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Subventricular Zone-Derived Neuronal Progenitors Migrate into the Subcortical Forebrain of Postnatal Mice

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ABSTRACT
The presence of a germinal layer and the capacity to generate neurons, once thought restricted to the embryonic brain, persists in the forebrain of both postnatal and adult mammals. The two regions in which this phenomenon has been extensively demonstrated are the hippocampal dentate gyrus and the lateral ventricle subventricular zone (SVZ). SVZderived cells migrate along the rostral migratory stream into the olfactory bulb, where they differentiate into local interneurons. In this study, using tracer injections into the SVZ at different postnatal ages, we investigated the occurrence of secondary migratory pathways in the mouse subcortical forebrain. During the course of the first week postnatal, in addition to the well-characterized rostral migratory stream, SVZ-derived progenitors migrate in a ventral migratory mass across the nucleus accumbens into the basal forebrain and along a ventrocaudal migratory stream originating at the elbow between the vertical and horizontal limbs of the rostral migratory stream. These cells give rise to granule neurons in the Islands of Calleja and olfactory tubercle pyramidal layer, respectively. In adult, a very small number of cells continue to migrate along the ventrocaudal migratory stream, whereas no migration was observed across the nucleus accumbens. These data demonstrate that in early postnatal and, to a minor extent in adult mice, SVZ-derived cells contribute new neurons to the subcortical forebrain.

Indexing terms: subventricular zone; olfactory bulb; cell migration; olfactory tubercle; island of Calleja

The forebrain is a highly complex and organized structure that has been the object of extensive studies on the migratory processes underlying its development, especially cerebral cortex migration (for review, see Marin and Rubenstein, 2003). Two main types of cell migration have been described during forebrain development: radial and tangential migration. In radial migration cells migrate along a radial glia scaffold from the germinal layer lining lateral ventricles to the outer surface. Radial migration is considered the primary mechanism establishing the general cytoarchitecture of the forebrain. In tangential migration, cells do not follow the direction of the radial glia, and instead migrate perpendicularly, dispersing to different regions. Tangential migration in the cortex permits dispersion of neuronal types arising in one location to other subdivisions of the forebrain. Tangential migration is thought to increase the complexity of forebrain circuitry. During embryonic development the basal forebrain contains three main proliferative regions: the lateral (LGE), medial, and caudal ganglionic eminences. These regions are the source of cells directed to specific areas of the developing ventral forebrain and via tangential migration to the cerebral cortex, hippocampus, and olfactory bulb (OB) (Wichterle et al., 1999, 2001; Nery et al., 2002). The LGE, in particular, gives rise to neurons of the dorsal and ventral striatum, including the nucleus accumbens (Acb) and olfactory tubercle (OT), and to interneurons of the OB (Wichterle et al., 2001). The occurrence of processes of neurogenesis persist in the OB of adult mammals

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(Hinds, 1968a, b; Altman, 1969; Bayer, 1983). In postnatal life, granule and periglomerular cells of the OB originate from a multipotent stem cell compartment located in the subventricular zone (SVZ) surrounding the ependymal layer of the dorsolateral wall of the lateral ventricle (Luskin, 1993; Lois and Alvarez-Buylla, 1994). Lineage studies and cell transplantation suggest that the SVZ is derived from residual precursors of the embryonic LGE (Wichterle et al., 2001; Marshall et al., 2003). Neonatal and adult SVZ newly generated cells migrate tangentially along the rostral migratory stream (RMS) toward the OB (Luskin, 1993; Lois and Alvarez-Buylla, 1994). Upon reaching the core of the bulb, these migrating cells turn radially and disperse over the granule and glomerular layers, differentiate, and integrate into the olfactory circuit (for review, see Carleton et al., 2003).

Recent studies carried out in primates reported the existence of newly generated neurons in other telencephalic areas, including neocortex, piriform cortex, olfactory tubercle, and amygdala (Gould et al., 1999; Bedard et al., 2002; Bernier et al., 2002). Moreover, the existence of SVZ-derived cell migration through the subcortical parenchyma has been recently demonstrated in the adult rabbit brain (Luzzati et al., 2003). These findings suggest that, in addition, the RMS secondary neurogenic pathways from the SVZ may be present in the rodent brain. In the early postnatal rat brain, SVZ progenitors with a gliogenic potential migrate along a dorsal radial pathway into the overlying white matter and cortex (Marshall et al., 2003).

To investigate the fate of SVZ-derived progenitor cells in the forebrain of early postnatal and adult mice, we labeled SVZ progenitors at different postnatal ages by stereotaxic dye injection and tracked the subcortical targets at different survival periods. Our results indicate that cells in the SVZ of early neonates migrate ventrally across the nucleus accumbens to give rise to subcortical neuronal structures, in particular, the Islands of Calleja. A subpopulation of SVZ-derived progenitors deviate from the rostral migratory stream and course ventral and caudal to the olfactory tubercle. Thus, in addition to migration of neuroblasts to the OB along the rostral migratory stream, the neonatal SVZ gives rise to additional subcortical forebrain neuronal populations.

MATERIALS AND METHODS

Stereotaxic surgery and tracer injection

Stereotaxic injections were performed on neonatal and 8-week-old adult CD1 strain mice (Charles River Laboratories, Wilmington, MA). Neonates were injected either at postnatal day 1 or 6 (P1; P6; the day of birth was designated P0). Animals were housed, cared for, and used strictly in accordance with NIH Publication No. 85-23.1985. Mice at P1 and P6 were deeply anesthetized by hypothermia and their heads immobilized in a custom neonatal stereotaxic apparatus maintained at 4°C during surgery. Approximately 100 nl of Cell Tracker Green CMFDA (CTG) (10 mM in dimetheylsulfoxide; Molecular Probes, Eugene, OR) was injected at stereotaxic coordinates of 0 mm bregma line, 0.8 mm lateral to sagittal sinus, and 1.5 mm depth by means of a glass micropipette and a pneumatic pressure injection apparatus (Picospritzer II, General Valve, Fairfield, IL). After the micropipette was removed, the skin was sutured and the pups quickly revived on a warm platform and subsequently returned to the dam. The total duration under hypothermic anesthesia for postnatal mice was 15–20 minutes.

Adult animals were anesthetized by intraperitoneal injection of Nembutal (4–5 mg / 100 mg body weight in 0.9% saline solution) and positioned in a stereotaxic apparatus (Stoelting, Wood Dale, IL). The mice were maintained on a warm platform at 35°C during anesthesia. The surgical procedure followed the same steps described for neonatal mice, using stereotaxic coordinates of 0.5 mm anterior to bregma, 1 mm lateral to the sagittal sinus, and a depth of 2 mm. Following surgery the animals were left on a warm platform and constantly monitored until recovery.

