Bayer, SA and J Altman (1991) NEOCORTICAL DEVELOPMENT Raven Press.

CHAPTER 11

Development of the Visual Areas

Differential connections of the rat visual cortex are the basis for Zilles' (1985) subdivision into four parts running from medial to lateral: OC2M, OC1M, OC1B, and OC2L (Fig. 11–1). Several methods, such as anterograde transneuronal transport of [^{3}H] amino acids injected into one eye (Zilles et al., 1984), retinotopic mapping (Montero, 1973, 1981), and 2-deoxyglucose uptake (Thurlow and Cooper, 1988) indicate that the primary visual cortex (OC1) is divided into a lateral part that receives binocular input (OC1B) and a medial part that receives monocular contralateral input (OC1M) via a relay in the thalamic dorsal lateral geniculate nucleus (Ribak and Peters, 1975; Peters and Saldanha, 1976; Hughes, 1977; Lund and Mustari, 1977; Woodward and Coull, 1984; Sefton and Dreher, 1985). That same relationship is found in other rodents such as the mouse (Dräger, 1974) and hamster (Tiao and Blakemore, 1976) in contrast to the alternating ocular dominance columns found in cats and primates (Hubel and Wiesel, 1969; Rakic, 1983; Shatz and Luskin, 1986; Löwel et al., 1988). OC1M and OC1B are the same as Krieg's (1946a, 1946b) area 17. Secondary visual areas (OC2) surround the primary areas both laterally (OC2L, similar to Krieg's area 18a) and medially (OC2M, similar to Krieg's area 18). There are multiple retinotopic maps in OC2L and OC2M (Espinoza and Thomas, 1983; Montero, 1981; Olavarria, 1979) relayed via projections from the lateral posterior thalamic nucleus (Hughes, 1977; Olavarria, 1979; Perry, 1980; Montero, 1981), which receives strong input from the superior colliculus (Altman and Carpenter, 1961; Perry, 1980; Montero, 1981). Thalamocortical afferents from either the lateral geniculate or the lateral posterior nucleus terminate heavily in layer IV, lightly in layers VI and I as part of the class I afferent system (Caviness and Frost, 1980; Frost and Caviness, 1980; Herkenham, 1980).

Of all the neocortical areas, the primary visual cortex has received the most attention in previous [^{3}H]thymidine autoradiographic studies; all of which have been done with the pulse labeling technique. There is a radial (inside-out) neurogenetic gradient between adjacent layers in a variety of species, including rat (Brückner et al., 1976; Lund and Mustari, 1977; Miller, 1985, 1986, 1988; Cavanagh and Parnavelas, 1988, 1989), hamster (Crossland and Unchwat, 1982), cat (Luskin and Shatz, 1985a), ferret (Jackson et al., 1989), and monkey (Rakic, 1977). A transverse gradient of neurogenesis between lateral and medial locations was found in cats (Luskin and Shatz, 1985a) and ferrets (Jackson et al., 1989). Finally, a longitudinal gradient of neurogenesis between anterior and posterior locations was reported in ferrets (Jackson et al., 1989).

Five frontal levels were analyzed quantitatively from A2.8 anteriorly to P0.4 posteriorly (Pellegrino et al., 1979). Cells were separately counted in layers VI, V, IV, III, and II (*dashed lines*, Fig. 11–1). Just as throughout the rest of the rat neocortex, layers VI and V are thick, forming the lower two-thirds of the total

