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Histogenesis of Ferret Somatosensory Cortex

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ABSTRACT

The ferret has emerged as an important animal model for the study of neocortical development. Although detailed studies of the birthdates of neurons populating the ferret visual cortex are available, the birthdates of neurons that reside in somatosensory cortex have not been determined. The current study used bromodeoxyuridine to establish when neurons inhabiting the somatosensory cortex are generated in the ferret; some animals also received injections of [^{3}H]thymidine. In contrast to reports of neurogenesis in ferret visual cortex, most neurons populating the somatosensory cortex have been generated by birth. Although components of all somatosensory cortical layers have been produced at postnatal day 0, the layers are not distinctly formed but develop over a period of several weeks. A small number of neurons continue to be produced for a few days postnatally. The majority of cells belonging to a given layer are born over a period of approximately 3 days, although the subplate and last (layer 2) generated layer take somewhat longer. Although neurogenesis of the neocortex begins along a similar time line for visual and somatosensory cortex, the neurons populating the visual cortex lag substantially during the generation of layer 4, which takes more than 1 week for ferret visual cortex. Layer formation in ferret somatosensory cortex follows many established principles of cortical neurogenesis, such as the well-known inside-out development of cortical layers and the rostro-to-caudal progression of cell birth. In comparison with the development of ferret visual cortex, however, the generation of the somatosensory cortex occurs remarkably early and may reflect distinct differences in mechanisms of development between the two sensory areas. J. Comp. Neurol. 387:179–193, 1997.

Indexing terms: cerebral cortex; development; bromodeoxyuridine; [^{3}H]thymidine; neuronal birthdates

Although the clearly described inside-out pattern of development in cortical neogenesis has been known for many years, details about the emergence of layers in the cerebral cortex are not available for many species (Bayer and Altman, 1991). The ferret is becoming an important animal model for studying development, and many recent studies focus on the developing ferret to understand mechanisms of maturation in the neocortex. Ferrets are especially good models for understanding development of the neocortex, because they have a protracted period of cortical neogenesis that continues after birth (Jackson et al., 1989).

To more clearly understand mechanisms contributing to development of the cerebral cortex, it is necessary to provide more detail regarding the date of birth of neurons residing in various regions of the cerebral cortex. A detailed study of cortical neogenesis of ferret visual cortex (areas 17 and 18) was completed by Jackson and colleagues (1989). Although this study provides a good basis for understanding the relative timing of formation of layers in ferret neocortex, it does not answer specific questions about cortical areas other than visual.

In the past several years, a number of studies suggest that different sensory regions of neocortex may also differ in their developmental time line and thus in response to deprivation. For example, application of tetrodotoxin (TTX) or *N*-methyl-D-aspartate (NMDA) antagonists to either the eye or visual cortex prevents formation of ocular dominance columns (Reiter et al., 1986; Stryker and

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Harris, 1986). Similar applications to peripheral or central sites in the rodent somatosensory system do not inhibit formation of cellular aggregates in the barrel system (Chiaia et al., 1992a,b; Hendersen et al., 1992; Chiaia et al., 1994). A partial explanation for the differences induced by pharmacologic blockade of activity in the two systems is the relative state of maturation in each cortical site. That is, the somatosensory system appears to mature before the visual system, thereby influencing the ability to display subsequent plastic changes in the neocortex. The details of cortical development have rarely been compared in different sensory regions, especially in a more highly developed mammal, such as the ferret. The current study was designed to determine the date of birth for neurons that populate each cortical layer in ferret somatosensory cortex. We present data indicating that the somatosensory cortex develops substantially earlier than the dates reported for ferret visual cortex. Much of this information was presented earlier in abstract form (Noctor et al., 1994).

TABLE 1. Dates of BRDU and [^{3}H]thymidine Injection and Subsequent Dates of Analysis

Dates of injection	Dates of analysis				
	P1	P7	P14	P28	P64
E22	●	●			●
E26	●◆		●◆	●◆	●◆
E28	●		●	●	
E30	●		●	●	●
E33	●		●	●	
E34	●◆		●◆	●◆	●◆
E35	●		●	●	
E38	●◆	●◆		●◆	●
P1	●◆	●		●	●
P2				●	
P4		●	●	●	
P7				●	

● Dates of analysis of BRDU injections.
◆ Dates of analysis of [^{3}H]thymidine injection.
Each symbol represents a date of analysis; a total of 19 pregnant ferrets were injected, and 89 kits were used for analysis.

MATERIALS AND METHODS

Pregnant or neonatal ferrets were injected with 5-bromo-2′-deoxyuridine (BRDU). BRDU has proven over the past several years to be a useful alternative to [^{3}H]thymidine for assessing the birthdates of neurons. BRDU is an analog of thymidine that becomes incorporated into the nucleus during cell division and can be subsequently localized immunohistochemically; it thereby eliminates the need for radioactivity and autoradiography (Gratzner, 1982; Miller and Nowakowski, 1988; Nowakowski et al., 1989). A total of 19 pregnant ferrets were injected with BRDU or [^{3}H]thymidine, and 89 of their offspring were included in this study. Timed pregnant ferrets were obtained from Marshall Farms, New Rose, NY. The day of conception is considered to be embryonic day 0 (E0) and the day of birth (consistently E41 or 42) considered postnatal day 0 (P0). Sixteen ferret kits were injected with BRDU and/or [^{3}H]thymidine postnatally. In these cases, the ferret kit received an IP injection of either BRDU (150 mg/k in 0.15 ml) or [^{3}H]thymidine (500 µCi in 0.15 ml). At selected time points, ferrets were killed, and their somatosensory cortex was examined for distribution of cells that incorporated BRDU into their nucleus (BRDU+) while dividing.

IV and IP methods of injection were used. Both methods resulted in good uptake of the thymidine analog and resulting label. Animals that received IV injections were anesthetized with halothane (2–3% through a nose cone). A small incision was made over the external jugular vein, and a catheter was inserted. The BRDU was injected (50–75 mg/kg in saline with 0.007 N NaOH) in a volume of 5 ml. Ferrets that received IP injections were also anesthetized with halothane, and the same dilution of BRDU was injected. To compare possible differences in uptake between BRDU and [^{3}H]thymidine, three animals received [^{3}H]thymidine injections IV (4 mCi in 4 cc of saline) at time points that corresponded with selected BRDU injections (see Table 1); three pregnant jills and two postnatal kits (P1) were injected with both BRDU and [^{3}H]thymidine. After different survival times (see Table 1) ferrets were given an overdose of pentobarbital Na (60 mg/kg IP) and perfused through the heart with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer, followed by 10% sucrose in the same buffer. The brain was removed and sunk in the 10% sucrose buffer, followed by 20% sucrose buffer at 4°C. Each brain was then blocked and frozen in isopentane and kept at −70°C until cut in a cryostat. Sections were cut at 40 µm thickness in the coronal plane and collected on subbed slides. Alternate series of sections were saved for staining with cresyl violet for visualization of Nissl substance, immunohistochemistry for BRDU, or for autoradiography (if [^{3}H]thymidine was injected). The Nissl-stained sections were used to assess the locations of cortical layers. All procedures involving animals were approved by the USUHS animal care and use committee and conformed to all guidelines established by the NIH.

The reaction for visualizing BRDU involved an adaptation of the procedure used by Miller and Nowakowski (1988). The sections were incubated in 95% EtOH and 5% acetic acid for 30 minutes at room temperature, and then in 2 N HCl with 1 mg/ml pepsin for 1 hour at 37°C. This was followed by one rinse for 3 minutes with phosphate-buffered saline (PBS) at pH 8.5. The sections were then incubated overnight in the primary antibody (anti-BRDU, Becton Dickson, San Jose, CA; concentration 1:20) in PBS (pH 7.4) with 0.05% Tween 20 at 4°C. After this, sections were rinsed in PBS, pH 7.4, three times for 5 minutes each and incubated in the secondary biotinylated antibody (concentration 1:100) in PBS with 1.5% normal horse serum. This was followed by three rinses in PBS at pH 7.4 for 5 minutes each. Sections were then incubated in ABC (avidin-biotin complex, Elite standard Vectastain kit, Vector Laboratories, Burlingame, CA) for 1 hour and rinsed in PBS pH 7.4 three times for 5 minutes each. The sections were then placed in diaminobenzidine (40 mg/ml) for 8 minutes, followed by a final rinse in PBS pH 7.4. for 5 minutes.

The sections used for visualizing [^{3}H]thymidine were dipped in Kodak NTB-2 emulsion and stored in the dark at 4°C for 4–6 weeks. After development in Kodak D-19, the sections were fixed and subsequently stained for appreciation of Nissl substance with cresyl violet. Cells were considered heavily labeled with [^{3}H]thymidine if they contained at least five times as many silver grains as those observed in background (i.e., unlabeled) regions.

The labeled cells were viewed on a light microscope and their locations drawn using a drawing tube. The cells incorporating BRDU were easy to view; in almost all instances they were clearly and darkly stained. For the vast majority of cases, the BRDU was injected days to

months before the animal was terminated, allowing enough time for the BRDU to be further diluted in nonneuronal cells that might have taken up the thymidine analog. Labeled cells were also identified by using morphological criteria to distinguish glia from neurons. Any lightly or ambiguously labeled cells were excluded from analysis. Adjacent sections stained for Nissl substance were used to identify cortical architecture. The photomicrographs shown here were digitized and enhanced slightly to increase contrast by using Adobe Photoshop.

A quantitative analysis was conducted on representative sections at different ages. A given section was digitized by using Image ProPlus (Media Cybernetics, Silver Spring, MD) and the optical density of five representative BRDU-labeled cells determined on each section to be analyzed. These cells were chosen by eye and always fell into a limited range of density values. A threshold value was set at the mean optical density value of the five labeled cells; this value always included clearly labeled cells but excluded lightly or ambiguously labeled cells. The threshold cells were counted in a 500-μm-wide band of cortex and assigned to bins according to their laminar position. After the counting, the file was transferred to Microsoft Excel and a histogram was created.