Tissue processing

The distribution of the CTG-positive cells was analyzed in coronal and parasagittal sections at several times postinjection (at 4 hours, 8 hours; and 1, 2, 3, 4, 5, 6, and 15 days). At least three animals were examined at each survival time. Mice were deeply anesthetized with intraperitoneal injection of sodium pentobarbital (6.5 mg /100 g body weight) and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After dissection all specimens were postfixed for a further 4 hours, washed in 0.1 M PB for 15 minutes, and placed in 30% sucrose solution in 0.1 M PB for 24 hours at 4Â°C. The brains were embedded in Tissue-Tek optimal cutting compound (OCT; Sakura Finetek, Torrance, CA), frozen on dry ice-cooled isopentane, and stored at –70°C until sectioned.

Tracers characteristics and visualization

Cell Tracker Green CMFDA freely passes through cell membranes, and once inside a cell undergoes a series of specific reactions, producing a cell-impermeant fluorescent dye that is susceptible to aldehyde fixatives. This probe is retained in living cells through several generations and is not transferred among adjacent cells in a population. Previous work with the dye showed that it is a highly efficient method for labeling SVZ progenitor cells (De Marchis et al., 2001). In cases where the dye is injected into an axon fiber we never observed dye uptake and transport by the fibers. Unlike a viral green fluorescent protein construct, which takes over 24 hours to become detectable, CTG can be visualized in migrating cells immediately. Thus, CTG is ideally suited for examining migration from the injection site during the first few hours postinjection. Tissue blocks were serially sectioned at 25 m in either coronal or parasagittal plane and collected as floating sections. Staining and cell position reconstructions were made on every sixth section (i.e., every 150 µm through the forebrain).

Immunohistochemistry

The mature neuronal phenotype adopted by SVZderived cells was confirmed by labeling with a monoclonal anti-NeuN antibody. Sections were sequentially incubated in 1% bovine serum albumin (BSA) in TBST (0.1 M Tris, pH 7.4, 0.9% saline, and 0.3% Triton X-100), mouse anti-NeuN (1:10,000; Chemicon International, Temecula, CA) in 1% BSA in TBST for 24 hours, washed in TBST (three washes, 10 minutes each), Cy-3 conjugated donkey antimouse antibodies (1:500; Jackson ImmunoResearch Laboratories, West Grove, PA) in 1% BSA/TBST for 60 minutes, washed in TBS (three washes, 10 minutes each), and coverslipped with a DABCO (1,4-diazobicyclo-(2,2,2) octane)-based anti-fade mounting media. All incubations were performed at 22Â°C. Some sections were counterstained by incubation in 5 nM Sytox Orange (Molecular Probes) for 30 minutes at room temperature.

Photography and cell counting

Digital microscopy images were captured on an Olympus FluoView confocal microscope fitted with standard excitation and emission filters for the visualization of CY2, CY3, and CY5. The digital images were brightness, color, and contrast-balanced, assembled into montages using CorelDraw 11 (Corel USA). No additional digital image manipulation was performed.
The proportion of cells in the rostral migratory stream (RMS), ventral migratory mass (VMM), and ventrocaudal migratory stream (VMS) were estimated from optically sectioned confocal image stacks (14–15 images through each 25-µm section). A counting grid was placed perpendicular to each pathway and cells intersecting the grid lines counted. A total of three grid lines per section were used for the RMS, four lines per section for the VMM, and 10 lines per section for the VMS.

Due to the infrequency of cells in the VMS, a higher number of grid lines were used to ensure sufficient numbers of cells for reliable statistics (at least 10 cells measured). Two dorsoventral levels through the VMM were assessed. Since there were no statistical differences between the levels of the VMM, these data were pooled. Three animals were examined (postnatal day 3–5) and two sections from each animal. The number of cell intersections in the RMS was standardized to 100 and the intersections in the VMM and VMS represented as a fraction of the cells in the RMS. All counts are presented as mean ± standard error of the mean.

Camera lucida reconstructions

Camera lucida reconstructions of the location of CTGpositive cells at different survival times following tracer injection were generated using CorelDraw 11. Sections were photographed first at low magnification from Sytox Orange-stained sections and used to delineate the outline of the tissue and major subdivisions. High-magnification photographs were taken along the SVZ-RMS-OB and basal regions of the brain. A black spot was drawn corresponding to each CTG-positive cell. Camera lucida reconstruction dramatically increases the contrast and ease of viewing of the positions of labeled cells.

RESULTS

The subventricular zone of rodent has been shown to give rise to numerous interneurons that follow a highly defined pathway rostral into the olfactory bulb; the rostral migratory stream. To investigate whether SVZ-derived progenitors migrate into other regions of the forebrain during development and in adult, stereotaxic injections of Cell Tracker Green (CTG) into the SVZ were undertaken in neonatal and adult mice. The large size of the injections ensures that most of the rostral SVZ is labeled by the dye, allowing for even small migratory pathways exiting this region to be analyzed. However, while these injections are essential for identifying low-frequency migratory pathways, they do no allow the potential for migration from different specific sub-SVZ regions to be assessed. CTG crosses into all cells, where it is cleaved by cytoplasmic esterases and glutathione transferases into a fluorescent insoluble reaction product. After tracer injections, mice were sacrificed at different survival times, from a few hours to 2 weeks, and the distribution of CTG-labeled cells analyzed in serial sections through the forebrain.

Ventral migration from the subventricular zone

Injections of CTG into neonatal brain (P1) at stereotaxic coordinates for the SVZ of the lateral ventricle robustly label progenitor cells in that region (De Marchis et al., 2001). At short survival times (4 hours postinjection) labeling was confined to the SVZ and surrounding tissue, including the ependymal layer (Fig. 1A–D). Diffuse staining was observed surrounding the core of the injection. In these regions elongated radial glial-like and astrocyticlike cell bodies and processes were labeled. This staining remained static throughout all survival times and did not affect our ability to visualize cells once they migrated out of the region of the injection. At 8 hours postinjection CTG-labeled cells were emerging from the injection site halo along the proximal segment of the RMS vertical limb (Fig. 1E,F). In addition to cells entering the RMS, other cells began to exit the injection site ventrally over the nucleus accumbens (Fig. 1E,F). These cells were clearly visible in coronal sections at the ventrolateral border of the lateral ventricle (LV) (Fig. 1G,H) in close contact with negative ependymal cells. Since the same zone was devoid of CTG-labeled cells at 4 hours, we infer that these cells migrated down from a dorsal portion of the SVZ, stained at the moment of tracer injection. At 24 hours postinjection a large number of cells are present along the RMS (Fig. 1I,J). Numerous CTG-labeled cells are distributed widely through the dorsal half of rostrocaudal axis of nucleus accumbens (Fig. 1I,J). These cells migrate ventrally in a confined zone