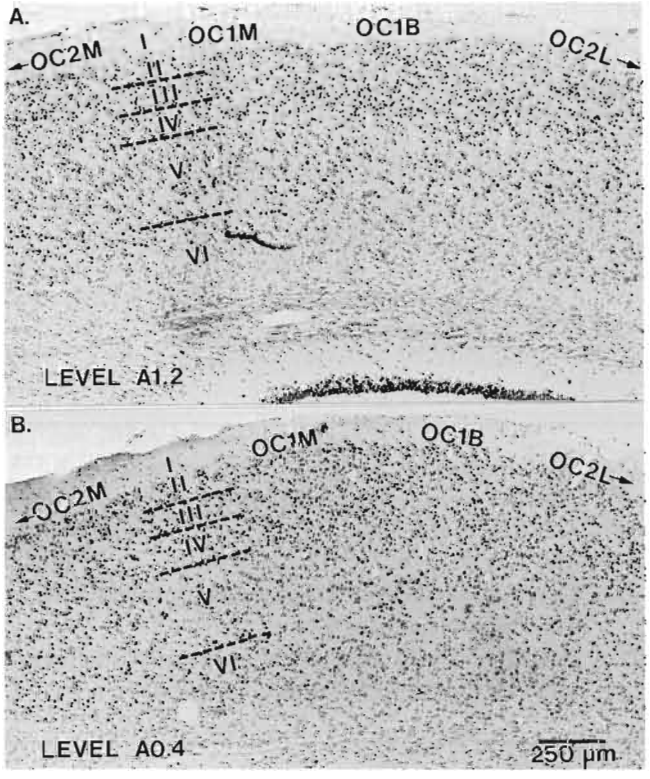


FIG. 11–1. **A** and **B** are low-magnification views of the visual cortex in the brain of a rat exposed to [^{3}H] thymidine on E17 and E18 and killed on P60 (6 μm paraffin sections, hematoxylin/eosin stain); dorsal is at the top, medial is to the left. Four visual areas are located in the dorsal cerebral wall at levels A1.2 **(A)** and A0.4 **(B)** of the Pellegrino et al. (1979) atlas. The anterior and posterior parts of the visual cortex have similar cytoarchitectonics. The primary visual areas (OC1M, OC1B) are surrounded by a medial secondary area (OC2M) and a lateral secondary area (OC2L; Zilles, 1985); the two secondary areas extend beyond the borders of each photograph (*arrows*). Roman numerals separated by dashed lines indicate how the cortex was subdivided into layers for the cell counts.

cortical depth. The transition between VI and V is clear even in the thin (6 μm) sections used for autoradiography, since layer VI cells are smaller and more densely packed than layer V cells (Zilles et al., 1980). Especially in the primary areas, there are many small granule cells in layer IV, which sets it off from both layers V and III (Zilles et al., 1980). However, the density of layer IV granule cells in the primary visual cortex is notably lower than in layer IV of the somatosensory cortex (Figs. 3–4, 3–5, and Chapter 13). The cells in layers II and III are very similar in appearance (Fig. 11–6); consequently, the cells between the upper limit of layer IV and the lower limit of layer I were divided into lower (designated layer III) and upper (designated layer II) halves for counting. The sign test indicated no differences in neurogenesis in the anterior-posterior axis, and therefore the data were combined from all three levels (A1.2, A0.4, P0.4, drawings, Figs. 11–4 and 11–5) where the primary (OC1M and OC1B) and secondary (OC2M and OC2L) areas are represented. Since far posterior transverse sections do not contain all of the cortical layers, we could not examine the occipital pole, and a longitudinal gradient (anterior to posterior) might exist in the rat as it does in the ferret (Jackson et al., 1989).

11.1 THE RADIAL NEUROGENETIC GRADIENT

Just as for the entire neocortex, cells in the visual areas are generated in a strong radial neurogenetic gradient (deep cells are older than superficial cells). The magnitude of the radial gradient is shown in Fig. 11–2, which was compiled by combining the data separately for each layer from all areas of the visual cortex that were analyzed: neurons in layer VI are generated mainly on E15–E17, in layer V on E16–E17, in layer IV on E17–E18, in layer III on E18–E19, and in layer II on E19–E20. The sign test showed that all comparisons between adjacent layers were significant (P = 0.0001); the repeated measures analysis of variance (SAS GLM procedure) also indicated significant differences (P = 0.0001, with values of F ranging from 697.18 to 247.6, df = 1). Our data confirm the many previous reports of the deep-to-superficial neurogenetic gradient in the visual cortex (Brückner et al., 1976; Rakic, 1977; Crossland and Unchwat, 1982; Luskin and Shatz, 1985a; Miller, 1986, 1988; Cavanagh and Parnavelas, 1988; Jackson et al., 1989). The full magnitude of the radial gradient can be appreciated by comparing how much of each population is generated before or after the selected embryonic ages (E15, E17, and E19) that are indicated by vertical dashed lines (Fig. 11–2). By E17 for example (*center dashed line*), the generation of layer VI is nearly completed (>70%) while the generation of layer II has not yet begun (<1%).

The stippled areas in the graphs (Fig. 11–2) represent the proportion of each layer that is generated concurrently with the adjacent layer above (four lower graphs) or with the layer below (top graph). For layers V/VI, IV/III, and III/II, the overlap is between 62% and 64%. There is less convergence in the generation of layers V and IV (only 54% overlaps). As we will show in the auditory areas (Chapter 12), somatosensory areas (Chapter 13), and motor areas (Chapter 14), the least amount of neuronal cogeneration occurs between layer V and layer IV. That may be related to the reversal from the early to the late labeling pattern with [^{3}H]thymidine 1 day after injection in the cortical ventricular zone (Chapter 4) that we postulate to occur after the generation of the deep layers (VI/V) and at the onset of generation of the superficial layers (IV/III/II).

