the ependymal lining was a hypocellular gap layer, thinner than that in the adult, composed of ependymal and astrocytic processes. This hypocellular layer was not continuous between the ependyma and the cellular layer in the medial and anterior levels. The third layer was composed of a heterogeneous cell population.

Cell types in the neonatal SVZ: Using the established cell morphological criteria for rodents (Doetsch et al., 1997; Mirzadeh et al., 2008) and primates (Gil-Perotin et al., 2009), we identified the predominant cell types in the neonatal marmoset SVZ: they were ependymal cells (type E), migrating neuroblasts (type A), astrocytes (type B), unciliated astrocytes that contacted the ventricle (type B1), and putative intermediate precursors (type C). Due to the high proliferation and likely rapid transitions from one cell type to another, it was not always easy to distinguish between cell types and especially between A and C cells. In addition to ependymal cells and astrocytes, neurons were occasionally seen in direct contact with the ventricle (Fig. 14C).

The ependymal cells were multiciliated and formed a continuous single-cell layer of pseudo-stratified epithelium (Fig. 13B). These cells had a polygonal shape and a radial projection that extended into the subjacent hypocellular layer. The ependymal cells were extensively interdigitated and displayed long, thin junctional complexes near the apical surface, where they exhibited abundant short, thick microvilli. In the neonate, the cellular organization was not fully developed, and some cells had not yet differentiated. In particular, the cilia had not been elaborated in all cases, so some cells contained basal bodies (without associated cilia) that were deep in the cytoplasm and oriented randomly (Fig. 13C).

Cilia (9+2) emerged from the cell surface toward the lumen, and in some cells the cilia were oriented parallel to one another. We did not find deuterosomes, a structure expected in cilia formation, but we did observe electron-dense granules near the basal bodies, which are typical of immature ependymal cells (Spassky et al., 2005). The ependymal nuclei were frequently extensively invaginated and contained dispersed and lax chromatin and scarce, small nucleoli. The cytoplasm of these cells was electron-translucent and rich in intermediate filaments. These cells had scant RER, the Golgi with many dictyosomes, but with few saculi, and few mitochondria.

We frequently observed a structure in ependymal cells, not present in other cell types, composed of an electron-dense substance surrounded by Golgi saculi and endoplasmic reticulum (ER). Curiously, the ER cisterns did not show ribosomes on the side facing this dense substance (Fig. 13D). Lipid droplets were not seen in the ependymal cells in the neonatal brain. The posterior SVZ showed profuse invaginations of the ventricular wall at

the ventral horn, which often formed rosette-like structures. Displaced ependymal cells at these invaginations exhibited cilia, microvilli, and junction complexes.

As observed in the adult SVZ, sporadic groups of astro-cyte-like cells (type B1 cells) contacted the ventricular lumen between the ependymal cells (Fig. 13E). At the ventricular surface of the type B1 cells we observed a single cilium (9+0) adjacent to a perpendicular centriole, without evidence of ciliary roots (Fig. 13F). The nucleus was large and deeply invaginated, with lax, disperse chromatin and 1–2 large globular nucleoli, similar to those of astrocytes or type B2 cells. The cytoplasm of these cells was scarce, electron-translucent, and rich in intermediate filaments.

Migratory neuroblasts (type A cells) were arranged as tightly packed chains close to the ependymal cell layer (Fig. 14A), ensheathed by both astrocytic and ependymal cytoplasmic expansions. These cells corresponded to the small cells observed in the semithin sections. The migrating chains were frequent in the anterior SVZ regions, but few to none were seen in the posterior levels, where they were replaced by the hypocellular or gap layer. The cell surfaces of the type A cells were smooth and showed typical intercellular spaces in transverse sections, suggesting this was an area of active cell movement (Doetsch et al., 1997). Contact with neighboring cells was through junctional complexes (not shown).

The marmoset type A cell nuclei were round to oval and contained clusters of heterochromatin. Transverse sections showed some nuclei surrounded by scarce cytoplasm, with elongated cytoplasmic expansions parallel to the ventricular surface. The cytoplasm contained microtubules and ribosomes and lacked intermediate filaments and ER; these features characterized the cells as neuroblasts and differentiated them from astrocytes.

Adjacent to the ependyma, we found wide ribbons of astrocytes (type B cells) (Fig. 14A). In the posterior regions, these astrocytes were located next to the hypocellular gap layer. Their nuclei were typically large and deeply invaginated. The nucleoli of the astrocytes were also different from those of the ependymal cells: they were more globular and had electron-translucent areas. In contrast to the neuroblasts, the astrocytes' cytoplasm contained abundant intermediate filaments and electron-dense bodies. Their membrane surface was deeply interdigitated.

We identified some cells with a morphology that was intermediate between that of the type A and type B cells (Fig. 14B). These cells showed an irregular, invaginated nucleus, with chromatin containing small, electron-dense clumps. The cytoplasm was abundant and electron-translucent. This morphology was comparable to that of rodent transit amplifying (type C) cells (Doetsch et al., 2002), and therefore these cells may be their marmoset equivalent, in which case they also correspond to the Mash1-positive cells identified above (Fig. 12G).

Occasionally we observed cells in the SVZ parenchyma that contacted the ventricular lumen through a cytoplasmic expansion with a swollen foot-like structure that contained vesicles (Fig. 14C). The cytoplasm of these cells was rich in mitochondria and RER, which are characteristics of neuronal somas. In addition, axons that were in contact with ependymal microvilli were observed in some places (Fig. 14D). These axons were identified by their

content of dense, pleomorphic vesicles and abundant mitochondria. Some axons extended within the ependymal cell layer (Fig. 14E). Other cell types, such as microglia and endothelial cells, were found sporadically. Mitoses were occasionally observed in the ribbon layer.

In vivo and in vitro chain migration—To further characterize neuroblasts and their migratory path, we examined the RMS at the level of the OT by EM. The core of the tract contained large accumulations of migratory neuroblasts, identified by their elongated, microtubule-rich cytoplasm (Fig. 15A, B). These cells were surrounded by extracellular gaps similar to those observed in areas where neuroblasts are actively migrating in mice. To observe the behavior of the migrating cells directly, we cultured fragments of the SVZ in Matrigel, as described previously for the rodent (Wichterle et al., 1997). Chains of migrating cells formed around the SVZ explants (Fig. 15C). Most of the chains were composed of neuroblasts expressing PSA-NCAM (Fig. 15D) and β III-tubulin (Fig. 15E), and GFAP-positive astrocytes were occasionally observed at the proximal part of the chains (Fig. 15D, E). Live imaging of these chains demonstrated that these neuroblasts migrated along each other, similar to the previously reported description in mice (Wichterle et al., 1997) (Supporting Information Movie 1).

