Cell Migration in the Rat Embryonic Neocortex

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ABSTRACT

Three-dimensional reconstructions of the normal rat embryonic (E) neocortex on days E15, E17, E19, and E21, using Skandha (software designed by J. Prothero, University of Washington, Seattle), show that the neocortical ventricular zone shrinks rapidly in the medial direction during cortical morphogenesis. [3H]thymidine autoradiography indicates that the shrinkage of the ventricular zone occurs before neurons in lateral and ventrolateral parts of layers IV-II are generated. Consequently, most of these neurons originate 400–1000 μm medial to their settling sites in the cortical plate. Embryos killed at daily intervals up to E21 after a single injection of [3H]thymidine on either E17 or E18 revealed the presence of a prominent migratory path, the lateral cortical stream, used by neurons migrating to the lateral and ventrolateral cortical plate; neurons migrating to the dorsal cortical plate follow a direct radial path. Arrival times of neurons in the cortical plate depend on the migratory path and are proportional to the overall distance travelled. Neurons that migrate only radially arrive in the dorsal cortical plate in two days (shortest route). Neurons that migrate laterally arrive in the lateral cortical plate in 3 days (longer route) and in the ventrolateral cortical plate in 4 days (longest route). [3H]thymidine autoradiography also shows that cells generated in the neocortical ventricular zone migrate in the lateral cortical stream for 5 or more days and accumulate in a reservoir. Cells leave the reservoir to enter the piriform cortex and destinations (as yet undetermined) in the basal telencephalon. The lateral cortical stream is found wherever the neocortical primordium surrounds the basal ganglia and is absent behind the basal ganglia. A computer analysis of nuclear orientation in anterior and posterior parts of the intermediate zone in the dorsal neocortex between days E17 and E22 shows that horizontally oriented nuclei are more common anteriorly where many cells are migrating laterally than posteriorly where most cells are migrating radially.

Key words: neocortical development, [3H]thymidine autoradiography, three-dimensional computer reconstructions

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However, earlier observations and more recent empirical evidence have cast doubt on the hypothesis that simple radial migration can account for all aspects of the translocation of cells from the ventricular zone to the cortical plate. It has been known for some time that *horizontally oriented cells* abound in the subventricular and intermediate zones of the cortex. Such horizontal cells have been visualized with the Golgi technique (Derer, '74; Stensaas, '87; Valverde et al., '89), electron microscopy (Shoukimas and Hinds, '78), histochemical marking after retroviral infection (Vogt and Cepko, '88; Austin and Cepko, '90), and quantitative Nissl studies (Bayer and Altman, '91; Bayer et al., '91). Even before the radial glia hypothesis was proposed, Hicks and D'Amato ('68) tracked the migration of neocortical neurons with \[^{3}H\]thymidine autoradiography and reported that the young neurons migrate via "complex paths . . . leaving the proliferative zones to follow along the curving corticopetal fibers, only to leave them as they arrived at the cortical plate" (p. 626).

In this paper we show with sequential survival \[^{3}H\]thymidine autoradiography that many of the young neurons in the lower intermediate zone of the embryonic neocortex are actively migrating to the lateral and ventrolateral cortex in a prominent stream that skirts the border between the striatum and the neocortex. The impetus to search for laterally migrating cells was the visualization of the entire embryonic neocortex in three-dimensional computer reconstructions. Those images show a progressive shrinkage of the anterior cortical ventricular zone from ventrolateral to dorsomedial. Since we knew from \[^{3}H\]thymidine dating studies (Bayer, '90; Bayer and Altman, '91) that neurons settling in layers IV–II of the lateral and ventrolateral cortex are being generated for some time after ventricular shrinkage has begun, we surmised that the neurons settling in the ventrolateral and lateral areas of the anterior cortex must be generated more medially and, therefore, could not reach their targets by simple radial migration. Our examination of sequential survival \[^{3}H\]thymidine autoradiograms not only reveals the existence of a distinct stream of laterally migrating cells, what we call the *lateral cortical stream*, but also shows that there is a *progressive delay* in the time of arrival of synchronously generated neocortical neurons in the dorsal, lateral, and ventrolateral parts of the cortical plate. We also present preliminary observations showing that the *neocortical* ventricular zone generates cells that will settle *outside of the neocortex* in the basal telencephalon.

**MATERIALS AND METHODS**

**Animal preparation and histological procedures**

**Normal animals.** Wistar female rats were transferred to the home cages of individually housed Wistar male rats from 4:00 P.M. to 7:00 A.M. the next morning. Vaginal smears were taken from each female, and those that contained sperm were placed into maternity cages. The day of sperm positivity was the first day of gestation or embryonic day (E) 1. Food and water were provided ad lib throughout gestation. Between 8:30 and 9:00 A.M. on each day from E13 to E22, two or more dams were deeply anesthetized with sodium pentobarbital (35 mg/kg) and the embryos were removed and killed by immersion in Bouin's fixative; from E17 on, the embryos were decapitated before fixation, and from E19 on, the calvarium was removed after the heads had been fixed for approximately 20 minutes to aid penetration of the fixative into the brain. The embryos remained in Bouin's fixative for 24 hours, then were transferred to 10% neutral formalin (pH 7.4) until the time of embedding either in paraffin (Tissue Prep, Fisher) or in methacrylate (JB-4, Polysciences) according to standard histological procedures. Only well-preserved specimens were cut serially at 10 μm (paraffin, every section saved) or 3 μm (methacrylate, every 10th section saved) in the three cardinal planes and were stained with hematoxylin (paraffin) or toluidine blue (methacrylate). The animals used in this study were part of our large collection (approximately 1,500 specimens) of normal rat embryos.