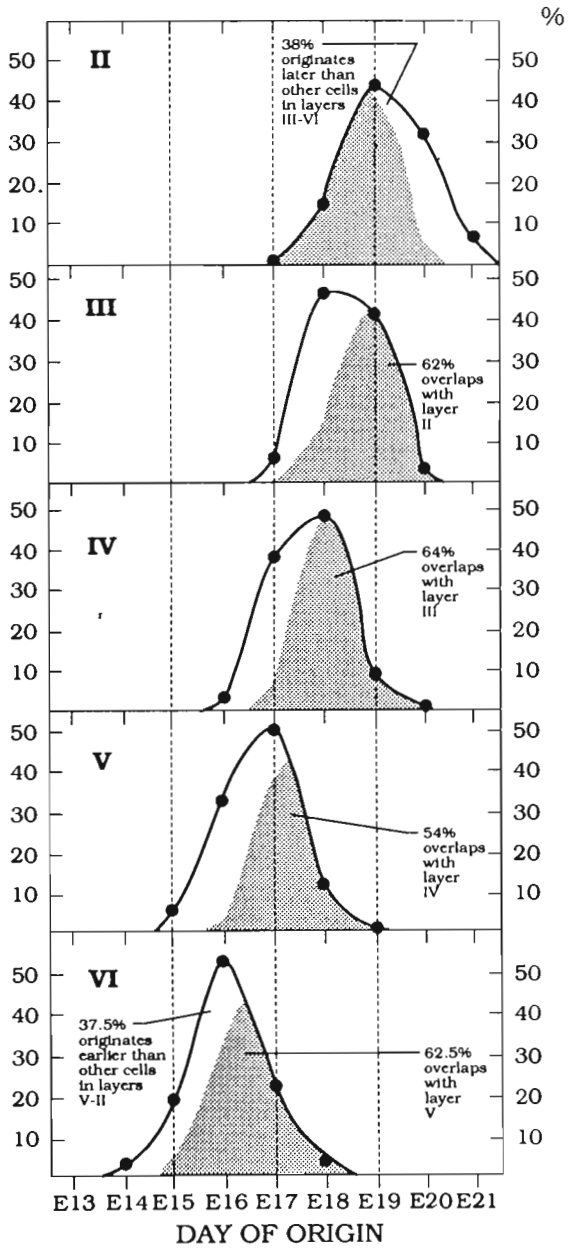


FIG. 11–2. The radial neurogenetic gradient in the visual cortex of animals that survived to P60 after two consecutive exposures to [^{3}H]thymidine during embryonic life. Each graph represents the proportion of cells generated from E13 to E21 in separate layers (II-top graph to VI-bottom graph) based on data combined from all the visual areas that were analyzed. Vertical hatched lines indicate relative amounts of cells that have been generated before and after E15 (*left line*), E17 (*center line*), and on or after E19 (*right line*). Proceeding from layer VI to layer II, the peaks of neurogenesis shift from early (E16 in VI) to late (E19 in II). Stippled areas represent the portion that is generated concurrently with the adjacent layer. Note that the least amount of concurrent generation occurs between layers V and IV (54% indicated in the graph second from the bottom).

11.2 TRANSVERSE NEUROGENETIC GRADIENTS IN THE DEEP LAYERS

11.2.1 Layer VI

Layer VI shows the global transverse neurogenetic gradient found throughout the neocortex: laterally situated neurons tend to be generated earlier than medial neurons (see Chapter 3). Figure 11–3 shows the labeling in the upper part of layer VI in lateral (B) and medial (A) subdivisions of the visual cortex in the brain of a rat exposed to [^{3}H]thymidine on E17–E18 and killed on P60. There is a higher proportion of labeled neurons in medial OC2M than in lateral OC2L. Neurogenesis in layer VI peaks on E16 in all visual areas, but there is a higher proportion of older neurons (generated on or before E15) laterally. By the end of E16, approximately 83–86% of layer VI neurons have been generated in OC2L and OC1B, 70–72% in OC1M and OC2M; from E17 onward, proportionally more neurons are generated medially (30% in OC2M) than laterally (14% in OC2L). There is an overall difference of 16% between the most lateral and most medial sites ($P < 0.0001$, sign test; $F = 32.78$, df $= 1$, $P < 0.0001$, SAS GLM procedure).

11.2.2 Layer V

There is a stepwise lateral (older) to medial (younger) neurogenetic gradient throughout all visual areas in layer V (*arrows* in drawings, Fig. 11–4). More neurons are generated on E16 than E17 in OC2L and OC1B, while more neurons are generated on E17 than on E16 in OC1M and OC2M. Statistical tests indicated significant differences between all adjacent areas ($P < 0.035$ to $P < 0.001$, sign test; $P < 0.0001$, repeated measures analysis of variance). The lateral-to-medial gradient is quite prominent when OC2L is compared with OC2M (bottom and top graphs, Fig. 11–4). From E17 to E19, only 47% of the neurons are generated in OC2L, while 70% of them are generated in OC2M.

11.3 TRANSVERSE NEUROGENETIC GRADIENTS IN GRANULAR LAYER IV

Neurons in layer IV are generated mainly on E17 and E18 in each visual subarea. There are significant lateral-to-medial neurogenetic gradients between the two secondary areas (OC2L, OC2M; $P < 0.039$, sign test; $F = 31.01$, df $= 1$, $P < 0.0001$, SAS GLM procedure) and between the two primary areas (OC1B, OC1M; $P < 0.006$, sign test; $F = 44.55$, df $= 1$, $P < 0.0001$, SAS GLM procedure). When the primary and secondary areas are compared however, the stepwise significant transverse neurogenetic gradient is weakened