Close examination of individual migrating cells revealed that they extended a leading process followed by nuclear movement, as described in mice (Schaar and McConnell, 2005). The average speed of the cells migrating in chains ($n = 11$) was $102.3 \pm 26.0 \mu\text{m/h}$. The active moving phase was interrupted by a resting phase. The average speed of the cells in the active moving phase was $162.7 \pm 21.1 \mu\text{m/h}$.

DISCUSSION

In this work we performed a cytoarchitectural analysis of the SVZ and RMS of the adult and neonatal common marmoset. We found that the marmoset SVZ was organized in layers, similar to that of humans (Sanai et al., 2004; Quinones-Hinojosa et al., 2006) and other nonhuman primates (Gil-Perotin et al., 2009). On the other hand, it shared some features with the rodent SVZ (Doetsch et al., 1997, 1999a) that are not preserved in the human brain (Sanai et al., 2004; Quinones-Hinojosa et al., 2006).

In the adult marmoset, very scarce neuronal migration was taking place, and dispersed cells resembling migrating neuroblasts were found along the OT instead of an evident RMS. Similarly, some authors have shown that the adult human RMS contains some immature neuroblasts that do not exhibit typical chain migration (Sanai et al., 2004). However, Curtis et al. (2007) described a human RMS in which the migrating cells were organized around an extension of the lateral ventricle. The discrepancies in these studies could be due to the age of the brain, to underlying pathologies, or to the preservation of the postmortem samples. The marmoset SVZ described in this article seemed to be most similar to the human SVZ described by Sanai et al. (2004), which also lacked an evident RMS or olfactory ventricle. However, in the neonatal marmoset we observed a prominent RMS that extended through the OT to the OB, as in the rodent brain.

Neurogenesis in the SVZ and RMS of adult nonhuman primates has been described most extensively using Old World primates such as the rhesus (*Macaca mulatta*) (Kornack and Rakic, 2001; Pencea et al., 2001), cynomolgus (*Macaca fascicularis*) (Kornack and Rakic, 2001), and Japanese monkey (*Macaca fuscata*) (Tonchev et al., 2003). We recently performed an EM analysis of the SVZ in *M. fascicularis* (Gil-Perotin et al., 2009). The squirrel monkey (*Saimiri sciureus*), a New World monkey, also contains an RMS and SVZ (Bedard et al., 2002) that have some features in common with those of other mammals, including the common marmoset.

McDermott and Lantos (1989, 1990) showed that proliferating cells exist in the marmoset SVZ, and that their number decreases during development. They reported that the marmoset SVZ contains several morphologically distinct cell types. However, the precise cell composition and organization were not described in these reports.

Here we provide detailed information about the marmoset SVZ and RMS at the level of the data available for humans and rodents. By using marker expression and analyzing the cell morphologies in fine detail, we identified multiciliated ependymal cells, astrocytes, single-ciliated astrocytes in contact with the ventricle, putative intermediate precursors (similar to type C cells in mice), and neuroblasts.

As described in the rodent brain, the ependymal cells were undergoing differentiation in the neonatal marmoset. Electron microscopy of the neonatal ependyma showed frequent electron-dense aggregates typical of forming cilia (Hagiwara et al., 2000). We did not observe deuterosomes, the spherical bodies that are thought to function as the core for the formation of cilia, although they have been described in the rodent ependyma (Spassky et al., 2005). Neonatal ependymal cells often contained a homogeneous electron-dense substance surrounded by Golgi saculi and ER. In the adult, we observed sporadic electron-dense crystals as cytoplasmic inclusions. We do not know the function of either of these structures.

The ependymal surface of the ventrocaudal regions of the neonatal lateral ventricle was invaginated, with an irregular contour. This could be the precursor for the adult organization, in which the surface was smooth, but with frequent displaced ependymal rosette-like structures in the parenchyma, predominantly at the caudal levels. A similar displacement of ependymal cells has been reported in the human (Quinones-Hinojosa et al., 2006) and in nonhuman primates (Gil-Perotin et al., 2009). In mice, displaced ependymal cells are only observed after ependymal denudation (Del Carmen Gomez-Roldan et al., 2008). Interestingly, the SVZ showed more invagination in the neonatal brain, suggesting that the origin of the displaced ependyma in the adult marmoset comes from the folding of the ventricular surface, as speculated for the human SVZ (Quinones-Hinojosa et al., 2006). In addition, neural stem cells might require the expanded ventricular surface during the abundant proliferation that takes place in the neonate. In the adult, the ventricular invaginations would be incorporated into the subjacent parenchyma, and the cerebrospinal fluid (CSF)-contacting surface would be greatly diminished.

Underneath the ependymal lining we observed in the adult a hypocellular gap layer composed of ependymal and astrocytic processes, resembling the human SVZ (Sanai et al., 2004). However, while the human hypocellular layer contains only intermediate filaments, in the marmoset it contained both intermediate filaments and microtubules. This could be due to the different fixation quality of the human samples, which could hinder the identification of microtubules. The presence of microtubules in the astrocytes could also be due to the extensive growth of the cytoplasmic expansions that form the hypo-cellular layer. The marmoset gap layer contained more myelin than that of the human. Below the gap layer, astrocytes (type B cells) formed a ribbon. Interposed between the ependymal cells were astrocytes with a single cilium (9+0) and a centriole (similar to the rodent type B1 cells), which was in contact with the ventricular lumen. In the rodent SVZ, type B1 cells function as stem cells (Doetsch et al., 1999b; Mirzadeh et al., 2008). The human SVZ contains GFAP-positive astrocytes that can proliferate and generate neurons and glia in vitro (Sanai et al., 2004; Quinones-Hinojosa et al., 2006). In the human, astrocytic extensions in contact with the ventricle have been observed, but a primary cilium has not been reported, and type B1 astrocytes have not been described in macaque monkeys either (Gil-Perotin et al., 2009). However, it is possible that type B1 astrocytes are present in both humans and macaques, but have not been observed because of their low frequency.

