

# THE DEVELOPMENT OF OCULAR DOMINANCE COLUMNS IN THE CAT

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In the striate cortex of cats and monkeys, cells responding preferentially to stimulation of one or the other eye are grouped together in columns that extend vertically through all the cortical layers (Hubel and Wiesel, 1965, 1968). The anatomical basis for the columnar arrangement is a segregation, in layer IV, of the terminals of the geniculocortical afferents serving the two eyes. These afferents arise in different laminae of the lateral geniculate nucleus. The segregation has been demonstrated by a number of techniques (Hubel and Wiesel, 1972; Wiesel, Hubel, and Lam, 1974; LeVay, Hubel, and Wiesel, 1975), of which the most useful has been the autoradiographic method, which depends on the transneuronal transport of tritiated proline injected into one eye (Wiesel et al., 1974). In surface view, the columns have the shape of bands or patches, about 400 microns wide, that alternate with each other to form a rather constant overall pattern in area 17 (LeVay et al., 1975; Shatz, Lindström, and Wiesel, 1977).

Until quite recently, it was thought that the ocular dominance system was already established in newborn animals and closely resembled that of adults (Hubel and Wiesel, 1963; Wiesel and Hubel, 1974; Blakemore, Van Sluyters, and Movshon, 1975). The first indication that this might not be the case came from autoradiographic observations on a monkey that had received an eye injection on the second day of life: instead of clear bands of label in layer IV, separated by unlabeled gaps, the label was distributed continuously on each side of the brain, with slight, wave-like fluctuations in labeling density visible on the ipsilateral side only (Hubel, Wiesel, and LeVay, 1977). Physiologically, too, the columnar pattern in layer IV was somewhat blurred in a week-old monkey, as compared with the very precise segregation found in adults. At the

same time, Rakic (1976) injected the eyes of fetal monkeys of various gestational ages and found completely uniform distribution of label in layer IV up until about 3 weeks before birth. These findings suggested that when the left- and right-eye afferents first grow into the cortex, they intermingle freely with each other and only later segregate out into the columnar arrangement.

### TIME COURSE OF SEGREGATION

Because of its immaturity at birth, the cat is a more convenient animal in which to study this developmental process. As may be seen from Figure 1, eye injections produced continuous, uniform labeling in layer IV up until 2 weeks of postnatal age. At 3 weeks, undulations in labeling density became apparent; at 39 days these undulations were sharper; and by 3 months the adult columnar pattern had been reached (LeVay, Stryker, and Shatz, 1978).

The autoradiographic picture is contaminated, to some extent, by an artifact that we refer to as "spillover." This is the leakage of radioactivity between laminae of the lateral geniculate nucleus, its uptake by the wrong set of geniculate neurons, and subsequent transport to their terminals in the cortex. We have measured spillover and calculated its effect on the cortical labeling pattern at each age. The results of these calculations indicate that, although the early uniform distribution of label reflects a genuine overlap of left- and right-eye fibers in the cortex, the process of segregation occurs faster than the autoradiographs suggest, being largely complete by about 6 weeks of age. The later increase in clarity of the columns is due almost entirely to a reduction in spillover.

Intermingling of left- and right-eye inputs is reflected in the physiology of the postsynaptic neurons (LeVay et al., 1978). Recordings made during long, tangential penetrations through layer IV in several 2-week-old kittens gave no hint of the regular alternations in eye preference that are so characteristic of this layer in adult cats (Shatz and Stryker, 1978). Instead, most cells were about equally influenced by the two eyes (Figure 2). This observation, besides supporting the anatomical evidence for an intermingling of the afferents, suggests that they form functional connections with cortical neurons in their early, overlapping pattern. This in turn suggests that, during the process of segregation, functional connections from the left eye are broken in the forming right-eye columns, and vice versa. Physiologically, the progression from