through the basal region of the ventricular zone adjacent to the lateral ventricle until they reach the base of the ventricle (Figs. 1K,L, 2A). Thus, rather than migrating radially away from the ventricular zone, these cells migrate tangentially through the ventricular zone to the base of the LV and then radially into the nucleus accumbens. Cells at the base of the lateral ventricle disperse to migrate both medially and laterally through the nucleus accumbens, forming a "shower" of cells descending into the ventral forebrain (Figs. 1K,L, 2A). Collectively, we refer to the ventrally migrating cells derived from the SVZ as the ventral migratory mass (VMM). These cells show the typical morphology of migrating neuroblasts, with a round-elongated cell body bearing a long leading and/or trailing process (Fig. 2B,C). The total distance traveled by cells in the RMS over 24 hours is greater than the distance covered by cells migrating ventral in the VMM. The slower migration of VMM cells is consistent with reported radial migration rates (25–35 μm/hr) of individual cells in other brain regions compared to the 100–150 μm/hr rates along the RMS (De Marchis et al., 2001). Cells in the VMM are only present as individual cells and not in the clear chain arrangements seen in the RMS, suggesting different migratory mechanisms between these pathways. To estimate the frequency of cells entering the VMM compared to the RMS in postnatal mice, we compared the number of cells intersecting a perpendicular counting grid line in each pathway. For every 100 cells intersecting the counting grid in the RMS there were 73.2 ± 9.3 VMM cells intersecting the counting grid (n = 3 animals). However, since the migratory rate in the RMS is greater than in the VMM, the total cell number entering the ventral forebrain is likely to be lower (assuming radial migration rate vs. RMS migration rate there would be ~20 VMM cells entering the ventral brain per 100 RMS cells).

To determine whether the ventral migratory mass is a single event occurring only at P1 or forms a continuous stream, we injected mice at postnatal day 6 and adult. Injections carried out in P6 mice still show the presence of ventrally migrating cells along the pathway described above; however, the number of CTG-positive cells labeled at P6 is significantly lower than that found in mice injected at P1. In adult, no SVZ-derived cells were observed to migrate along the path of the VMM (not shown). This suggests that the VMM is a developmental pathway present only in early neonates and is absent in adults.

Destination of the ventral migration

The ventral forebrain contains a variety of functionally segregated regions including piriform cortex, anterior olfactory nucleus, tenia tecta, olfactory tubercle, and others. Cells descending in the VMM could contribute to any of these structures. To investigate the target sites of these cells we examined a series of coronal and parasagittal sections of P1-injected forebrains at 24-hour intervals from 1 day to 1 week postinjection. Sections at every 150 m were examined and the position of all labeled cells on selected sections reconstructed as camera lucida drawings (Fig. 3A–F). From this analysis we conclude that there are three stages to cell migration in the VMM: 1) Tangential ventricular migration (<24 hours postinjection) — As described above, during this phase cells migrate from the SVZ tangentially through the ventricular layer to the base of the lateral ventricle (Figs. 1, 3A,B). 2) Dispersion (1–4 days postinjection) — Migrating cells upon reaching the base of the lateral ventricle disperse in a ventromedial to ventrolateral arch through the nucleus accumbens (Figs. 2A, 3A–D). At 1–2 days postinjection most of these dispersing cells are found in the dorsal half of the accumbens, with only a few cells reaching the olfactory tubercle (Figs. 2A, 3A,B). At 3–4 days postinjection CTG-labeled cells are scattered throughout the nucleus accumbens, with numerous cells reaching the olfactory tubercle (Fig. 3C,D). At this stage there are reduced cells migrating down from the SVZ, probably due to the bulk of cells labeled by the initial injection having migrated out of the SVZ. 3) Reorganization (5–7 days postinjection) — The dispersed CTG cells gradually reorganize into a few tightly packed spherical or ellipsoidal clusters sharply delimited from the surrounding neuropil (Figs. 3E,F, 4). CTG cells in the clusters are small and