We identified cells in the SVZ with a morphology intermediate between that of astrocytes and neuroblasts, a feature of rodent type C cells, by EM. Mash1 expression confirmed the presence of such intermediate precursors. These cells were more abundant in the neonatal brain, where they constituted the vast majority of the active proliferating cell population. However, the proportion of precursor cells in the marmoset SVZ was much lower than in the rodent. In the macaque SVZ, we did not find intermediate precursors (Gil-Perotin et al., 2009). It seems that the transition between astrocytes and neuroblasts occurs directly in the macaque, as has been proposed for the rat SVZ (Danilov et al., 2009). In the mouse SVZ, most of the precursor cells differentiate into neuroblasts that migrate toward the OB (Doetsch et al., 1997). Interestingly, 1 month after BrdU injection the majority of the BrdU+ cells were distributed in the SVZ and RMS and expressed Dcx. We did not find BrdU-labeled neurons in the OB 1 month after the injection. These data suggest that these cells are maintained as immature cells, and only a small percentage reach the OB. Alternatively, neuroblasts could take longer than 1 month to reach the OB and acquire a mature phenotype. However, we cannot rule out the possibility that migrating neuroblasts remain in the OT and differentiate there. It is also possible that some of the adult SVZ-generated cells migrate to other brain regions and/or differentiate into non-neuronal cells including oligodendrocytes. For example, in the rabbit, glia-independent chains of neuroblasts migrate from the SVZ to telencephalic areas, such as the amygdala or cortex (Luzzati et al., 2003).

Chains of neuroblasts converge to form the RMS in the rodent brain. Using immunostaining for PSA-NCAM or Dcx, semithin sections, and EM, we observed the beginning of the RMS in the anterior ventral SVZ, as described for other nonhuman primates (Kornack and Rakic, 2001; Pencea et al., 2001; Gil-Perotin et al., 2009). Whereas chain migration in the neonate evidently occurred in an RMS through the OT to the OB, in the adult marmoset a clear RMS was not observed and, if present, neuroblasts appeared to migrate as independent cells, similar to the process suggested in the adult human brain (Quinones-Hinojosa et al., 2006).

As described in the rodent SVZ, marmoset neuroblasts (type A cells in the rodent) contained microtubules and showed typical intercellular spaces in transverse sections, indicating cell movement. Neuroblasts accumulated in the rostral SVZ, and tended to be rare to absent in the caudal regions. In rodents, migrating neuroblasts form elongated cell aggregates called chains (Lois et al., 1996). We found that marmoset SVZ cells embedded in Matrigel showed a chain migration similar to that of mouse SVZ cells (Wichterle et al., 1997). The marmoset cells exhibited a long leading process that pointed toward their destination. The average migration speed of the cultured neuroblasts was $102.3 \pm 26.0 \mu\text{m/h}$, and that of cells that were actively moving was $162.7 \pm 26.0 \mu\text{m/h}$, comparable to the migration speed of mouse neuroblasts cultured using a similar method ($122 \mu\text{m/h}$) (Wichterle et al., 1997). Whole-mount staining of the lateral wall of the lateral ventricles revealed an extensive network of chains of migrating neurons in adult marmosets. In mice, such networks can be observed in almost all regions of the lateral wall (Doetsch and Alvarez-Buylla, 1996). However, the chains were restricted to the anterior regions in the marmoset brain. Our data suggest that chain migration is a conserved mechanism between rodents and primates.

Previous studies have demonstrated that the marmoset OB is similar to that of other mammals, with a distribution of various peptides resembling that of other mammalian species. For example, the distribution of tyrosine hydroxylase-positive neurons in the marmoset OB is similar to that of other mammals studied (Jeong et al., 2003), marmoset neuropeptide Y-positive neurons are distributed similar to those in cats and rats (Sanides-Kohlrausch and Wahle, 1990), and substance P-positive neurons are distributed much as in rats, guinea pigs, and cats (Sanides-Kohlrausch and Wahle, 1991). Curiously, marmosets are reported to have a better olfactory memory than other primates (Laska and Metzker, 1998), but their adult RMS is smaller.

Interestingly, we observed some cells in the SVZ that might correspond to CSF-contacting neurons, which have been well described in the lateral ventricles of birds and reptiles (Vigh and Vigh-Teichmann, 1998) and in the rodent and monkey central canal (Vigh et al., 2004; Marichal et al., 2009). We also observed axons throughout the SVZ and at the ependymal surface. PSA-NCAM+ cells can be found along the ventricular surfaces in the rat (Alonso, 1999). It is unclear whether the axons and the CSF-contacting neurons we observed are related.

Because of its advantages as an experimental animal, the common marmoset has been used to develop animal models of neurological diseases, including a marmoset middle cerebral artery occlusion (MCAO) model of stroke (Freret et al., 2008). Interestingly, MCAO increases the number of newborn neurons in the ischemic tissue in marmosets (Bihel et al., 2010), similar to the findings in rodents (Arvidsson et al., 2002; Parent et al., 2002; Yamashita et al., 2006). Successful attempts to manipulate endogenous neural stem cells to develop new brain-repair strategies will require a deep understanding of the mechanisms that regulate this neurogenesis (Kaneko and Sawamoto, 2009). The detailed description of the composition and organization of the marmoset SVZ presented here will be useful for future studies using marmoset models to evaluate new therapies for neurological diseases as well as for basic biological research aimed at elucidating the mechanisms and significance of the adult neurogenesis in primates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LITERATURE CITED