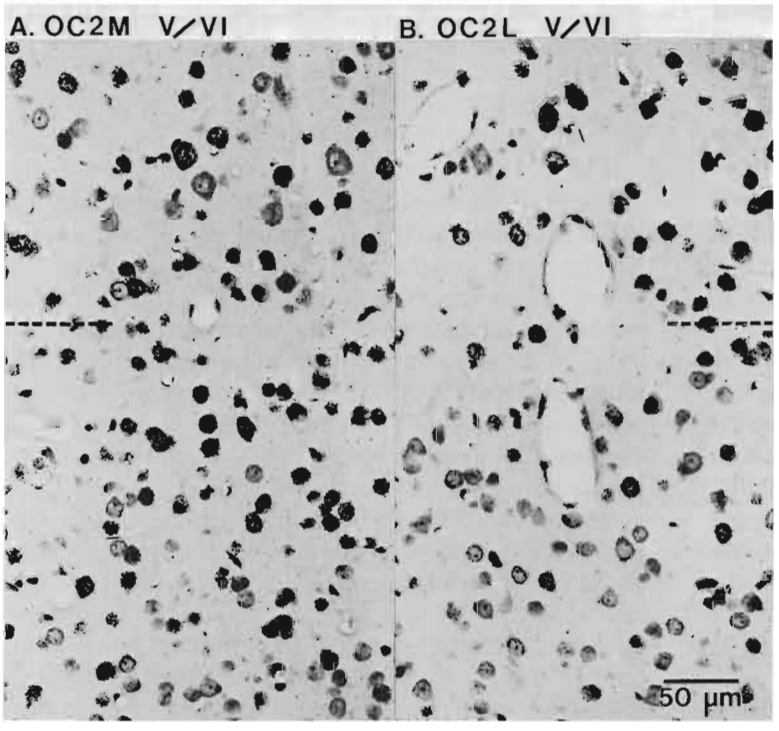
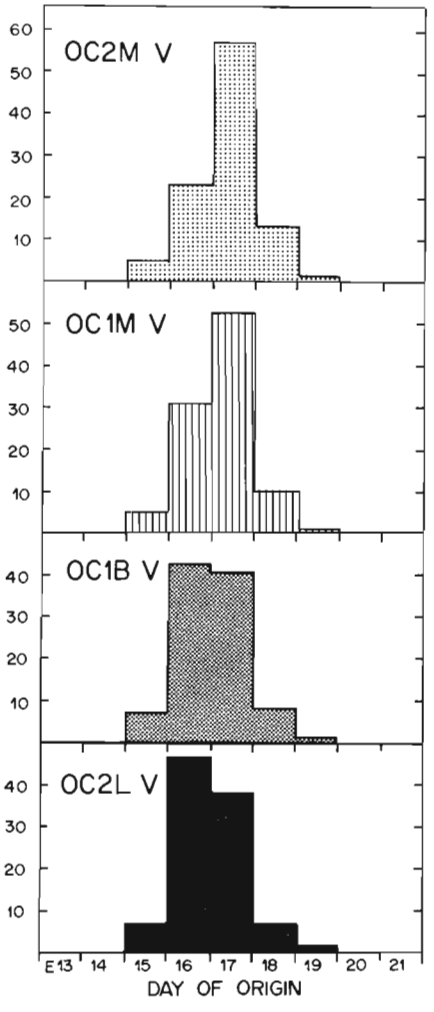


FIG. 11–3. High-magnification views of the lateral **(B)** and medial **(A)** parts of the layer V/VI junction (*dashed lines*) in the lateral secondary (OC2L) and medial secondary (OC2M) visual areas. The animal was given two consecutive exposures to [^{3}H]thymidine on E17 and E18 and survived to P60. In lateral layer VI there is a majority of unlabeled cells (birthdays before E17), while medial layer VI still has a majority of labeled cells (birthdays on or after E17). The deep layers in the primary areas show an intermediate labeling pattern between these two extremes. In layer V, only a few cells are unlabeled both laterally and medially. In the E19–E20 injection group, there is a lateral-to-medial progression of labeling in layer V, just as is shown here for layer VI one day earlier.



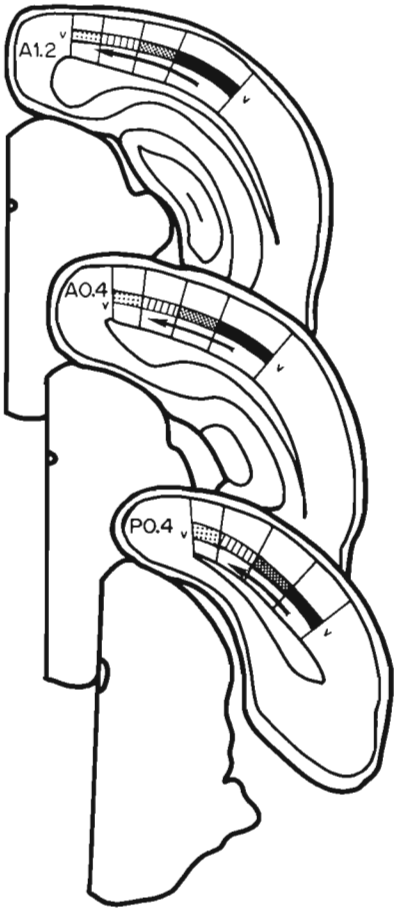


FIG. 11–4. Time of neuron origin in layer V of the lateral secondary visual area (OC2L), binocular recipient primary visual area (OC1B), monocular recipient primary visual area (OC1M), and medial secondary visual area (OC2M) in animals that survived to P60 after two consecutive daily exposures to [^{3}H]thymidine during embryonic life. The bar graphs represent the proportions of neurons generated on single embryonic days. Since neurogenesis occurs simultaneously in the rostrocaudal plane, all graphs are combined data from each level for specific areas. Lateral neurons originate earlier than medial neurons throughout the entire extent of the visual areas (*arrows* in drawings) and separate neurogenetic gradients do not distinguish primary from secondary areas.