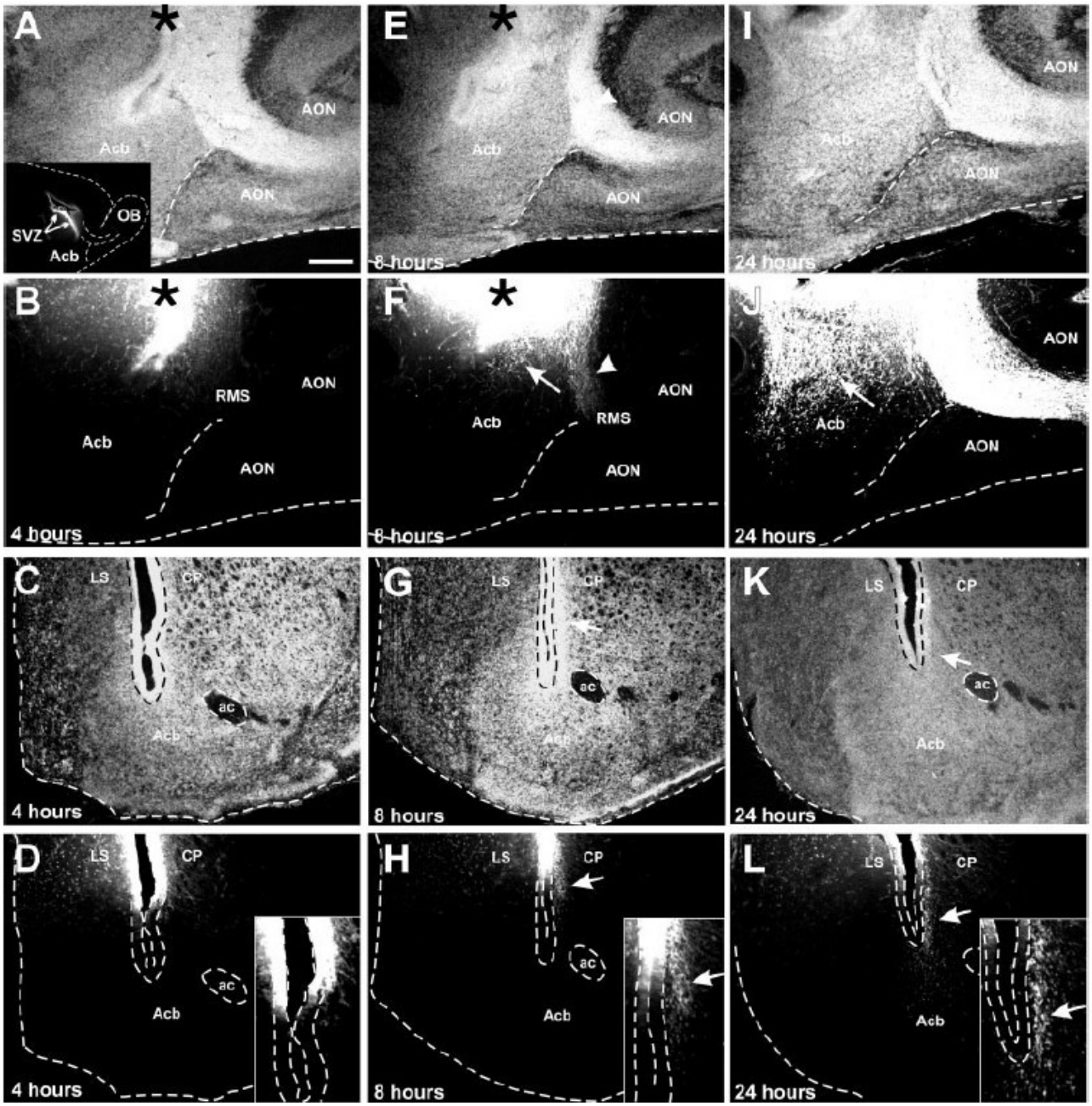

Fig. 1. The ventral migratory mass derives from the SVZ. A: Sagittal section at 4 hours postinjection (injections all performed at P1) showing the rostral migratory stream (RMS), nucleus accumbens (Acb), and anterior olfactory nucleus (AON) stained with the nonspecific nuclear counterstain Sytox Orange. The insert panel shows an SVZ injection site (arrows, 1 hour postinjection). ac, anterior commissure. B: Cell Tracker Green (CTG) labeling in the same section as A. No CTG-labeled cells have exited the halo of staining around the injection site (the edge of the injection site is indicated by an asterisk, the ventral staining along the ventricle derives from the injection). C: Sytox Orange-stained coronal section at 4 hours postinjection, showing the RMS, Acb, AON, and anterior commissure (ac). D: CTG labeling in the same section as C. Staining radiating lateral and medial from the lateral ventricle is due to dye labeling of radial glia cells, which have processes entering the injection site. E: Sytox Orange-stained sagittal section at 8 hours postinjection showing the RMS, Acb, and AON. F: CTG labeling in the same section as E, showing CTG-labeled cells along the proximal RMS (arrowhead) and migrating a short distance ventral into the Acb region (arrow). G: Sytox Orange-stained coronal section at 8 hours postinjection showing the RMS, Acb, AON, and ac. H: CTG labeling in the same section as C, showing SVZ progenitors migrating ventral along the lateral ventricle (tangential to the ventricular layer, arrow). I: Sytox Orangestained sagittal section at 24 hours postinjection showing the RMS, Acb, and AON. J: The same section as I, showing CTG-labeled cells migrating in the RMS and ventral across the Acb (arrow). K: Sytox Orange-stained coronal section at 24 hours postinjection showing the RMS, Acb, AON, and ac. L: In the same section as K, CTG-labeled cells migrate tangential to the lateral ventricle (arrow) and are dispersing ventral across the Acb as a migratory mass. Scale bar in A = 200 μm (applies to A–L).

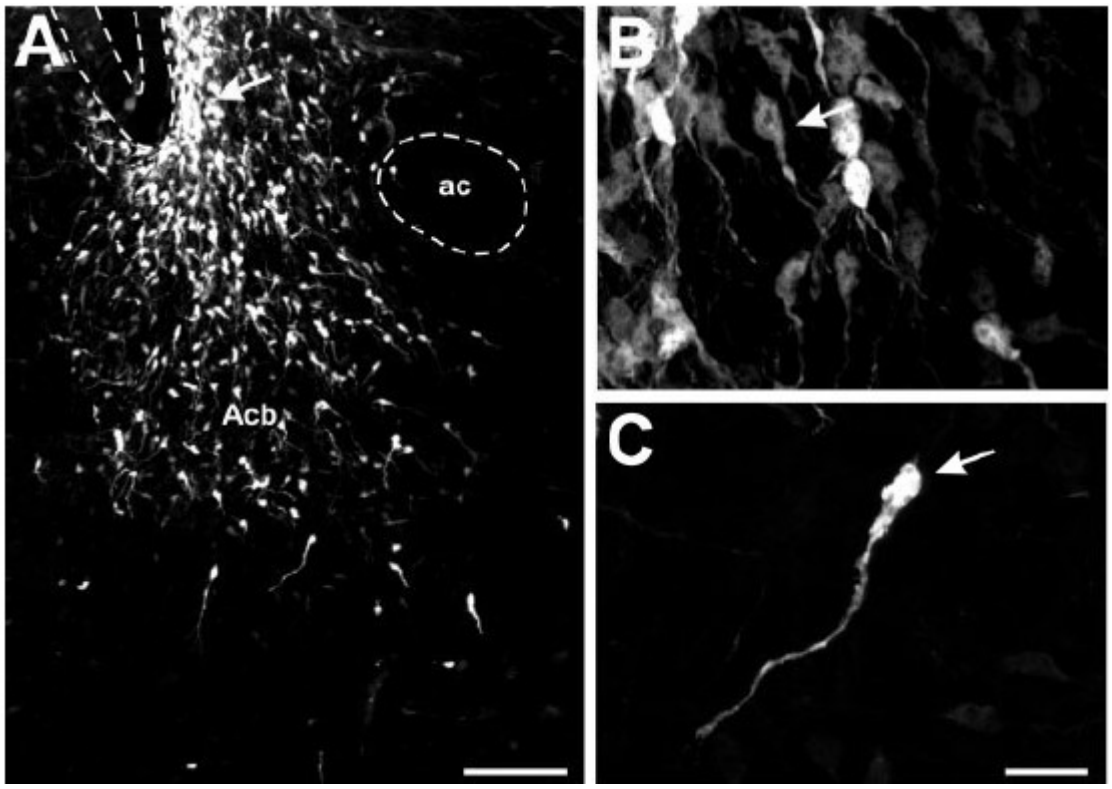

Fig. 2. Dispersion of ventral migratory mass cells across the nucleus accumbens (Acb). A: Cell Tracker Green-labeled cells 24 hours postinjection (injection performed at P1) have migrated to the base of the lateral ventricle and are dispersing across the Acb. Ac, anterior commissure. B: Cells in the ventral migratory mass are present as single cells radiating across the Acb (arrow) rather than a chain orientation, as seen in the RMS. C: Individual CTG-labeled cells have an elongated cell body with a short trailing process and a longer leading process and growth cone typical of migrating cells (arrow). Scale bars = 100 μm in A; 20 μm in C (applies to B).

have a bipolar morphology, with fine dendrites showing varicosity, but not spine-like processes (Fig. 4C). The clusters found in the nucleus accumbens were larger than that found in the olfactory tubercle polymorphic layer (Fig. 3E,F). The reorganization could be due to selective loss of cells not in these locations or migration of cells into these clusters. There were no apparent reductions in the number of cells in the basal forebrain at 7 days postinjection compared to earlier time points, and the tightly packed clusters gradually emerged from loosely organized, dispersed cells present at 1–4 days postinjection. Thus, while we cannot exclude some cell loss, we conclude that the clustering is due to migration and reorganization of SVZ-derived cells. CTG-labeled cells were still present at 15 days postinjection and the majority were colabeled for NeuN, a marker of differentiated neurons (Fig. 4G–I). The location and cellular organization of these spherical/ellipsoidal clusters correspond to descriptions of the granular Islands of Calleja, which include the small island structures in the olfactory tubercle and the larger insular magna of Calleja in the nucleus accumbens (Fallon et al., 1978; Fallon, 1983; Meyer et al., 1989). Thus, SVZ-derived neuroblasts migrate ventral and give rise to Island of Calleja cells. Many cells in the Islands of Calleja are GABAergic interneurons (unpubl. obs.), suggesting a common phenotype with the RMS neuroblasts that contribute to GABAergic interneurons in the olfactory bulb. In addition to organization into the Islands of Calleja, individual CTG-labeled cells are present in the nucleus accumbens and olfactory cortical regions.