RESULTS

Because the development of the neocortex proceeds in a well-known lateral-to-medial and rostral-to-caudal progression, our analysis focused on a limited area of the somatosensory cortex. The forelimb region of area 3b in the somatosensory cortex is located in a precise position that can be located by using a number of cytoarchitectural and morphologic criteria. In young ferrets, the cortical architecture is not mature, but the position of the forelimb can be recognized grossly by the location of the postcruciate dimple, and in tissue sections by the dimple, the placement of the anterior commissure, and the coronal sulcus (Fig. 1). As the animal matures, delineation of the different cytoarchitectural fields becomes evident (Fig. 2; McLaughlin et al., 1995, 1996; Juliano et al., 1996). At younger ages (postnatal day 1; P1–P7) the cortical plate could be delineated; it consisted of the poorly differentiated layers 5 and 6, and the forming layers 2–4. Layer 1 was also present as well as a distinct subplate (Fig. 2). By P14 each cortical layer could be distinguished, although the cytoarchitecture in somatosensory cortex was not mature until P28. Through comparison of the BRDU label with adjacent sections stained for Nissl substance, each labeled cell could be assigned a laminar position. Injections were made in pregnant ferrets or postnatal animals at time points ranging from E22 to P7 (see Table 1).

Injections on E22–E26

BRDU incorporation after injections at this age labels cells in the subplate (Figs. 2, 3, and 4). Labeled cells are clearly observed in young animals from P1 to P7 in the subplate; they are diminished by P14, and by P28, only very few scattered labeled cells are seen in mature cortex (Figs. 3 and 5). These observations confirm earlier reports that many subplate cells do not survive into adulthood (e.g., see Allendoerfer and Shatz, 1994 for review). Cells labeled after injection at E22 are positioned slightly deeper within the subplate than cells labeled after injection at E26 (Fig. 3).

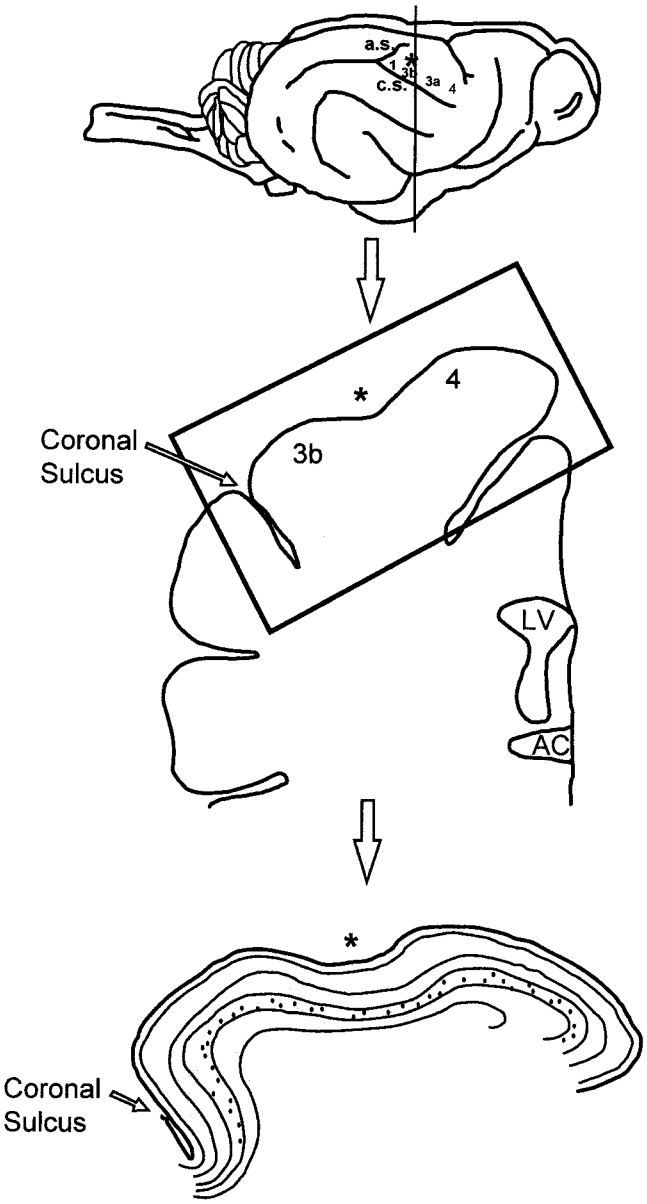

Fig. 1. Top: Schematic dorsolateral view of a ferret brain; the plane of section is indicated by a vertical line and the postcentral dimple is denoted by an asterisk. The cytoarchitectural fields in the region of interest that can be viewed from the surface are indicated (areas 4, 3a, 3b, 1). Middle: The appearance of a coronal section through the marked plane. The region of interest is shaded in the middle and bottom. Bottom: A typical region in a section used for analysis in the adult. a.s., ansate sulcus; c.s., coronal sulcus.

Injections on E28–E30

Injections of BRDU on these days result in labeled cells located in the deeper layers. After E28 injections, the labeled cells are distributed in the immature layers 5 and 6 on P1, with the strongest concentration located in the deepest part of the cortex, near the junction with the subplate (Fig. 6). E30 injections also result in BRDU+ cells in deep portions of immature cortex, although shifted slightly superficial compared with cells labeled after E28 injections (Fig. 6). Injections at both times result in labeled

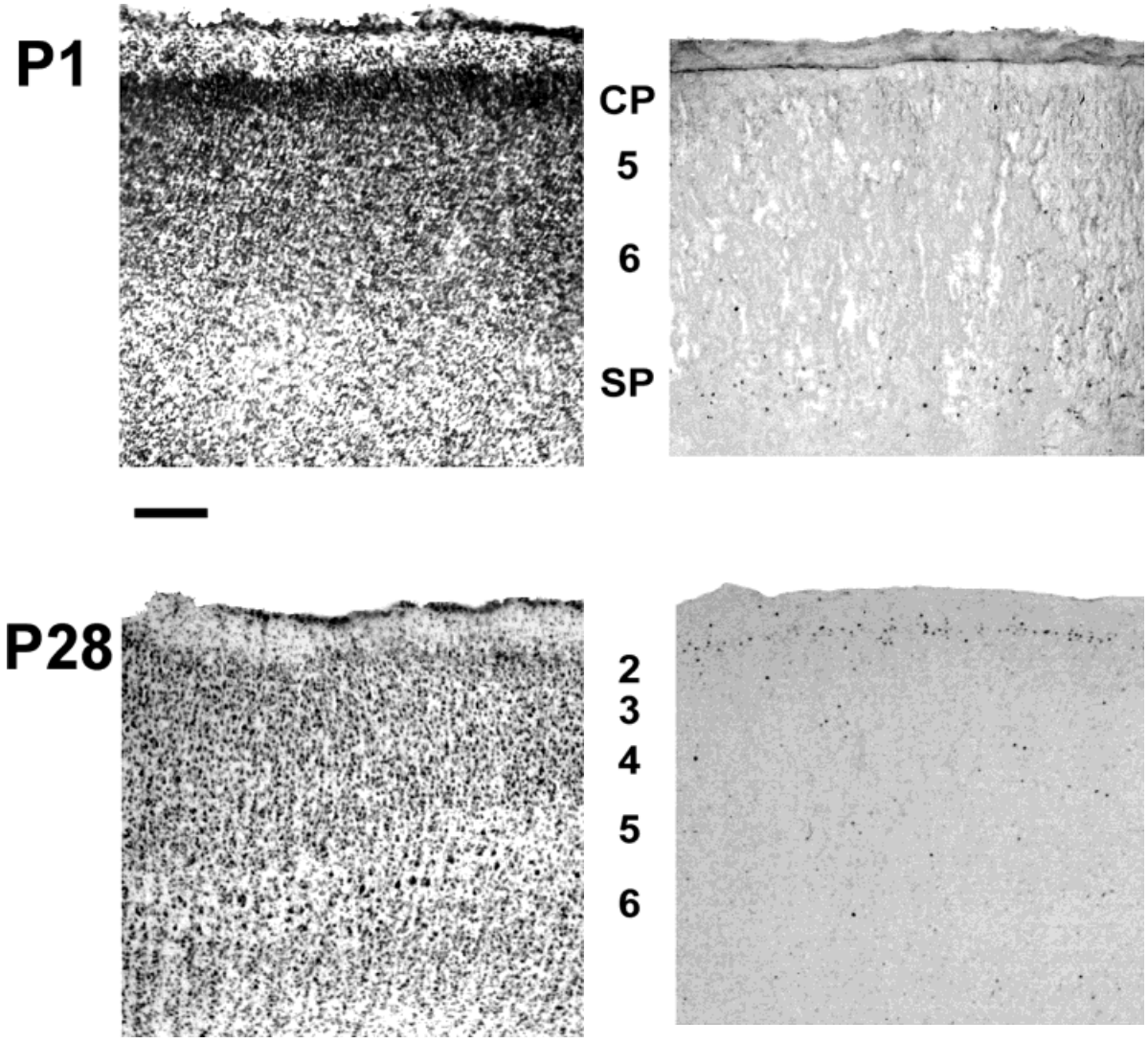

Fig. 2. Photomicrographs of adjacent sections either stained for Nissl substance (left) or immunohistochemical visualization of BRDU (right). The localization of BRDU+ cells to cortical layers is shown in the adjacent Nissl-stained sections. Two examples are illustrated, one in which BRDU was injected into the pregnant dam on embryonic day (E24) and labeled cells appear in the subplate on postnatal day 1 (P1; top) and another in which BRDU was injected into a ferret kit on P2, which produced label in the cytoarchitecturally mature cortex in upper layer 2 on P28 (bottom). Cortical layers are indicated with numbers (CP, cortical plate; SP, subplate). Scale bar = 100 μm, top; 250 μm, bottom.

cells throughout the thickness of layers 5 and 6, although even at P1, a distinct stratification of label is evident, with injections at E28 resulting in an overall deeper pattern of label than injections at E30. In mature cortex, the label is more clearly stratified and E28 BRDU injections label cells found in layer 6, whereas E30 injections result in layer 5 BRDU+ cells.