**Animals used for \[^{3}H\]thymidine autoradiography.** The experimental animals were the embryos (prepared as described above) from Wistar timed-pregnant rats that were given a single subcutaneous injection of \[^{3}H\]thymidine (Schwarz-Mann; sp. act. 6.0 Ci/mM; 5 μCi/g body wt) between 9 and 11 A.M. Several dams were injected for each day between E13 and E21. Survival times in each injection group varied from 2 hours (short survival series) to several days (sequential survival series). For example, one dam in the E15 injection group was killed 2 hours after the injection, another was killed 1 day later on E16, another two days later on E17, and so on until the last dam was killed on E22. All groups were treated as the E15 group. The dams were anesthetized with pentobarbital before the embryos were removed and killed by immersion in Bouin's fixative. After 24 hours, the embryos were transferred to 10% neutral formalin until the time of embedding in methacrylate following standard histological techniques. The blocks were serially sectioned (every 10th saved) at 3 μm in the coronal, sagittal, and horizontal planes. For \[^{3}H\]thymidine autoradiography, the slides were coated with Kodak NTB-3 emulsion, exposed for 12–18 weeks, developed in Kodak D-19 and post-stained with hematoxylin.

**Three-dimensional reconstructions of the embryonic neocortex**

**Choosing the specimens and photographing the sections.** The entire neocortex and a portion of the lateral limbic cortex was reconstructed in three dimensions from paraffin sectioned embryos (part of our normal collection described above) on days E15, E17, E19, and E21. Because of the lengthy procedures involved in each reconstruction, only one representative embryo was chosen from each age group. Depending on the age of the embryo, every other section (E15) or every third section (E17–E21) through the cortex was photographed at low magnification using a Leitz/Wild microscope equipped with a zoom lens (6.3× to 35×) and an automatic exposure camera unit. The entire telencephalon and diencephalon on the right side of the brain, including the midline, was in each photograph. To assure that each section was photographed at exactly the same magnification, the zoom lens was marked at the magnification needed for the largest section, and all other sections were photographed at that magnification mark. A stage micrometer was also photographed at the set magnification at the beginning, midway, and at the end of the photographic session for each embryonic brain.

**Aligning the photographed brain sections.** In small embryonic brains, extensive tissue damage results from placing pinholes or lengthwise lesions that could serve as guidelines for alignment of the sections into a three-dimensional structure. We considered putting pinholes for
orientation into the opposite hemisphere, but this requires taking the photographs at a much lower magnification so that fine details (such as the border between the ventricular and subventricular zones) are difficult to see. Consequently, the photographed sections were aligned using the following method. By examining midline sagittal and parasagittal sections of embryos of the same age, the height of the dome of the cortex as it curves over the diencephalon was determined. Starting with the photograph of the middle anteroposterior section of the cortex, at approximately the highest point on the dome, we marked three fiducial points so as to form a triangle; that section was named the "orientation section." Using a light board, we transilluminated the photograph of the orientation section and placed the photograph of the section in front of it, matching up several anatomical landmarks. Once the section was aligned with the one used for orientation, we marked the fiducials on that photograph. Then, we removed the photograph of the orientation section and used the section that was just marked as a template to place fiducials on the next section in front. Periodically, we checked the fiducials of the photograph now being aligned with the fiducials on the orientation section to see how they conformed to the curvature in the sagittally sections brain. After sections in front of the orientation section were aligned, we used the same procedure to align the sections behind the orientation section.

**Delineating the neocortex and collecting the raw data.**

To delineate the ventrolateral edge of the neocortex, a straight (or slightly curved) line was drawn from the most lateral extension of the ventricular zone above the ganglionic eminence to the ventrolateral edge of the cortical plate, taking care not to include any part of the striatum. To delineate the dorsomedial edge, a line angling approximately 45° from the midline was drawn through cortex as it curves ventrally to form the medial cerebral wall. The prelimbic, cingulate, and retrosplenial areas in the medial cortical wall were not included in the reconstruction so that we could more easily visualize the changing thickness of the various layers of the developing neocortex from the medial side. Using a Rapidograph Pen (#00 point), the outline of the wedge of neocortex (within the defined borders) was drawn on each photograph, and lines were drawn at the interface between the ventricular and subventricular zones, at the base of the cortical plate, and at the surface of the cortical plate. Those delineations allowed us to reconstruct the entire neocortex, the ventricular zone, and the cortical plate. Since boundaries between the subventricular zone, intermediate zone, upper intermediate zone, and the subplate are not definite, all these were reconstructed together and were collectively called the transition field.

