Cortical Target Depletion and Ingrowth of Geniculocortical Axons: Implications for Cortical Specification

T. U. Woo and B. L. Finlay

Department of Psychology, Cornell University, Ithaca, New York 14853

During the early development of the neocortex, thalamocortical axons arrive potentially in time to instruct migrating cortical neurons in several aspects of local differentiation, such as number of layer IV neurons and efferent connectivity. Migration of layer IV neurons into the cortical plate just precedes thalamocortical invasion, suggesting that *these neurons could cue or tropically direct thalamic ingrowth.* To explore the interactions of layer IV neurons and their thalamocortical input, we administered a mitotic inhibitor methylazoxymethanol acetate (MAM) intraperitoneally to timed pregnant hamsters on E14 when layer IV neurons are normally being generated in striate cortex. Reduced numbers of cortical neurons overall, the absence of small-diameter granule neurons, and the absence of the zone of reduced density of callosally projecting neurons suggest that neither the depletion of layer IV cells in the ventricular zone nor thalamic afferents in the subplate or cortical plate respecify the later generated cohort of neurons (presumptive layer II/III neurons) to acquire morphological and connectional properties of layer IV. DiI injections into the dorsal lateral geniculate nucleus (LGd) of animals from embryonic (E15) and postnatal (P7) ages show that the final position of thalamic axons with respect to layer V is not affected by the absence of E14 neurons. In the normal visual cortex, geniculocortical axons have begun their arborization in their presumptive target layer in the upper cortex immediately below the undifferentiated cortical plate on P4, while in MAM animals, this process occurs 1 d later. The extent and density of arborization is much reduced in the thinner cortex of the MAM animals. We thus find no evidence for instruction of migrating neurons by thalamocortical axons to assume the layer IV phenotype; if instruction does occur, it must take place in a very restricted time window. Thalamic axons can also find their laminar position in the absence of cells of this phenotype.

As is well known, the vast majority of neurons in the cerebral cortex are generated in the ventricular zone, and the final disposition of cortical neurons and their morphological and functional properties are largely predictable by their birthdates (McConnell, 1988b, 1991, 1992). This pattern of neuronal generation and disposition is reminiscent of the development of another highly laminated but more accessible structure, the retina, in which the mechanisms of cell fate determination have been more fully studied (Reh and Radke, 1988; Reh, 1989, 1992a,b; Altshuler et al., 1991; Cepko, 1993). Local environment in the retinal neuroepithelium is believed to be important in determining a particular cell fate. For example, selective removal of a particular class of neurons during retinal development results in increased neuronal output from the neuroepithelium to replace the depleted neurons (Negishi et al., 1982, 1985, 1987; Reh and Tully, 1986; Reh, 1992a). In the cortex, there has also been evidence suggesting the importance of the microenvironment in the ventricular zone in determining the laminar fate of postmitotic neurons (McConnell, 1985, 1988a, 1990; McConnell and Kaznowski, 1991).

One important feature regarding the development of the cortex is that it receives inputs from subcortical structures during the very early phase of development. The most noticeable among these inputs is probably the thalamocortical system, which is the earliest arriving afferent pathway and has been strongly implicated in many aspects of cortical development and differentiation (Kennedy and Dehay, 1993; O'Leary et al., 1994). Peripheral information from sensory organs through the thalamus has been demonstrated to be crucial in organizing the cytoarchitecture of the barrel cortex in the rodent (Welker and Van der Loos, 1986a,b; Jensen and Killackey, 1987a,b; Erzurumlu and Jhaveri, 1990; Schlaggar and O'Leary, 1991, 1994; Killackey et al., 1994). Prenatal removal of the majority of geniculocortical inputs by bilateral enucleation in utero in monkeys results in the shift in borders between area 17 and its neighboring areas with the reduction in the tangential extent of area 17 (Rakic, 1988; Dehay et al., 1991; Rakic et al., 1991). Complete ablation of the lateral geniculate nucleus in newborn hamsters when geniculocortical axons are in residence in the subplate results in the apparent loss of layer IV (Windrem and Finlay, 1991).

Studying the development of the thalamocortical pathway has also provided insight into the possible functional significance of the thalamus in cortical specification and development. Thalamocortical axons arrive early in the subplate and presumptive layer IV neurons have to migrate through the thalamic afferents in the subplate compartment to arrive in the cortical plate (monkeys: Rakic, 1976, 1977, 1982; cats: Shatz and Luskin, 1986; rodents: Erzurumlu and Jhaveri, 1990, 1992; Catalano et al., 1991; De Carlos and O'Leary, 1992; Kageyama and Robertson, 1993; Miller et al., 1993; marsupials: Sheng et al., 1990, 1991). After the arrival of their target neurons in the cortex, thalamic axons commence their ingrowth process into the cortical plate. The relative timing is appropriate for thalamic axons to specify layer IV cell fate as these cells migrate through the subplate. Further, it also raises the possibility that mutual recognition between the two neural elements within the subplate might be the determinant of the initiation of thalamic ingrowth.

One approach to further explore the nature of these specification events within the ventricular zone and the subplate would be to remove the population of presumptive layer IV cells from the respective compartments and challenge the later generated cohort of cells (presumptive layer II/III cells) to change their original fate to acquire layer IV neuron characteristics. In the present study, we applied a potent mitotic inhibitor, MAM (methylazoxymethanol acetate), to timed-pregnant hamsters at the time when the major population of presumptive layer IV neurons are normally being generated. This manipulation depletes the ventricular zone of the presumptive layer IV population. It also massively reduces the population of migrating presumptive layer IV cells in the subplate and thereby disrupts the presumptive interactions in this layer. Our results demonstrate the apparent loss of layer IV after the removal of a major population of neurons generated at the time when layer IV cells normally become postmitotic and the failure of the microenvironment in the ventricular zone or the thalamic axons in the subplate to respecify the

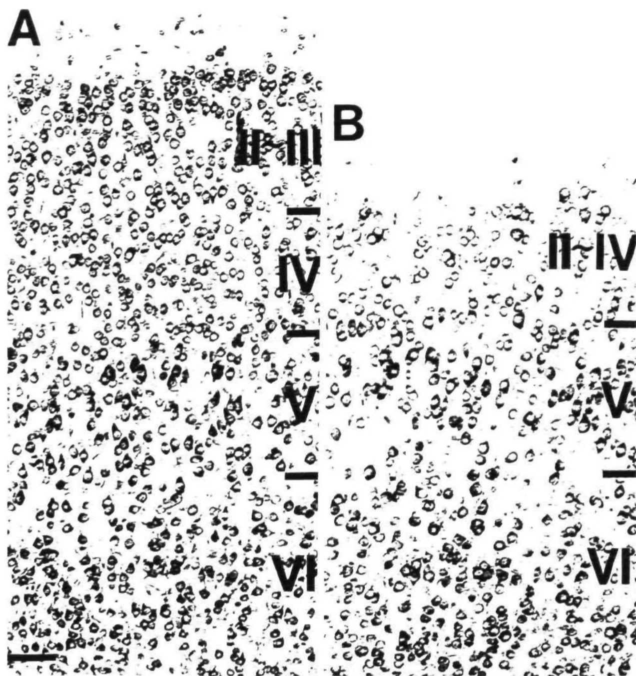


Figure 1. Photomicrographs of coronal sections of a P20 normal (*A*) and MAM (*B*) visual cortex. Note that while layer IV granule neurons are distinct in the normal cortex, they are no longer apparent in the MAM cortex. Also, the thickness of the whole cortex, especially that of upper cortical layers, is severely reduced. Layers V and VI, which were generated before the application of MAM, are relatively unaffected. Scale bar, 50 μm.

later generated population of neurons to adopt layer IV cell fate. Further, the apparent absence of this particular population of neurons does not affect the time course of development and ingrowth of geniculocortical axons.

Preliminary results of these experiments have been presented in abstract form (Woo et al., 1992, 1994).

Materials and Methods

Injections of Mitotic Inhibitor

A total of 119 golden hamsters of various embryonic and postnatal ages were used in this study. A potent mitotic inhibitor, MAM (methylazoxymethanol acetate, Sigma), was used to deplete layer IV and supragranular neurons [Johnston and Coyle, 1982; Woo et al., 1996 (preceding companion article)]. Timed-pregnant hamsters were injected intraperitoneally (i.p.) with single doses of 30 mg/kg of MAM on embryonic day 14 (E14) and the timing of MAM injections was based on previous tritiated thymidine studies showing that E14 is the day when the major cohort of layer IV cells in visual cortex is being generated (Shimada and Langman, 1970; Crossland and Uchwat, 1982; unpublished observations). The choice of dosage was based on our previous study (Woo et al., 1996, preceding companion article) as the highest dosage that consistently results in the apparent loss of layer IV with simultaneously massive cell loss in layer II/III, without significant mortality or morbidity of either the dams or the pups. Only animals (80% of the original set) born following the normal gestational schedule were included in this study.

Characterization of Layer IV Depletion

After 30 mg/kg of MAM treatment, layer IV is morphologically indistinguishable from other cortical layers (Fig. 1). However, because layer IV cells in the rodent do not possess any wholly unique morphological or connectional characteristics, this statement refers to the distribution of properties in layer IV compared to neighboring layers, and not to any unique characteristics of layer IV neurons. To justify the morphological impression of the apparent loss of layer IV, we performed two analyses based on two criteria that distinguish layer IV from other layers in the normal visual cortex. First, layer IV is normally characterized by its major composition of small granule neurons, while neurons in layer II/III and V are mainly larger pyramidal cells. Second, the normal visual cortex has a distinct distribution pattern of callosally projecting neurons. These neurons mainly originate from layers II/III and V at the border between area 17 and 18a with layer IV containing a relatively smaller number of callosal cells (Dursteler et al., 1979; Rhoades and Dellacroce, 1980; Diao and So, 1991). If layer IV is produced or induced in MAM-treated animals later in development, cells generated on E15, which would normally form layers II–III, should have these size and connectivity distributions.

Quantification of Soma Area Distribution

Two normal and two MAM-depleted brains from previous experiments (see Woo et al., 1996) stained with cresyl violet were used in this part of the study. Dorsal reconstructions were performed to outline the rostrocaudal and mediolateral extent of visual cortex in each brain based on cytoarchitectonic criteria in reference to the rat brain atlas by Paxinos and Watson (1986) and our own hamster brain atlas (unpublished observations; see Woo et al., 1996, for details and methodological concerns). Only neurons within the extent of Ocl (area 17) were included in the assessment. Neurons were sampled according to the following paradigm. We first identified three equally spaced coordinate sets from the two-dimensional dorsal reconstructions of visual cortex and each coordinate set corresponded to a vertical column of neurons in the corresponding coronal section (section thickness, 10 μm). Each column was measured in the dimensions of about 600–700 μm in depth and 120 μm in width in the coronal plane. Within each column, only neurons with well-delineated nuclei and nucleoli were sampled to minimize the potential confounding effect due to the asymmetrical sectioning of cell bodies. Soma areas were measured using a computer-assisted imaging system attached to a Leitz Diaplan microscope and the relative change in soma size was the interest of this analysis.