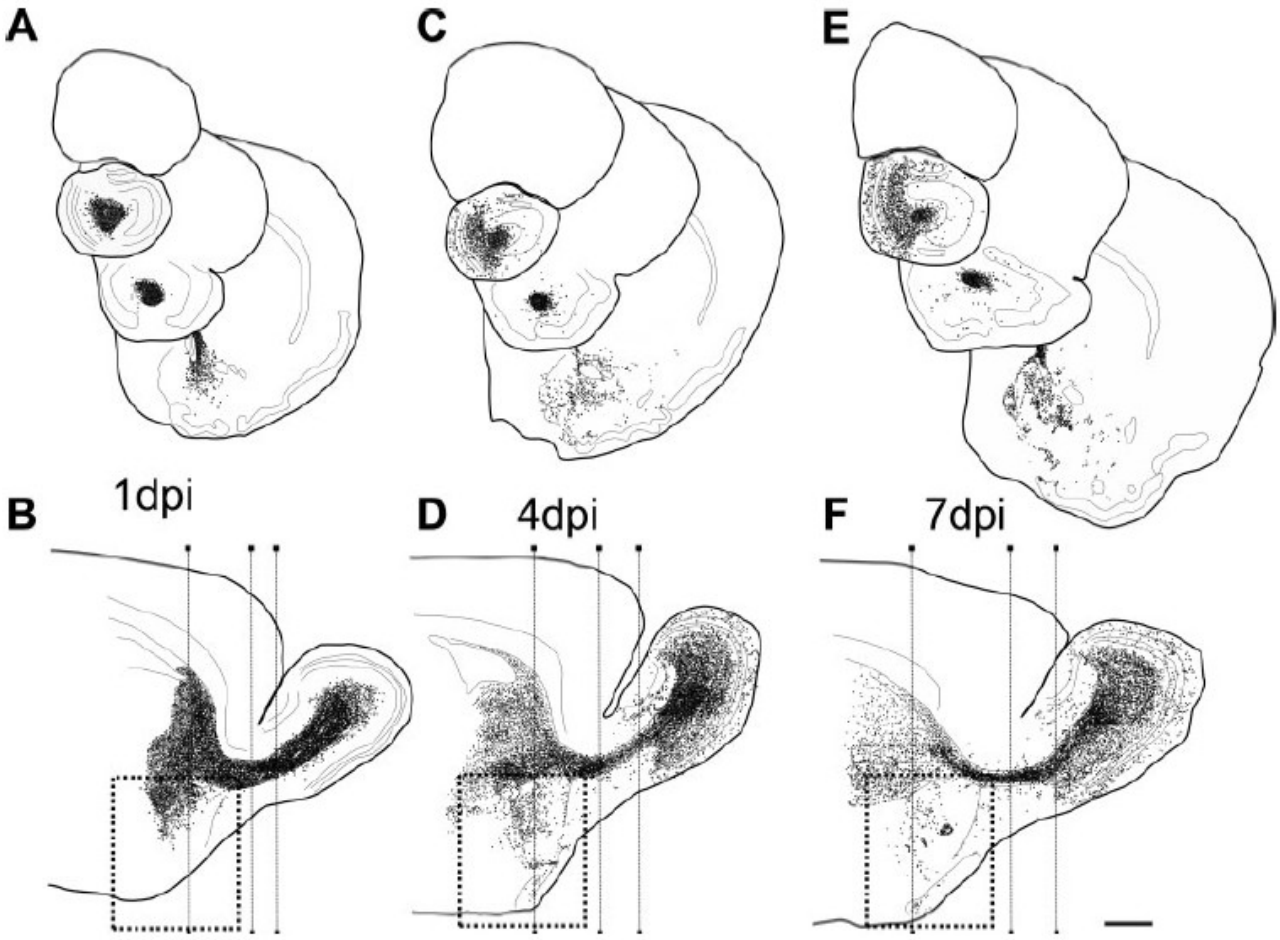

Fig. 3. Camera lucida reconstruction of the position of subventricular zone progenitors in the basal forebrain following a CTG injection in P1 mouse; (A,B) at 1 days postinjection, (C,D) at 4 days postinjection, and (E,F) at 7 days postinjection. At 1 and 4 days postinjection cells are dispersing ventral across the nucleus accumbens into the ventral forebrain. At 7 days postinjection there are clusters of labeled cells corresponding to the Islands of Calleja as well as individual cells scattered in ventral forebrain structures. Scale bar = 500 μm in F.

Rostral migration into the anterior piriform cortex and anterior olfactory nucleus

The rostral migratory stream is often considered a highly precise pathway for cells migrating from the SVZ into the olfactory bulb. In adult, the vast majority of migrating neuroblasts travel precisely to the olfactory bulb (with one exception, described below). However, during early postnatal development we have observed a small number of cells “shedding” off from the stream and migrating into the anterior piriform cortex and anterior olfactory nucleus. Individual CTG-labeled SVZ-derived progenitors exited the RMS and migrated radially into the piriform cortex and anterior olfactory nucleus (Fig. 3C–F). These cells represent a small fraction of the total population of RMS cells that reach the olfactory bulb and could be the result of migration errors, or a specific pathway providing additional neurons to the anterior olfactory nucleus and anterior piriform cortex during early postnatal development.

Ventrocaudal migratory stream; a branch of the rostral migratory stream

The RMS consists of a vertical, or descending, limb which turns rostral, forming an “elbow,” and courses into the central regions of the olfactory bulb. Mice injected at P1 and sectioned at 24-hour intervals from 1–7 days consistently show a group of CTG-labeled cells forming a pathway coursing from the elbow of the RMS towards the olfactory tubercle (Fig. 5A–C). Due to the location and direction of these migratory cells we refer to this pathway as the ventrocaudal

migratory stream (VMS). Several days postinjection these cells are present along the boundary between the nucleus accumbens and anterior olfactory nucleus. Individual cells have an elongated cell body and a leading process oriented parallel to the borderline between nucleus accumbens and anterior olfactory nucleus, typical of migratory neuroblasts. Often small groups of 2–3 cells are arranged in a linear order reminiscent of the "chain migration" organization found along the RMS. At 5 days postinjection the VMS pathway delineated by these migrating cells becomes more defined (Fig. 5C). The cells forming the VMS originating at the elbow between the vertical and the horizontal limbs of the RMS and coursing caudally are clearly distinct from the cells lying over the Acb and in the Islands of Calleja. This stream established a continuous pathway from the RMS to the olfactory tubercle, where numerous labeled cells were localized in the tubercle (Fig. 5C). This pattern strongly suggests the olfactory tubercle as a target of the VMS. However, we cannot exclude that cells distributed over the OT pyramidal layer derive at least in part from the VMM migration across the nucleus accumbens. The large number of labeled cells apparent in the RMS compared to the VMS suggests that only a very small fraction of SVZ-derived progenitors turn down the VMS towards the olfactory tubercle. To measure this we counted the number of cells intersecting a counting grid placed perpendicular to each pathway. For every 100 cells intersecting the counting grid in the RMS, there were 2.9 ± 0.1 VMS cells (n = 3 animals). At 15 days postinjection CTG-positive cells are present in the OT expressing NeuN (Fig. 5D–F). These results suggest that SVZ-derived cells migrate to the olfactory tubercle along the VMS, and possibly with the VMM, where they differentiate into new neurons.