Injections on E33–E35

In neonates (P1–P3), BRDU injections on E33–E35 produce labeled cells throughout the immature cortical plate above layer 5 (Fig. 4). Even among BRDU+ cells resulting from injections at ages E33, E34, or E35, a slight stratification of labeled cells exists, with more cells located superficially in the cortical plate after E34 injections, and a greater number of cells found deeply after E33 injections (Fig. 7). By P28, in the cytoarchitecturally mature somatosensory cortex, the majority of labeled cells produced from injections at E33–E35 occurred in layer 4. At P28, the label was also slightly stratified, in that injections at E33 resulted in labeled cells found in slightly deeper portions of layer 4 and in upper layer 5, and injections at E35 caused BRDU+ cells in more superficial parts of layer 4 and in lower layer 3. Injections at E33–E34 generated labeled cells almost totally confined to layer 4 (Figs. 5 and 7).

Injections on E38–P1

Injections at E38–P1 caused label in more superficial portions of the cortex. After E38 injections, the BRDU+ cells were less stratified on P1 than after any of the previous injection times. Many BRDU+ cells were ob-

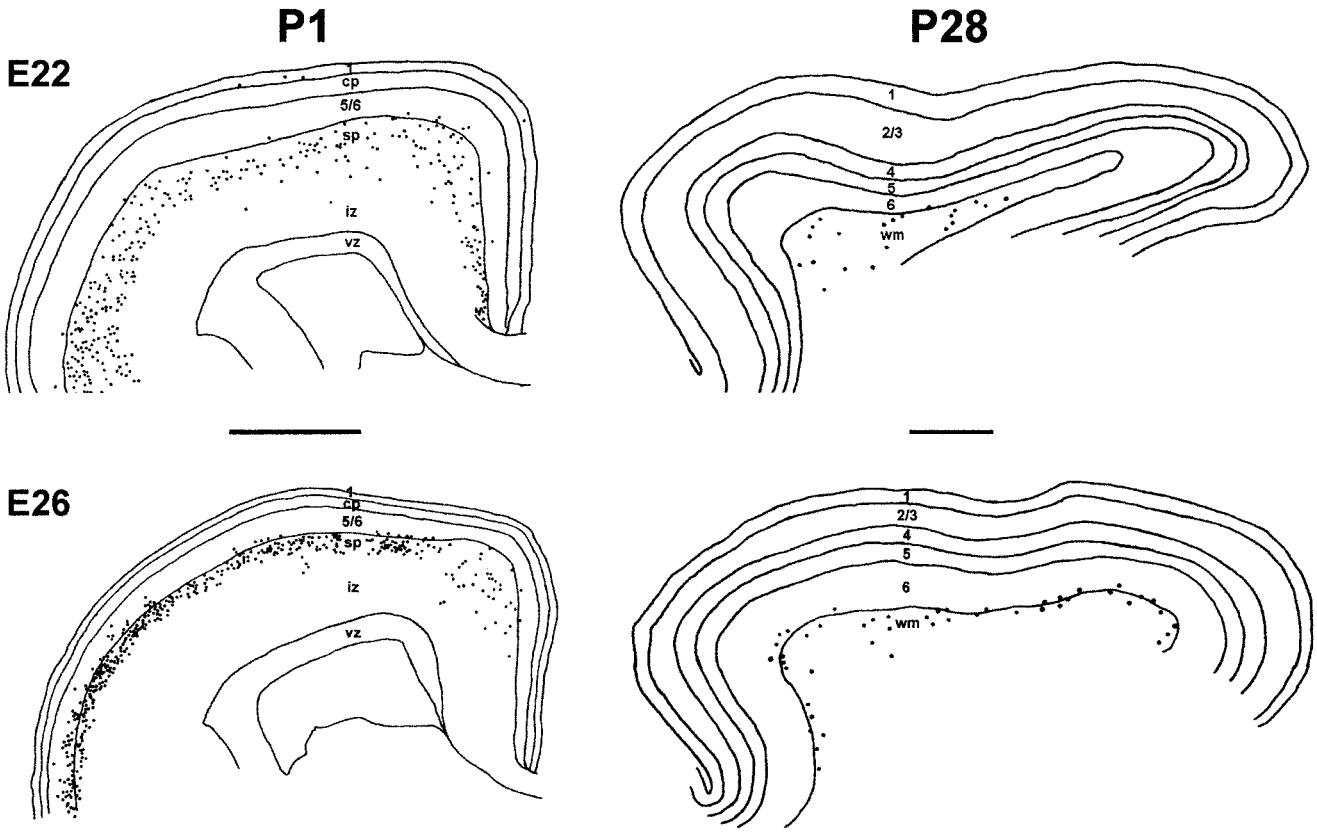

Fig. 3. Drawings of the positions of BRDU+ cells after injections on E22 or E26. Illustrated are labeled cells observed on P1 (left) or in mature cortex (right). Injections on either of these days result in labeled cells in the subplate region on P1 (left). By P28, however, only a few labeled cells remain, which are roughly concentrated near the remnants of the subplate region. The labeled cells are in a more superficial position after E26 injections, compared with E22 injections. Both scale bars = 1 mm; the bar on the left pertains to the P1 drawings, whereas the bar on the right pertains to the drawings of P28 sections. (VZ, ventricular zone; IZ, intermediate zone; SP, subplate; CP, cortical plate; wm, white matter). Other cortical layers are indicated with numbers.

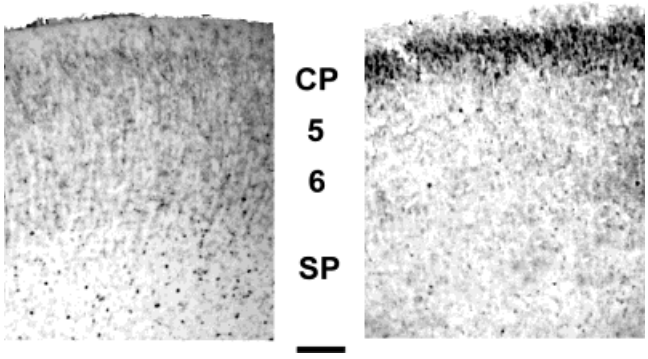

Fig. 4. Examples of BRDU immunoreactivity on P1 of two different animals, one (on the left) with labeled cells in the subplate (after BRDU injection on E24), and one with BRDU+ cells in the cortical plate (after BRDU injection on E33). CP, cortical plate; SP, subplate. Scale bar = 100 μm.

served in all parts of the cortical plate and in the intermediate zone. These cells were presumed to be newly generated neurons migrating to their appropriate site in the cortex. Cells were also observed throughout the thickness of the immature cortical plate. By maturity, E38 injections identified cells clearly located in layer 2 (Figs. 5 and 8). Figure 9 also illustrates images of cells labeled by injection of ^{3}H-thymidine on E38, indicating that the distributions of labeled cells resulting from this method and by BRDU injection were similar. In this experiment, cells incorporating [^{3}H]thymidine, as evidenced by silver grains occurring at least five times above background, were also distributed in all layers of the cortical plate.

After BRDU injections on P1, labeled cells were observed several days later, on P7, but these BRDU+ cells concentrated in the ventricular zone and the intermediate zone, and very few labeled cells occurred in the cortex, indicating that neurons incorporating BRDU on P1 have not yet migrated to the cortex (Fig. 8). By P28, however, very few BRDU+ cells were found in the somatosensory cortex, in comparison with the label observed after injections at other ages, although sparse label could be seen in upper layer 2.

Injections on P2–P7

We examined the somatosensory cortex of cytoarchitecturally mature animals (on P28) after BRDU injections into kits on postnatal days 2–7; a number of animals were assessed at intervening time points as well (see Table 1). BRDU injections on P2 led to a small amount of labeled cells in the upper part of layer 2, near the layer 2–layer 1 border in adults (Figs. 2, 10, and 11). Injections after this

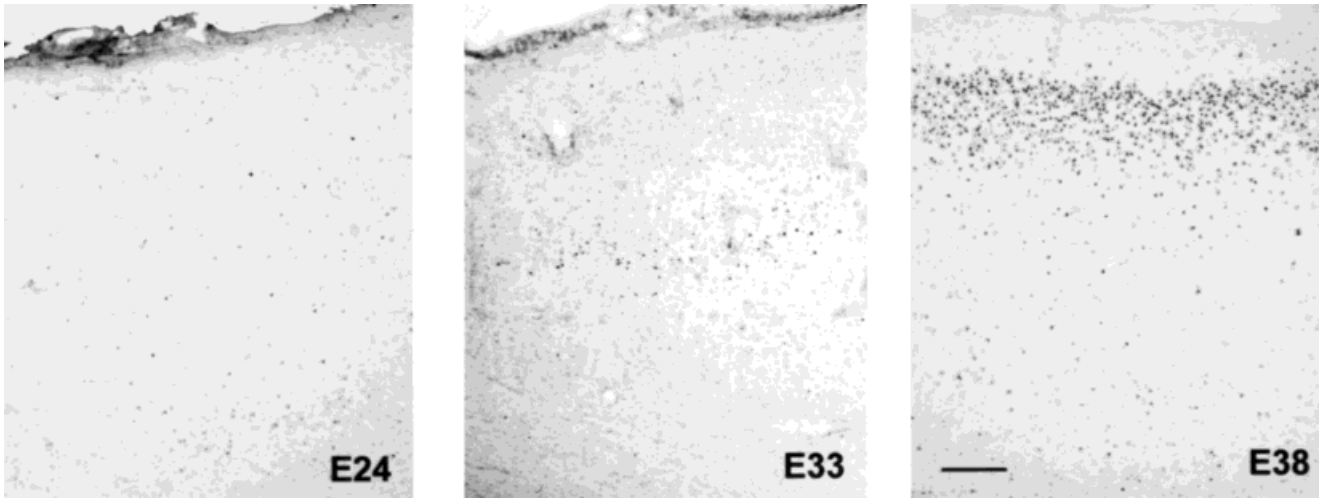

Fig. 5. Examples of BRDU+ cells found in mature cortex (P28 or older) after in utero injections on different days: E24, E33, or E38. E24 injections (left) label subplate cells; as a result, few immunoreactive cells are seen in mature cortex, although a few scattered BRDU+ cells can be seen. Injections on E33 result in labeled cells in layer 4 in the adult (middle). E38 injections resulted in label found predominantly in layer 2 in the mature animal (right), although BRDU+ cells can also be seen in layer 3. Scale bar = 200 μm.