Quantification of Callosal Distribution Pattern

Labeling of Callosal-Projecting Neurons in Cortex. A total of six normal and MAM-treated animals were used in this part of the study. We labeled callosal-projecting neurons in visual cortex by massive injections of HRP (horseradish peroxidase) into the contralateral cortex at the border between area 17 and 18a. Briefly, on P50 or older, animals were anesthetized by intraperitoneal injections of pentobarbital sodium (0.16 mg/100 gr b.wt.). The skull over the right posterior cortex was carefully removed. Part of the right visual cortex including the area 17 and 18a border was exposed and 30% HRP solution was then injected stereotaxically into multiple sites of the cortex at and around the border. The total volume of HRP injected in each animal was about 0.5 μl. The wound was then rinsed with normal saline and sutured. Animals were closely monitored after surgery until fully recovering from anesthesia before being returned to the colony. Animals were allowed to survive for 3 d to allow for HRP transport. They were then again deeply anesthetized by intraperitoneal injections of an overdose of pentobarbital sodium and were perfused through the heart first with cold normal saline for 10 min and then with a mixture of 2.5% glutaraldehyde and 1.5% paraformaldehyde at 4°C for another 20 min. Brains were removed from the skull and postfixed at 4°C for 6 hr before putting into 30% sucrose solution for cryoprotection until they sank. The brains were then embedded in albumin-gelatin and cut frozen at 25–35 μm on a freezing microtome. Sections were divided into two alternate sets and were collected in phosphate buffer (0.1 M, pH 7.4). One set of sections were processed directly for HRP histochemistry (Adams, 1977, 1981) and the alternate set stained with cresyl violet to identify laminar boundaries.

Data Analysis. Distributions of HRP retrogradely labeled callosal neurons in the contralateral cortex in both normal and MAM brains were plotted by employing a computer-assisted camera lucida system. Three consecutive sections were usually analyzed for each animal and were superimposed to get a summary plot for each animal (see Fig. 4). From each summary plot, a histogram was constructed by plotting the percentage of labeled cells as a function of cortical depth (see Fig. 5).

Tracing of Development of Geniculocortical Afferents

The age and number of animals used are listed in Table 1. Experimental procedures are described below.

Table 1
Animals used for DiI experiments

Age of injection	Number of pups used	
	MAM (30 mg/kg)	Normal
E15	11	4
P0	7	3
P1	10	3
P2	6	7
P3	9	4
P4	9	3
P5	11	3
P6	5	4
P7	10	4

E15 Fetal Animals

MAM-injected and normal pregnant hamsters were deeply anesthetized by overdose injections of pentobarbital sodium (i.p.). Nonsurvival cesarean sections were performed and fetal animals were removed. They were then decapitated and heads were placed immediately into phosphate-buffered (0.1 M, pH 7.4) 4% paraformaldehyde. After 3–4 d of fixation, brains were removed from skulls and were fixed for another 5–6 d before tracer injections.

Postnatal Animals

Pups were deeply anesthetized by either hypothermia or intraperitoneal administration of an overdose of pentobarbital sodium. They were then perfused through the heart first with 0.9% NaCl for 5 min followed by phosphate-buffered (0.1 M, pH 7.4) 4% paraformaldehyde for 10–15 min. Brains were removed from skulls and were placed into the same fixative for about a week before tracer injections.

Tracer Injections

The fluorescent lipophilic tracers DiI or fast DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, 1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine *p*-chlorobenzenesulfonate, respectively; Molecular Probes) were used in this study. A small crystal of either DiI or fast DiI was placed in the LGd. The brains were then returned to 4% paraformaldehyde and were stored in the fixative in the dark for at least 6 weeks, depending on the size of the brain, before examining. For fast DiI, incubation time was considerably less (about 50%) than that needed for DiI. Brains were cut coronally at 70–150 μm on a vibratome and sections were collected into phosphate buffer (0.1 M, pH 7.4), stained with bisbenzimide (Sigma, 1 mg/ml distilled water), mounted and coverslipped with either phosphate buffer or a special mounting medium (made by dissolving 5 gm of *n*-propyl gallate in 90 ml of glycerol and 10 ml of 0.1 M phosphate buffer (pH 7.4) under low heat). Sections were then examined under Rhodamine optics of a Leitz fluorescent microscope. In bisbenzimide-stained sections, the immature cortical plate can be identified by the high cell packing density and vertically elongated nuclei whereas differentiated neurons exhibit round nuclei and reduced cell packing density.

Results

General Effects of MAM on Cortical Development

As described in the preceding companion article (Woo et al., 1996), the cortical depletion effect of MAM is dose dependent. The most severe depletion was achieved by injections of 30 mg/kg of MAM on E14. After MAM treatment of this dosage, layer IV is no longer recognizable cytoarchitectonically in cresyl violet-stained sections, and the thickness of cortex above layer V is severely reduced (Fig. 1). We collectively refer to those neurons above layer V as layer II–IV neurons because of the difficulty identifying them as belonging to particular functional or laminar classes after the apparent disappearance of layer IV. Total loss of these neurons is 71%, which results in 31% loss of geniculate neurons (Woo et al., 1996).

Change in Soma Area Distribution

In the normal visual cortex, layer IV is composed mainly of small granule neurons with high neuronal density. We demonstrate the distinct distribution pattern of soma areas in the normal cortex by plotting the mean soma areas against cortical depth (Fig. 2). Layer IV consists of neurons with relatively small soma size (mean area ± SD = 86.56 ± 9.13 μm^2), whereas the mean soma areas of layer II/III, V, and VI neurons are larger (110.15 ± 10.29, 129.51 ± 15.06, 100.15 ± 12.53 μm^2, respectively). In the MAM cortex, this distinct distribution pattern characterized by the middle stratum of cells with small soma size is no longer present (Fig. 2). The mean soma areas of layer V and VI neurons are not statistically different from normal (136.41 ± 15.63 μm^2, $n = 40$, $t = -1.36$, $p = 0.18$; 96.77 ± 11.80 μm^2, $n = 40$, $t = 0.93$, $p = 0.36$; respectively). The mean soma area of layer II–IV neurons is 104.57 ± 10.36 μm^2, which is significantly different from that of layer IV neurons in the normal brain (Fig. 3; $n = 26$, $t = -7.12$, $p < 0.00001$) but not different from that of normal layer II/III neurons (Fig. 3; $n = 26$, $t = 1.14$, $p = 0.26$). Further, examination of the distribution of the entire population of layer II/IV neurons revealed no evidence of the existence of any remaining population of small cells (data not shown).

Change in Distribution of Callosal-Projecting Neurons

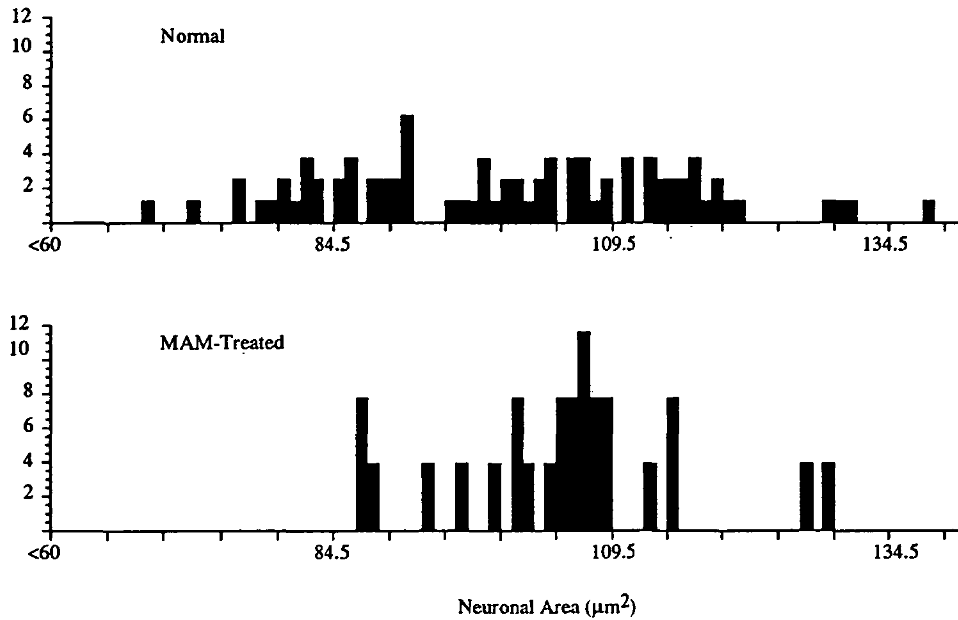
In the normal hamster brain, visual callosal neurons originate from all cortical layers except layer I at the border between area 17 and 18a as previously demonstrated by other groups (Rhoades and Dellacroce, 1980; Diao and So, 1991). Our data in the normal brain are in agreement with these previous findings. Callosal projecting neurons are highly concentrated in layer II/III and layer V, whereas density of these neurons in layer IV is relatively low. Nevertheless, layer IV is never completely devoid of callosal neurons. In fact, HRP labeled-neurons in layer IV could be numerous in some cases, making it sometimes difficult to appreciate any distinct distribution pattern in a single section. Therefore, we superimposed three consecutive sections to get a conglomerate distribution plot of HRP labeled-neurons and by this method layer IV becomes very visible as the layer of low density of callosal-projecting cells (Fig. 4*A*). However, in the MAM cortex, this bilaminar pattern of distribution of HRP label is no longer apparent (Fig. 4*B*). We also plotted the percentage of HRP-labeled neurons as a function of cortical depth of the two animals shown in Figure 4 (Fig. 5). Besides the apparent loss of the bilaminar distribution pattern in the MAM cortex, there also seems to be some redistribution of callosal neurons, especially in the bottom part of layer II–IV, in which the density of callosal cells has become quite high (Fig. 5). Nonetheless, in this particular animal, callosal cells in the upper part of the cortex (corresponding to layer II–IV) still constitute 51% of the total number of labeled neurons, which is not very different from the value of 56% (of which 12% are in layer IV) in the normal case.

Effects of Cortical Depletion on Development of Geniculocortical Afferents

The normal development of geniculocortical afferents in the hamster has been studied in great detail (Naegele et al., 1988; Miller et al., 1993). In the present study, we investigated the effects of depletion of the major targets of this pathway, for instance, neurons generated on E14, on its development.

The LGd in the hamster is generated from E9.5 to E12.5 (Crossland and Uchwat, 1982). As has been demonstrated by Miller et al. (1993), on E13 during normal geniculocortical development axons from the lateral geniculate have just arrived at the internal capsule and have begun to accumulate beneath the lateral cortex. On E14, most of the axons have already arrived at the lateral cortex, and occasionally some of

A.