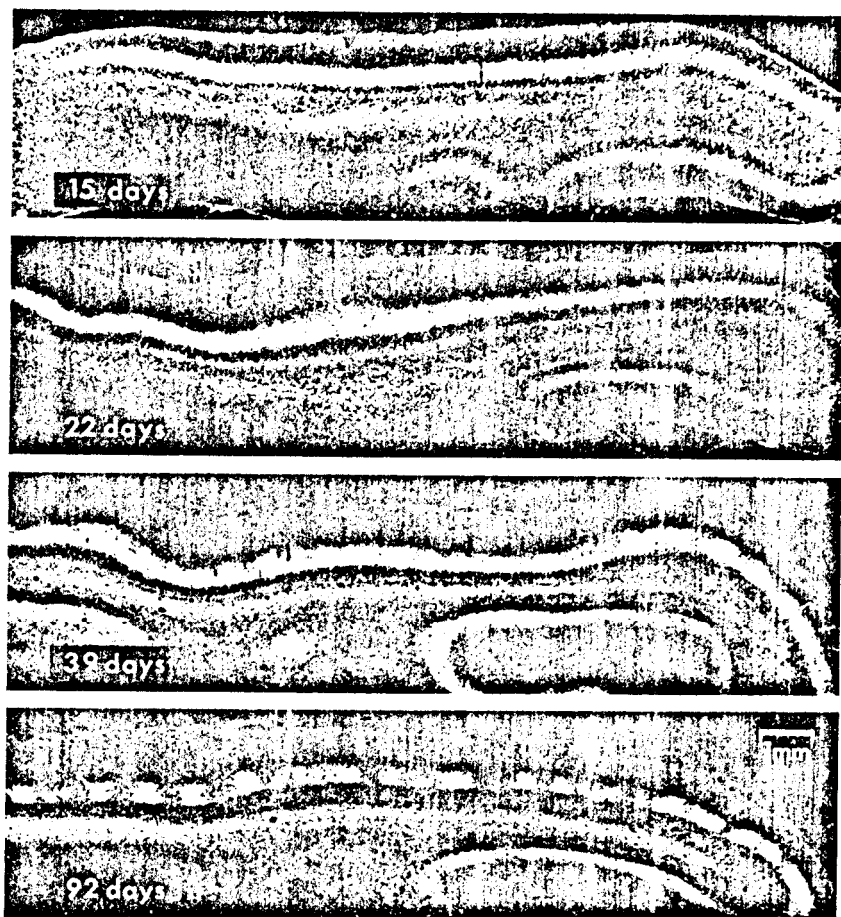


FIGURE 1. Postnatal development of ocular dominance columns in the cat as shown by transneuronal transport of [ $^3\text{H}$ ]proline injected into one eye. These are darkfield autoradiographs of the visual cortex at four different ages, ipsilateral to the injected eye. Horizontal sections, midline at the top of each figure, anterior to the left. At 15 days of age the afferents are spread uniformly along layer IV, completely intermingled with the (unlabeled) afferents serving the contralateral eye. At later ages the afferents progressively aggregate into clumps—the anatomical basis for the physiologically defined ocular dominance columns. The gaps are occupied by unlabeled afferents serving the other eye. (From LeVay, S., M. P. Stryker, and C. J. Shatz [1978]. Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study, *J. Comp. Neurol.* 179:223–244. With permission of The Wistar Press.)

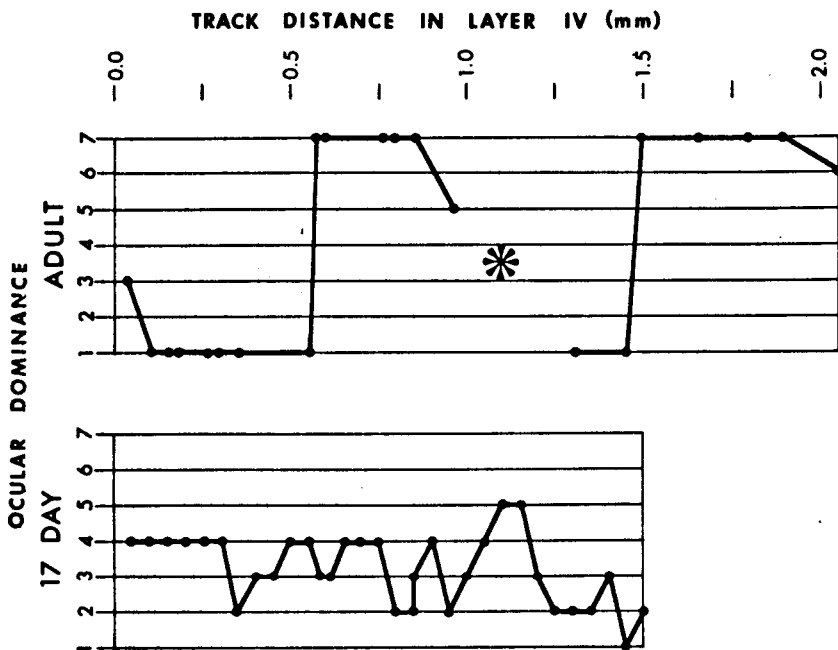


FIGURE 2. Ocular dominance distribution of cells encountered during the passage of an electrode tangentially through the fourth layer in two cats: an adult (above) and a 17-day-old kitten (below). Each point represents an isolated single unit whose ocular dominance, on the 7-point scale of Hubel and Wiesel (1962), is plotted against its position along the electrode track. In the kitten most cells could be driven from either eye, with on the whole a slight preference for the contralateral eye. In the adult there is a periodic variation from exclusively contralateral (Group 1) to exclusively ipsilateral (Group 7) eye dominance. The asterisk marks the position of a lesion made for the purpose of the histological reconstruction of the track; cells could not be recorded for a short distance after the lesion. The transitions in ocular dominance are more abrupt in this penetration than is commonly observed: more often, one or several binocular neurons are encountered at column borders. (From LeVay, S., M. P. Stryker, and C. J. Shatz [1978]. Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study, *J. Comp. Neurol.* 179:223-244. With permission of The Wistar Press.)