because OC2M has a tendency to be generated slightly earlier (nearly 40% of the population arises on or before E17) than OC1M (33% of the population arises on or before E17). While this difference is not significant ($P > 0.05$, sign test and SAS GLM procedure), it indicates a deviation from the global transverse neurogenetic gradient (lateral to medial).

11.4 TRANSVERSE AND SANDWICH NEUROGENETIC GRADIENTS IN THE SUPRAGRANULAR LAYERS

11.4.1 Layer III

Neurons in layer III are produced mainly on E18 and E19 in each visual subarea. There are significant lateral-to-medial neurogenetic gradients between the two secondary areas ($P < 0.0001$, sign test; $F = 50.55$, df $= 1$, $P < 0.0001$, SAS GLM procedure) and between the two primary areas ($P < 0.022$, sign test; $F = 21.97$, df $= 1$, $P < 0.0001$, SAS GLM procedure). When the primary and secondary areas are compared however, OC2M has significantly more neurons generated on or before E18 (58%) than OC1M (43%; $P < 0.003$, sign test; $F = 42.56$, df $= 1$, $P < 0.0001$, SAS GLM procedure). The transverse neurogenetic gradient shifts to a "sandwich" gradient where younger neurons are surrounded by older neurons both medially and laterally. That is a violation of the global transverse gradient in the neocortex. OC2M also has a tendency to be generated earlier than the more lateral primary area (OC1B), but the differences in neurogenesis are separated only by about 6% (42% on or after E19 in OC2M, 48% in OC1B; $P > 0.05$, sign test and SAS GLM procedure).

11.4.2 Layer II

Layer II neurons are generated mainly on E19 and E20 in each visual subarea (Fig. 11–5). The dominant neurogenetic gradient is again the sandwich type, where younger neurons prevail in the two primary areas (OC1B and OC1M) and older neurons prevail in the two secondary areas (OC2L and OC2M; $F = 60.03$ and 17.62 respectively, df $= 1$, $P < 0.0001$, SAS GLM procedure). That is a violation of the transverse neurogenetic gradient seen throughout the neocortex. The transverse gradient is still seen between the two secondary areas (OC2L and OC2M, bottom and top graphs, Fig. 11–5; $P < 0.001$, sign test; $F = 34.11$, df