B.

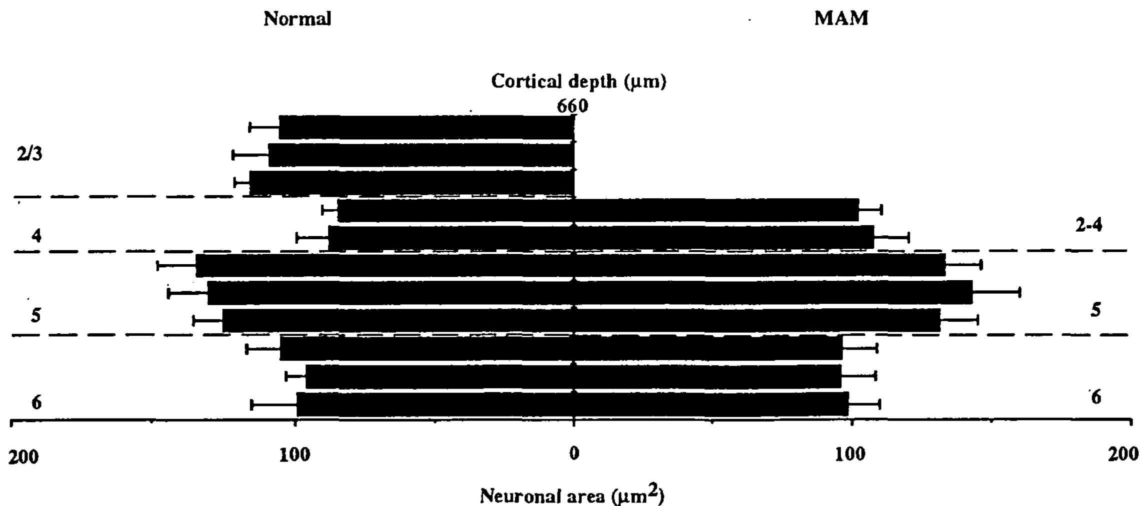


Figure 2. *A*, Histogram of the distribution of cell areas (μ^2) in layers II–IV for normal [*n* (cells) = 80; *n* (animals) = 2] and MAM-treated animals [*n* (cells) = 26; *n* (animals) = 1] Data are expressed as percentage of the total distribution of cells to facilitate comparison of the two histograms. The population of cells with small somas is missing in the MAM neocortex. *B*, Histograms of distribution of soma size across cortical depths in the normal and MAM P20 visual cortex. In the normal visual cortex, the bilaminar pattern of distribution of soma area is distinct with layer IV composed of neurons with small soma size. This bilaminar distribution pattern is no longer apparent in the MAM cortex. Note that there is no difference in soma areas of layers V and VI.

them can be seen to have already reached the intermediate zone beneath visual cortex. This is also the age when MAM was injected; after the complete generation of the geniculate nucleus but before the majority of geniculocortical axons arrive at visual cortex.

On E15, 24 hr after MAM injections, many more axons have arrived and continue to accumulate in the intermediate zone beneath visual cortex (Fig. 6). This is also the age when the subplate has just begun to differentiate (Bayer and Altman, 1990; Miller et al., 1993). By E16 (P0, day of birth) the subplate is fully differentiated (Fig. 6*A*). Virtually all of the axons have already arrived and accumulate within the subplate (Fig. 6*B*). Occasionally, fibers would extend to reach the marginal zone (future layer I), whereas the vast majority of thalamic axons remain in the subplate. On this age, the cortical plate is still totally undifferentiated. During normal development, neurons generated on E14 should have arrived at the subplate by now. In the present study, massive depletion of E14 neurons would mean that the subplate would now be relatively devoid of migrating presumptive layer IV neurons.

On P1, as geniculocortical axons continue to accumulate and arborize within the subplate, the very bottom part of the cortical plate has just begun to differentiate into layer VI and axons begin to invade this region (Fig. 6*C*). Thalamic axon ingrowth thus occurs in tandem with the differentiation of the cortex, as in normal development (Fig. 6*D*; Miller et al., 1993). More importantly, even without the presence of the majority of the E14 neurons in the subplate zone, thalamic axons invade the cortex following essentially the normal developmental schedule.

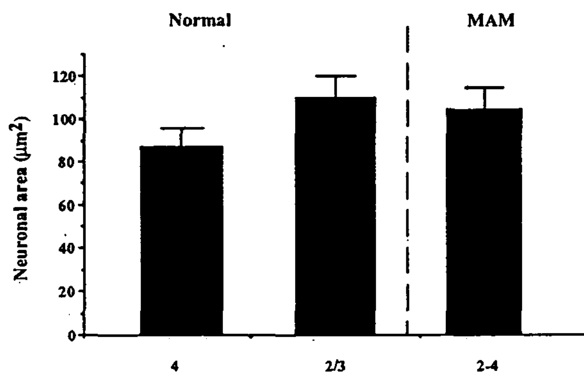


Figure 3. Mean soma area of layer II–IV cells in the MAM cortex is significantly different from that of layer IV cells in the normal cortex (MAM = 104.57 ± 10.36 μm^2, normal layer IV = 86.56 ± 9.13 μm^2, $n = 26$, $t = 7.12$, $p < 0.00001$) but is not different from that of normal layer II/III cells (110.15 ± 10.29 μm^2, $n = 26$, $t = 1.14$, $p = 0.26$). These results suggest that the majority of small granule cells in layer IV have been depleted.

By P2, the bottom half of the cortex has differentiated and the border between layers V and VI can first be recognized. Thalamic axons continue to arborize quite extensively in the differentiated region and at the same time arbor density in the subplate has reduced considerably by this age (data not shown). Occasional axons can be traced to penetrate the cortical plate to reach the marginal zone. Up to this age, we do not yet discern any differences in either the time course of development or the growth pattern of geniculocortical axons.

On P3, although thalamic axons continue to arborize in layer VI, the arbor density is significantly reduced (Fig. 7*C*, compared with the normal cortex in Fig. 7*B*). Nevertheless, there is no evidence of retardation of growth of these axons. In some of the normal brains examined, thalamic axons have already begun to arborize in the presumptive future layer IV immediately below the undifferentiated cortical plate, which now comprises only the upper one-fourth of the cortex, giving rise to the laminar-like segregation of arborizations (Miller et al., 1993). However, in none of the MAM brains have we seen any evidence of bilaminar segregation on this age. Again, occasional fibers can be seen to penetrate the undifferentiated cortical plate, but they seldom give off any collaterals. Cortical thickness, in particular, the thickness of the undifferentiated cortical plate, is obviously reduced (Fig. 7*D*).

By P4, the bilaminar pattern of thalamic arborizations was evident in all the normal brains that were examined (Fig. 8*B*). The upper lamina of arborization is located right below the undifferentiated cortical plate and the lower lamina is in the region between upper layer VI and lower layer V. Arbor density in the upper lamina is either lower than or the same as that in the lower lamina. On the contrary, in the MAM cortex, there is still no evidence of any laminar-like segregation of arborizations on this age (Fig. 8*C*). Axonal arbors remain confined to the border of layer V/VI. Arbor density has increased compared to the P3 cortex, but it is not different from that of the normal cortex of the same age (compare Fig. 8*B*,*C*).

On P5, in the normal cortex, thalamic axons continue to arborize extensively in the upper lamina immediately below the cell dense cortical plate, which is now only a very thin layer immediately below layer I (Fig. 9*A*). At the same time arbor density in the lower lamina has begun to reduce so that by this age thalamic arborization in the upper lamina is much more dense than that in the lower lamina (Fig. 9*B*). In the MAM cortex, this is the age when bilaminar segregation of arborization has first become evident (Fig. 9*C*). Compared to the normal cortex, both the extent and density of axonal arbors in the upper lamina is much reduced (compare Fig. 9*B*,*C*). The fact that thalamic axons arborize above layer V, their normal termination zone, is further demonstrated by the relative position of the axonal arbors and the retrogradely

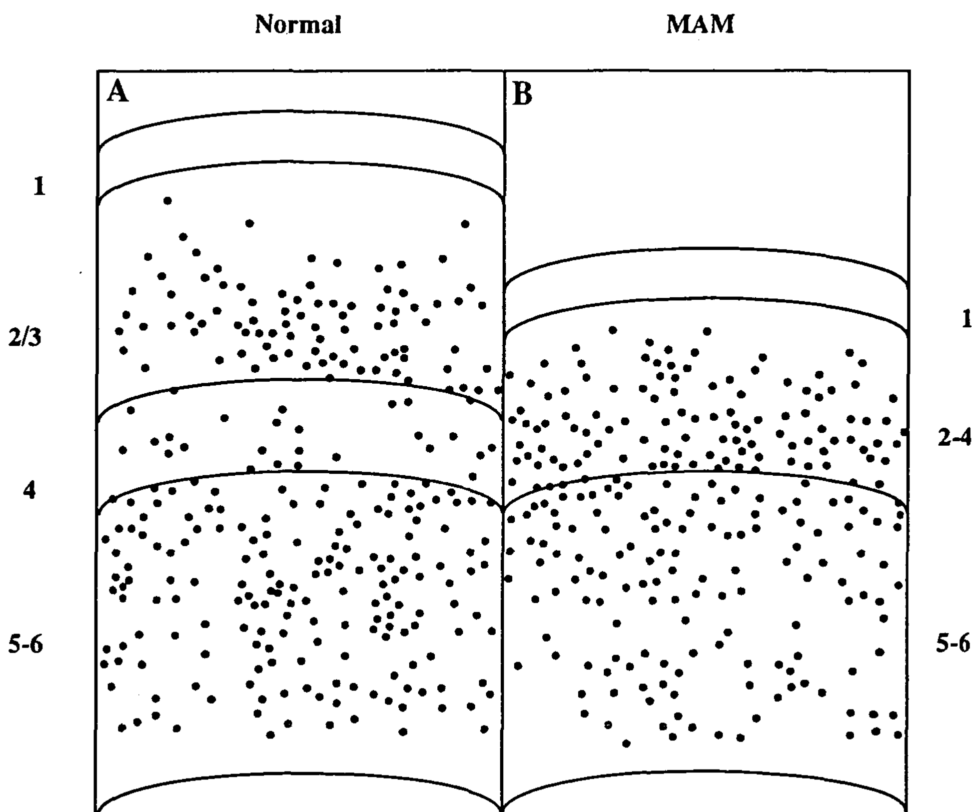
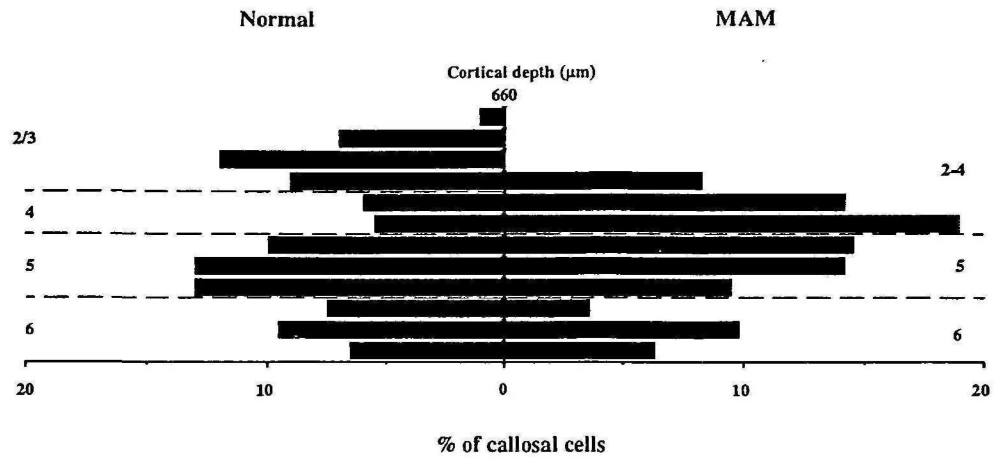