The marked photographs were placed onto a Summagraphics Digitizer and interfaced to an IBM PS/2 Model 80 computer with an 8514 monitor (resolution 1,024 x 780). First, the fiducials were digitized, followed by successively digitizing the outlines of the whole wedge of neocortex, the ventricular zone, the transitional field, and the cortical plate. The computer stored the X-Y coordinates outlining the profile of each structure and displayed the drawn outlines on the monitor. If the outlines were not accurately digitized, the program was set up to re-enter data for a particular section. Besides storing the X-Y coordinates, the computer also calculated the area of each structure and stored that along with the section number, which was used to determine the Z coordinate. After all of the sections had been digitized, a separate segment of the computer program aligned the data from each section in the file using the fiducial marks. The volume of each structure was also calculated. The final result was a large file (close to 2 megabytes in the E21 brain) that would serve as the data base for the reconstruction of either the entire neocortex, or specific layers.

**Final reconstruction.** The three-dimensional images were visualized by a software package, Skandha, developed by Dr. J. Prothero and his coworkers at the University of Washington, Seattle. The data from selected sections in the large raw data file were sent to a Silicon Graphics IRIS work station, and were prepared for Skandha by a file conversion program (Mor2dat). We used the raster display option in the menu so that images were reconstructed with solid surfaces. Rotation of the image in the X, Y, or Z coordinates to any desired vantage point is quickly and easily accomplished by using Skandha’s interactive format. The “sun” altitude, ranging from –90° to +90°, and “sun” azimuth, ranging from –180° to +180°, could be changed to bring out depth by using the light source to cast shadows on image surfaces. Especially helpful in reconstruction of the neocortex, Skandha also has a feature that allows deeper structures (shown at full opacity) to be viewed beneath or inside of superficial structures (shown at 50% capacity); there is also the option to make certain features completely transparent (0% opacity). In the images that we show, the most satisfactory views of the neocortex came from using a sun altitude of +4° and a sun azimuth of –15°. By making the subventricular and intermediate zones completely transparent, we were able to reconstruct the ventricular zone and the cortical plate in their exact spatial relationship to each other.

**Determination of cell orientation**

The same computer analysis that was used to determine nuclear orientation in the preceding companion paper (Bayer et al., ’91) was used to determine nuclear orientation in anterior and posterior sections of the intermediate zone in the dorsomedial neocortex. For each animal, photographic montages were constructed of two strips of dorsal neocortex (anterior and posterior) from the ventricular to the pial surfaces at a final magnification of 790x (anterior strips) or 810x (posterior strips). The vertical meridian (a line perpendicular to the ventricular and pial surfaces) was drawn on the montage. The outlines of nuclear profiles in the intermediate zone were drawn on the photographs with a Rapidograph #00 pen. Nuclear orientation was measured on a Summagraphics digitizer interfaced to an IBM PS/2 Model 80 computer. In each photographic montage, the end points of the vertical meridian were digitized first, next the nuclear profiles were traced. The vertical meridian was considered to be rotated exactly 90° above the horizontal axis, which was set at 0°. From the X-Y coordinates that outlined the profile of the cell nucleus, the computer program determined the long axis of each nucleus by drawing a straight line between the two most distant points in the profile. Then the program calculated the degrees that the long axis deviated from the horizontal (0°). The very few nuclei that were perfectly round were assumed to deviate 90°. The computer program sorted the data into histograms contain-
ing three groups. The vertical group (V) contained nuclei deviating from 60° to 90° from the horizontal, the oblique (O) group from 30° to 59.9°, the horizontal group (H) from 0° to 29.9°. Next, proportions were divided by calculating the number of nuclei in an orientation group (V, O, H) by the total number of nuclei measured in each animal. By representing the data as proportional, differences between animals in the number of nuclear profiles measured were minimized so that the focus was more clearly directed to the number of nuclear profiles measured were representing the data as proportional, differences between animals in the number of nuclear profiles measured were minimized so that the focus was more clearly directed to.

RESULTS

Changing spatial relationship between the ventricular zone and the cortical plate

Figure 1 is a medial view of the Skandha-generated images that reconstruct the ventricular zone (VZ, bright structure) and the cortical plate (CP, dark structure) on E15 (A, CP not present), E17 (B), E19 (C), and E21 (D). The spatial relationship between the two layers has been faithfully maintained by making the subventricular and intermediate zones transparent (0% opacity). That transparency causes the cortical plate and the ventricular zone to appear separated by a thin gap on E17, and by progressively wider gaps on E19 and E21.