early binocularity to more monocular responses was seen not only in layer IV but also in the other cortical layers, although in these layers the degree of ocular segregation in normal adult cats is always less than that found in layer IV. Columnar development in these layers probably

depends both on the columnar segregation of the direct geniculate inputs to these layers (LeVay and Gilbert, 1976) and on the increasing monocularity of the input relayed through layer IV.

### STRUCTURE OF AXONS BEFORE SEGREGATION

The changing autoradiographic picture seen in layer IV between 2 weeks and maturity seems likely to result from roughly synchronous changes in the arborizations of thousands of overlapping geniculocortical axons. An idea of the cytological processes involved can be obtained by reconstructing the entire arborizations of single afferent axons, before and after columnar segregation. In young kittens, when the afferents are not yet myelinated, it is possible to impregnate them in their entirety with the Golgi method. Figure 3 is an example of one such arborization in camera lucida reconstruction. It is one of the larger type of geniculocortical afferents, which terminates in the upper half of layer IV with some extension into the bottom of layer III. Most impressive about this arborization is its size: it extends for over 2 millimeters in both the dorsoventral and the anteroposterior directions. When one considers that the kitten's cortex is about 30% smaller, in linear dimensions, than the adult cat's, and that ocular dominance columns are not more than 0.5 mm wide in adults, it is clear that this arborization extends over a territory that is destined to become a number of columns. Within this area, the axon branches profusely, without any obvious local clumping.

Looked at more closely (Figure 4), the individual branches of the axonal tree had an irregular outline, marked by countless bumps and bulges, but with relatively few obvious boutons. It was hard to guess, from the Golgi picture, where the synapses might be located. In order to answer this question, we have injected the lateral geniculate nucleus with tritiated proline and studied the cortex with electron microscopic (EM) autoradiography. As in the Golgi picture, afferents identified in this way had a notably irregular outline as they coursed through the neuropil (Figure 5). It could now be seen that synapses were formed with neighboring dendritic elements along the course of the axon. These synapses were, for the most part, quite elementary in construction, consisting of apposed membrane thickenings and a very small number of synaptic vesicles. The glial wrapping, which is prominent in the adult geniculocortical synapse, was either absent or poorly developed. Although the EM autoradiographic method does not permit us to recon-