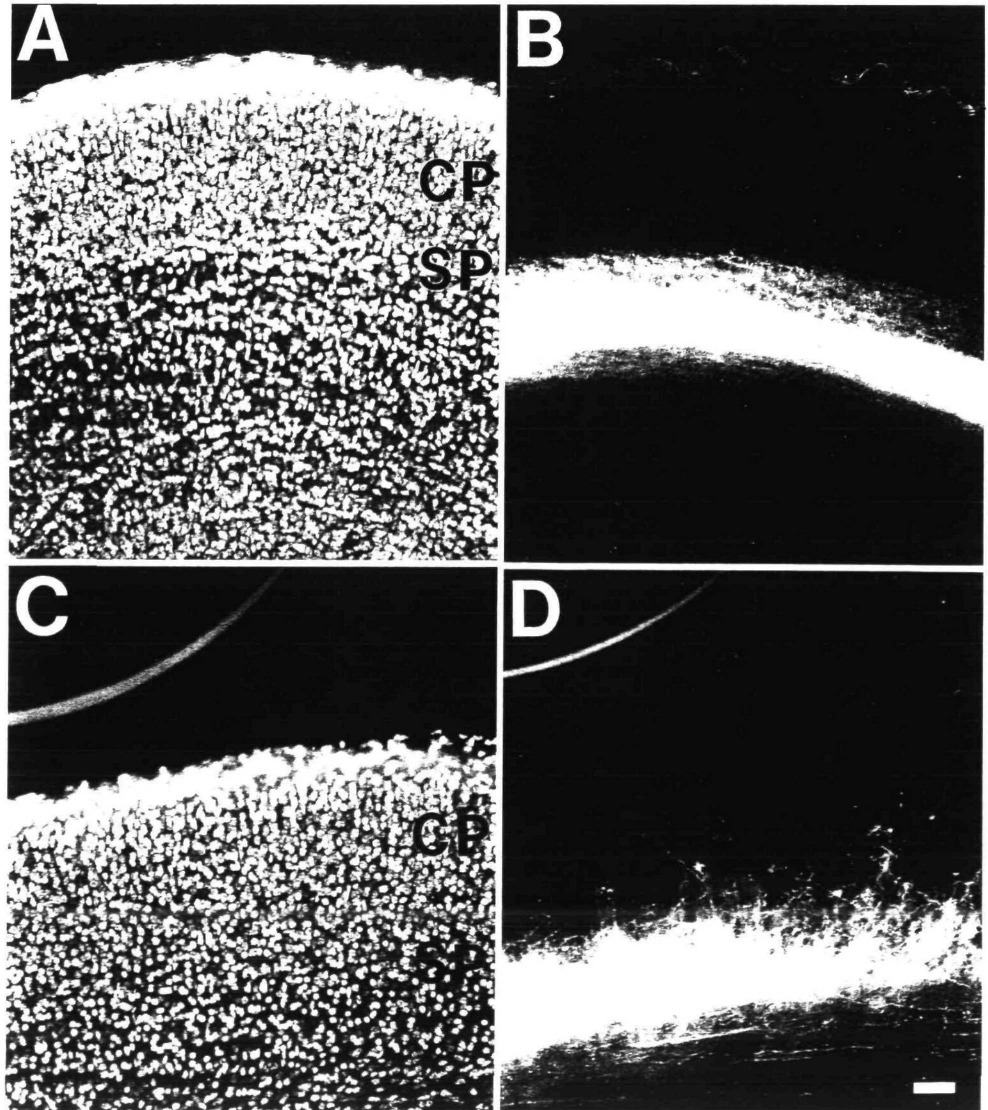
Figure 4. Composite camera lucida drawings from three superimposed adjacent sections showing the distribution pattern of callosally projecting cells in visual cortex in a normal and a MAM P50 animal. Laminar boundaries were identified by comparing with adjacent Nissl sections. Note that the bilaminar pattern of distribution is characteristic of the normal cortex, with layer IV containing relatively less number of callosal neurons. This bilaminar distribution pattern is not apparent in the MAM cortex, demonstrating the severe depletion of layer IV.

Figure 5. Histograms of distribution of callosally projecting cells as a function of cortical depth in visual cortex in the same animals shown in Figure 4. As in Figure 4, note that in the normal cortex, the bilaminar pattern of distribution is apparent, with layer IV containing relatively less callosal cells. This bilaminar distribution is no longer present in the MAM cortex.



labeled layer V cells in Figure 9C. These cells were labeled while projecting through the lateral geniculate to their targets in the ventral lateral geniculate nucleus and the superior colliculus (Lund, 1966; Sefton et al., 1981; Miller et al., 1993). Arbor density in the lower lamina has also been reduced as in normal development. There is no difference in density of axonal arbors in the lower lamina between normal and MAM cortices, thereby showing no evidence of compensatory retainment of thalamic axons in layer VI and subplate after depletion of a large proportion of their normal targets.

Figure 6. Fluorescence photomicrographs of DiI-labeled geniculocortical axons in P0 (*B*) and P1 (*D*) MAM visual cortices. The same sections were counterstained with bisbenzimide (*A* and *C*, respectively). In the P0 cortex, subplate (*SP*) is well differentiated and is distinguishable from the undifferentiated cortical plate (*CP*) above (*A*). Geniculocortical axons accumulate and have begun to arborize within the subplate (*B*). No invasion into the cortical plate is obvious on this age. On P1, geniculocortical axons have begun to invade and arborize within the very bottom part of the cortical plate, which has just begun to differentiate to become the future layer VI (compare *C* and *D*). Scale bar, 50 μm.



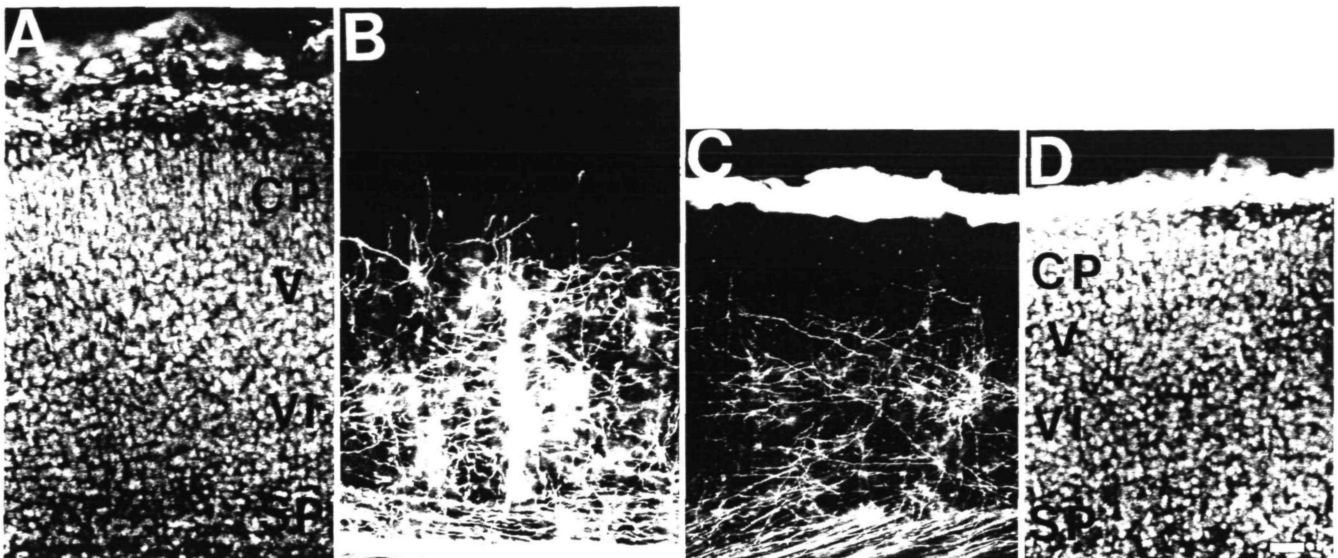


Figure 7. In the normal P3 visual cortex (*A*, bisbenzimide stained), layers V and VI are well differentiated and the undifferentiated cortical plate (*CP*) comprises only the upper one-fourth of the cortex (note the high cell packing density and the vertically elongated appearance of nuclei). Geniculocortical axons labeled with DiI (*B*) have arborized extensively up to the bottom of the cortical plate, which corresponds to the differentiating presumptive layer IV. In some animals, laminar-like segregation of arborization has become evident at this age. In the MAM cortex of the same age (*C*), geniculocortical axonal arbors have also reached the bottom part of the undifferentiated cortical plate (*D*), although arbor density is reduced compared to normal. Laminar-like segregation was not apparent in any of the animals examined. Layers V and VI (*D*) are well differentiated, as in the normal cortex, but the whole cortex, especially the cortical plate, is much reduced in thickness. Scale bar, 50 μm.

On P7 the cortical plate in the normal cortex is completely differentiated and laminar borders are clearly distinguishable. Density of axonal arbors in the upper lamina (located in layer IV and the bottom part of layer 3) continues to increase while that in the lower lamina has been greatly reduced (Fig. 10*A*,*B*). In the MAM cortex, thickness of layers V and VI is comparable to that in normal cortex, but thickness of layer II–IV is much reduced, with layer IV not identifiable (Fig. 10*C*,*D*). Thalamic axons arborize within a thin stratum above layer V with much reduced arbor density. In no cases is there evidence of these axons overgrowing to reach layer I, suggesting that the loss of the majority of E14 neurons does not prevent thalamic axons from recognizing their correct termination lamina in the cortex. There is also no evidence of compensatory increase in arborization in layer VI and subplate (compare Fig. 10*B*,*C*). In fact, by this age arbor density in the lower lamina has been reduced to the normal level.