- Alonso G. Neuronal progenitor-like cells expressing polysialylated neural cell adhesion molecule are present on the ventricular surface of the adult rat brain and spinal cord. *J Comp Neurol.* 1999; 414:149–166. [PubMed: 10516589]
- Altman J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J Comp Neurol.* 1969; 137:433–457. [PubMed: 5361244]
- Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol.* 1965; 124:319–335. [PubMed: 5861717]
- Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med.* 2002; 8:963–970. [PubMed: 12161747]
- Bayer SA. 3H-thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. *Exp Brain Res.* 1983; 50:329–340. [PubMed: 6641865]
- Bayer SA, Yackel JW, Puri PS. Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. *Science (New York).* 1982; 216:890–892.
- Bedard A, Levesque M, Bernier PJ, Parent A. The rostral migratory stream in adult squirrel monkeys: contribution of new neurons to the olfactory tubercle and involvement of the antiapoptotic protein Bcl-2. *Eur J Neurosci.* 2002; 16:1917–1924. [PubMed: 12453055]
- Bihel E, Pro-Sistiaga P, Letourneur A, Toutain J, Saulnier R, Insausti R, Bernaudin M, Roussel S, Touzani O. Permanent or transient chronic ischemic stroke in the non-human primate: behavioral, neuroimaging, histological, and immunohistochemical investigations. *J Cereb Blood Flow Metab.* 2010; 30:273–285. [PubMed: 19794396]
- Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of double-cortin during adult neurogenesis. *J Comp Neurol.* 2003; 467:1–10. [PubMed: 14574675]
- Curtis MA, Kam M, Nannmark U, Anderson MF, Axell MZ, Wikkelsø C, Holtas S, van Roon-Mom WM, Bjork-Eriksson T, Nordborg C, Frisen J, Dragunow M, Faull RL, Eriksson PS. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science (New York).* 2007; 315:1243–1249.

- Danilov AI, Gomes-Leal W, Ahlenius H, Kokaia Z, Carlemalm E, Lindvall O. Ultrastructural and antigenic properties of neural stem cells and their progeny in adult rat subventricular zone. *Glia*. 2009; 57:136–152. [PubMed: 18709646]
- Debus E, Weber K, Osborn M. Monoclonal antibodies specific for glial fibrillary acidic (GFA) protein and for each of the neurofilament triplet polypeptides. *Differentiation*. 1983; 25:193–203. [PubMed: 6198232]
- Del Carmen Gomez-Roldan M, Perez-Martin M, Capilla-Gonzalez V, Cifuentes M, Perez J, Garcia-Verdugo JM, Fernandez-Llebrez P. Neuroblast proliferation on the surface of the adult rat striatal wall after focal ependymal loss by intracerebroventricular injection of neuraminidase. *J Comp Neurol*. 2008; 507:1571–1587. [PubMed: 18236450]
- Doetsch F, Alvarez-Buylla A. Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc Natl Acad Sci U S A*. 1996; 93:14895–14900. [PubMed: 8962152]
- Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the sub-ventricular germinal zone in the adult mammalian brain. *J Neurosci*. 1997; 17:5046–5061. [PubMed: 9185542]
- Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. 1999a; 97:703–716. [PubMed: 10380923]
- Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Regeneration of a germinal layer in the adult mammalian brain. *Proc Natl Acad Sci U S A*. 1999b; 96:11619–11624. [PubMed: 10500226]
- Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM, Alvarez-Buylla A. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron*. 2002; 36:1021–1034. [PubMed: 12495619]
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. Neurogenesis in the adult human hippocampus. *Nat Med*. 1998; 4:1313–1317. [PubMed: 9809557]
- Freret T, Bouet V, Toutain J, Saulnier R, Pro-Sistiaga P, Bihel E, Mackenzie ET, Roussel S, Schumann-Bard P, Touzani O. Intraluminal thread model of focal stroke in the non-human primate. *J Cereb Blood Flow Metab*. 2008; 28:786–796. [PubMed: 18000514]
- Gil-Perotin S, Duran-Moreno M, Belzunegui S, Luquin MR, Garcia-Verdugo JM. Ultrastructure of the subventricular zone in *Macaca fascicularis* and evidence of a mouse-like migratory stream. *J Comp Neurol*. 2009; 514:533–554. [PubMed: 19350672]
- Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E. Hippocampal neurogenesis in adult Old World primates. *Proc Natl Acad Sci U S A*. 1999; 96:5263–5267. [PubMed: 10220454]
- Hagiwara H, Ohwada N, Aoki T, Takata K. Ciliogenesis and ciliary abnormalities. *Med Electron Microsc*. 2000; 33:109–114. [PubMed: 11810467]
- Jeong YG, Lee NS, Lee KY, Chung SH, Hwang IK, Suh JG, Kang TC, Hyun BH, Oh YS, Won MH. Morphological characteristics of dopaminergic immunoreactive neurons in the olfactory bulb of the common marmoset monkey (*Callithrix jacchus*). *Ann Anat*. 2003; 185:543–547. [PubMed: 14703999]
- Kaneko N, Sawamoto K. Adult neurogenesis and its alteration under pathological conditions. *Neurosci Res*. 2009; 63:155–164. [PubMed: 19118585]
- Kim KK, Adelstein RS, Kawamoto S. Identification of neuronal nuclei (NeuN) as Fox-3, a new member of the Fox-1 gene family of splicing factors. *J Biol Chem*. 2009; 284:31052–31061. [PubMed: 19713214]
- Kohwi M, Osumi N, Rubenstein JL, Alvarez-Buylla A. Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. *J Neurosci*. 2005; 25:6997–7003. [PubMed: 16049175]
- Kornack DR, Rakic P. Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proc Natl Acad Sci U S A*. 1999; 96:5768–5773. [PubMed: 10318959]
- Kornack DR, Rakic P. The generation, migration, and differentiation of olfactory neurons in the adult primate brain. *Proc Natl Acad Sci U S A*. 2001; 98:4752–4757. [PubMed: 11296302]
- Laska M, Metzker K. Food avoidance learning in squirrel monkeys and common marmosets. *Learn Mem*. 1998; 5:193–203. [PubMed: 10454364]