The major pattern that emerges from the images in Figure 1, and by different rotations of those images in Figures 2, 3, is that the spatial relationship between the cortical plate and the ventricular zone changes during development. On E15 (Fig. 1A), a thick ventricular zone is the major component of the neocortex and its domed shape predicts the future appearance of the cortex; the cortical plate has not yet emerged. By E17 (Fig. 1B), the ventrolateral edge of a thin cortical plate (CPvl) extends farther down than that of the still thick ventricular zone (VZvl). The disparity between the ventrolateral edges of the two layers progressively increases on E19 (Fig. 1C) and E21 (Fig. 1D) as the ventricular zone continually thins and the cortical plate thickens. Figure 2 shows that E17 (A), E19 (B), and E21 (C) Skandha-generated images from the lateral aspect (Y axis rotation, 180°). The cortical plate is the gray "veil" (shown at 50% opacity) draped over the white ventricular zone (shown at full opacity). The numbers in the photographs refer to the distance between the lateral edge of the ventricular zone and the ventrolateral edge of the cortical plate (measured in the middle anteroposterior part of the neocortex). Figure 3 shows these images rotated +90° in the Y axis so that one can see the posterior edges of the ventricular zone (VZp) and the cortical plate (CPP). The images are also rotated -20° in the X axis so that the frontal poles are tipped down, the occipital poles up; that allows visualization of the widening gap between the cortical plate and the ventricular zone (filled by the subventricular and intermediate zones). The narrowing of the anterior ventricular zone (V Za) becomes progressively more prominent from E17 (A) through E21 (C), while the posterior edge of the ventricular zone is nearly coextensive (connected with black lines) with the span of the posterior cortical plate at all ages.

Taken together, the images in Figures 1–3 show the dramatic shrinkage of the neocortical part of the ventricular zone. That is caused by rapid growth of the basal ganglia (not reconstructed) in a dorsomedial direction from primordia in the ventrolateral telencephalon that obliterates the contiguity between the lateral ventricle and the lateral neocortical primordium. As a result, the neocortical ventricular zone is displaced dorsomedially. Since this happens from the earliest appearance of the cortical plate (there is even a slight medial shift on E16; Bayer and Altman, '91), many of the young neurons that will settle permanently in the dorsolateral and ventrolateral neocortex must be generated as much as 400 to 1000 µm more medially. That implies that young neurons could not reach the dorsolateral and ventrolateral parts of the cortical plate by following a strict radial migratory path.

The lateral cortical stream in thymidine autoradiograms

Choice of injection groups for observation. Since cells in the superficial layers of the neocortex are generated later than deep cells (Angevine and Sidman, '61), the superficial cells in the lateral cortex are more likely to have lateral migratory paths. Consequently, our aim was to find an injection group that would maximally label these young neurons. Table 1 shows the peak days of neurogenesis in each one of the superficial layers (IV–II) in laterally and ventrolaterally situated cortical areas (Bayer and Altman, '91). E17 is the peak day for layer IV neurogenesis in most areas; E18 is the peak for layer III, E19 for layer II. The days when the last neurons are generated in layer II are also listed and range from E19 (secondary auditory cortex) to E21 (primary somatosensory cortex). Since E17 is also the time when many layer V cells are generated in the laterally situated primary somatosensory cortex, a maximal number of heavily labeled young neurons migrating to the lateral cortical plate should be evident in 24 hour intervals after an E17 injection. A few observations will also be presented from the E18 injection group because many neurons destined to settle in the lateral cortex are also generated on that day.

Cell migration in the lateral cortical stream and the delayed settling of neurons in the lateral cortical plate

Following a single injection of [3H]thymidine on E17, the locations of intensely labeled cells are shown in the anterior neocortical primordium in animals killed from E18 through E21 (Figs. 4, 5). On E18 (Fig. 4A), 1 day after injection, there are as yet no heavily labeled cells in the cortical plate. Instead, young neurons generated on E17 are concentrated in two bands: 1) the first inferior band (ib1) in the deepest part of the subventricular zone, and 2) the second superior band (sb2) in the center of the intermediate zone. Each of these bands is described elsewhere (Altman and Bayer, '91; Bayer and Altman, '91). Heavily labeled cells are also densely distributed in what we call the "head" (h) of the lateral cortical stream (lcs). The head spans the lateral edges of ib1 and sb2. The lateral cortical stream contains unlabeled cells (generated before E17) migrating (double arrow) along the lateral border of the basal ganglia (BG). There is also a dense accumulation of unlabeled cells in what we call the "reservoir" (r) at the base of the lateral cortical stream.

By E19 (Fig. 4B), two days after injection, the first neurons generated on E17 reach the dorsal neocortical plate (single arrows), but none have yet penetrated the
Fig. 1. Medial views of computer generated (Skandha) 3-dimensional reconstructions of ventricular zone (white structure) and cortical plate (gray structure) in the embryonic neocortex from E15 to E21 (A–D). For all photographs, anterior is left, posterior is right, dorsal is at the top, and ventral is at the bottom (scale bar = 1 mm). The space between the dorsomedial cortical plate (CPdm) and the dorsomedial ventricular zone (VZDM) that widens between E17 (B) and E21 (D) is taken up by the subventricular zone and intermediate zones, which have been made transparent (0% opacity) so that the CP and VZ can be seen in their spatial relationship to each other. VL, VL, ventrolateral.
lateral cortical plate. Bands of heavily labeled cells are no longer seen in the intermediate and subventricular zones, but a concentrated group of heavily labeled cells, presumably generated on E17, is now in the head (h) and in the lateral cortical stream (lcs) as it arches around (double arrow) the border of the basal ganglia (BG). Cells in the reservoir (r) are still mainly unlabeled, except for a few dorsal ones.