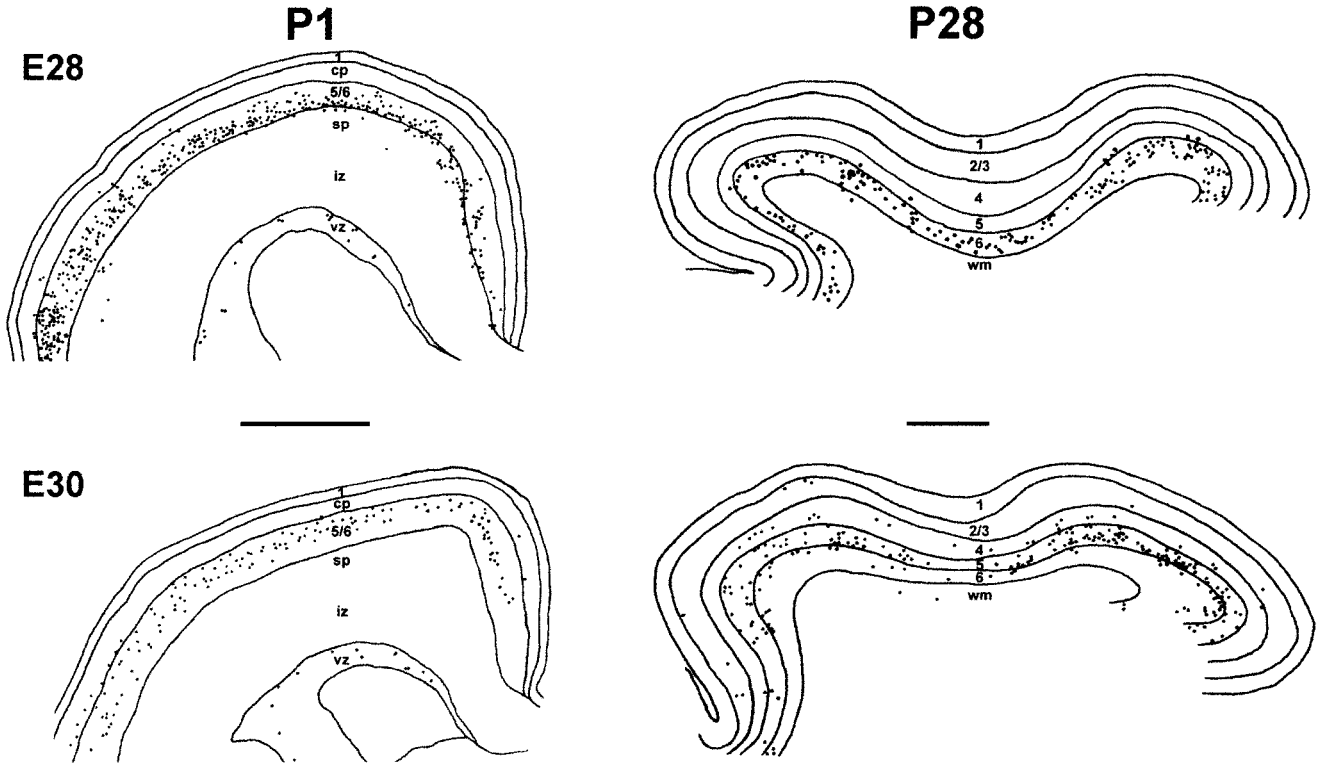

Fig. 6. Drawings of the positions of BRDU+ cells after injections on E28 or E30. Illustrated are labeled cells observed on P1 (left) or in mature cortex (right). Injections on E28 result in labeled cells located near the base of the forming cortical layers (i.e., presumptive layer 6) on P1 and are found in layer 6 in the mature cortex (top right). The E30 injection results in labeled cells shifted somewhat more superficially in the cortex (i.e., presumptive layer 5) and are found in layer 5 in adult cortex (bottom right). VZ, ventricular zone; IZ, intermediate zone; SP, subplate; CP, cortical plate. Scale bars = 1 mm.

date, including postnatal days 4 or 7, did not label cells with a distinct laminar pattern but only resulted in a few scattered darkly stained neurons within the somatosensory cortex (Figs. 10 and 11).

Previous investigations assessing birthdates of neurons populating ferret visual cortex concluded that visual cortical neurons were born up to two or more weeks after birth (Jackson et al., 1989). Because in our hands, the somatosen-

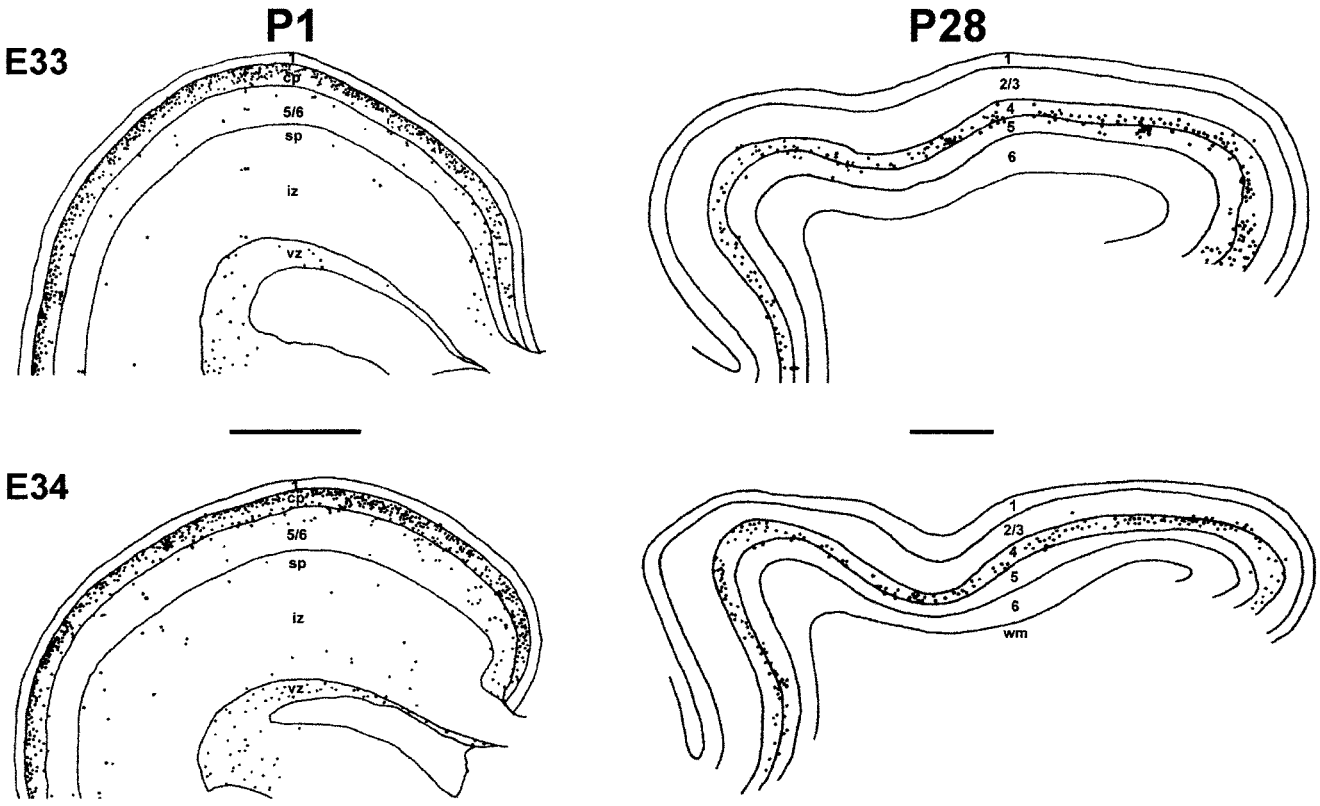

Fig. 7. Drawings of the positions of BRDU+ cells after injections on E33 or E34. Illustrated are labeled cells observed on P1 (left) or in mature cortex (right). Injections on E33 result in labeled cells in the differentiating cortical plate on P1, and they are found in layer 4 in the mature cortex (top right). The E34 injection results in labeled cells shifted somewhat more superficially in the cortical plate and are found in layer 4 in adult cortex (bottom right). The bar on the left applies to the P1 drawings, whereas the bar on the right applies to the P28 drawings. VZ, ventricular zone; IZ, intermediate zone; SP, subplate; CP, cortical plate. Scale bars = 1 mm.

sory cortical neurons completed their generation by 2 days of postnatal life, we assessed several time points to determine the presence of BRDU+ cells in visual cortex (conducted on the same animals used for somatosensory cortex assessment). Neurons populating the visual cortex were generated for at least several days after only occasional scattered BRDU+ cells were observed in somatosensory cortex. Examples can be seen in Figures 10 and 11, in which the ferret kit received an injection of BRDU at P7, resulting in labeled cells in layer 2 of primary visual cortex. Injections at the same age resulted in no above background BRDU label in the somatosensory cortex.