- Lee MK, Tuttle JB, Rebhun LI, Cleveland DW, Frankfurter A. The expression and posttranslational modification of a neuron-specific beta-tubulin isotype during chick embryogenesis. *Cell Motil Cytoskeleton*. 1990; 17:118–132. [PubMed: 2257630]
- Leuner B, Kozorovitskiy Y, Gross CG, Gould E. Diminished adult neurogenesis in the marmoset brain precedes old age. *Proc Natl Acad Sci U S A*. 2007; 104:17169–17173. [PubMed: 17940008]
- Lois C, Alvarez-Buylla A. Long-distance neuronal migration in the adult mammalian brain. *Science (New York)*. 1994; 264:1145–1148.
- Lois C, Garcia-Verdugo JM, Alvarez-Buylla A. Chain migration of neuronal precursors. *Science*. 1996; 271:978–981. [PubMed: 8584933]
- Luzzati F, Peretto P, Aimar P, Ponti G, Fasolo A, Bonfanti L. Glia-independent chains of neuroblasts through the subcortical parenchyma of the adult rabbit brain. *Proc Natl Acad Sci U S A*. 2003; 100:13036–13041. [PubMed: 14559968]
- Mansfield K. Marmoset models commonly used in biomedical research. *Comp Med*. 2003; 53:383–392. [PubMed: 14524414]
- Marichal N, Garcia G, Radmilovich M, Trujillo-Cenoz O, Russo RE. Enigmatic central canal contacting cells: immature neurons in “standby mode”? *J Neurosci*. 2009; 29:10010–10024. [PubMed: 19675235]
- McDermott KW, Lantos PL. The distribution of glial fibrillary acidic protein and vimentin in postnatal marmoset (*Callithrix jacchus*) brain. *Brain Res Dev Brain Res*. 1989; 45:169–177.
- McDermott KW, Lantos PL. Cell proliferation in the subependymal layer of the postnatal marmoset, *Callithrix jacchus*. *Brain Res Dev Brain Res*. 1990; 57:269–277.
- Mirzadeh Z, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell*. 2008; 3:265–278. [PubMed: 18786414]
- Nacher J, Crespo C, McEwen BS. Doublecortin expression in the adult rat telencephalon. *Eur J Neurosci*. 2001; 14:629–644. [PubMed: 11556888]
- Okano H, Sawamoto K. Neural stem cells: involvement in adult neurogenesis and CNS repair. *Philos Trans R Soc Lond B Biol Sci*. 2008; 363:2111–2122. [PubMed: 18339601]
- Panganiban G, Rubenstein JL. Developmental functions of the Distal-less/Dlx homeobox genes. *Development*. 2002; 129:4371–4386. [PubMed: 12223397]
- Panganiban G, Sebring A, Nagy L, Carroll S. The development of crustacean limbs and the evolution of arthropods. *Science*. 1995; 270:1363–1366. [PubMed: 7481825]
- Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Ann Neurol*. 2002; 52:802–813. [PubMed: 12447935]
- Parras CM, Galli R, Britz O, Soares S, Galichet C, Battiste J, Johnson JE, Nakafuku M, Vescovi A, Guillemot F. Mash1 specifies neurons and oligodendrocytes in the post-natal brain. *EMBO J*. 2004; 23:4495–4505. [PubMed: 15496983]
- Pencea V, Bingaman KD, Freedman LJ, Luskin MB. Neurogenesis in the subventricular zone and rostral migratory stream of the neonatal and adult primate forebrain. *Exp Neurol*. 2001; 172:1–16. [PubMed: 11681836]
- Perez-Martin M, Cifuentes M, Grondona JM, Bermudez-Silva FJ, Arrabal PM, Perez-Figares JM, Jimenez AJ, Garcia-Segura LM, Fernandez-Llebrez P. Neurogenesis in explants from the walls of the lateral ventricle of adult bovine brain: role of endogenous IGF-1 as a survival factor. *Eur J Neurosci*. 2003; 17:205–211. [PubMed: 12542656]
- Quinones-Hinojosa A, Sanai N, Soriano-Navarro M, Gonzalez-Perez O, Mirzadeh Z, Gil-Perotin S, Romero-Rodriguez R, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. *J Comp Neurol*. 2006; 494:415–434. [PubMed: 16320258]
- Sakaguchi M, Shingo T, Shimazaki T, Okano HJ, Shiwa M, Ishibashi S, Oguro H, Ninomiya M, Kadoya T, Horie H, Shibuya A, Mizusawa H, Poirier F, Nakauchi H, Sawamoto K, Okano H. A carbohydrate-binding protein, Galectin-1, promotes proliferation of adult neural stem cells. *Proc Natl Acad Sci U S A*. 2006; 103:7112–7117. [PubMed: 16636291]
- Sanai N, Tramontin AD, Quinones-Hinojosa A, Barbaro NM, Gupta N, Kunwar S, Lawton MT, McDermott MW, Parsa AT, Manuel-Garcia Verdugo J, Berger MS, Alvarez-Buylla A. Unique

- astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature*. 2004; 427:740–744. [PubMed: 14973487]
- Sanai N, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. Comment on “Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science* (New York). 2007; 318:393. author reply 393.
- Sanides-Kohlrausch C, Wahle P. Morphology of neuro-peptide Y-immunoreactive neurons in the cat olfactory bulb and olfactory peduncle: postnatal development and species comparison. *J Comp Neurol*. 1990; 291:468–489. [PubMed: 2298945]
- Sanides-Kohlrausch C, Wahle P. Distribution and morphology of substance P-immunoreactive structures in the olfactory bulb and olfactory peduncle of the common marmoset (*Callithrix jacchus*), a primate species. *Neurosci Lett*. 1991; 131:117–120. [PubMed: 1724304]
- Sasaki E, Suemizu H, Shimada A, Hanazawa K, Oiwa R, Kamioka M, Tomioka I, Sotomaru Y, Hirakawa R, Eto T, Shiozawa S, Maeda T, Ito M, Ito R, Kito C, Yagihashi C, Kawai K, Miyoshi H, Tanioka Y, Tamaoki N, Habu S, Okano H, Nomura T. Generation of transgenic non-human primates with germline transmission. *Nature*. 2009; 459:523–527. [PubMed: 19478777]
- Schaar BT, McConnell SK. Cytoskeletal coordination during neuronal migration. *Proc Natl Acad Sci U S A*. 2005; 102:13652–13657. [PubMed: 16174753]
- Seki T, Arai Y. Expression of highly polysialylated NCAM in the neocortex and piriform cortex of the developing and the adult rat. *Anat Embryol (Berl)*. 1991; 184:395–401. [PubMed: 1952111]
- Seki T, Rutishauser U. Removal of polysialic acid-neural cell adhesion molecule induces aberrant mossy fiber innervation and ectopic synaptogenesis in the hippocampus. *J Neurosci*. 1998; 18:3757–3766. [PubMed: 9570806]
- Spassky N, Merkle FT, Flames N, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A. Adult ependymal cells are postmitotic and are derived from radial glial cells during embryogenesis. *J Neurosci*. 2005; 25:10–18. [PubMed: 15634762]
- Stephan, H. The brain of the common marmoset (*Callithrix jacchus*): a stereotaxic atlas. Berlin: Springer; 1980.
- Stuhmer T, Anderson SA, Ekker M, Rubenstein JL. Ectopic expression of the *Dlx* genes induces glutamic acid decarboxylase and *Dlx* expression. *Development*. 2002a; 129:245–252. [PubMed: 11782417]
- Stuhmer T, Puelles L, Ekker M, Rubenstein JL. Expression from a *Dlx* gene enhancer marks adult mouse cortical GABAergic neurons. *Cereb Cortex*. 2002b; 12:75–85. [PubMed: 11734534]
- Tonchev AB, Yamashita T, Zhao L, Okano HJ, Okano H. Proliferation of neural and neuronal progenitors after global brain ischemia in young adult macaque monkeys. *Mol Cell Neurosci*. 2003; 23:292–301. [PubMed: 12812760]
- Vigh B, Manzano e Silva MJ, Frank CL, Vincze C, Czirok SJ, Szabo A, Lukats A, Szel A. The system of cerebro-spinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. *Histol Histopathol*. 2004; 19:607–628. [PubMed: 15024719]
- Vigh B, Vigh-Teichmann I. Actual problems of the cerebrospinal fluid-contacting neurons. *Microsc Res Tech*. 1998; 41:57–83. [PubMed: 9550137]
- Wichterle H, Garcia-Verdugo JM, Alvarez-Buylla A. Direct evidence for homotypic, glia-independent neuronal migration. *Neuron*. 1997; 18:779–791. [PubMed: 9182802]
- Yamashita T, Ninomiya M, Hernandez Acosta P, Garcia-Verdugo JM, Sunabori T, Sakaguchi M, Adachi K, Kojima T, Hirota Y, Kawase T, Araki N, Abe K, Okano H, Sawamoto K. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. *J Neurosci*. 2006; 26:6627–6636. [PubMed: 16775151]
- Yang Z, Covey MV, Bitel CL, Ni L, Jonakait GM, Levison SW. Sustained neocortical neurogenesis after neonatal hypoxic/ischemic injury. *Ann Neurol*. 2007; 61:199–208. [PubMed: 17286251]
- Zhao Y, Flandin P, Long JE, Cuesta MD, Westphal H, Rubenstein JL. Distinct molecular pathways for development of telencephalic interneuron subtypes revealed through analysis of *Lhx6* mutants. *J Comp Neurol*. 2008; 510:79–99. [PubMed: 18613121]