By E20 (Fig. 4C), 3 days after their generation, heavily labeled neurons first arrive in the lateral cortical plate (top two single arrows). Many heavily labeled cells are still in the intermediate zone beneath the thin portion of the cortical plate that will presumably differentiate into the insular area (ICP, lowest single arrow). By now, the head (h) of the lateral cortical stream contains only lightly labeled cells.

Fig. 2. The same images in Figure 1B–D rotated 180° in the Y axis so that the observer is viewing the cortical plate (gray) and the ventricular zone (white) from the lateral aspect (anterior, left; posterior, right; dorsal, top; ventral, bottom; scale bar = 1 mm). The cortical plate is shown at 50% opacity so that the ventricular zone can be seen beneath it. Numbers in the photographs indicate the distance between the lateral edges of the ventricular zone and the cortical plate.

Fig. 3. The same images in Figure 2 rotated +90° in the Y axis and −20° in the X axis so that the observer is viewing the ventricular zone (white wedge) and the cortical plate (gray shell) from their posterior edges (VZp, CPp) and is looking slightly downward toward the frontal pole (medial is right, lateral is left, dorsal is top, ventral is bottom; scale bar = 1 mm). The coextensive span of the posterior cortical plate and ventricular zone is readily apparent (connected by black lines), while the prominent narrowing of the anterior ventricular zone (VZa) can be seen as one looks toward the frontal pole. DM, dorsomedial; VL, ventrolateral; CPA, anterior cortical plate.
TABLE I. Peak Days of Neurogenesis of the Superficial Cells (LIV–LII) in Lateral and Ventrolateral Cortical Areas1

<table>
<thead>
<tr>
<th>Cortical area</th>
<th>Layer</th>
<th>Last cells reached</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV</td>
<td>III</td>
</tr>
<tr>
<td>TE1 AS4</td>
<td>E17</td>
<td>E16</td>
</tr>
<tr>
<td>TE2 AS4</td>
<td>E17</td>
<td>E17</td>
</tr>
<tr>
<td>PARI A5.4</td>
<td>E17–E18</td>
<td>E16</td>
</tr>
<tr>
<td>PARI A5.4</td>
<td>E17</td>
<td>E17–E18</td>
</tr>
<tr>
<td>PARI A7.4</td>
<td>E17–E18</td>
<td>E16–E19</td>
</tr>
<tr>
<td>PARI A7.4</td>
<td>E17</td>
<td>E17–E18</td>
</tr>
<tr>
<td>A3 A9.2–A9.4</td>
<td>E16</td>
<td>E17</td>
</tr>
</tbody>
</table>

1These data are from Bayer and Altman (‘91) and Bayer (‘90). "Peak" is defined as the day when the highest proportion of cells is generated in the entire span of neurogenesis for each layer. Auditory areas: TE1-primary; TE2-secondary; Somato sensory areas: PARI-primary; PARI-secondary; AI-agranular insular area; AI-GU-gustatory area. The numbers (A3.4 to A9.2) refer to levels of coronal sections in the Pellegrino et al. (‘79) stereotaxic atlas of the rat brain.

The many heavily labeled cells that were in the head on E19 are migrating in the lateral cortical stream (les) and are now distributed throughout the reservoir (r).

On E21 (Fig. 5), 4 days after their generation, an oblique coronal section of the anterior neocortical primordium shows heavily labeled cells (presumably E17 birthdays) begin to penetrate the thin insular cortical plate (ICP, two top arrows). By this time, there are no heavily labeled cells in the lateral cortical stream (now indistinct) but many are still in the reservoir (r). Some of these cells appear to be migrating into the deep layers of the piriform cortex primordium (PCP).

High magnification views of the delayed time of arrival of neurons in the lateral and ventrolateral cortical plate (Figs. 6, 7)

By E19, two days after [3H]thymidine injection, heavily labeled neurons are distributed throughout the dorsal cortical plate (Fig. 6A). Some have already reached the superficial border, others are still scattered in the upper (izu) and lower (izl) parts of the intermediate zone. In the lateral cortical plate (Fig. 6B), most of the labeled cells are still in the intermediate zone but a few are crossing through the subplate (SP) and are reaching the deep border of the cortical plate. By E20, three days after [3H]thymidine injection, heavily labeled neurons have fully penetrated the lateral cortical plate (Fig. 7A). However, further ventrolaterally in the insular cortical plate (ICP, Fig. 7B) most of the heavily labeled cells are still in the lateral cortical stream (Fig. 7B, les), while a few are migrating radially (arrow); they will not reach the superficial parts of the insular cortical plate until E21 (Fig. 5).

High magnification views of migration in the lateral cortical stream and the accumulation of cells in the reservoir (Figs. 8, 9)

On E18 (Fig. 8), cells heavily labeled by a single [3H]thymidine injection on E17 are concentrated in the head (h) of the lateral cortical stream (outlined by dashed lines). Some heavily labeled cells are sparsely scattered in the upper part of the lateral cortical stream but none have yet reached its lower part or its reservoir (r). By E19 (Fig. 9A), the heavily labeled cells have left the head, and most appear to have migrated into the lateral cortical stream (large double arrow) and are just penetrating the dorsal part of the reservoir. On E20 (Fig. 9B), heavily labeled cells are leaving the lateral cortical stream and the reservoir (single arrows). Many of them are in the lateral cortical plate; others are migrating toward the ventrolateral cortical plate. However, heavily labeled cells are still abundant in the reservoir (r, double arrow).