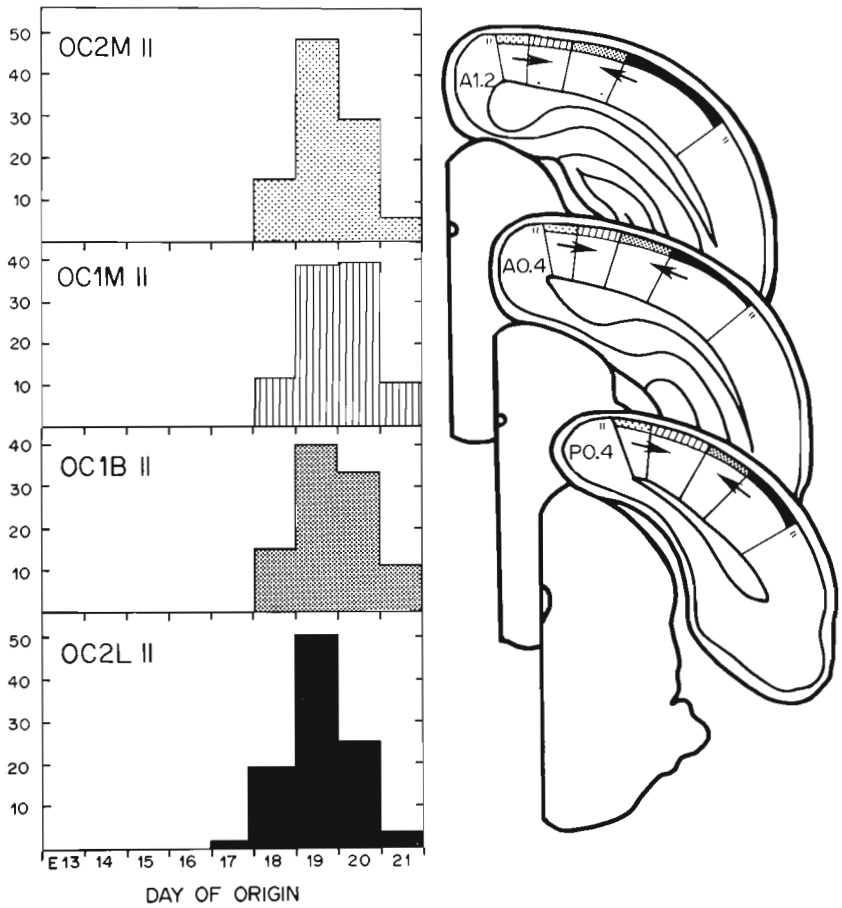


FIG. 11–5. Time of neuron origin in layer II of the lateral secondary visual area (OC2L), binocular recipient primary visual area (OC1B), monocular recipient primary visual area (OC1M), and medial secondary visual area (OC2M) in animals that survived to P60 after two consecutive daily exposures to [^{3}H]thymidine during embryonic life. The bar graphs represent the proportions of neurons generated on single embryonic days. Since neurogenesis occurs simultaneously in the rostrocaudal plane, all graphs are combined data from each level for specific areas. The neurogenetic gradients have shifted from a gradual lateral-to-medial progression in layer V (see Fig. 11–4) to a young centered "sandwich" where the primary areas are generated later than the secondary areas, medially and laterally (*arrows* in drawings). That is a violation of the normally strong transverse neurogenetic gradient throughout most of the neocortex.

= 1, $P < 0.0001$, SAS GLM procedure), but the two primary areas are generated simultaneously (graphs OC1B and OC1M, Fig. 11–5; $P > 0.05$, sign test and SAS GLM procedure). The sandwich gradient is illustrated in layer II from the visual cortex of an animal exposed to [^{3}H]thymidine on E21 and E22 and killed on P60 (Fig. 11–6). There are no labeled cells judged to be neurons in the lateral secondary area (Fig. 11–6C), only one labeled neuron in the medial secondary area (*arrow*, Fig. 11–6A), but there are several labeled neurons in the primary area (*arrows*, Fig. 11–6B). The shift from a transverse gradient in the deep layers to a sandwich gradient in the superficial layers has not been reported in any previous [^{3}H]thymidine autoradiographic study of neurogenesis in the visual cortex. We will report a similar phenomenon in the limb areas of the primary somatosensory cortex (Chapter 14).

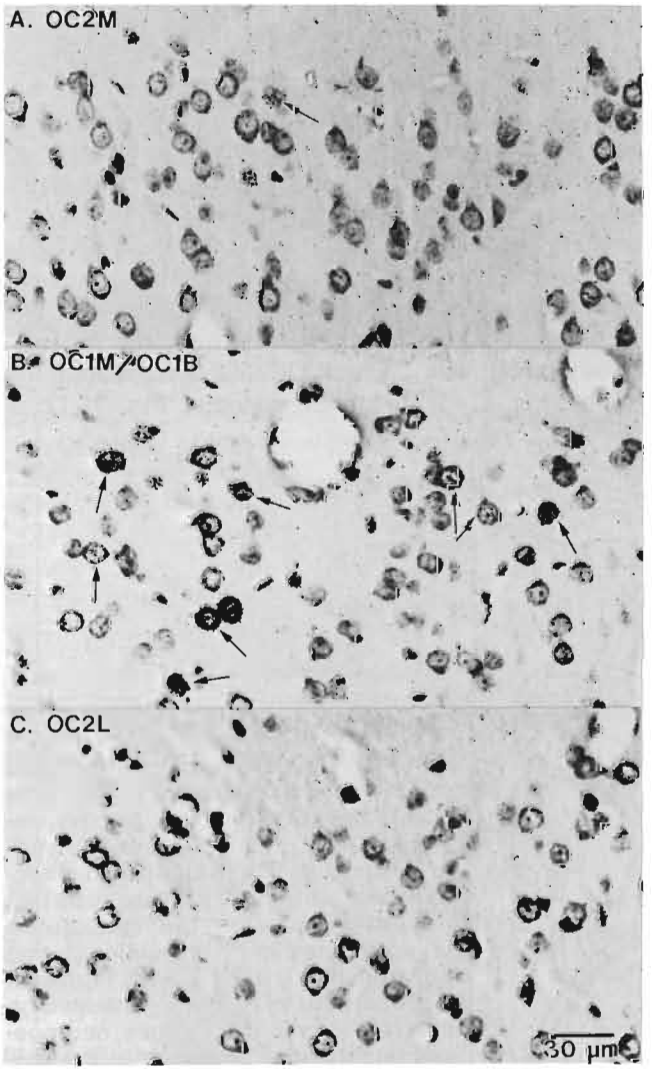


FIG. 11–6. An illustration of the sandwich gradient in the superficial layers of the visual cortex in the brain of an animal exposed to [^{3}H]thymidine on E21 and E22 and killed on P60. There is one labeled cell in the medial secondary area (*arrow* in **A**), no labeled cells in the lateral secondary area **(C)**, and many labeled cells in the junction between the two primary areas (*arrows* in **B**). That pattern is found in all animals of the E21–E22 injection group and indicates that more cells are born later in the primary areas than in either secondary area.