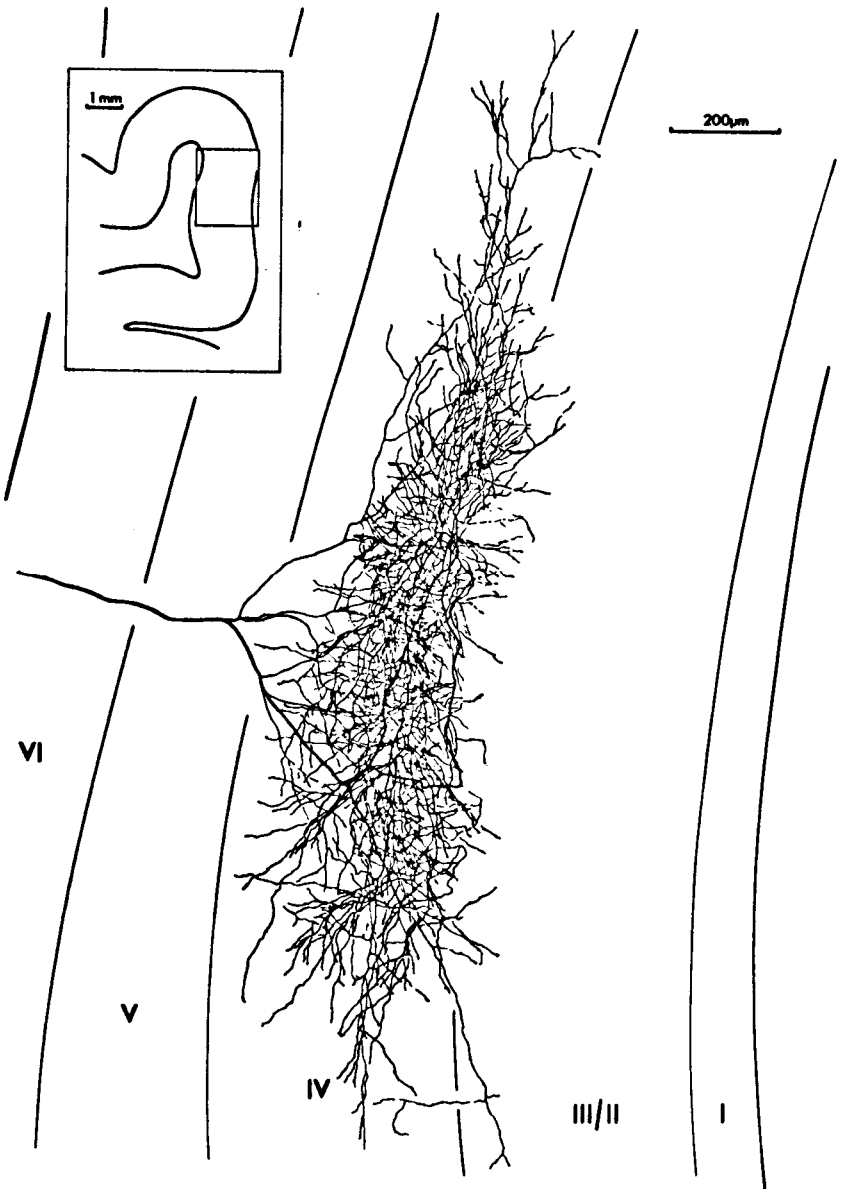


FIGURE 3. Arborization of a single geniculocortical afferent in the visual cortex of a 17-day-old kitten, i.e., just prior to the beginning of columnar segregation. This is a camera lucida reconstruction made from 25 successive coronal sections, each 100  $\mu\text{m}$  thick, of an axon impregnated with the rapid Golgi method and em-

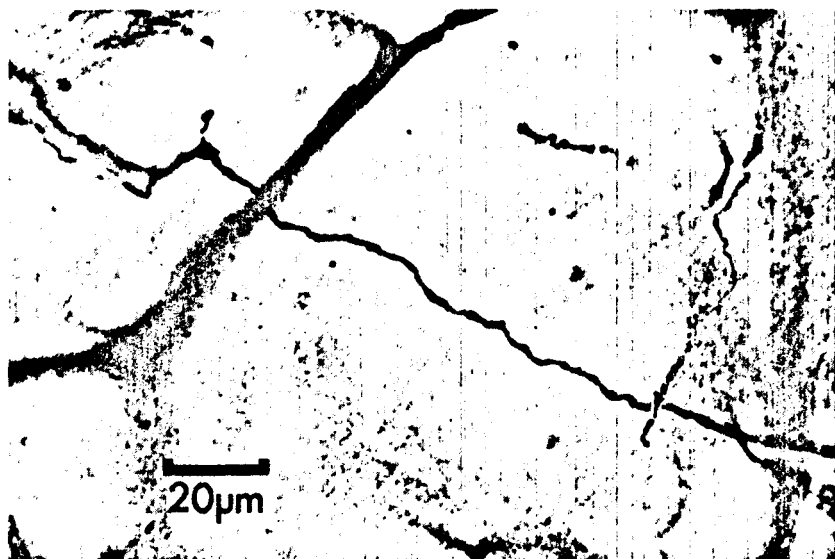


FIGURE 4. Detail from a Golgi-impregnated arborization in a 17-day-old kitten, similar to the one reconstructed in Figure 3. The immaturity of the arborization is revealed by a lack of clear differentiation into boutons (synaptic region) and connecting segments (compare with the adult pattern illustrated in the inset to Figure 6). A single bouton on a side twig is seen at left.

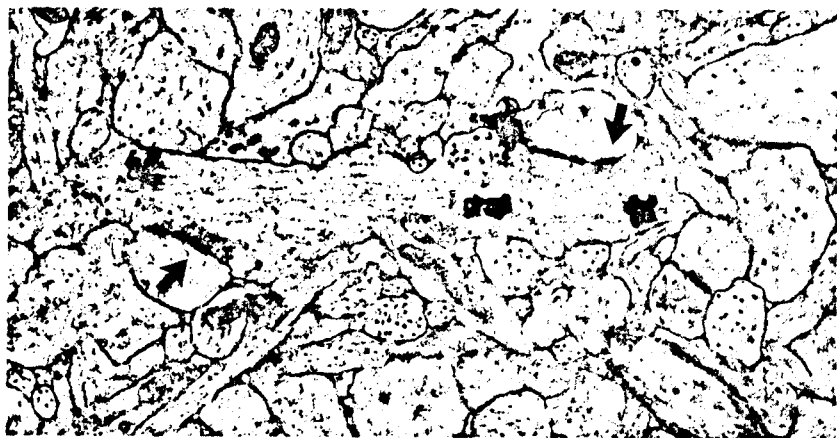
struct the synapses over a whole axonal tree, it seems likely, taking the EM and Golgi pictures together, that these rather simple *en passage* synapses are found over the entire arborization.