The major findings regarding the development of geniculocortical axons after the massive depletion of E14 neurons, their principal future target neurons, are presented schematically in Figure 11. In summary, there are several major observations. First, there is no delay of thalamic axons in invading

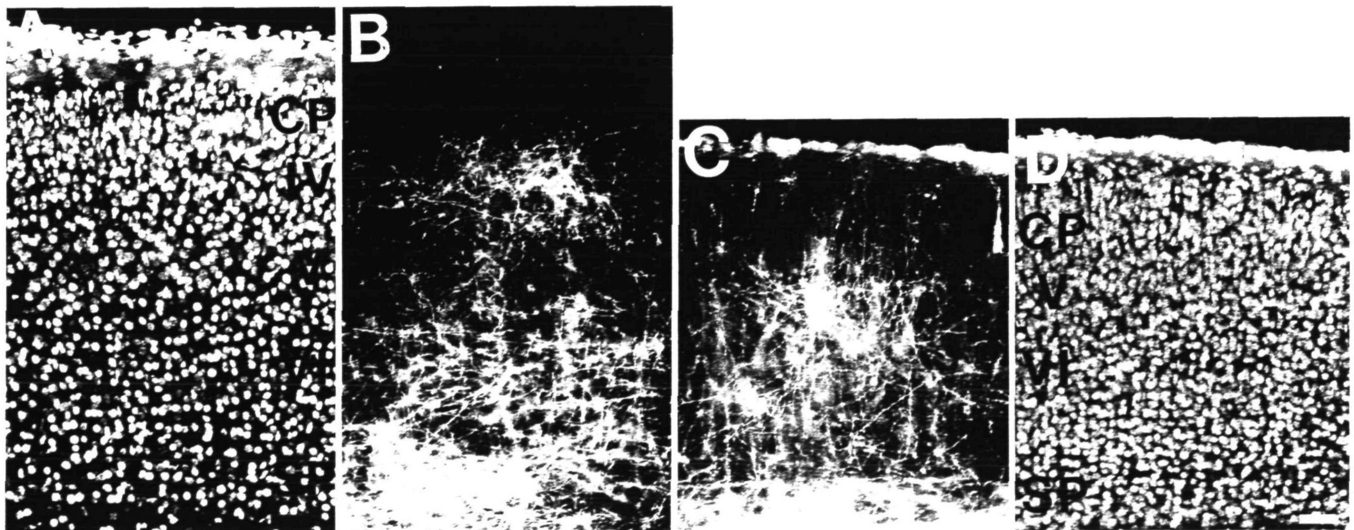


Figure 8. In the normal P4 visual cortex (*A*, bisbenzimide stained), the border between layer IV and V has become distinguishable. Bilaminar segregation of arborization of geniculocortical axons labeled with DiI is apparent (*B*). The upper lamina of the arborization is located immediately below the undifferentiated cortical plate (*CP*) and the lower lamina is in the region between upper layer VI and lower layer V. Arbor density in the lower lamina has become reduced compared to P3. In the MAM cortex of the same age (*C*), no bilaminar segregation of arborization is evident. Geniculocortical axonal arbors remain confined to layers V and VI and the arbor density is very similar to that of the lower lamina in the normal cortex. In the bisbenzimide-stained MAM cortex (*D*), note that the thickness of the whole cortex, in particular the cortical plate, is reduced compared to normal. Scale bar, 50 μm.

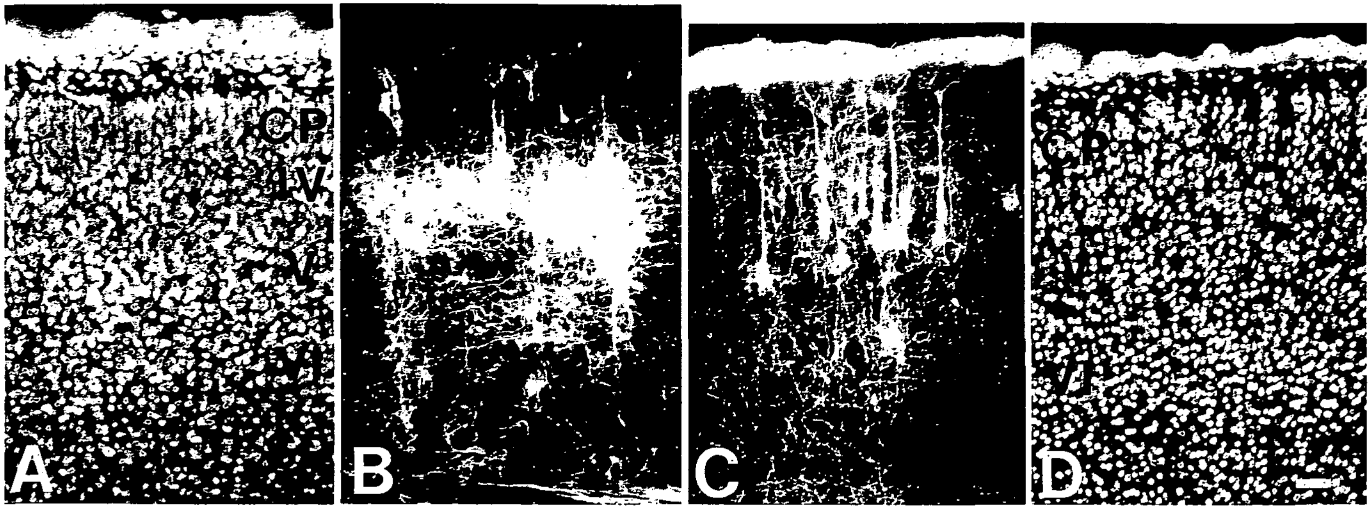


Figure 9. In the normal P5 visual cortex (*A*, bisbenzimide stained), layer IV is well differentiated and the undifferentiated cortical plate (*CP*) comprises only a very thin layer immediately below layer I. *B*, Geniculocortical axons labeled with DiI continue to arborize extensively in the upper lamina immediately below the cell dense cortical plate, while arbor density in the lower lamina is reduced. *C*, In the MAM cortex of the same age, bilaminar segregation of geniculocortical arborization has first become evident. Thalamic axons have begun to arborize in the region above layer V, their correct termination zone. The fact that they arborize in the correct lamina is also demonstrated by the relative location of the axonal arbors and the retrogradely labeled layer V cells (*arrows*), which were labeled while their axons passing through the LGd. However, both the extent and density of arborization in the upper lamina are much reduced compared to normal. Note also that arbor density in the lower lamina has now been significantly reduced, although it might still be higher compared to normal. *D*, Both thickness and cell density of cortical plate are reduced in the MAM cortex and layer IV is apparently absent. Scale bar, 50 μm.

the cortex on P1, and the time course of development of these axons is essentially normal until P3/P4. Second, in the normal cortex, laminar-like segregation of thalamic arborizations is first seen at P3 and usually becomes prominent at P4. In the depleted cortex, there is no evidence of segregation until P5. Third, during the whole course of development, thalamic arbor density tends to be lower compared to normal. After bilaminar segregation of thalamic axons, both the extent and density of arborization in the upper lamina is very much reduced compared to normal. Further, there is no evidence of any compensatory increase in arborization in the lower lamina.

Discussion

The Nature of Layer IV Loss

After 30 mg/kg of MAM treatment, cytogenesis on E14 was severely, but not completely depressed, and layer IV is no lon-

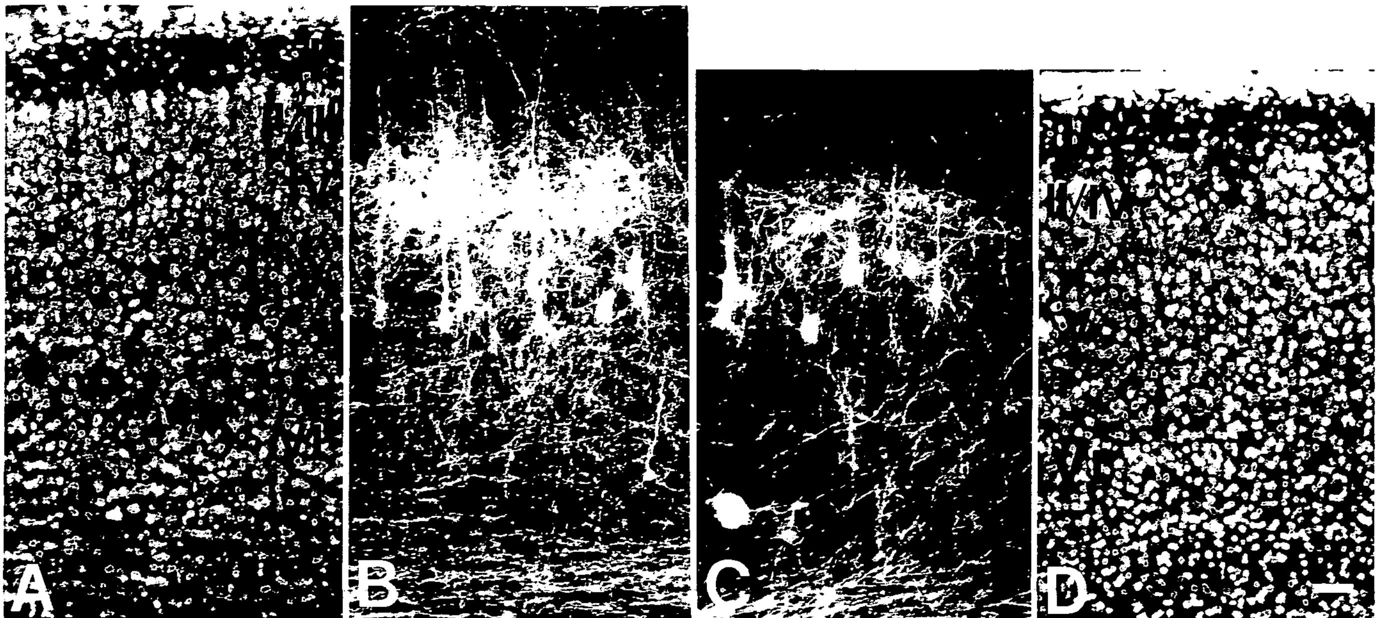


Figure 10. *A*, Bisbenzimide-stained normal P7 visual cortex. The whole cortex is completely differentiated. *B*, Arbor density of DiI-labeled geniculocortical axons in the upper lamina continues to increase while that in the lower lamina has been greatly reduced compared to previous ages. *C*, In the MAM cortex of the same age, both the extent and density of geniculocortical arborization in the upper lamina are severely reduced compared to normal (note the location of the lamina of axonal arbors above the retrogradely labeled layer V cells). Arbor density in the lower lamina has been greatly reduced by this age and is very comparable to the normal cortex, showing no evidence of compensatory retainment of thalamic axons in layer VI and subplate. *D*, Thickness and cell density of layers V and VI are similar to normal. Layer IV is apparently absent and the remaining layer above layer V is much reduced in thickness compared to normal. Scale bar, 50 μm.