Quantitative analysis

Histograms of the distribution of labeled cells were calculated for each animal (see Materials and Methods). In Figures 12 and 13, the number of labeled cells are indicated as a percent of the total cells and plotted as a function of their distance from the pia. The representative histograms indicate that for most cortical layers, the distribution of labeled cells in postnatal animals are roughly located in sites appropriate to their adult locations in the cortical plate or subplate. (The exceptions to this are the results of injections on or after E38, which label cells that ultimately reside in layer 2, see below.) Figure 12 illustrates that the early generated cells (E22 and E26) belonging to the subplate are present at birth in an appropriate site, although somewhat less focused than the later born layers. No histograms are shown for adults after BRDU injections on days E22–E26, because very few BRDU+ neurons remain into maturity. Layer 6 and layer 5 cells (E28 and E30; Fig. 12) also reside in a roughly appropriate location at birth, although they are distributed through more bins than labeled cells found in the adult somatosensory cortex. For example, on P1 after E30 injections, no more than 30% of the total labeled cells are found in a single bin (Fig. 12), whereas more than 65% of the total labeled cells born on E30 are located in specific bin on P28 (Fig. 13). The cells belonging to the later-born layers 4 and 3 are also distinctly situated by P1 (Fig. 12). Similar to the earlier generated layers, the majority of labeled cells in mature cortex reside in fewer bins than in newborn cortex (E33 and E34; Fig. 13). Fifty percent of BRDU+ cells generated on E34 are found in a single bin by P64, whereas only 40% of the BRDU+ cells born on the same date are in a single bin on P1. Cells belonging to layer 2, however, are not clearly positioned in a discrete cortical site by P1 (Fig. 12). In this case, early after birth, the BRDU+ cells are still migrating to the cortex and occupy the intermediate zone and all layers of the cortical plate. When the cortex reaches cytoarchitectural and laminar maturity, the distributions of BRDU-labeled cells are more focused and clearly reside in a discrete cortical layer. A small number of neurons that populate the somatosensory cortex continue to be generated postnatally. Histograms made on P7 after P1 injections indicate that many BRDU+

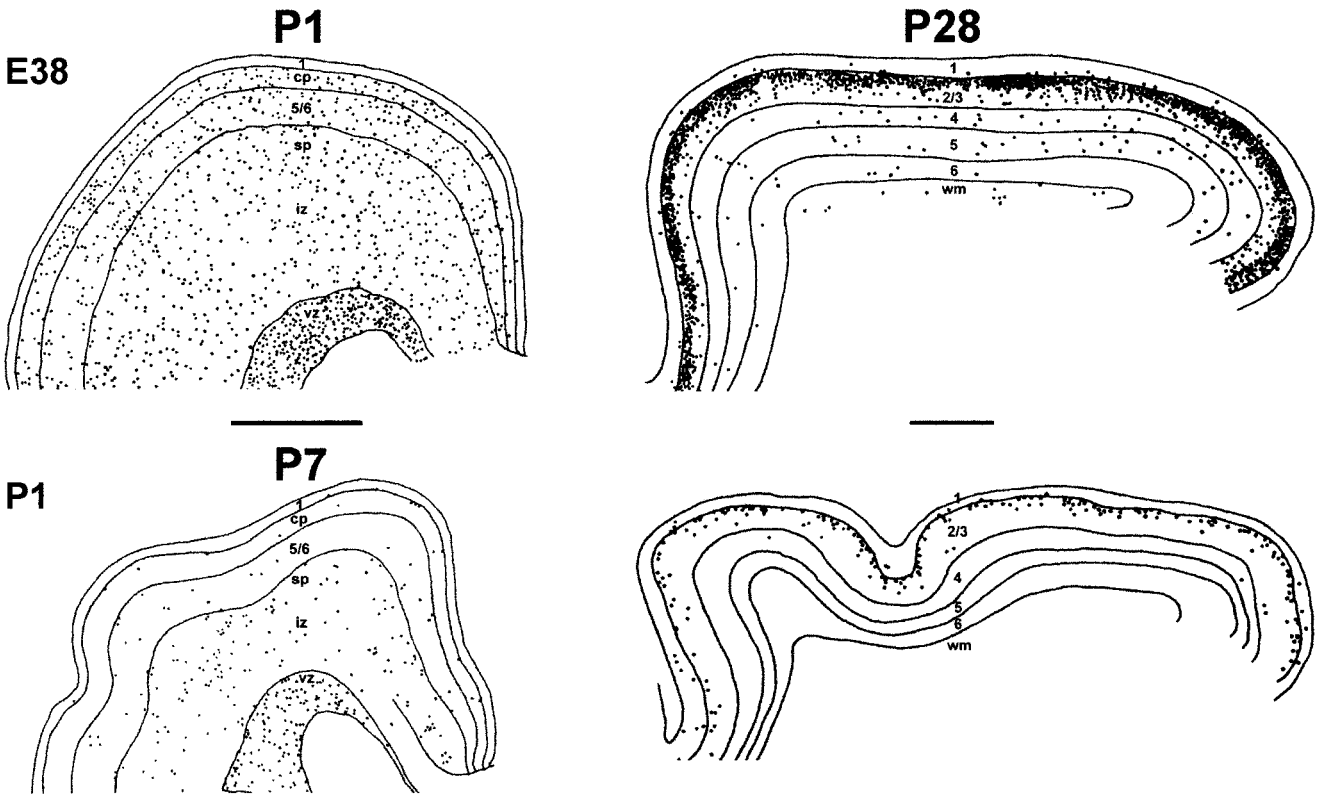

Fig. 8. Drawings of the positions of BRDU+ cells after injections on E38 or P1. Illustrated are labeled cells observed on P1 (upper left) or in mature cortex (right). Injections on E38 result in labeled cells located throughout the VZ, the IZ, and the cortex on P1; BRDU+ cells are found in layer 2 in the mature cortex (top right). The P1 injection results in labeled cells shifted somewhat more deeply toward the VZ on P7, and they are found in layer 2 in adult cortex (bottom right). In the examples on the left, the BRDU+ cells were still migrating toward the cortex on either P1 or P7 and had not yet arrived at their cortical destinations. In the case of the P1 injection evaluated on P7 (bottom left), the labeled cells were located closer to the VZ, because they had less time to travel to their destination. After injections on P1 and evaluations on P28, the BRDU+ cells appeared quite superficially within layer 2 of the cortex and were few in number. VZ, ventricular zone; IZ, intermediate zone; SP, subplate; CP, cortical plate. Scale bars = 1 mm; the bar on the left corresponds to the P1 and P7 drawings; the bar on the right corresponds to the drawings on the right.

cells disperse throughout the intermediate zone and are, presumably, on their way to the cortex (Fig. 12). When the cortex reaches its mature laminar pattern, BRDU+ cells occur predominantly in a single bin after P2 injections (Fig. 13). Very few somatosensory cortical neurons are born after P2.

DISCUSSION

Summary and formation of layers

A summary of results can be seen in Figure 14. Our findings indicate that the well-known inside-out development of cerebral cortex also occurs in the development of ferret somatosensory cortex. By birth, components of all layers have been generated. A few cells destined to populate the somatosensory cortex continue to be born postnatally, but they are not great in number and are not generated much after P2. Although components of each layer have already been produced, the layers are not distinctly formed at birth. At P1, the layers formed early during corticogenesis (layers 5 and 6) are relatively organized. The layers born later (layers 4, 3, and 2) are not yet clearly formed on P1, although layers 4 and 3 have largely migrated into the cortex. Cells populating layer 2 continue migrating to their destination for several days after birth. The overall picture suggests that the distinct pattern of cortical layers emerges over a period of several weeks, leading to each lamina being clearly separate from its neighbor by 4 weeks of age.

The majority of cells populating a given layer of somatosensory cortex are generated over a period of approximately 3 days, although neurons populating the subplate and layer 2 take somewhat longer. This is in contrast to neurons generated in ferret primary visual cortex (area 17) in which (several) neocortical layers are born over more protracted periods. Figure 15 compares developmental time lines for neurons generated in somatosensory vs. visual cortex (Jackson et al., 1989; Johnson and Casagrande, 1993). The development of both cortical regions begins along similar time lines; the birth of neurons populating the somatosensory cortex proceeds somewhat earlier than visual cortex, as would be expected from the rostrocaudal gradient of development. The two patterns of cortical generation begin to diverge, however, during birth of layer 4 in the visual cortex. The generation of neurons in layer 4 of area 17 occurs over a substantially greater number of days than the birth of layer 4 in area 3b of the somatosensory cortex. This differential period of neuronal