In adult, the ventral migratory mass is no longer present and all the SVZ-derived progenitors enter the rostral migratory stream. However, while the majority of SVZ progenitors reach the olfactory bulb, a small fraction turn at the RMS elbow and migrate along the VMS between the nucleus accumbens and anterior olfactory nucleus (Fig. 6). Cells entering the VMS in adult are uncommon (on the order of 0.1% of RMS cells), making a statistical comparison with the RMS unreliable. Adult VMS cells also have the typical long leading process and occasional trailing process of migratory neuroblasts. In adult, the VMS represents only a tiny fraction of SVZderived cells.

DISCUSSION

Neural generation in the lateral, medial, and caudal ganglionic eminence germinal zone and migration into forebrain cortical and subcortical regions is critical for the formation of the highly laminated cortical structures and for generating the bulk of neurons in the early brain (Wichterle et al., 2001; Nery et al., 2002). The major neurogenic area of the postnatal and adult brain is the subventricular region, from which neuroblasts migrate primarily to the olfactory bulb. To investigate alternate migratory pathways for SVZ progenitors into the forebrain, we reconstructed the position of dye-labeled cells following injections into postnatal SVZ. Our data show that in addition to the well-characterized rostral migratory stream, SVZ-derived progenitors migrate in a ventral migratory mass across the nucleus accumbens into the basal forebrain and along a ventrocaudal migratory stream. Cells from the VMM and VMS give rise to neurons in the basal forebrain Islands of Calleja and olfactory tubercle, respectively.

The SVZ is defined as the layer of cells generated superficial to the embryonic ventricular layer following the formation of the cortical plate (Boulder Committee, 1970). This structure is thought to

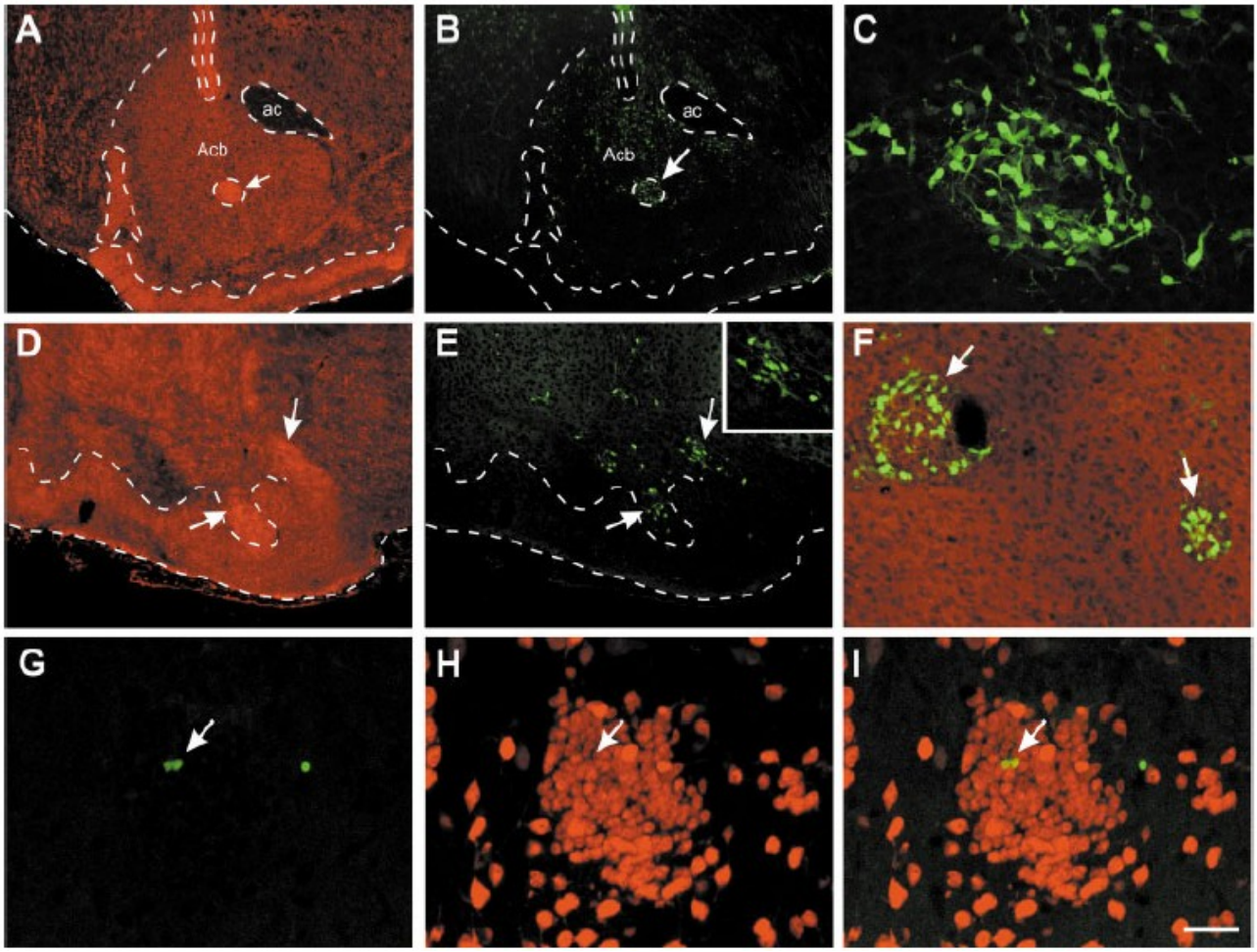

Fig. 4. Cells from the ventral migratory mass form Islands of Calleja. A: Coronal section through the nucleus accumbens showing the insular magna of Calleja (one of the Islands of Calleja) counterstained with the nuclear dye Sytox Orange (arrow). ac, anterior commissure. B: CTG-labeled cells form a cluster in the insular magna of Calleja (arrow). Tracer injections performed at P1. C: Higher magnification showing the cluster of CTG cells reorganizing in the insular magna of Calleja. D: Coronal section through the olfactory tubercle showing the smaller cellular aggregates of the Islands of Calleja counterstained with the nuclear dye Sytox Orange (arrows). E: CTG cells 6 days postinjection reorganizing into Islands of Calleja (arrows). The insert shows a higher magnification of one of the cell clusters. F: Reorganized Islands of Calleja incorporate numerous SVZ-derived CTG-labeled progenitor cells (arrows). G: CTG-labeled cells 15 days postinjection in an Island of Calleja (arrows). H: The same section as G stained with the neuronal marker NeuN. I: Overlay of G and H showing the CTG-labeled cells in the Island colabeled with NeuN (arrows). Scale bar = 200 μm in I (applies to A,B); 100 μm for D,E; 50 μm for F; 20 μm for C,G,H,I.