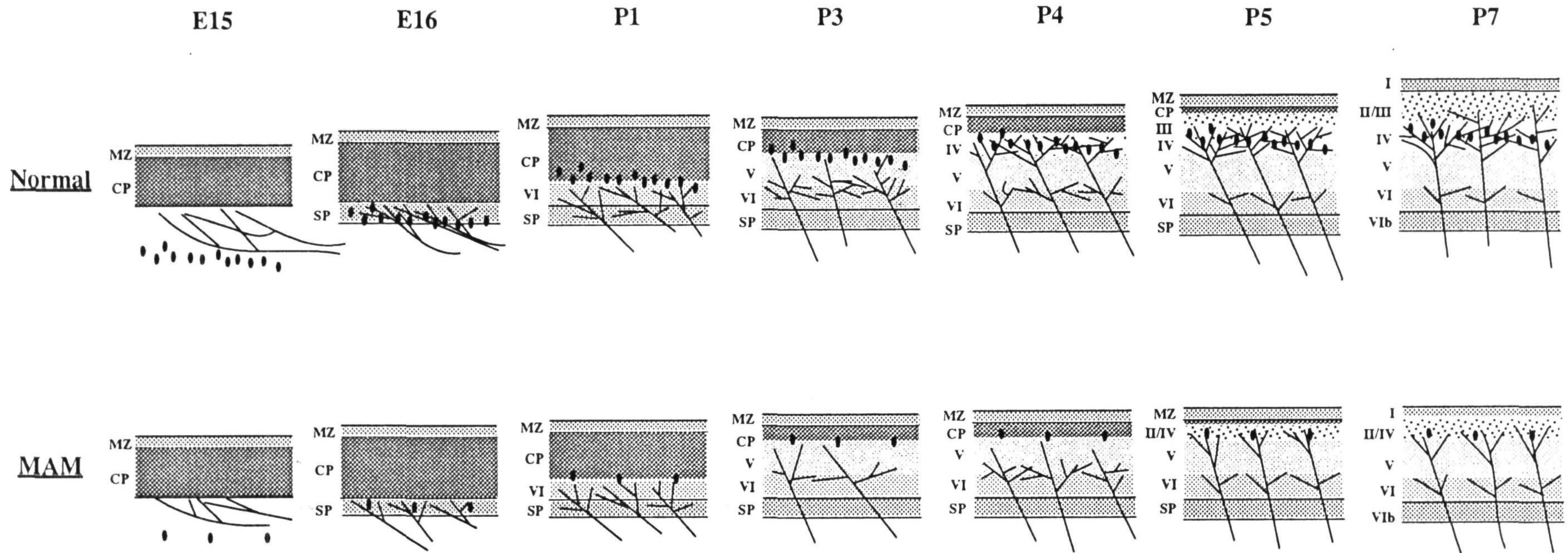


Figure 11. Schematic drawings summarizing the development of geniculocortical axons in the normal cortex and in the MAM cortex after severe depletion of E14 neurons (*filled elliptical circles*). On E15, in both the normal and MAM cortex, geniculocortical axons have arrived in the vicinity of visual cortex. E14 neurons are still on the way migrating to the developing cortical plate (*CP*). In the MAM cortex, the number of migrating E14 neurons would be much reduced. On the day of birth, geniculocortical axons continue to accumulate and arborize within the subplate, which is completely differentiated by this age. E14 neurons have also just arrived in the subplate and presumably interact with the residing thalamic axons. After MAM treatment, the number of migrating neurons arriving in the subplate on this age and thus any presumptive interactions with thalamic axons would be much lessened. On P1, geniculocortical axons have started to invade the cortex and arborize in layer VI, which has just begun to form. The depletion of the majority of E14 neurons has no effect on the timing of thalamic invasion. By P3, in the normal brain, layer V has become distinguishable. Geniculocortical axons reach immediately below the cortical plate and arborize extensively within layers V and VI. E14 neurons have also just arrived at the cortical plate. At this age, bilaminar segregation of thalamic arborization can be seen in some animals. In the MAM cortex, geniculocortical axons continue to grow up to the line of differentiation. Arbor density in layers V and VI is reduced. On P4, in the normal cortex, layer IV has begun to form and bilaminar segregation of arborization has become obvious. However, no segregation can be seen in the MAM cortex; neither can layer IV be distinguished. By P5, arbor density in the upper lamina in the normal cortex continues to increase while that in the lower lamina has been reduced. In the MAM cortex, after a 1-d delay, bilaminar segregation of arborization has become visible. However, both the extent and density of arborization in the upper lamina are reduced while arbor density in the lower lamina is very comparable to that in the normal brain, suggesting that there is no compensatory increase in arborization in layer VI and subplate. By P7, the cortex is completely differentiated. Arbor density in the upper lamina in the normal cortex continues to increase and is now much higher than that in the lower lamina. In the MAM cortex, both the extent and density of axonal arbors are still reduced. Again, there is no evidence of compensatory increase in arbor density in the lower lamina.

ger morphologically recognizable (Fig. 1). The indiscriminability of layer IV at the cytoarchitectonic level certainly does not imply the loss of every "layer IV neuron." The properties that constitute a single "layer IV neuron" are diverse and nonexclusive; layer IV is a feature of gross morphology associated with a particular time in neurogenesis, a zone of thalamic input, a relative absence of callosal and intracortical efferent connections, and small, nonpyramidal neurons with principally local connections. None of these features are entirely unique to layer IV, and there is no specific cell marker (but see Cohen-Tannoudji et al., 1994). Therefore, we do not claim to remove all presumptive "layer IV neurons," although we may be certain we have depleted this population severely. The majority of layer IV neurons in the hamster visual cortex are generated on E14 (Shimada and Langman, 1970; Crossland and Uchwat, 1982). In the preceding companion article (Woo et al., 1996), BrdU (5-bromo-2′-deoxyuridine) labeling at different time points after 20 mg/kg of MAM injections quantified the time course of action of MAM and also the populations of neurons most severely affected. These BrdU labeling experiments have shown that cell loss is most severe in the middle cortical lamina including layer IV and the bottom part of layer 3, together constituting the normal thalamic recipient zone, within the first 24 hr after MAM injections.

We then ask if a later-generated cohort assumes the distributional properties of size and connectivity of the original layer IV. Our data indicate that the later generated population of neurons (presumptive layer II/III neurons) fail to be "respecified" to acquire these properties. The following discussion explores the possible mechanisms involved in layer IV cell fate determination.

Layer IV Cell Fate Determination

Why is it that the population of neurons generated at E14 are statistically more likely to have smaller soma size, to become targets for thalamic axons, and less likely to send out callosal axons? More generally, what are the developmental mechanisms that determine a particular population of neurons generated at a particular time are more probable to manifest certain morphological and connectional properties? The most straightforward hypothesis is that layer IV cell fate has already been imposed either intrinsically through some genetic mechanisms of cell lineage determination or extrinsically through epigenetic interactions with their microenvironment while postmitotic cells are still in the ventricular zone. A second possibility is that some other epigenetic mechanisms such as interactions between migrating neurons and their immediate environment taking place beyond the neuroepithelium may be involved in cell fate determination. Finally, cell fate could be determined *in situ* after neurons have arrived in the cortical plate through interactions with local cortical microenvironment or with incoming thalamic afferents.

The Role of the Ventricular Zone

Very little is known about the ventricular zone, from the kinetics of cell production and output to the mechanisms of neuronal fate determination. Studies in the retina might provide some insight into the role of the cortical ventricular zone in neuronal fate determination. In the retina of the fish and the frog, after selective removal of a particular population of amacrine cells, the dopaminergic amacrine neurons, by applying the neurotoxin 6-hydroxydopamine (6-OHDA), there is a selective increase in the production of dopaminergic amacrine cells in the neuroepithelium (Negishi et al., 1982, 1985, 1987; Reh and Tully, 1986). Application of kainic acid also results in the degeneration of the inner nuclear layer and the ganglion cell layer (Hampton et al., 1981; Ingham and Morgan, 1983; Beazley et al., 1987). It has been demonstrated that this manipulation induces an upregulation of neuronal proliferation in the periphery and thus the replacement of the removed neurons (Reh, 1992a). Among the first postmitotic cells in the retinal neuroepithelium are the ganglion cells (Altshuler et al., 1991). Exposure to x-irradiation at the time when ganglion cells are being generated results in virtually the complete loss of the ganglion cell layer and the destroyed population is replaced in a similar manner (Rugh and Wolff, 1955). All of these experimental findings argue against the notion that neuronal fate is determined following a strictly programmed intrinsic temporal sequence of mitoses. Instead, a certain feedback/feedforward or inhibitory/inductive mechanisms (Reh, 1987, 1992b; Altshuler and Cepko, 1992) seem to be operating such that previously generated neurons, while they are still in the neuroepithelium, might signal the dividing progenitors to inhibit the generation of the same class of cells but induce the production of other cell types. Removal of a particular cell class during a particular developmental time point is equivalent to removal of the normally present inhibitory and inductive factors provided by this cell class. Later-generated cells will now remain responding to the originally present environmental factors to develop into the old cell type and thus replace the depleted cells. According to this scenario, in the present study, we would expect the later-generated cohort of neurons, which are normally destined to become layer II/III cells, to develop into layer IV cells and replace the lost population. Our results appear to refute that this retinal model of cell fate determination operates in the ventricular zone of the neocortex. Furthermore, from the BrdU labeling studies in the animals receiving 20 mg/kg of MAM (Woo et al., 1996), we do not find any evidence of increased proliferation in the ventricular zone. On the other hand, our data by no means rule out the role of the ventricular zone in cell fate specification. Indeed, the importance of interactions between postmitotic cells in the ventricular zone and their microenvironment in cortical laminar fate specification has been demonstrated (McConnell, 1988, 1990, 1991; McConnell and Kaznowski, 1991), although it should be emphasized that laminar position is but one particular aspect of a collection of morphological and functional attributes that constitute a particular neuronal fate. It seems probable that cell fate determination is a multistep process with the involvement of different cellular compartments in the developing cortex in different aspects of cell identity specification.

The Role of the Thalamus

There are several interesting quantitative and qualitative relationships between thalamic inputs and layer IV granule neurons. Primary sensory cortical areas, which receive inputs from specific thalamic nuclei, have a well-differentiated granular layer IV. In the cat visual cortex, the number of cells in the binocular region, which receive more thalamic inputs, is higher than that in the monocular region (Beaulieu and Colonier, 1983). A direct influence of thalamocortical afferents on the differentiation and selection of spiny stellate neurons in layer IV during phylogeny has been postulated (Valverde, 1985, 1991). Perhaps one of the best examples of the importance of thalamocortical afferents in organizing layer IV cytoarchitecture is the emergence of "barrels" in the rodent somatosensory cortex, which is characterized by the clustering of layer IV stellate cells (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974; Woolsey et al., 1975a,b).