Observations in the E18 injection group

The same events that are seen following an E17 injection are also seen in sequential-survival [3H]thymidine autoradiograms after an E18 injection (Figs. 10, 11). Figure 10 shows that the heavily labeled cells, presumably generated on E18, have penetrated the dorsal cortical plate by E20; that is shown at higher magnification in Figure 11A. However, the lateral cortical plate and the insular cortical plate (ICP) have only a few labeled cells (Figs. 10, 11B).

While some of these could be young neurons, most of them appear to be locally multiplying glial cells and endothelial cells. The difference in concentration between the labeled cells in the dorsal cortex (Fig. 11A) versus the lateral cortex (Fig. 11B) indicates that many more young neurons have reached their destination dorsally than laterally, where many labeled young neurons are still in the lateral cortical stream (large curved arrow, Figs. 10, 11B). In subsequent survival times (not shown), most neurons generated on E18 were found to have the same delay in arrival as those generated on E17. It takes 3 days to reach the lateral cortical plate and 4 days to reach the insular cortical plate.

Cell orientation in anterior and posterior parts of the intermediate zone in the dorsal neocortex

In the sequential survival autoradiograms described above, the lateral cortical stream is most prominent in the anterior three-fourths of the neocortical primordium where the basal ganglia occupy a large part of the lateral cortical wall. In more posterior coronal sections, the lateral cortical stream becomes progressively less noticeable, and disappears as soon as the sections are behind the basal ganglia. As that change is taking place, the cortical ventricular zone is becoming broader, extending farther beneath the lateral cortical wall. In the most posterior sections, the ventricular zone and the cortical plate have the same mediolateral span (Fig. 3). Heavily labeled cells arrive simultaneously at both dorsal and lateral parts of the cortical plate in sections behind the basal ganglia (not shown), indicating that lateral cell migration probably does not occur in the most posterior neocortical primordium. In the preceding companion paper (Bayer et al., ‘91), we reported that horizontally oriented nuclei predominate in the intermediate zone at a middle anteroposterior level of the dorsal neocortex where the ventricular zone has shifted dorsoomedially and where cells are migrating laterally. Would cells with horizontally oriented nuclei predominate in a posterior level of the dorsal neocortex where the ventricular zone has not shifted dorsoomedially and where cells are not migrating laterally?

To answer that question, we quantitatively investigated whether or not nuclear orientation was different in the intermediate zone of the anterior vs. posterior dorsal neocortex (drawings, Fig. 12). The anterior section examined was at the decussation of the anterior commissure, while the posterior section examined was the one where the hippocampal primordium extended as far down as the ventrolateral edge of the cortical plate. Only the anterior section contained a medially displaced ventricular zone.
Figure 4
Fig. 5. An oblique coronal section of the anterior neocortex in an E21 rat embryonic brain that was exposed to a single injection of $[^3]$H$/$thymidine on E17. Four days after injection, heavily labeled cells have now penetrated the insular cortical plate (ICP) and are widely distributed in the reservoir (r). Some cells appear to be migrating from the reservoir into the piriform cortical plate (PCP). (3 $\mu$m methacrylate section, hematoxylin stain. Other labels: BG, basal ganglia; CC, corpus callosum; CP, cortical plate; h, head; lv, lateral ventricle.)

Fig. 4. Coronal sections of the anterior neocortical primordium in rat embryonic brains at daily intervals after a single injection of $[^3]$H$/$thymidine on E17. The black bands or dots represent heavily labeled cells. One day after injection on E18 (A), the cortical plate (CP) contains no labeled cells. Heavily labeled cells arrive in the dorsal CP 2 days after injection on E19 (B) and arrive in the lateral CP 3 days after injection on E20 (CP), but have not yet reached the insular cortical plate (ICP). Between E18 (A) and E20 (C) heavily labeled cells also migrate into the head (h) of the lateral cortical stream (lcs) and eventually fill the reservoir (r). (3 $\mu$m methacrylate sections, hematoxylin stain. Other labels: AC, anterior commissure; BG, basal ganglia; IB1, inferior band 1; lv, lateral ventricle; sb2, superior band 2.)
Fig. 6. The dorsal (A) and lateral (B) parts of the cortical plate (CP) in a rat embryo exposed to a single injection of [3H]thymidine on E17 and killed on E19. Intensely labeled cells have reached the most superficial part of the CP in A, but are just reaching its deepest part in B. (3 μm methacrylate sections, hematoxylin stain. Other labels: I, layer I; izl, lower intermediate zone; izu, upper intermediate zone; SP, subplate.)