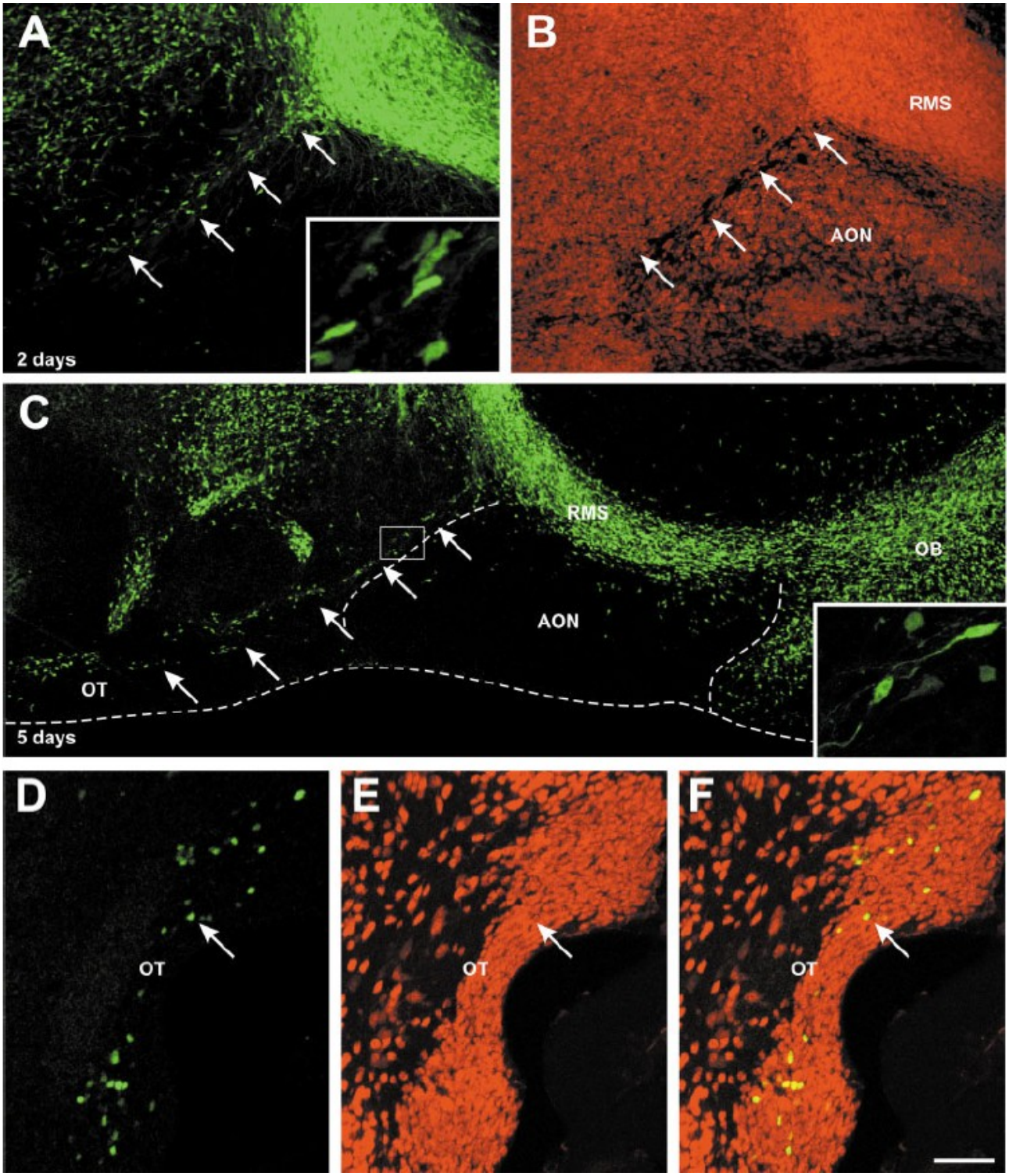

Fig. 5. The ventrocaudal migratory stream is a branch of the rostral migratory stream. A: In neonates a small population of cells migrates ventral and caudal from the elbow region of the rostral migratory stream towards the olfactory cortex (arrows). Tracer injections performed at P1. B: Sytox Orange staining of the same section as A. C: Montage showing the rostral migratory stream (RMS), olfactory bulb (OB), anterior olfactory nucleus (AON), olfactory tubercle (OT), and labeled cells migrating along the ventrocaudal migratory stream (arrows). These VMS cells form a continuous pathway towards the olfactory tubercle. D: CTG-labeled cells are present in the olfactory tubercle 15 days postinjection (arrow) and double-labeled for the neuronal marker NeuN (E,F). Scale bar = 100 μm in F (applies to A,B); 200 μm for C; 50 μm for D–F.

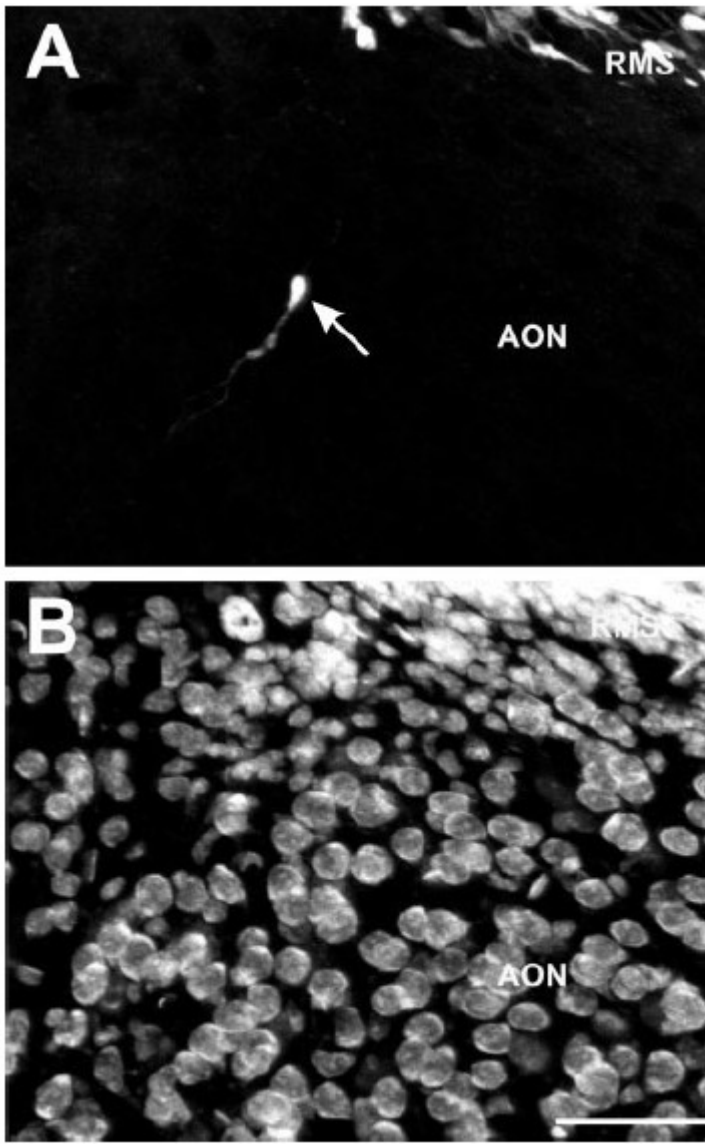

Fig. 6. The ventrocaudal migratory stream is present as a minor pathway in adult. A: A CTG-labeled cell (arrow) turning from the rostral migratory stream (RMS) down into the ventrocaudal migratory stream at the boundary of the anterior olfactory nucleus (AON). In adults, only a very small number of cells course down this pathway. B: Sytox Orange staining of the same section as A. Scale bar = 50 μm in B.