### STRUCTURE OF AXONS IN ADULT

In the adult, the Golgi method was no longer successful in revealing entire arborizations. Instead, Ferster and LeVay (1978) were able to fill afferents with horseradish peroxidase (HRP) from an extracellular injection in the optic radiation. Figure 6 is an example of one of the larger afferents, which arborizes in the upper half of layer IV and the bottom of layer III, in an adult cat. As in the young kitten, the extent

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bedded in Epon according to the method of Nevin, Tanaka, and Cruce (1978). The axon arborizes profusely and uniformly over a disc-shaped area that is more than 2 mm in diameter. The entire arborization is unmyelinated; the myelin sheath of the afferent trunk probably leaves off in layer VI at the point where the impregnation begins.



**FIGURE 5.** Part of a geniculocortical arborization in cortical layer IV from a 17-day-old kitten, identified by EM autoradiography after injection of [ $^3\text{H}$ ]proline into the A laminae of the lateral geniculate nucleus. The axon forms two *en passage* synapses with processes that are probably dendritic spines (arrows). Note the small number of synaptic vesicles and the lack of mitochondria in the axon, which is mainly filled with microtubules.  $\times 30,000$ .

of the arborization in layer IV is quite large—a little under 2 mm in both directions. In the adult, however, the terminal arborization is broken up into a number of clumps, connected by thicker axonal branches that run tangentially within layer IV. These clumps and gaps, though somewhat variable in size, have the appropriate dimensions to correspond to individual ocular dominance columns. Although this conclusion has not been verified by recording or by combining the HRP fillings with transneuronal autoradiography, it is hard to doubt that the clumps of dense arbor do correspond to columns serving the same eye.

Besides this clumping of their terminals, the adult arborizations differ from those seen in young kittens in being myelinated. Myelin ensheaths all but the finest, terminal-bearing segments of the arborizations. The axonal branch-points occur, of course, at nodes of Ranvier. A notable difference between the axonal branching patterns in adult cats and in kittens is that whereas in the kitten the branching is mostly dichotomous, in the adult many branch-points, particularly the higher-order ones, involve a splitting of the parent axon into a spray of three to six daughters. A curious feature of the daughter branches is that they often hug the outside of the myelin sheath of the parent trunk for a variable

distance before turning off sharply in a new direction (see inset to Figure 6). It seems likely that this configuration, as well as the multiple branching, arises from a tendency of the myelinating oligodendrocytes to sweep the randomly located branchpoints to common sites—the definitive nodes—as sketched in Figure 7.

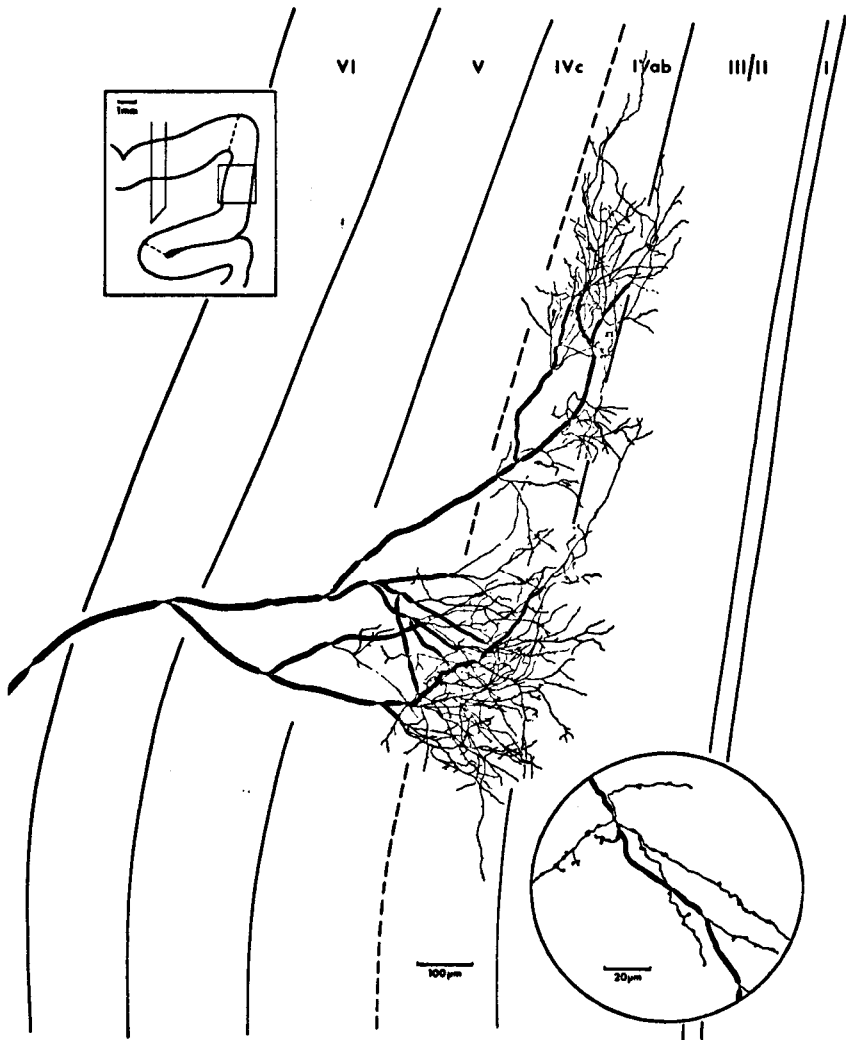
The boutons on the mature arborizations are very distinct bulbous structures that occur either as varicosities or as side twigs on the smooth, very fine preterminal axons (see inset to Figure 6). It is a simple if tedious task to count them: one axon of the same type as illustrated in Figure 6 possessed 1,543 boutons in layers IV and III (Ferster and LeVay, 1978). The boutons are, of course, the sites of synaptic specialization. This was shown by autoradiography (Figure 8). The swellings were packed with synaptic vesicles and mitochondria, formed synapses with one or several postsynaptic elements, and were enveloped in an astrocytic lamella. No synapses were found on any other parts of the axon tree (LeVay and Gilbert, 1976).