The data of three age groups (bar graphs, Fig. 12) were selected to show the orientations of cell nuclei in the intermediate zone at early (E17), middle (E19), and late (E21) stages. On E17 (left column of graphs, Fig. 12), the anterior intermediate zone has a predominance of cells with horizontally oriented nuclei (approximately 63%), while the posterior intermediate zone has only 31% of its cells with horizontally oriented nuclei, more (36%) are vertically oriented. On E19 (center column of graphs, Fig. 12), cells with vertically oriented nuclei predominate in the posterior intermediate zone (54%), while the anterior intermediate zone still contains more cells with horizontally oriented (38%) than vertically oriented (28%) nuclei. By E21 (left bank of graphs, Fig. 12), the cells at both anterior and posterior levels have a broad distribution of nuclear orientations. The repeated measures analysis of variance indicated that the anterior and posterior strips have significantly different means (F = 30.68; df = 1, 6; P = 0.0015), best shown by the data on E17 and E19. There is a significant interaction between means and age (F = 23.22; df = 5.6; P = 0.0007), best shown by the similarities in the data on E21.
Fig. 7. The lateral cortical plate (A, CP) and ventrolateral insular cortical plate (B, ICP) in a rat embryo exposed to a single injection of \(^{3}H\)thymidine on E17 and killed on E20. Intensely labeled cells have reached the most superficial part of the CP in A (arrow), but are just reaching its deepest part in B (arrow) and many are still in the lateral cortical stream (lcs). Note that only the lateral cortical plate (A) has an underlying subplate. However, some of the sparsely scattered separated large cells at the base of the insular cortical plate (B) may be infiltrated subplate neurons (Bayer, '90). (3 μm methacrylate sections, hematoxylin stain. Other labels: I, layer I; SP, subplate.)

DISCUSSION
Lateral cell migration and delayed arrival of neurons in the lateral cortical plate

The progressive shrinkage of the ventricular zone from ventrolateral to dorsomedial that the Skandha-generated images show (Figs. 1–3) is a prominent structural feature of the developing neocortex, noticeable as soon as the cortical plate appears on E16 (Bayer and Altman, '91), and quite evident on E17 (Figs. 1B, 2A). Since ventricular zone shrinkage starts during the generation of cells for layers VI and V and becomes progressively more pronounced before and during generation of cells for lateral layers IV–II (Table 1), it follows that many neurons will settle in the cortical plate a considerable distance (400–1,000 μm) lateral to their site of origin. Correlated \(^{3}H\)thymidine autoradiographic studies (summarized in Fig. 13) showed two migratory paths: 1) a radial one to the dorsal cortical plate and 2) a lateral one to the lateral and ventrolateral cortical plate. The latter path was inferred by observing sequential
shifts in the positions of heavily labeled cells in the lateral cortical stream (Figs. 4, 5). A radial path in the dorsal cortex and a lateral path in the lateral cortex have also been inferred after tracking the dispersal of clonally related cells in the developing mouse cerebral cortex after retroviral infection (Austin and Cepko, '90). Depending on the migratory path and the distance traveled, there is a progressive delay in the time of arrival of neurons in the cortical plate. Neurons that migrate only radially arrive in 2 days in the dorsomedial neocortex (shortest distance). Those that migrate laterally arrive in 3 days in the lateral neocortex (longer distance) and in 4 days in the ventrolateral neocortex (longest distance). A surprising finding is that the neocortical ventricular zone generates cells that settle (5 or
Fig. 9. The lateral cortical stream and reservoir (outlined) in rat embryonic brains exposed to a single injection of \(^{[3]}H\)thymidine on E17 and killed on E19 (A) and E20 (B). Heavily labeled cells are in the dorsal part of the reservoir by E19 and are throughout its extent by E20. Heavily labeled cells leave both the stream and the reservoir (lateral arrows in B) to penetrate the lateral cortical plate and move toward the insular cortical plate. (3 μm methacrylate sections, hematoxylin stain. Other labels: h, head; r, reservoir; SP, subplate; CP, cortical plate.)
Fig. 10. The anterior neocortex in a rat embryo that was exposed to a single injection of \[\text{H}\]thymidine on E18 and was killed on E20. Two days after injection, heavily labeled cells have penetrated the dorsal cortical plate (CP) (upright arrows), but have not reached the lateral cortical plate or the ventrolateral insular cortical plate (ICP). Heavily labeled cells are migrating into the lateral cortical stream (large curved arrow) around the basal ganglia (BG). (3 KRN methacrylate section, hematoxylin stain. Other label: SE, septum.)

more days later) outside of the neocortex, in the piriform cortex and possibly other sites in the basal telencephalon (question mark, Fig. 13). The possibility that neurons generated in the neocortical ventricular zone migrate outside of the neocortex can be inferred from a recent study of the ontogeny of cholinergic neurons in the mouse (Schambra et al., '89) where young cholinergic neurons appear to be leaving the lateral cortical stream and penetrating the basal telencephalon (see Figs. 6-1D, 7-1C, and 7-2E and F in Schambra et al., '89).

In the preceding companion paper (Bayer et al., '91), we presented quantitative evidence that a majority of the cells in the intermediate and subventricular zones have horizontally oriented nuclei. Here we show (Fig. 12) that cells with horizontally oriented nuclei are more common in the intermediate zone of the anterior dorsal neocortex (where the ventricular zone is displaced) than in the posterior dorsal neocortex (where the ventricular zone is not displaced). These findings indicate that many of the horizontally oriented cells in anterior and middle parts of the developing neocortex must be actively migrating laterally to parts of the cortical plate devoid of an underlying ventricular zone.