Results from several experiments suggest the direct role of thalamocortical inputs in specifying or trophically supporting cortical cytoarchitecture. First, after early bilateral enucleation in the monkey *in utero* before the arrival of geniculocortical axons in visual cortex, greatly reducing the amount of geniculocortical innervation, a shift in the borders between area

17 and its adjacent areas that reduces the tangential extent of area 17 has been described, producing a novel cytoarchitectonic area (Rakic, 1988; Dehay et al., 1989, 1991; Rakic et al., 1991). Second, ablation of the dorsal lateral geniculate nucleus in the hamster at the time when geniculocortical axons are in residence in the subplate but have not yet invaded the cortical plate results in the loss of layer IV (Windrem and Finlay, 1991). Finally, ablation of the subplate and thus preventing thalamic axons from invading the cortex in the cat by kainic acid injections, results in the loss of layer IV stellate neurons (Ghosh and Shatz, 1993). These experiments demonstrate the importance of thalamic input in stabilization of neocortical architecture and cell number, but do not address whether thalamocortical input trophically sustains a set of prespecified thalamocortical recipient neocortical cells, or specifies an undifferentiated cell group to become thalamorecipient.

There is ample opportunity for thalamocortical axons to specify neuronal fate of migrating cells in the subplate. The earliest arriving axons in the developing cortex are from the dorsal thalamus (Erzurumlu and Jhaveri, 1990, 1992; Catalano et al., 1991; De Carlos and O'Leary, 1992; Miller et al., 1993, Bicknese, et al., 1994). The arrival of these axons and their potential targets, the presumptive layer IV neurons, in the subplate in the rodent are synchronous. As the presumptive layer IV neurons migrate through the subplate, thalamic axons simultaneously commence their innervation of the cortex, as if they are following their future targets (Miller et al., 1993).

Our results suggest that cell fate has already been restricted by late stages of migration, and that the role of the thalamus is trophic, not instructional. One important qualification to make is that if thalamic "instruction" can only occur in a limited time window in cortical development, less than 24 hr, these experiments could not distinguish the "instructional" and "trophic" hypotheses. If the thalamus supplies trophic support, it has importance greater than the density of innervation it supplies; recent studies have shown that in the cat and monkey, the thalamus supplies a minority of the inputs to stellate neurons (Peters and Payne, 1993; Peters et al., 1994).

Target Recognition of Layer IV by Thalamic Afferents

One of the defining features of cortical structures is layering of their afferents. During development, after arriving in the subplate in the cortex, thalamic afferents have to invade the cortex, recognize their termination zone and arborize.

Our experiments were designed to remove the population of neurons, the presumptive layer IV neurons, at the developmental time when they are normally migrating through the subplate zone and thus encountering thalamic axons. By doing this we could test the hypothesis that it is the mutual interactions or recognition between thalamic axons and their potential target neurons generated at a particular time that is responsible for the successful ingrowth of thalamic axons into the developing cortex. In fact, the "waiting" of thalamic axons in the subplate in the cat and monkey (Rakic, 1977; Shatz and Luskin, 1986) and the fact that they do not invade the cortex until the arrival of layer IV cells in the cortical plate lend support to the notion that thalamic axons are waiting for signals from their future targets to commence their ingrowth process. Our results do not support this hypothesis. Even in the absence of the population of neurons generated on E14, thalamic axons follow the normal developmental time course to invade the cortex, suggesting that this ingrowth process and the migration and arrival of the presumptive layer IV neurons are two independent developmental events. Thus, what are thalamic axons waiting for in bigger brains in which the arrival of thalamic axons and that of layer IV neurons has become temporally discrepant? Perhaps the temporally regulated expressions of other cellular or extracellular factors in the local cortical environment are responsible (see below). It could be that expressions of these factors occur in orchestration with corticogenesis and thus "waiting" is simply epiphenomenonal of their delayed expressions in bigger brains.

After invading the cortex, thalamic axons would normally stop and arborize in the middle lamina of the cortex (presumptive layer IV). Therefore, it has been suggested that thalamic axons recognize a "stop signal" expressed by their target neurons, layer IV neurons, after these neurons have reached their correct cortical location (Molnar and Blakemore, 1991). In the MAM animals, the removal of the population of neurons generated at E14 does not prevent thalamic axons from recognizing their "correct" termination zone, although there is a 1-d delay in arborization within this lamina. These results demonstrate that the process of ingrowth of thalamic axons and the recognition of the middle cortical stratum as their correct termination zone do not depend on the presence of a particular neuronal population generated on E14. This notion is further supported by observations on thalamocortical termination in the hedgehog. In this species, although layer IV is not apparent, thalamic axons are still able to recognize and terminate in the middle cortical stratum and bilaminar segregation is obvious (Gould et al., 1978). Recent studies have identified a molecular marker that labels infragranular but not supragranular layers, particularly layer V (Frantz et al., 1994); perhaps layer V cells retard the arborization of thalamocortical afferents.

Alternatively, the changing local environment in the cortex may be responsible. Bolz and his colleagues have found evidence suggesting that it is the developmental upregulation of a particular extracellular growth-permissive lectin-binding glycoprotein, peanut agglutinin (PNA), that signals the beginning of innervation of thalamic axons (Bolz and Gotz, 1992; Gotz et al., 1992). On E17 in the rat visual cortex, PNA expression is mainly confined to the subplate and the marginal zone. On E19, PNA can be detected in the very bottom of the cortical plate, showing the first sign of upregulation. At the same time, thalamic axons have also just begun to invade the cortex. At all the subsequent ages, the growth of thalamic axons follows closely the sequential expression of this molecule until they reach layer IV where they stop and arborize. Nevertheless, PNA expression continues to spread upward until finally merging with the PNA-positive band in the marginal zone. By P2, the whole cortex has become PNA positive. These experimental results suggest that the upregulation of PNA may provide a permissive environment for the ingrowth of thalamic axons. However, they still do not address the questions as to how and why thalamic axons recognize their normal termination zone. The fact that these axons stop and arborize in layer IV instead of continuously growing towards the top of the cortex following the continuous expression of PNA suggests that other mechanisms must be involved in target recognition. In coculture experiments, when thalamic explants are placed next to the pial surface of cortical explants, they are still able to recognize and arborize at the correct cortical lamina (Bolz et al., 1992). When cortical explants and thalamic explants are placed lateral to each other forcing thalamic axons to invade the cortex from the side, thalamic axons initially spread through the whole vertical extent of the cortex but gradually they become confined to layers I, IV, and VI but avoiding layers II/III and V, suggesting further that some promotive or attractive signals are provided by the normal thalamic recipient layers (Yamamoto et al., 1992).

Finally, the observation of the 1-d delay in thalamic arborization is interesting because this time period corresponds

precisely with the temporal delay between the arrival of thalamic axons in their cortical terminal zone and the arrival of E15 (layers II/III) neurons. Perhaps neurotrophic or arborization promoting factors derived from target neurons are necessary to induce and sustain arborization and in the absence of E14 neurons, these factors are not available until the arrival of E15 cells, thus creating the novel "waiting" phenomenon. Comparative studies correlating the relative timing of expressions of various cellular and molecular substrates of thalamocortical development with respect to corticogenesis would provide important insight into the general principles and strategies underlying cortical development and expansion.

Notes

We thank John Niederer for excellent technical assistance. This work was supported by NIH Grant RO1 NS19245.

T. U. Woo is now at Department of Psychiatry, BST W1650, Western Psychiatric Institute and Clinic, 3811 O'Hara Street, Pittsburgh, PA 15213.

Address correspondence to Barbara L. Finlay, Department of Psychology, Cornell University, Ithaca, NY 14853.

References

Adams JC (1977) Technical considerations in the use of horseradish peroxidase as a neuronal marker. Neuroscience 2:141-145.

Adams JC (1981) Heavy metal intensification of DAB-based HRP reaction product. J Histochem Cytochem 29:775.

Altshuler D, Cepko C (1992) A temporally regulated, diffusible activity is required for rod photoreceptor development *in vitro*. Development 114:947-957.

Altshuler D, Turner DL, Cepko CL (1991) Specification of cell type in the vertebrate retina. In: Development of the visual system (Lam DM-K, Shatz CJ, eds), pp 37-58. Cambridge, MA: MIT Press.

Bayer SA, Altman J (1990) Development of layer I and the subplate in the rat neocortex. Exp Neurol 107:48-62.

Beaulieu C, Collonier M (1983) The number of neurons in the different laminae of the binocular and monocular regions of area 17 in the cat. J Comp Neurol 217:337-344.

Beazley LD, Perry VH, Baker B, Darby JE (1987) An investigation into the role of ganglion cells in the regulation of division and death of other retinal cells. Dev Brain Res 11:117-129.

Bicknese AR, Sheppard AM, O'Leary DDM, Pearlman AL (1994) Thalamocortical axons extend along a chondroitin sulfate proteoglycan-enriched pathway coincident with the neocortical subplate and distinct from the efferent path. J Neurosci 14:3500-3510.

Bolz J, Gotz M (1992) Mechanisms to establish specific thalamocortical connections in the developing brain. In: Development of the central nervous system in vertebrates (Sharma SC, Goffinet AM, eds), pp 179-192. New York: Plenum.

Bolz J, Novak N, Staiger V (1992) Formation of specific afferent connections in organotypic slice cultures from rat visual cortex cocultured with lateral geniculate nucleus. J Neurosci 12:3054-3070.

Catalano SM, Robertson RT, Killackey HP (1991) Early ingrowth of thalamocortical afferents to the neocortex of the prenatal rat. Proc Natl Acad Sci USA 88:2999-3003.

Cepko CL (1993) Retinal cell fate determination. Prog Retina Res 12: 1-12.

Cohen-Tannoudji M, Babinet C, Wassef M (1994) Early determination of a mouse somatosensory cortex marker. Nature 368:460-463.

Crossland WJ, Uchwat CJ (1982) Neurogenesis in the central visual pathways of the golden hamster. Dev Brain Res 5:99-103.

De Carlos JA, O'Leary DDM (1992) Growth and targeting of subplate axons and establishment of major cortical pathways. J Neurosci 12:1194-1211.

Dehay C, Horsburgh G, Berland M, Killackey H, Kennedy H (1989) Maturation and connectivity of the visual cortex in monkey is altered by prenatal removal of retinal input. Nature 337:265-267.

Dehay C, Horsburgh G, Berland M, Killackey H, Kennedy H (1991) The effects of bilateral enucleation in the primate fetus on the parcellation of visual cortex. Dev Brain Res 62:137-141.