### MYELINATION

The intermediate stages of columnar development have not yet been studied in detail. One aspect of cortical maturation that has been followed, however, is the process of myelination (Figure 9). At 22 days, when the segregation of the afferents is getting under way, there was no myelin in the cortex, except for a very occasional trunk in layer VI. At 49 days, when segregation is essentially complete, there were lightly myelinated axons running vertically and obliquely through layers VI and V. These were probably the parent trunks of the geniculocortical arborizations. There was still virtually no myelin in layer IV. By 3 months, there was a lightly myelinated tangential plexus in layer IV (which probably included the arborizations of the geniculate afferents) and in layer I. The density of myelinated fibers was still far lighter than in the adult, however, particularly in the upper cortical layers. It is clear then that the myelination of the afferent arborizations occurs *after* their segregation into ocular dominance columns is complete.

### DISCUSSION

It has been suggested previously that, in forming ocular dominance columns, the geniculocortical afferents express a compromise between two opposing instructions—one for both sets of afferents to occupy the entire visual cortex according to a single retinotopic map, and the



**FIGURE 6.** Arborization of a large geniculocortical afferent in an adult cat. This axon was filled with horseradish peroxidase from an extracellular injection into the optic radiation (see inset, top left). Camera lucida reconstruction from 16 successive coronal sections, each  $100\ \mu\text{m}$  thick. In its overall form and laminar distribution, this axon resembles the one reconstructed from a young kitten (Figure 3). It differs from it in four respects: (i) the arborization is not uniform but is divided into four clumps (two are superimposed in the lower part of the reconstruction), (ii) the arborization is myelinated, (iii) the mode of branching is no longer mainly dichoto-

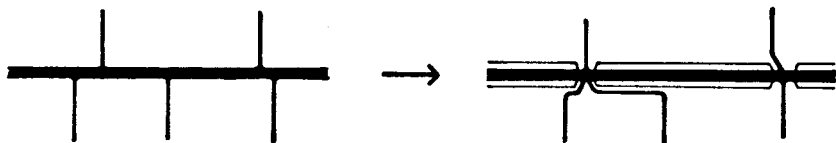


FIGURE 7. Typical axonal branching patterns in young kittens (left) and adult cats (right), arranged to illustrate the hypothesis that axonal branch-points are translocated to nodes of Ranvier during the process of myelination (see text).

other for fibers serving one eye to remain apposed to each other, rather than intermingling with those for the other eye (LeVay et al., 1975). These two instructions will generate a pattern of alternating bands, if the numbers of fibers serving the two eyes are about equal, because such an arrangement minimizes the length of interface between the two sets of fibers. The finding that the two sets of afferents are at first intermingled suggests that these two instructions are expressed at different stages of development. The first, retinotopic innervation of the cortex, is expressed, one may guess, during the initial ingrowth of the geniculocortical afferents. The second, sorting according to eye preference, is not expressed until 2–3 weeks postnatally in the cat. What the signal for this sorting process may be remains to be determined. In the monkey, at least, it does not seem to be dependent on visually evoked activity in the retina, since the process of segregation begins prenatally (Rakic, 1976) and goes to completion postnatally, even in conditions of dark-rearing or binocular lid-suture (Wiesel and Hubel, 1974; Hubel, Wiesel, and LeVay, unpublished).