Figure 1.

MRI observation of the adult ventricles. **A, B:** Dorsal (A) and lateral (B) views of the 3D shape of the lateral ventricle (blue) reconstructed from serial MRI images of a postmortem marmoset brain. Anterior is to the top in A and to the left in B. **C, D:** High-resolution 2D T₂-weighted coronal imaging, with the first slice positioned at the anterior horn of the lateral ventricle (indicated by a yellow line in A and B) in the brain of a living marmoset. D is a higher-magnification view of the boxed area in C. Arrow indicates the small ventricular space containing cerebrospinal fluid. **E:** Coronal brain section stained for PSA-NCAM (from Fig. 2) with an arrow indicating the ventricular space corresponding to the area in D. **F:** Levels of the subventricular zone studied in the adult and neonatal *Callithrix jacchus* brain. Left, 3D shape of the lateral ventricle (blue) reconstructed from serial MRI images of a postmortem marmoset brain. Diagrams show representative coronal sections of the three lateral ventricle levels indicated by lines a (anterior), m (medial), and p (posterior), modified from (Stephan, 1980). Scale bar = 500 μ m in E (applies to A–D).

Figure 2.

Neuroblasts in the adult SVZ. **A–F:** Distribution of PSA-NCAM-positive neuroblasts in a coronal section of the anterior forebrain. **B–D:** Higher-magnification views of the boxed areas in **A**. **E,F:** Distribution of PSA-NCAM-positive neuroblasts in a coronal section of the posterior forebrain. **F:** Higher-magnification view of the boxed area in **E**. **G, H:** Whole mount of the lateral wall of the lateral ventricle stained for PSA-NCAM. **H:** Higher magnification of the anterior horn, from the specimen shown in **G**. Scale bars = 500 μ m in **A, E**; 25 μ m in **B** (applies to **C, D, F**); 1 mm in **G**; 100 μ m in **H**.

Figure 3.

PSA-NCAM-positive neuroblasts in the adult OB and OT. **A, B:** Sagittal section of the OB. PSA-NCAM-positive cells were found in the OB core. **B:** Higher-magnification view of the boxed area in **A**. **C:** Sagittal section of the OT. **D–F:** Coronal section of the OB. Dorsal is to the left. **E:** Higher-magnification view of the boxed area in **D**. Neuroblasts were found in the core (**D**) and granule cell layer (**F**). Scale bars = 50 μm in **A** (applies to **D**); 10 μm in **B** (applies to **C**, **E**, **F**).

Figure 4.

Cell proliferation in the adult brain. **A–H:** BrdU-labeled cells in coronal sections of the OB (A, E), the dorsolateral aspect of the lateral ventricle (B, F), the lateral wall of the lateral ventricle (C, G), and the ventral aspect of the lateral ventricle (D, H) at 2 hours (A–D) or 1 month (E–H) after BrdU injection. **I–N:** Double immunofluorescence for BrdU (I, L, magenta), Dcx (J, M, green) and their merged images (K, N) showing labeled neuroblasts in the OB (I–K) and SVZ (L–N) 1 month after BrdU injection. Scale bars = 50 μ m in A (applies to B–H); 10 μ m in I (applies to J–N).

Figure 5.

Ultrastructure of the SVZ in the adult. **A:** Typical layered organization of the adult SVZ. Layer I: ependyma; Layer II: hypocellular gap; Layer III: astrocyte ribbon. Inset shows a cross-section of an astrocytic expansion from the hypocellular gap layer, which contains both intermediate filaments in the center (asterisk) and microtubules in the periphery (arrows). **B:** Ependymal cells showed an interdigitating cytoplasm and a thin radial projection. **C:** Occasional electron-dense crystals were observed in the cytoplasm of ependymal cells. **D:** Displaced ependymal cells formed rosette-like structures that included cilia, basal bodies, and microvilli. Scale bars = 7 μm in A; 200 nm in A, inset; 2 μm in B; 0.5 μm in C; 2 μm in D.

Figure 6.