**Comments on the head and reservoir of the lateral cortical stream**

We have shown earlier (Altman, 1966) that a portion of what we now describe as the head of the lateral cortical stream (a cluster of primitive cells near the lateral edge of
the lateral ventricle above the striatum) contains proliferative cells in the adult rat cortex. These cells were interpreted to be locally multiplying glia. From this finding we infer that heavily labeled glia as well as neurons coexist in the head of the lateral cortical stream in the embryonic cortex. Since all heavily labeled cells move out of the head (Figs. 4, 8, 9), it is quite likely that from the beginning of its appearance, the lateral cortical stream contains not only young migrating neurons but also glial cells that accompany the neurons. We have shown that the cortical germinal matrix becomes transformed from being a source of both neurons and glia to one that produces only nonneuronal cells after cortical neurogenesis is completed (Altman and Bayer, '90). The lateral migratory stream could be understood as a pathway for both neurons and glia in embryonic brains, but only a pathway for glia in adult brains. Since glia retain their proliferative capacity, the remnants of the head of the lateral cortical stream become "pockets" of locally multiplying cells in the adult brain.

The reservoir is large during late embryonic stages (E19 to E21, Figs. 4B, 4C, and 5) but it shrinks considerably after birth (unpublished observations). In adults, the few cells that remain in the reservoir constitute the most anterolateral intercalated mass in the amygdala (Bayer, '80). It is interesting that the reservoir tends to retain heavily labeled cells for several days after birth. For example, the cells labeled on E17 and E18 represent 65% of the total cells in the reservoir on P5, but by P60, only 34% of these cells are labeled by the same injection schedule, indicating that many of these cells move out to as yet unknown destinations in the basal telencephalon (in preparation).

Theories of cell migration in the neocortex

For close to 20 years, the literature on cell migration in the neocortex has been dominated by the radial glia hypothesis proposed by Rakic ('72, '78, '82, '88). The basic assumption is that the radial glia link particular sites in the ventricular zone to specific columns in the cortical plate so that young neurons migrating on radial glia are guided to their exact destinations in the cortical plate (Rakic, '88). Indeed, those neurons that settle in the dorsal cortical plate follow a radial path (Figs. 4B, 6A, 10, 11A). But the same sections show that neurons settling in the lateral and ventrolateral cortical plate follow a lateral path rather than a radial one (Figs. 4–11). Many studies concerned with the radial glia fiber system have examined the dorsal neocortex where a ventricular zone is always present (Meller and Tetzlaff, '75; Pinto-Lord et al., '82; Goffinet, '84; Gadisseux and Evrard, '85; Misson et al., '88a; Gadisseux et al., '89). However, two studies (Misson et al., '88b; Edwards et al., '90) used RC1 and RC2, monoclonal antibody markers for radial glia, to examine the lateral and ventrolateral neocortex in mice. They found a dense immunoreactive band in the same position as the lateral cortical stream that we describe here. The low magnification photographs in these publications do not allow us to distinguish between glial cells with short processes (which we postulate migrate with neurons) and true radial glia, which have cell bodies in the ventricular zone and long processes extending to the pial surface. A Golgi study by Smart and Sturrock ('79) in the mouse telencephalon presented evidence of a circumferential fiber band around the basal ganglia extending from the head of the lateral cortical stream to the reservoir. The band consists of scattered perikarya with shorter and longer processes, described by Smart and Sturrock as

Fig. 11. High magnification views of the dorsal cortical plate (A) and ventrolateral cortical plate (B) from the same section shown in Figure 10. Heavily labeled cells (young neurons) are in the cortical plate dorsally, but laterally they are still in the lateral cortical stream (large curved arrow, B). The sparsely scattered labeled cells in the cortical plate in B are most likely locally multiplying glial cells.
Figure 12
NEOCORTICAL CELL MIGRATION

Obviously, the original radial glia hypothesis requires some modification. First, the cells migrating laterally follow a circumferential rather than a radial path. Second, the presumed glial cells in the lateral cortical stream, as visualized with the Golgi technique (Smart and Sturrock, '79), do not have the exact topographic features of radial glial cells with perikarya located in the germinal matrix. Further research with immunohistochemical techniques that can distinguish between glial cells and migrating neurons is needed to clarify the identity of the cells with shorter and longer processes.

Lateral migration of neocortical neurons has been suggested by others. Hicks and D'Amato ('68) proposed that the cells migrate along the incoming thalamocortical axons. The Golgi studies of Valverde et al. ('89) prompted the authors to suggest that the horizontal orientation of cells in the lower intermediate zone could be lateral migration. After infecting ventricular zone cells with a histochemically detectable retrovirus Walsh and Cepko ('88) noted that clusters of marked cells, presumably clones, appear to be migrating tangential to the surface of the ventricular zone. More recently, Austin and Cepko ('90) tracked clones of cells produced after retroviral infection in the developing mouse neocortex and essentially have come to similar conclusions about the lateral migration of cortical neurons that we describe here and in more detail elsewhere (Bayer and Altman, '91).

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