The reconstruction of complete terminal arbors of geniculocortical afferents, before and after columnar development, has strengthened the autoradiographic evidence for a sorting process. More importantly, it has given us a clearer idea of the anatomical changes that may be involved (Figure 10). For the larger axons, at least, columnar development does not seem to require any major movement of entire arbors. Since, before segregation begins, afferent arbors span a cortical terri-

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mous: as seen in the inset, lower right, several daughter branches can arise from each node of Ranvier, and (iv) the boutons—either of the terminal or *en passage* variety—are easily recognizable specializations on the terminal axon branches. (From Ferster, D. and S. LeVay [1978]. The axonal arborizations of lateral geniculate neurons in the striate cortex of the cat, *J. Comp. Neurol.* 182:923–944. With permission of The Wistar Press.)

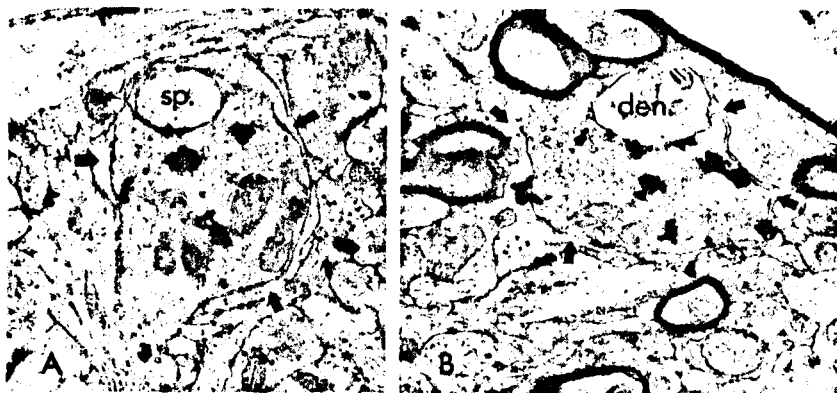


FIGURE 8. Geniculocortical terminals in an adult cat, identified by EM autoradiography. In contrast to the early synapses (Figure 5), those in the adult are situated at specialized swellings (boutons) that are ensheathed in astrocytic lamellae (arrows). The boutons contain large numbers of synaptic vesicles and mitochondria, but few or no microtubules. The synapses are made onto spines (sp.) or small dendritic shafts (den.).  $\times 30,000$ . (From LeVay, S. and C. D. Gilbert [1976]. Laminar patterns of geniculocortical projection in the cat, *Brain Res.* 113:1-19. With permission of Elsevier/North Holland Biomedical Press.)

tory that is large enough to house a number of neighboring columns, the sorting process may involve nothing more than local changes in density of the terminal branches on a static arbor.

The physiological observations suggest that the left- and right-eye afferents establish functional connections with cortical neurons in their early, overlapping pattern, and hence that synapses in the appropriate columns are likely to be broken during the process of segregation. It should not be thought, however, that these temporary connections have the elaborate structure of mature geniculocortical synapses. On the contrary, the EM autoradiographic observations show that most of these connections are very simple *en passage* contacts, with only small numbers of synaptic vesicles, just as has been described for early synapses in other parts of the developing nervous system. We have not yet applied the EM autoradiographic method to a study of the geniculocortical synapses during the process of segregation. Given the elementary structure of the contacts and the obvious limitations of electron microscopy in the third and fourth dimensions, it will not be easy to catch any synapses unmistakably in the act of dissolution, even though it is probably a commonplace event.