form from the embryonic lateral ganglionic eminence (Wichterle et al., 2001; Marshall et al., 2003). Postnatal SVZ progenitors give rise to both interneurons and glia. Viral tagging of SVZ cells shows that glial and neuronal progenitors take distinct migratory pathways. Progenitors migrate out of the SVZ into the dorsal and lateral white matter/cortex and striatum, following the direction of radial glia fibers, and develop into astrocytes and oligodendrocytes (Levison and Goldman, 1993; Luskin and McDermott, 1994; Zerlin et al., 1995). Progenitors migrate tangentially along the RMS into the olfactory bulb, where they switch to a radially oriented migration and eventually develop into olfactory interneurons (Luskin, 1993). These gliogenic and neurogenic progenitors, once thought to originate from a separated specialized compartment of the SVZ, the posterior and anterior SVZ, respectively (Luskin, 1993), were recently demonstrated to arise from the entire rostrocaudal extent of the SVZ, including the RMS (Suzuki and Goldman, 2003). Here we show that a mass of cells, the “ventral migratory mass,” migrate ventral from the SVZ into the basal forebrain and contribute to the formation of the Islands of Calleja. In a previous study, Kakita and Goldman (1999) found some cells that migrate tangentially from the dorsolateral SVZ into the striatal SVZ of postnatal forebrain slices in culture; however, the target of these cells was unclear. The ventral migratory

mass forms from a population of SVZ cells migrating ventrally, parallel to the surface of the ventricle, and may represent the same cells observed migrating in vitro by Kakita and Goldman (1999).

SVZ progenitor migration tangential to the lateral ventricle into the VMM does not appear to follow radial glia or chain migratory mechanisms; rather, individual neuroblasts migrate in a ventral direction spreading out through the nucleus accumbens. This migration may be more similar to the migration of adult SVZ progenitors radially in the olfactory bulb, where there are no glia scaffolds available to the cells. Unlike cortical migration, where cells migrate directly to a final target layer, cells in the VMM migrate in a dispersed pattern that then reorganizes into spheroidal and ellipsoidal cell aggregates.

This “ectopic migration” of SVZ progenitor cells then reorganizes into specific cell aggregates. Ectopic dispersion of axonal projections that then reorganize into specific targets is a common occurrence in neurodevelopment; however, similar ectopic migration that then reorganizes into specific targets is a novel phenomenon.

Islands of Calleja are aggregations of granule cells in the basal forebrain, originating from the ventral recess of the lateral ventricle (Sanides, 1958; Creps, 1974; Fallon et al., 1978; Fallon, 1983; Meyer et al., 1989). Interestingly, the ontogenetic development of these structures has been demonstrated to be protracted in early postnatal life in mouse and hamster (Creps, 1974; ten Donkelaar and Dederen, 1979). Based on the location of CTG-positive cells at different survival times and colocalization of CTG with neuronal marker NeuN, we show that these islands are derived from SVZ progenitor migration during postnatal development. The Island of Calleja structures in this region receive strong dopaminergic inputs from the substantia nigra / ventral tegmental area and project to several basal regions, including the nucleus accumbens and amygdala (Fallon et al., 1978; Fallon, 1983); thus, the Islands of Calleja have neural projections that are consistent with a role in the amphetamine/cocaine reward pathway. Interestingly, recent studies suggest the primary structure involved in the reward effects of amphetamine and cocaine is the olfactory tubercle (Ikemoto, 2003).

In addition to the ventral migratory mass, we observed a subpopulation of SVZ-derived progenitors migrate along the initial vertical segment of the rostral migratory stream, then deviate down a “ventrocaudal migratory stream” towards the olfactory tubercle, where numerous CTG and NeuN-labeled cells were localized in the pyramidal layer. This ventrocaudal pathway towards the olfactory tubercle, as well as the occurrence of newborn neurons into this region, are also present in the adult squirrel monkey (Bedard et al., 2002). However, we cannot exclude that the VMM contribute, at least in part, to the neurogenesis in the olfactory tubercle. In adult mouse, migration along the ventrocaudal migratory stream persists, but it is strongly reduced compared to that observed in early postnatal animals. This decrease could reflect the general reduction of the SVZ-RMS system observed in adult animals or a specific reduction in the VMS.

Our findings show postnatal SVZ progenitors take varying migratory routes to cortical and subcortical structures. These varying migratory routes suggest the presence of different extrinsic cues regulating migration along the different pathways. Although there are a wide range of secreted and membrane-bound molecules that have roles in regulating cell migration/fate in cortex and other structures, comparatively little is known about their role in SVZ-derived progenitor migration. Recent tests on a wide range of growth factors (18 different factors, several of which induce cortical and other cell migration) and several neurotransmitter/activity antagonists suggest that none of these factors influence the migration of SVZ progenitors in vitro (Mason et al., 2001). However, the polysialic acid-bearing form of the neural cell adhesion molecule (PSA-NCAM) (Tomasiewicz et al., 1993; Ono et al., 1994; Hu et al., 1996), ephrinB2/EphB2 interactions (Conover et al., 2000), slit/Robo interactions (Wu et al., 1999; Chen et al., 2001), reelin (Hack et al., 2002), and an uncharacterized astrocyte-derived factor (Mason et al., 2001) can all influence SVZ progenitor migration. Putatively, selective responses to these and other cues could direct different groups of migrating SVZ progenitors down different migratory pathways along the VMM, VMS, or RMS.

Distinct subpopulations within the SVZ may give rise to cells with specific predetermined migratory pathways, or alternatively, the same population of cells make stochastic choices for the different pathways at junction regions. The neonatal SVZ consists of at least two separate lineages, a gliogenic and a neurogenic population that selectively choose between migratory pathways. As noted above, only cells in a gliogenic lineage migrate into the cortical white matter and striatum (Levison and Goldman, 1993; Zerlin et al., 1995), whereas other SVZ cells appear to commit to a neurogenic lineage and migrate along the rostral migratory stream to the olfactory bulb (Luskin, 1993; Lois and Alvarez-Buylla, 1994). However, multipotent stem cells can still be isolated from along the rostral migratory stream and in the olfactory bulb (Law et al., 1999; Gritti et al., 2002; Liu and Martin, 2003). While neurogenic and gliogenic populations take segregated migratory pathways, it remains to be shown whether the neurogenic SVZ population subdivides into groups predestined for the VMM, VMS, or RMS.

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