11.5 CORRELATIONS BETWEEN NEUROGENETIC GRADIENTS AND THALAMOCORTICAL CONNECTIONS

The primary visual cortex receives retinotopic input through a relay in the thalamic dorsal lateral geniculate nucleus. Although the dorsal lateral geniculate nucleus is not a laminated structure in the rat as it is in the cat and monkey (Polyak, 1957; Walls, 1953; Gilbert and Wiesel, 1979), there is segregation of retinal afferents into ipsilateral and contralateral zones (Lashley, 1934; Hayhow et al., 1962) in both pigmented (Lund et al., 1974, 1976; Hickey and Spear, 1976; Bunt et al., 1983; Jeffrey, 1984; Reese and Cowey, 1987; Reese, 1988) and albino strains (Lund et al., 1974, 1976; Hickey and Spear, 1976; Bunt et al., 1983; Manford et al., 1984). The overall pattern of retinal input is such that the external parts of the nucleus as well as much of the dorsomedial and ventrolateral borders receive afferents from the contralateral eye, while either a single internal central area (in pigmented rats) or smaller multiple internal foci (in albino rats) receive afferents from the ipsilateral eye (Hickey and Spear, 1976). The dorsal lateral geniculate nucleus has an external (older) to internal (younger) neurogenetic gradient (indicated by the arrow in Fig. 11–7) that is very prominent on P5 (Altman and Bayer, 1989c); however, by P60, the neurogenetic gradient is no longer obvious (Altman and Bayer, 1979). Developmental studies of the retinogeniculate projection in rats indicate that ipsilateral and contralateral zones begin to segregate around E21 (Bunt et al., 1983). The segregation process can be retarded by exposing fetal cats to tetrodotoxin to block nerve transmission (Shatz and Stryker, 1988), or facilitated by the spontaneous firing of retinal ganglion cells (recorded in fetal rats; Galli and Maffei, 1988). By P5, there is a concentrated ipsilateral projection zone in the internal core of the dorsal lateral geniculate nucleus (Manford et al., 1984), where the youngest neurons settle. On the other hand, the contralateral retinal input is heaviest in the external part of the lateral geniculate nucleus, where the oldest neurons settle. Between P6 and P12, retinal axons start to make synaptic contacts on dendrites, and synaptic maturity is reached by P20 (Aggelopoulos et al., 1989). Although no combined [^{3}H]thymidine autoradiographic and tract

tracing studies have yet been done, it seems likely that ipsilateral retinal axons establish contacts with younger lateral geniculate neurons, while contralateral retinal axons establish contacts with older lateral geniculate neurons (diagrammed in Fig. 11–7).

The projection of the lateral geniculate nucleus to the primary visual cortex (Ribak and Peters, 1975; Peters and Saldanha, 1976; Hughes, 1977; Lund and Mustari, 1977; Woodward and Coull, 1984; Sefton and Dreher, 1985) results in a lateral-to-medial segregation of input from the ipsilateral and contralateral eyes (Montero, 1973, 1981; Zilles et al., 1984; Thurlow and Cooper, 1988). Thalamic axons terminate heavily in layer IV, lightly in layers VI and I as part of the class I afferent system (Caviness and Frost, 1980; Frost and Caviness, 1980; Herkenham, 1980). The neurogenetic gradients in the primary visual cortex are such that younger cells in layers IV and VI of OC1M receive an input only from the presumably older lateral geniculate neurons that relay optic input from the contralateral eye (cells with lightly stippled axons, Fig. 11–7), while *older* cells in layers IV and VI of OC1B receive input from two sources: (1) the *older* contralateral eye relay neurons (lightly stippled axons, Fig. 11–7), and (2) the presumably younger ipsilateral eye relay neurons (with darkly stippled axons, Fig. 11–7). Due to the latter projection, the average age of lateral geniculate neurons terminating in OC1B is probably younger than the average age of lateral geniculate neurons terminating in OC1M. When one considers the projection from the lateral posterior nucleus to the lateral extrastriate cortex (OC2B; Montero, 1981; Olavarria, 1979) together with its neurogenetic gradients (Altman and Bayer, 1989c), younger thalamic neurons in medial parts of the nucleus project to older lateral cortical neurons in OC2L, while older thalamic neurons in lateral parts project to younger medial cortical neurons in OC2L.

In both cases, the neurogenetic gradients between source cells and target cells are chronologically reversed: older thalamic neurons send axons to cortical areas populated by younger cells. That reversal has a constant relationship to the length of thalamic axons (Fig. 11–7). Thalamic axons in the visual radiation, for example, first go forward and curve laterally into the posterior limb of the internal capsule; next, the axons enter the ventrolateral cortex and join the white matter just beneath layer VI where they curve back in a dorsomedial direction, staying in the white matter until they reach specific targets in primary visual cortex (Fig. 11–7; Caviness and Frost, 1980; Frost and Caviness, 1980). Given that general trajectory, an axon that has a medial target in the cortex will be longer than one that has a lateral target (note axon lengths, Fig. 11–7). Perhaps it is not coincidental that areas of