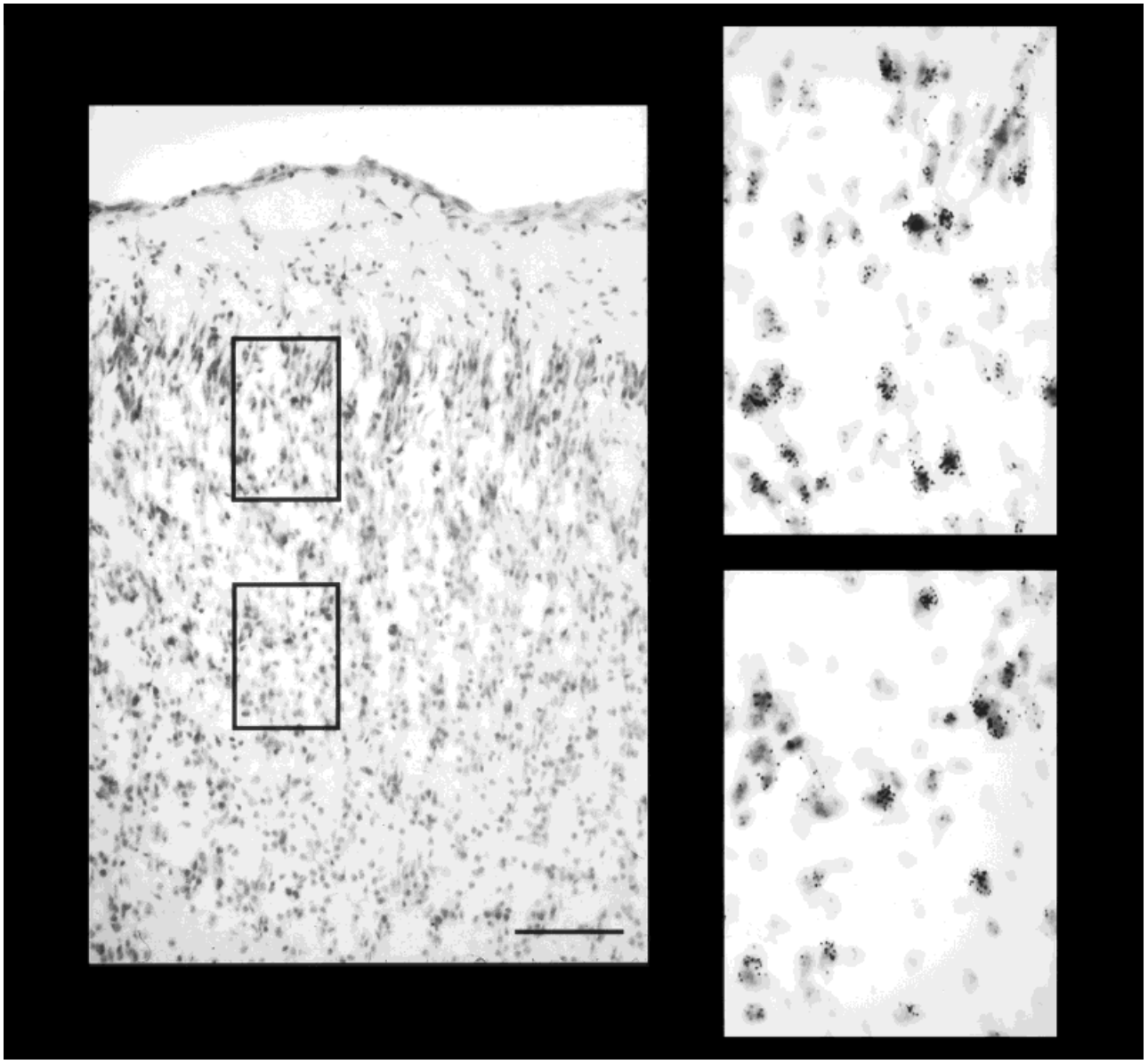

Fig. 9. An example of [^{3}H]thymidine label on P1 after injection on E38; the section is Nissl counterstained. The boxed areas are shown at higher power on the right for visualization of silver grains overlying cells incorporating [^{3}H]thymidine. This figure shows that, after E38 injections, labeled cells are present throughout the full thickness of the cortical plate. Scale bar = 60 μm for the left image and 30 μm for the photomicrographs on the right.

birth extends the period of neogenesis in the visual cortex, so that many cortical neurons are generated after the animal is born. In some ways this is not surprising, because layer 4 of area 17 is known to have more neurons than layer 4 of other cortical regions (e.g., Hendry, 1987).

The finding that the birthdates for neurons of somatosensory and visual cortex are different may in part reflect the different techniques used. The previous study evaluating birthdates of ferret visual cortex used [^{3}H]thymidine to determine when neurons in areas 17 and 18 are generated (Jackson et al., 1989), whereas the current study used BRDU-birthdating. In several animals of this study, we used [^{3}H]thymidine incorporation to determine when a cell was generated to supplement the BRDU analysis and found no differences in the distributions of labeled cells using either method. In three pregnant ferrets and two postnatal kits BRDU and [^{3}H]thymidine were injected together and confirmed that similar populations of cells incorporate both markers. In addition, Miller and Nowakowski (1988) report in their study using sections double-labeled with both [^{3}H]thymidine and BRDU, cells were either labeled with both markers or neither marker, supplying strong evidence that both thymidine analogs label very similar, if not identical, populations of neurons. Other studies also report similar distributions of labeled cells using either marker (DeFazio et al., 1987). The method used in this study (BRDU incorporation and immunohistochemical identification) yielded results com-

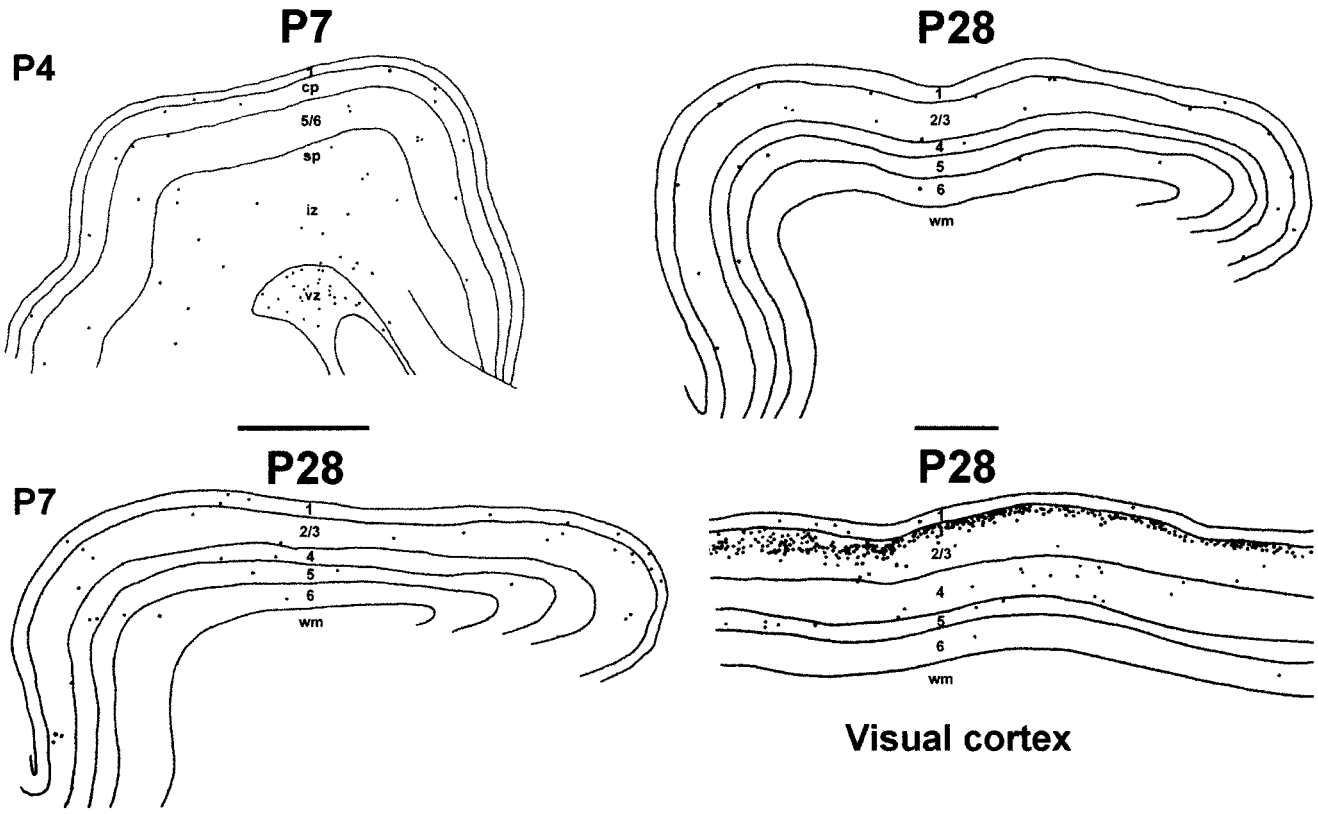

Fig. 10. Drawings of the positions of BRDU+ neurons after injection of BRDU on P4 (top) or P7 (bottom). After injection on P4, there are few labeled cells in the cortical plate by P7 (upper left); on P28 very few BRDU+ cells are found in the cortex, aside from a few scattered BRDU+ cells. Injections on P7 also result in very few BRDU+ cells in mature cortex (bottom left), whereas in primary visual cortex many labeled cells can be seen on P28 in layers 2 and 3 after a P7 BRDU injection (bottom right). Scale bars = 1 mm.

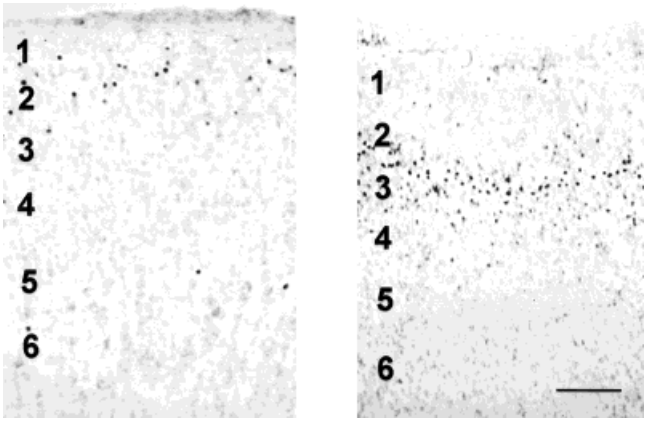

Fig. 11. Examples of BRDU+ cells in cytoarchitecturally mature somatosensory and visual cortex after BRDU injection on P2 (left, somatosensory cortex) or on P7 (right, visual cortex). Scale bar = 200 μm.

parable with those used in previous studies using [^{3}H]thymidine to determine the birthdates of cells in ferret visual cortex (Jackson et al., 1989; Johnson and Casagrande, 1993). Although we did not complete an exhaustive study comparing the BRDU label occurring in visual versus somatosensory cortex, we evaluated the label distributed in area 17 on several dates. We found, for example, that injections on P7 yielded no label in somatosensory area 3b, whereas many labeled cells were observed in the visual cortex. The current results therefore confirm previous findings that neurons populating ferret visual cortex continue to be generated for considerable periods postnatally, whereas neurons in ferret somatosensory cortex undergo their last mitosis, for the most part, prenatally.