Ultrastructure of astrocytes in the adult SVZ. **A:** Stem cell-like astrocytes (B) in the ribbon layer. Cytoplasmic expansions from these astrocytes formed the gap layer. The astrocytes were rich in intermediate filaments. The inset shows a large nucleolus, less compact than that of other cell types, with electron-translucent areas. Beside the astrocyte, an oligodendrocyte (O) with a round, dark nucleus and scarce cytoplasm is visible. **B:** Structural astrocyte, located above the GAP layer and the ribbon. It contained fewer intermediate filaments. **C:** Astrocyte (type B1) in contact with the ventricle. **D:** Type B1 cells displaying a single short cilium (arrow). **E:** GFAP staining in a Toluidine-blue-stained semithin section showing labeled cells in the ribbon and ependymal layers. **F:** Immunostaining for GFAP at the ultrastructural level confirmed the labeling of astrocytic intermediate filaments (inset). B, astrocytes. Scale bars = 2 μm in A; 500 nm in A, inset; 5 μm in B,C; 1 μm in D; 10 μm in E; 2 μm in F; 200 nm in F, inset.

Figure 7.

Ultrastructure of the SVZ cell types in the adult. **A:** Ultrastructure of the chains of migrating neuroblasts (A), surrounded by ependymal cells (E) and astrocytic processes. **B:** Neuroblasts (A) were elongated and showed a sparse, dark cytoplasm with cytoplasmic expansions rich in microtubules (black asterisk). The neuroblasts were surrounded by astrocytic expansions rich in intermediate filaments (white asterisks). **C:** Putative precursor cells were large with long processes. Inset shows a higher-magnification image of the cytoplasm, rich in microtubules, mitochondria, RER, and free ribosomes. Scale bars = 2 μ m in A; 500 nm in B; 2 μ m in C; 500 nm in C, inset.

Figure 8.

Diagram showing the cell organization in the anterior and posterior SVZ in the adult marmoset.

Figure 9.

Cell types at the beginning of the adult RMS at the ventral anterior SVZ. **A:** Migrating neuroblasts (A) forming the RMS were elongated and densely packed. **B:** Astrocyte (B) cytoplasmic projections rich in intermediate filaments (black asterisks) ensheath neuroblasts (A) along the RMS. Scale bars = 5 μm in A; 2 μm in B.

Figure 10.

Light microscopy and immunohistochemistry of the neonatal SVZ. **A–D:** Toluidine-blue-stained semithin sections of the ventricular wall of the lateral ventricles (V). **A:** Smooth ventricular surface at the rostral level (indicated by the square in the inset). **B:** Irregular surface at the caudal level, with deep invaginations at the ventral horn (the ventral horn is indicated by the square in the inset). **C:** The rostral SVZ showed compact groups of small, dark cells (neuroblasts) surrounded by light cytoplasmic processes. These groups of neuroblasts were irregularly distributed along the dorsoventral axis and formed up to 10 layers (double-headed arrow). **D:** The caudal SVZ was composed of 2–3 cell layers or fewer (double-headed arrow). **E–G:** Dorsolateral corner of the lateral ventricle stained for GFAP (**E**), Dlx (**F**), and β III-tubulin (**G**). **H:** Distribution of Dcx-positive neuroblasts in the dorsal SVZ (coronal section). **I, J:** Distribution of Dcx-positive neuro-blasts in the SVZ (sagittal views). **J:** Higher-magnification view of the position indicated by the box in **I**. Inset shows the morphology of the DCX-positive cells in the boxed area in **J**. Scale bars = 100 μ m in **B** (applies to **A**); 20 μ m in **D** (applies to **C**); 25 μ m in **E** (applies to **F, G**); 25 μ m in **H** (applies to **J**); 500 μ m in **I**.

Figure 11.

Light microscopy and immunohistochemistry of the neonatal RMS, olfactory tract (OT), and olfactory bulb (OB). **A–C:** Distribution of Dcx+ neuroblasts in a coronal section of the RMS. **B,C:** Higher-magnification views of the boxed areas in **A**. **D, E:** Distribution of Dcx+ neuroblasts in a sagittal section of the RMS. **E:** Higher-magnification view of the boxed area in **D**. **F, G:** Distribution of Dcx+ neuroblasts in a coronal section of the OT. **G:** Higher-magnification view of the boxed area in **F**. **H, I:** Distribution of Dcx+ neuroblasts in a sagittal section of the OT and OB. **I:** Higher-magnification view of the boxed area in **H**. **J, K:** Semithin section stained with toluidine blue showing the OT close to the OB. Dark cells (neuroblasts) are accumulated in the central zone of the tract. **K:** Higher-magnification view of the boxed area in **J**. The schematic drawing of a sagittal section of the neonatal marmoset brain (between **C** and **J**) shows the position of the RMS (red line) and the areas shown in **D** and **H**. Rostral is to the right. LV, lateral ventricle; CC, corpus callosum. Scale bars = 500 μ m in **A** (applies to **D, F, H**); 25 μ m in **B** (applies to **C, E, G, I**); 50 μ m in **J**; 10 μ m in **K**.

Figure 12.

Proliferation in the neonatal SVZ and OB. **A–F:** Coronal sections of the anterior SVZ (A, D), posterior SVZ (B, E), and OB (C, F) of a neonatal marmoset brain perfused and stained for BrdU, 2 hours after BrdU injection. **D–F:** Higher-magnification views of the boxed areas in A–C, respectively. **G–I:** Double immunofluorescence with anti-BrdU (G, magenta), anti-Mash1 (H, green), and their merged image (I). Eighty-eight percent of the BrdU-positive cells were positive for Mash1. Scale bars = 500 μ m in A (applies to B, C); 25 μ m in D (applies to E, F); 10 μ m in G–I.

Figure 13.

Ultrastructure of the ependymal layer in the neonate. **A:** Irregular cell organization in the neonatal anterior SVZ. The ependymal cells (E) formed a continuous single-cell layer of a pseudo-stratified epithelium. Astrocytic and ependymal cytoplasmic expansions formed a thin hypocellular gap layer (dashed lines). A third layer was formed by a heterogeneous cell population containing neuroblasts (A) and astrocytes (B). **B:** Ependymal (type E) cell in contact with the ventricle. These cells were multiciliated and displayed abundant microvilli on their apical surface. **C:** Randomly oriented, internalized basal bodies (arrows) without cilia, and electron-dense particles (arrowheads) in a cell undergoing ependymal differentiation. **D:** Electron-dense substance (asterisk) surrounded by Golgi saculi and endoplasmic reticulum (arrows) in the cytoplasm of an ependymal cell. **E:** Astrocyte-like (type B1) cell with a single cilium (not visible in this section) and centriole (arrowhead) in contact with the ventricular lumen through an expansion intercalated between ependymal cells (pseudocolored blue). **F:** Single cilium (arrow) of a different type B1 cell. This cilium is adjacent to a perpendicularly orientated centriole (arrowhead). Scale bar = 5 μ m in A; 2 μ m in B, E; 1 μ m in C, F; 500 nm in D.