Diao Y-C, So K-F (1991) Dendritic morphology of visual callosal neurons in the golden hamster. Brain Behav Evol 37:1-9.

Dursteler MR, Blakemore C, Garey LJ (1979) Projections to the visual cortex in the golden hamster. J Comp Neurol 183:185-204.

Erzurumlu RS, Jhaveri S (1990) Thalamic axons confer a blueprint of the sensory periphery onto the developing rat somatosensory cortex. Dev Brain Res 56:229-234.

Erzurumlu RS, Jhaveri S (1992) Emergence of connectivity in the embryonic rat parietal cortex. Cereb Cortex 2:336-352.

Frantz GD, Weimann JM, Levin ME, McConnell SK (1994) Otx1 and Otx2 define layers and regions in developing cerebral cortex and cerebellum. J Neurosci 14:5725-5740.

Ghosh A, Shatz CJ (1993) A role for subplate neurons in the patterning of connections from thalamus to neocortex. Development 117:1031-1047.

Gotz M, Novak N, Bastmeyer M, Bolz J (1992) Membrane-bound molecules in rat cerebral cortex regulate thalamic innervation. Development 116:507-519.

Gould JJ, Hall WC, Ebner FF (1978) Connections of the visual cortex in the hedgehog (*Paraechinus hypometas*). I. Thalamocortical projections. J Comp Neurol 177:445-471.

Hampton CK, Garcia C, Redburn DA (1981) Localization of kainic acid sensitive cells in the mammalian retina. J Neurosci Res 6:99-111.

Ingham CA, Morgan IG (1983) Dose-dependent effects of intravitreal kainic acid on specific cell types in chicken retina. Neuroscience 9:151-181.

Jensen KF, Killackey HP (1987a) Terminal arbors of axons projecting to the somatosensory cortex of the adult rat. I. The normal morphology of specific thalamocortical afferents. J Neurosci 7:3529-3543.

Jensen KF, Killackey HP (1987b) Terminal arbors of axons projecting to the somatosensory cortex of the adult rat. II. The altered morphology of thalamocortical afferents following neonatal infraorbital nerve cut. J Neurosci 7:3544-3553.

Johnston MV, Coyle JT (1982) Cytotoxic lesions and the development of transmitter systems. Trends Neurosci 5:153-156.

Kageyama, GH, Robertson RT (1993) Development of geniculocortical projections to visual cortex in rat: evidence for early ingrowth and synaptogenesis. J Comp Neurol 335:123-148.

Kennedy H, Dehay C (1993) Cortical specification of mice and men. Cereb Cortex 3:171-186.

Killackey HP, Chiaia NL, Bennett-Clarke CA, Eck M, Rhoades RW (1994) Peripheral influences on the size and organization of somatotopic representations in the fetal rat cortex. J Neurosci 14: 1496-1506.

Lund RD (1966) The occipitotectal pathway of the rat. J Anat 100: 51-62.

McConnell SK (1985) Migration and differentiation of cerebral cortical neurons after transplantation into the brains of ferrets. Science 229:1268-1271.

McConnell SK (1988a) Fates of visual cortical neurons in the ferret after isochronic and heterochronic transplantation. J Neurosci 8: 945-974.

McConnell SK (1988b) Development and decision-making in the mammalian cerebral cortex. Brain Res Rev 13:1-23.

McConnell SK (1990) The specification of neuronal identity in the mammalian cerebral cortex. Experientia 46:922-929.

McConnell SK (1991) The generation of neuronal diversity in the central nervous system. Annu Rev Neurosci 14:269-300.

McConnell SK (1992) The determination of neuronal identity in the mammalian cerebral cortex. In: Determinants of neuronal identity (Shankland M, Macagno ER, eds), pp 391-432. New York: Academic.

McConnell SK, Kaznowski CE (1991) Cell cycle dependence of laminar determination in developing neocortex. Science 254:282-285.

Miller B, Chou L, Finlay BL (1993) The early development of thalamocortical and corticothalamic projections. J Comp Neurol 335: 16-41.

Molnar Z, Blakemore C (1991) Lack of regional specificity for connections formed between thalamus and cortex in coculture. Nature 351:475-477.

Naegele JR, Jhaveri S, Schneider GE (1988) Sharpening of topographical projections and maturation of geniculocortical axon arbors in the hamster. J Comp Neurol 277:593-607.

Negishi K, Teranishi T, Dato S (1982) New dopaminergic and in-

doleamine-accumulating cells in the growth zone of goldfish retinas after neurotoxic destruction. Science 216:747-749.
Negishi K, Teranishi T, Dato S (1985) Growth rate of a peripheral annulus defined by neurotoxic destruction in the goldfish retina. Dev Brain Res 20:291-295.
Negishi K, Teranishi T, Dato S, Nakamura Y (1987) Paradoxical induction of dopaminergic cells following intravitreal injection of high doses of 6-hydroxydopamine in juvenile carp retina. Dev Brain Res 33:67-79.
O'Leary DDM, Schlaggar BL, Tuttle R (1994) Specification of neocortical areas and thalamocortical connections. Annu Rev Neurosci 17:419-439.
Paxinos G, Watson C (1986) The rat brain. New York: Academic.
Peters A, Payne BR (1993) Numerical relationships between geniculocortical afferents and pyramidal cell modules in cat primary visual cortex. Cereb Cortex 3:69-78.
Peters A, Payne BR, Budd J (1994) A numerical analysis of the geniculocortical input to striate cortex in the monkey. Cereb Cortex 4:215-229.
Rakic P (1976) Prenatal genesis of connections subserving ocular dominance in the rhesus monkey. Nature 261:467-471.
Rakic P (1977) Prenatal development of the visual system in the rhesus monkey. Philos Trans R Soc Lond [Biol] 278:245-260.
Rakic P (1982) Early developmental events: cell lineages, acquisition of neuronal positions, and areal and laminar development. Neurosci Res Prog Bull 20:439-451.
Rakic P (1988) Specification of cerebral cortical areas. Science 241: 170-176.
Rakic P, Suner I, Williams RW (1991) A novel cytoarchitectonic area induced experimentally within the primate visual cortex. Proc Natl Acad Sci USA 88:2083-2087.
Reh TA (1987) Cell-specific regulation of neuronal production in the larval frog retina. J Neurosci 7:3317-3324.
Reh TA (1989) The regulation of neuronal production during retinal neurogenesis. In: Development of the vertebrate retina (Finlay BL, Sengelaub DR, eds), pp 43-68. New York: Plenum.
Reh TA (1992a) Generation of neuronal diversity in the vertebrate retina. In: Determinants of neuronal identity (Shankland M, Macagno ER, eds), pp 433-468. New York: Academic.
Reh TA (1992b) Cellular interactions determine neuronal phenotypes in rodent retinal cultures. J Neurobiol 23:1067-1083.
Reh TA, Radke K (1988) A role for the extracellular matrix in retinal neurogenesis *in vitro*. Dev Biol 129:283-293.
Reh TA, Tully T (1986) Regulation of tyrosine hydroxylase containing amacrine cell number in larval frog retina. Dev Biol 114:463-469.
Rhoades RW, Dellacroce DD (1980) Visual callosal connections in the golden hamster. Brain Res 190:248-254.
Rugh R, Wolff J (1955) Reparation of the fetal eye following radiation insult. Arch Ophthalmol 54:351-359.
Schlaggar BL, O'Leary DDM (1991) Potential of visual cortex to develop arrays of functional units unique to somatosensory cortex. Science 252:1556-1560.
Schlaggar BL, O'Leary DDM (1994) Early development of the somatotopic map and barrel patterning in rat somatosensory cortex. J Comp Neurol 346:80-96.
Sefton AJ, Mackay-Sim A, Baur LA, Cottee LJ (1981) Cortical projections to visual centers in the rat: an HRP study. Brain Res 215:1-13.
Shatz CJ, Luskin MB (1986) The relationship between the geniculocortical afferents and their cortical target cells during development of the cat's primary visual cortex. J Neurosci 6:3655-3668.
Sheng X-M, Marotte LR, Mark RF (1990) Development of connections to and from the visual cortex in the wallaby (*Macropus eugenii*). J Comp Neurol 300:196-210.
Sheng X-M, Marotte LR, Mark RF (1991) Development of the laminar distribution of thalamocortical axons and corticothalamic cell bodies in the visual cortex of the wallaby. J Comp Neurol 307: 17-38.
Shimada M, Langman J (1970) Cell proliferation, migration and differentiation in the cerebral cortex of the golden hamster. J Comp Neurol 139:227-244.
Valverde F (1985) The organizing principles of the primary visual cortex in the monkey. In: Cerebral cortex, Vol 3 (Peters A, Jones EG, eds), pp 207-252. New York: Plenum.
Valverde F (1991) Aspects of phylogenetic variability of neocortical intrinsic organization. In: The neocortex: ontogeny and phylogeny (Finlay BL, Innocenti G, Scheich H, eds), pp 87-102. New York: Plenum.
Welker C, Woolsey TA (1974) Structure of layer IV in the somatosensory neocortex of the rat: description and comparison with the mouse. J Comp Neurol 158:437-454.
Welker E, Van der Loos H (1986a) Is areal extent in sensory cerebral cortex determined by peripheral innervation density? Exp Brain Res 63:650-654.
Welker E, Van der Loos H (1986b) Quantitative correlation between barrel-field size and the sensory innervation of the whisker-pad: a comparative study in size strains of mice bred for different patterns of mystacial vibrissae. J Neurosci 6:3355-3373.
Windrem MS, Finlay BL (1991) Thalamic ablations and neocortical development: alterations of cortical cytoarchitecture and cell number. Cereb Cortex 1:230-240.
Woo TU, Niederer JK, Finlay BL (1992) Effect of depletion on layer IV on dorsal lateral geniculate cell number and ingrowth of geniculocortical axons. Soc Neurosci Abstr 18:771.
Woo TU, Niederer JK, Finlay BL (1994) Effect of layer IV removal on the development of geniculocortical afferents. Soc Neurosci Abstr 20.
Woo TU, Niederer JK, Finlay BL (1996) Cortical target depletion and the developing lateral geniculate nucleus: implications for trophic dependence. Cereb Cortex 6:446-456.
Woolsey TA, Van der Loos H (1970) The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. Brain Res 17:205-242.
Woolsey TA, Welker C, Schwartz RH (1975a) Comparative anatomical studies of the SmI face cortex with special reference to the occurrence of barrels in layer IV. J Comp Neurol 164:79-94.
Woolsey TA, Dierker ML, Wann DF (1975b) Mouse SmI cortex: qualitative and quantitative classification of Golgi-impregnated barrel neurons. Proc Natl Acad Sci USA 72:2165-2169.
Yamamoto N, Yamada K, Kurotani T, Toyama K (1992) Laminar specificity of extrinsic cortical connections studied in coculture preparations. Neuron 9:217-228.