One surprising observation was the relative lack of BRDU+ cells in layer 1 after injections on any of the dates used in this study. There were occasional cells labeled after injections at all time points, but none of the selected dates resulted in a distinct distribution in layer 1. The most likely explanation is that cells populating layer 1 are generated earlier than the injection dates of this study. The marginal zone, composed of layer 1 and the subplate, is known to be the earliest born component of the neocortex. The layers of cortex that develop subsequently insinuate themselves between the components of the marginal zone, the subplate, and layer 1. Evidence also exists supporting the idea that at least one component of the layer 1, the Cajal Retzius cells, are born before the subplate cells (see Bayer and Altman, 1991, for review). This may account for our inability to conspicuously label cells residing in layer 1.

Differences between visual and somatosensory development

Although these findings globally confirm the well-known rostral-to-caudal development of the neocortex, they are

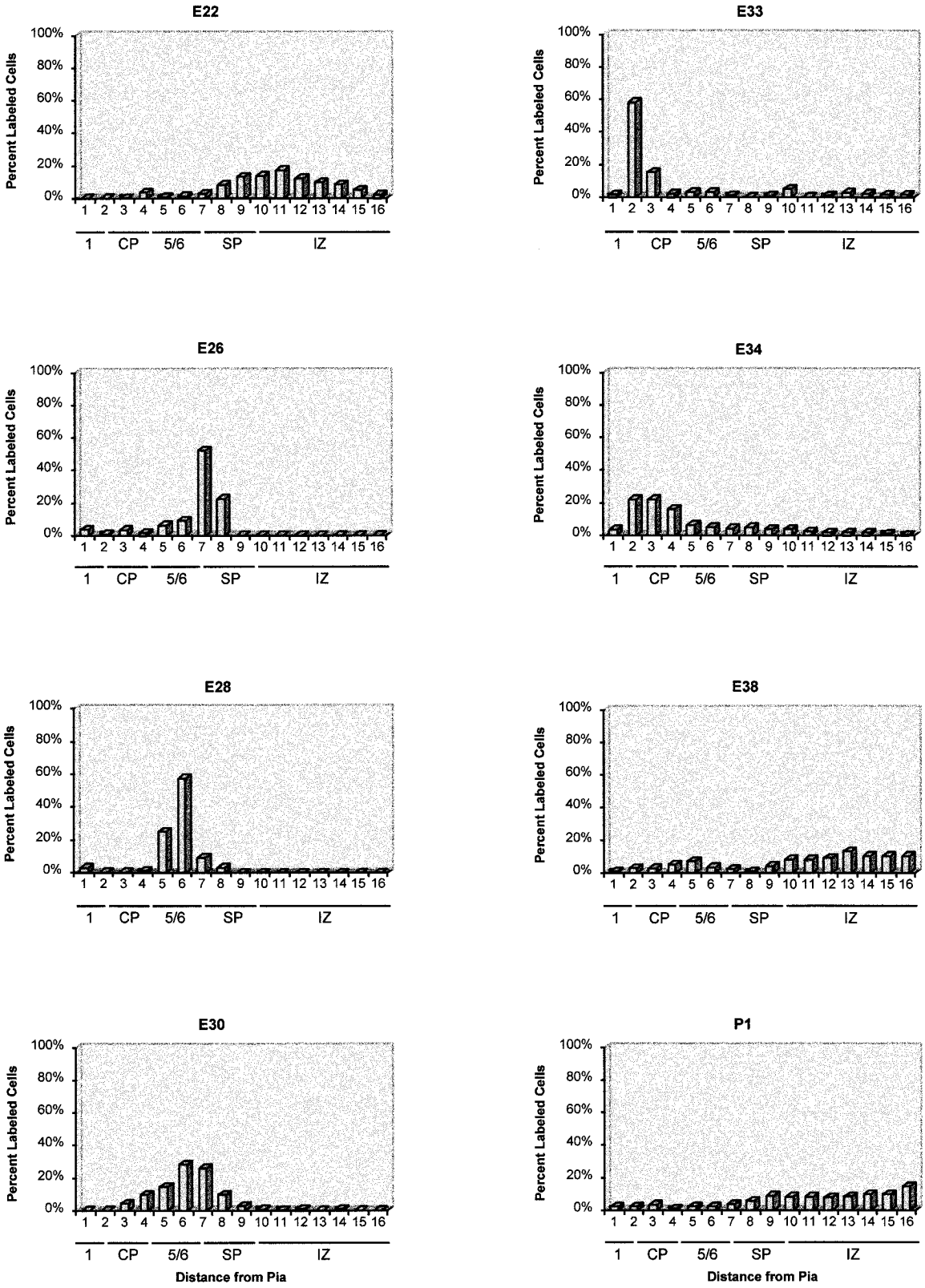

Fig. 12. Histograms of the distribution of labeled cells plotted in relation to their depth from the pial surface. The distributions are normalized to reflect the total number of cells in each section; therefore, absolute numbers of cells are not indicated. The plots shown here reflect the distribution of P1 neocortical cells that were generated from E22 (i.e., those belonging to the subplate, shown in upper left) to P1 (upper layer 2, shown in lower right). The BRDU+ cells are located in positions appropriate to their mature distribution by P1; the label shifts superficially after sequential injections on subsequent embryonic days. Injections on E38 and P1, however, label cells that are not focused in a specific bin but are distributed throughout the intermediate zone and cortical plate. Presumably, these cells did not have time to migrate to their appropriate locations by the date of analysis. See text for details. The locations of labeled cells to CP, SP, or IZ were determined by correlation with individual sections and are indicated below each histogram. Each bin represents an increment of 50 μm as measured from the pia. CP, cortical plate; SP, subplate; IZ, intermediate zone.

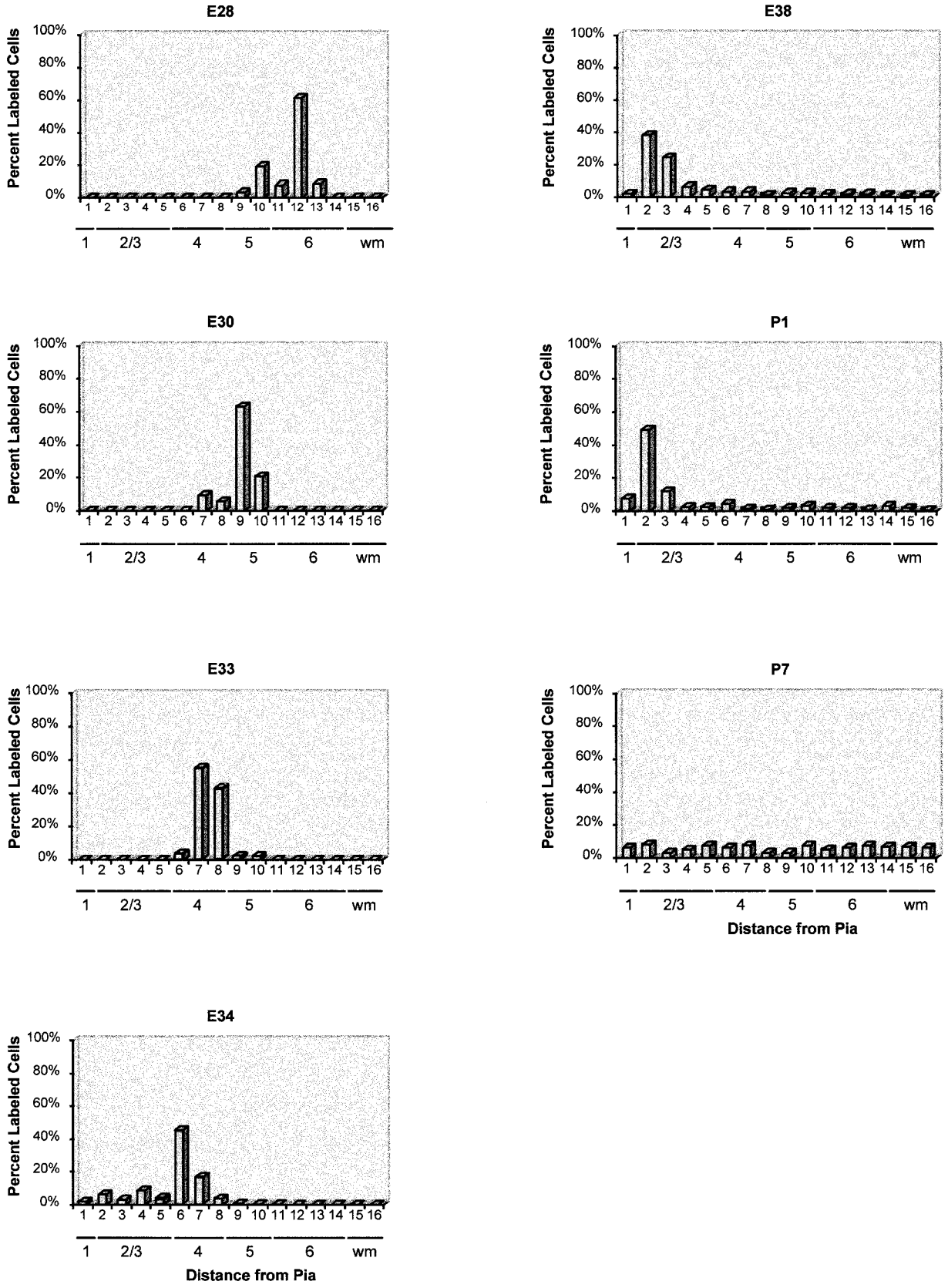

Fig. 13. Histograms of the distribution of labeled cells in cytoarchitecturally mature somatosensory cortex after BRDU injections on different embryonic days. Conventions are as for Figure 12. The distribution of BRDU+ cells is more focused in mature cortex (a greater percentage of cells in a single bin) than at P1. These histograms do not represent absolute numbers of cells, but normalized percentages per section. The layers in which the labeled cells are found are indicated along the x-axis of each histogram. Each bin represents a 100-μm increment as measured from the pia. wm, white matter.