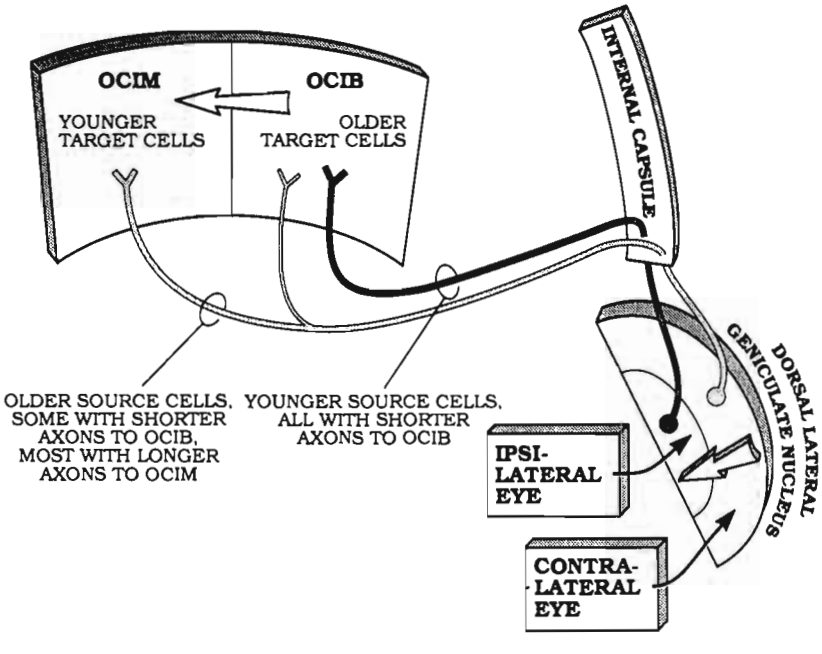


FIG. 11–7. A diagrammatic representation of the neurogenetic gradients and anatomical connections between the dorsal lateral geniculate nucleus and the primary visual cortex. The large arrows point to areas containing younger neurons in each structure. Retinal projections from the ipsilateral eye terminate in the core of younger neurons in the dorsal lateral geniculate, while the larger contralateral projection terminates in the external shell containing older neurons. There is an exact reversal of ages in source and target cells that may be related to axonal length. Most of the older source neurons in the external shell of the dorsal lateral geniculate body have longer axons (*thick light stipple*) that terminate in the monocular recipient cortical area (OC1M) that contains younger target neurons; fewer of the older source neurons project shorter axons (*thin light stipple*) to the binocular recipient area (OC1B). All of the younger source cells in the internal core of the dorsal lateral geniculate have shorter axons (*thick dark stipple*) that project to older target neurons in the binocular recipient area (OC1B).

the cortex with younger neurons are in most cases either medial or posterior to the entry points of thalamic afferents and are thereby contacted by longer axons from older source neurons.

The differential lengths and times of arrival of thalamic axons in the neocortex may be synchronized to interact with the layer IV neurons as they are migrating through the intermediate zone on their way to the cortical plate. It has been known for some time that geniculocortical afferents arrive early and remain in the subplate layer for several days before growing radially into the cortical plate (Lund and Mustari, 1977; Shatz and Rakic, 1981; Rakic, 1983; Shatz and Luskin, 1986; Shatz et al., 1988; Friauf et al., 1990). Recent evidence in the embryonic rat visual cortex indicates that geniculocortical afferents are in the intermediate zone and subplate as the layer IV cells migrate through them, and the thalamic afferents invade the cortical plate only 3 days after the layer IV cells have settled there (Reinoso and O'Leary, 1990); we will more fully discuss the synchrony in arrival of thalamic axons and the migration of neocortical neurons in Chapter 16. In the following chapters, we will show that specific thalamocortical projections relaying auditory (Chapter 12), somatosensory (Chapters 13 and 14), and gustatory (Chapter 15) information follow the same pattern. Moreover, anterior thalamic nuclear afferents traveling in the cingulum to targets in the cingulate and retrosplenial areas also follow the same pattern (Chapter 15; Bayer, 1990b).

11.6 POSSIBLE SIGNIFICANCE OF THE SHIFT IN GRADIENTS BETWEEN THE SUPERFICIAL AND DEEP LAYERS

It is remarkable that the primary visual areas have younger neurons in layers II and III than those in OC2M, violating the nearly universal lateral (older) to medial (younger) neurogenetic gradient throughout the cortex. Indeed, one of the themes that will recur in the following Chapters 12–14 is that primary sensory areas are always characterized by younger superficial neurons regardless of the locations of their respective secondary areas. Since there are rich associational connections between layers IV–II (Burkhalter, 1989), the unusually late neurogenesis of the supragranular layers in primary sensory areas may serve as a neurogenetic marker to single them out. That may be related to another peculiarity in the development of the primary sensory areas. As the specific thalamocortical axons invade the cortical plate in rats, all of the primary sensory areas are distinguished by transient cholinergic activity, while the surrounding secondary areas and the motor area do not show this activity. The primary visual area stains intensely for acetylcholinesterase (Robertson et al., 1985) and nicotinic receptor binding (Prusky et al., 1988; Fuchs, 1989) just at the time that the lateral geniculate projection is being established, but then disappears during the second postnatal week and is not seen in the mature cortex (Robertson et al., 1985).