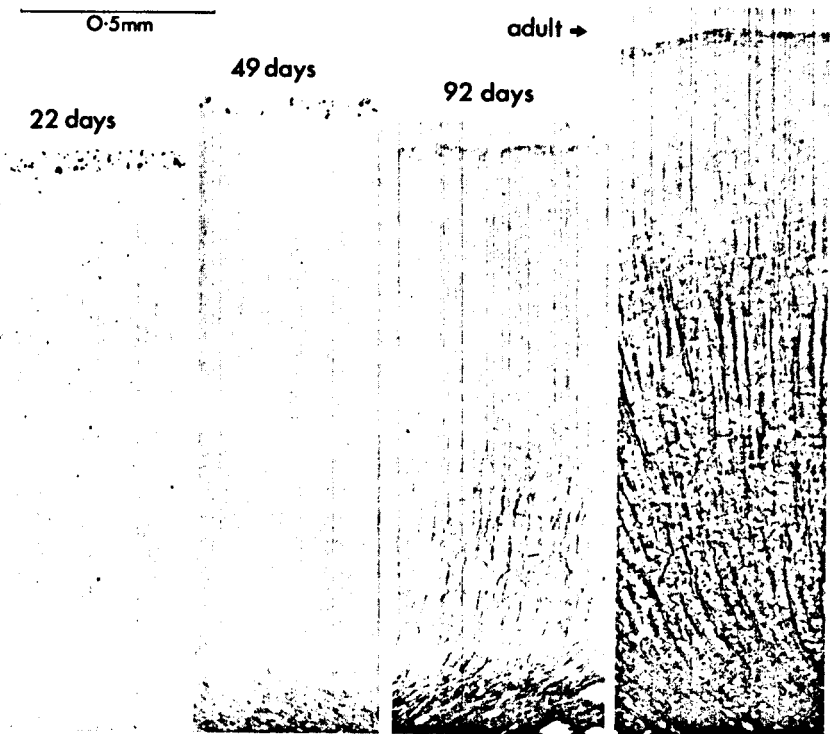
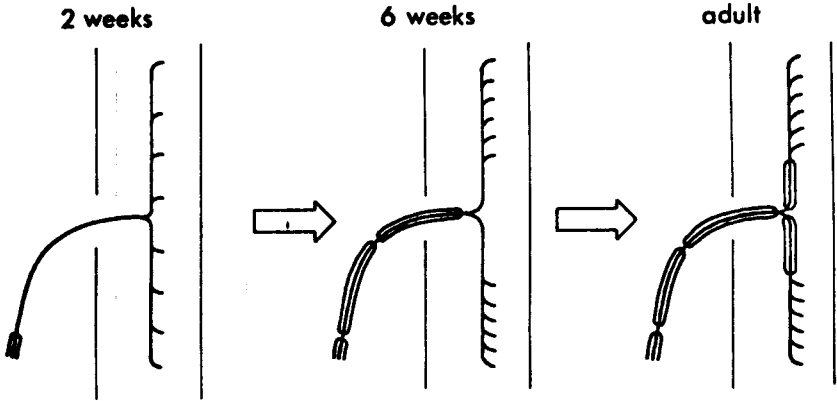


FIGURE 9. Time course of myelination in cat visual cortex. These are coronal sections stained by the method of Jebb and Woolsey (1977). The series illustrates that most of the myelin—except for that ensheathing large axonal trunks in the lower layers—is laid down after completion of columnar development (compare with Figure 1).

The rearrangement of axonal arbors in normal development envisioned here takes place during a "critical period" when the visual cortex and its afferents are susceptible to the effects of monocular deprivation (Wiesel and Hubel, 1963). During the fourth week of life in the cat (Hubel and Wiesel, 1970), or perhaps slightly earlier (Van Sluyters and Freeman, 1977; Van Sluyters, personal communication), a few days of monocular suture lead to a reduced influence of the deprived eye on cortical cells. Similar deprivation at younger ages does not reduce the influence of the deprived eye. Thus, in the cat, the beginning of the critical period roughly coincides with the onset in normal development of columnar segregation. Columnar segregation has begun by



**FIGURE 10.** Probable sequence of events in the maturation of a large geniculocortical afferent. Each part of the drawing represents an axonal trunk entering the cortex and arborizing in layer IV. At 2 weeks the terminal branches are distributed uniformly. By 6 weeks branches in the inappropriate columns have degenerated or have been reabsorbed, and branches in the appropriate columns strengthened, but the overall structure and position of the axonal tree is unchanged. After completion of columnar development the arborization is myelinated (see text).

birth in the monkey (Rakic, 1976; Hubel et al., 1977), and the critical period is advanced accordingly (Hubel, Wiesel, and LeVay, unpublished). The end of the critical period for the cortex as a whole occurs well after the end of columnar segregation in layer IV in both the monkey (Hubel, Wiesel, and LeVay, unpublished) and in the cat (Hubel and Wiesel, 1970; LeVay et al., 1978). In the monkey, however, the critical period for layer IV ends earlier than for the other layers, although its exact timing with respect to the end of the columnar segregation is not yet clear, and similar data are as yet lacking for the cat. If plasticity does persist in the afferent axons for a period after the end of columnar segregation, it will be tempting to blame the end of this plastic period on some later-occurring process such as myelination.

For the maturation of the afferents is by no means complete with the completion of columnar segregation. A number of events follow, of which the myelination of the arborizations is the most dramatic. This is not merely the addition of myelin to an axon. As argued above, myelination seems likely to involve some local restructuring of the axonal branching pattern. More significantly, there is likely to be a further loss of synapses associated with myelination. Since the early

synapses appear to be situated throughout the arborization of an axon, and since, in the adult, all but the finest terminal branches are myelinated, and hence nonsynaptic, it seems probable that the contacts made by the more proximal branches on the connecting trunks are either removed prior to myelination or are actually stripped off by the myelinating oligodendrocytes. Unlike the situation during columnar segregation, where synapses lost by one axon are likely to be reformed by another, breakage of connections during myelination would lead to a net reduction in synaptic number. Cragg (1972) has indeed reported an apparent drop in total synaptic number in the cat's visual cortex between 6 weeks and maturity, the period during which most of the myelination occurs.

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