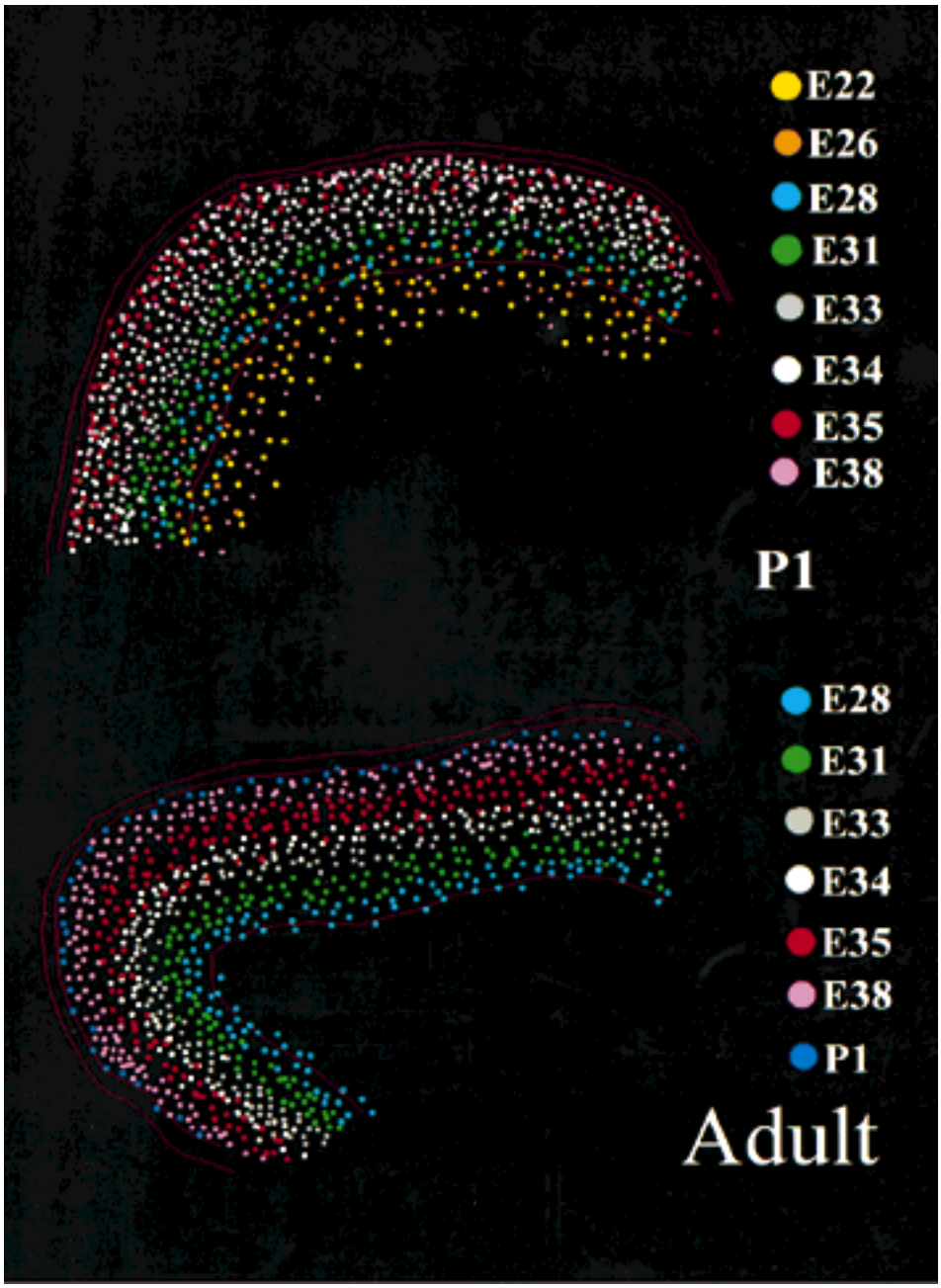

Fig. 14. Summary diagram of where BRDU+ neurons are found on P1 and P28 in the somatosensory cortex after BRDU injections on different embryonic and early postnatal days. Many neurons generated on a specific day are roughly in their appropriate layers by P1, although the laminar arrangement is not as distinct as observed in the cytoarchitecturally mature somatosensory cortex.

somewhat surprising because of the magnitude of the timing difference between generation of the two populations of neurons. This is of interest because a number of other recent studies indicate that features of somatosensory cortex mature considerably earlier than visual cortex. In cats, thalamocortical and corticocortical terminations are relatively mature by the first week of life in the somatosensory cortex, and kittens possess a crude somatotopic arrangement on P1 (Rubel, 1971; Wise et al., 1977; Juliano et al., 1996; Sonty and Juliano, 1997). This contrasts with the architecture and connectional patterns of visual cortex in cats, which do not mature until approximately 8–10 weeks (LeVay et al., 1978). Functional properties in the visual cortex of cats and ferrets are reported to mature in conjunction with the architecture and connectional patterns (LeVay and Stryker, 1979; Chapman and Stryker, 1992, 1993; Nelson and Katz, 1995).

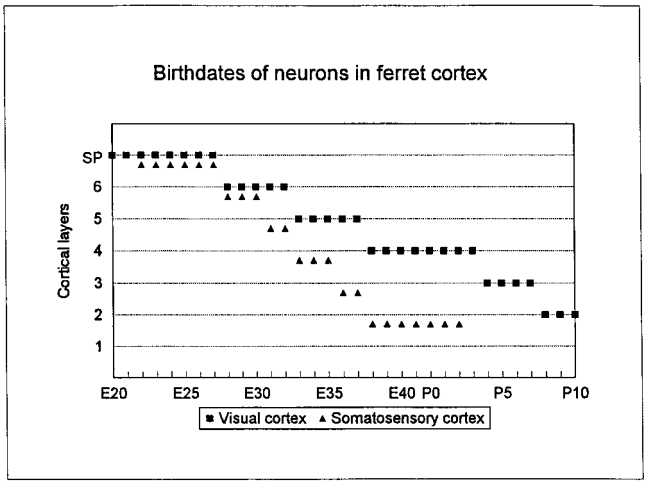

Fig. 15. Graph representing the birthdates of neurons populating the somatosensory cortex vs. visual cortex of the ferret. The neuronal birthdates are indicated on the x-axis, and the cortical layers are indicated on the y-axis. Dates for generation of neurons populating visual cortex were obtained from Jackson et al. (1988) and Johnson and Casagrande (1993). Although both the somatosensory and visual cortex begin generation of neurons at approximately similar dates, the visual cortex lags behind, especially upon birth of layer 4, which takes over 1 week for visual cortex, and approximately 3 days for somatosensory cortex. The subplate and layer 2 of the somatosensory cortex are generated over a period slightly longer than 3 days. Most neurons that populate the somatosensory cortex are born by P2, whereas neurons that reside in primary visual cortex continue to be generated for over a week postnatally.

Other studies show that, in the visual system, geniculocortical axons 'wait' for protracted periods at the level of the subplate, before entering the cortex (Lund and Mustari, 1977; Rakic, 1977; Shatz and Luskin, 1986). During this waiting period, the thalamocortical projections are not topologically organized but rearrange more precisely as the cortex matures (Rakic, 1977; LeVay et al., 1978; LeVay and Stryker, 1979; Antonini and Stryker, 1993). In a rodent model, a waiting period does not appear to exist for thalamocortical projections into the somatosensory cortex, and topographic precision occurs early during development of thalamocortical axons (Catalano et al., 1991; Agmon et al., 1993; O'Leary et al., 1994; Schlagger and O'Leary, 1994). A similar precision occurs early in the development of thalamocortical projections in ferret somatosensory system (Juliano et al., 1996).

In addition, the development of visual cortex appears more susceptible to the effects of deprivation than comparable losses in the somatosensory system. Blockade or interference with afferent activity by peripheral or central application of TTX, or NMDA antagonists, results in the failure of ocular dominance columns to form. These substances also interfere with plastic rearrangements that normally occur following monocular deprivation (Reiter et al., 1986; Stryker and Harris, 1986; Shatz and Stryker, 1988; Antonini and Stryker, 1993). In the rodent somatosensory system, application of TTX or NMDA antagonists do not hinder the formation of the whisker-related patterns in the neocortex (Chiaia et al., 1992; Hendersen et al., 1992; Chiaia et al., 1994a). These substances also fail to block the usually occurring rearrangements of whisker-related patterns in the CNS after deprivation of input from a defined set of whiskers (Chiaia et al., 1994a,b).

These studies, in conjunction with the results presented here showing a substantial timing difference in the generation of neurons populating the somatosensory and visual cortices, suggest that tangible distinctions exist between the development of each area. The fact that somatosensory cortex is generated so early, compared with visual cortex, may encourage a more mature and rigid cortical architecture, which resists changes induced from the periphery, unless they occur very early during development. For example, to interfere with the normal segregation of geniculocortical afferents in the visual system, it is necessary to block transmission early in the development of these fibers. The appropriate time to conduct a blockade varies with the species; in the case of cats, the appropriate time is before birth (Shatz and Stryker, 1988), whereas in ferrets, it is possible to block activity in this regard postnatally (Hahm et al., 1991). In the somatosensory system, experiments by Chiaia et al. (1994b) using hamsters, which are also altricial animals, found that blockade of activity by TTX failed to interfere either with development of whisker-related patterns in the somatosensory cortex, or with the plastic arrangements known to occur following sensory deprivation (Chiaia et al., 1994b). Applying substances that interfere with afferent activity very early during development, therefore, does not disrupt normal formation of the somatosensory architecture, whereas such treatments would dramatically interfere with the architecture of the visual system. These differences suggest that distinct developmental mechanisms may distinguish between sensory cortical areas.

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