Figure 14.

Ultrastructure of different SVZ cells in the neonate. **A:** Migratory neuroblasts (type A cells) in a chain (pseudocolored red) close to the ependymal cell layer and ensheathed by both astrocytic and ependymal expansions. Type A nuclei were round to oval and contained clusters of heterochromatin. **B:** Intermediate cell (C) between types A and B, possibly similar to mouse type C cells. These cells showed an irregular nucleus, chromatin containing small electron-dense clumps and abundant cytoplasm. An astrocyte (B) exhibiting an electron-light cytoplasm and a nucleus with lax chromatin. **C:** Possible neuron in contact with the ventricle through a cytoplasmic projection (pseudocolored brown). **D:** Axon in the ventricular lumen in contact with the ependymal surface (arrow). The axon contained dense, pleomorphic vesicles and abundant mitochondria. **E:** Axon in the ependymal layer (arrow), in contact with several ependymal cells. Scale bars = 5 μm in A; 2 μm in B; 5 μm in C; 1 μm in E (applies to D).

Figure 15.

In vivo and in vitro chain migration. **A:** Ultrastructure of the RMS at the level of the OT. Figure shows the core of the tract, where we observed an accumulation of migratory neuroblasts (type A cells), identified by their elongated cytoplasm rich in microtubules. **B:** Detail of an astrocytic expansion surrounding chains of migrating neuroblasts. The astrocyte contains intermediate filaments and some RER (white asterisk). Contrast this astrocytic process with a neighboring projection from a migrating neuroblast, which contains microtubules (black asterisk). **C–E:** SVZ cells cultured in Matrigel for 48 hours (see also Supporting Information Movie 1). **C:** Phase-contrast image of chain-forming neuroblasts. **D:** The migrating cells expressed neuronal markers PSA-NCAM (green) and β III-tubulin (magenta). **E:** Double immunofluorescence with anti-GFAP (green) and β III-tubulin (magenta). Scale bars = 2 μ m in A; 500 nm in B; 25 μ m in C–E.

TABLE 1**Primary Antibodies Used**

Target	Immunogen	Manufacturer, catalog number, species, type	Dilution
PSA-NCAM	Living cell suspensions in PBS obtained from the forebrain of an embryonic day 18 Wistar rat	Gift from Dr Tastsunori Seki, 12E3, mouse monoclonal IgM	1:300
Dcx	The C-terminus of synthetic Dcx peptide (amino acids 385–402 at the C-terminus of human Dcx)	Santa Cruz Biotechnology, sc-8066, goat polyclonal IgG	1:100
GFAP	GFAP from pig spinal cord	Sigma, G3893, mouse monoclonal IgG	1:100
Pan-Dlx	N-terminal 200 amino acids and 61-amino-acid homeodomain of butterfly Dlx protein.	Gift from Dr. Grace Boekhoff-Falk, rabbit polyclonal IgG	1:300
β III-tubulin	Synthesized peptide corresponding to amino acids 443–450 of human β -tubulin isotype III conjugated to BSA	Sigma, T8660, mouse monoclonal IgG	1:200
Mash1	Rat MASH1 full-length recombinant protein	BD Pharmingen, 556604, mouse monoclonal IgG	1:200
BrdU	BrdU	Abcam, ab6326 rat monoclonal IgG	1:100
NeuN	Purified cell nuclei from mouse brain	Chemicon, mouse monoclonal IgG, #MAB377	1:200

TABLE 2

Percentage of Dcx+ BrdU+ Cells among All BrdU+ Cells, 1 Month after BrdU Injection

SVZ	37% (37/101)
RMS	25% (4/16)
OT	27% (4/15)
OB	17% (1/6)

TABLE 3

Morphological Characteristics of Different Cell Types in the Adult Marmoset SVZ

	Migrating neuroblast		Astrocyte		Astrocyte	Putative precursor	Ependymal
Equivalent cell type in rodents	A	B1	B			C	E
Contour	Elongated, smooth	Irregular	Irregular			Smooth and elliptical	Interdigitating
Cytoplasm	Dark	Light	Light			Dark	Dark
Nucleus	Elongated, occasional invagination	Irregular, invaginated	Irregular, invaginated			Large spherical-irregular	Spherical, invaginated
Chromatin	Lax, some clumps	Lax	Some clumps			Mostly lax, some clumps	No clumps
Nucleoli	2 to 3	1 to 2	1 to 2			2 to 3	1 to 2
RER	+	++	+			++	+
Golgi apparatus	Small	Medium	Medium			Medium	Small
Mitochondria	+	++	++			++	+++
Free ribosomes	+++	++	+			++	+
Int. filaments	NO	YES ++	YES ++++			NO	YES +++
Microtubules	++++	+	+			+++	+
Lipid droplets	NO	NO	YES			NO	YES

These characteristics are based on transmission EM images of at least 10 cells of each type. RER, rough endoplasmic reticulum; +, few; ++, intermediate; +++ abundant; ++++ very abundant.

TABLE 4

Percentage of Cells of Different Types in the Lateral Wall of the Lateral Ventricle at the Anterior, Medial, and Posterior Levels

Cell type	Anterior	Medial	Posterior
Ependyma	65.78	64.06	72.64
Astrocyte	19.92	20.40	9.41
Displaced ependyma	3.44	13.07	16.32
Neuroblast	3.91	0.18	0.00
Type C	1.06	0.03	0.22
Oligodendrocyte	1.93	0.87	0.73
Microglia	0.28	0.00	0.06
Neuron	0.97	0.23	0.06
Pycnosis	0.28	0.03	0.00
Not identified	1.82	1.06	0.57

Percentages are of the total number of counted cells. The number of each cell type was counted in individual ultrathin sections ($n = 2$ marmosets, 3 sections per region) of the intermediate SVZ, examined by electron microscopy.