

Ocular Dominance Columns and Their Development in Layer IV of the Cat's Visual Cortex: A Quantitative Study

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ABSTRACT The distribution of geniculocortical afferents serving the left and right eyes was studied in adult cats and in kittens of various ages. Methods used were autoradiography of transneuronally transported ³H-proline injected into one eye, and physiological recordings.

In the adult cat, patches of label in layer IV corresponding to ocular dominance columns were seen both ipsilateral and contralateral to the injected eye. Between the patches, however, grain density was substantially above background, especially on the contralateral side. Similarly, in the contralateral lateral geniculate nucleus (LGN) there was substantial labelling of neuronal cell bodies in lamina A1, which receives no innervation from the injected eye. Such spillover of radioactivity into the inappropriate laminae of the LGN was measured in autoradiographs of semithin sections, and its effect on the cortical labelling pattern calculated. Spillover appeared to account quantitatively for the labelling seen between patches in the cortex. It was concluded that the geniculocortical afferents for the ipsilateral and contralateral eyes are equally and almost completely segregated from each other at the centers of columns, although there is extensive overlap at the borders. This pattern is consistent with the physiological pattern of ocular dominance in layer IV.

In kittens studied at one to two weeks of age, radioactive label formed a continuous band in layer IV on both sides of the brain. On the ipsilateral side, this appearance could not be accounted for by spillover in the LGN, but reflected a continuous, non-columnar distribution of afferents. Physiological recordings at this age showed most cells in layer IV to be nearly equally responsive to stimulation of either eye, in contrast to the adult pattern. Periodic variations in grain density were first noted at three weeks, and a pattern similar to that of the adult was reached by about six weeks of age. On the contralateral side the uniform labelling pattern seen in the 1- to 2-week-old kittens was uninterpretable, owing to the very great spillover of radioactivity in the contralateral LGN, but the physiology suggested that the contralateral afferents, too, were uniformly distributed in layer IV.

The results suggest that the earliest functional connections formed by geniculocortical afferents have a uniform, non-columnar arrangement in layer IV, and that the formation of the adult pattern is likely to involve the breakage and reformation of synaptic connections. This process appears to be similar to that described for the monkey (Hubel et al., '77; Rakic, '76), except that the beginning of segregation is postponed until postnatal life.

The visual cortex of the cat, like that of the monkey, is subdivided into regions dominated by the left or the right eye. These regions, known as ocular dominance columns (Hubel

and Wiesel, '62, '68), have an anatomical basis in the segregation of the geniculocortical af-

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ferents serving each eye into a system of alternating bands in the cortex. This segregation is most readily evident in layer IV, the principal recipient zone (Hubel and Wiesel, '72; Wiesel et al., '74; LeVay et al., '75; Shatz et al., '77), but it can also be seen in layer VI, which receives a subsidiary geniculate projection (LeVay and Gilbert, '76). Microelectrode recordings have shown that the afferents determine the eye preference not only of cells in the fourth-layer bands (LeVay et al., '75; Shatz and Stryker, '78), but also, by virtue of secondary connections, of cells in the other layers that lie directly above and below them (Hubel and Wiesel, '68).

How this system arises during development is the subject of the present study. In the monkey, the left- and right-eye afferents entering the cortex during prenatal development are initially intermixed. This has been demonstrated autoradiographically using the transneuronal transport of radioactive material following an injection of tritiated proline and focuse into one eye (Rakic, '76). Segregation into separate bands has begun by about three weeks before birth (Rakic, '76), continues in the immediate postnatal period, and is complete by about three weeks of age (Hubel et al., '77).

If a similar process of segregation should occur in the cat, we suspected that it might not begin until some time after birth, on account of the relative immaturity of the newborn kitten's visual system. The present study, which also employs the transneuronal autoradiographic method, shows that such is indeed the case. We found, however, that a reliable interpretation of the autoradiographic picture, both in young and adult animals, required a quantitative assessment of a significant technical artifact, namely the leakage of radioactive material between laminae of the lateral geniculate nucleus.

A further question, which we have studied with physiological techniques, is whether, prior to the beginning of segregation, functional connections are established in a pattern of ocular dominance distinctly different from that of the adult. We felt that this was an important issue, since conceivably much of the radioactive label might be present in growth cones, fibers of passage, or non-functional synapses. Recordings from an 8-day-old monkey have shown the cells in the fourth layer are somewhat less sharply grouped according to eye preference than they are in

the adult (Hubel et al., '77), indicating that some functional connections are established before segregation is complete. Using kittens, it has been possible to extend this study to earlier stages of cortical maturation.

METHODS

For the autoradiographic study ten cats were used. Their ages at death were 2, 2, 8, 15, 22, 33, 39, 50 and 92 days, and one was an adult of unknown age. Each received, under halothane-N₂O anesthesia, a single injection into the posterior chamber of the left eye of 2 mCi of 2,3-[³H]L-proline (specific activity 37.3 Ci/mmole) in 25-100 μ l of 0.9% NaCl. The isotope was injected rapidly (3-5 minutes) through the lateral margin of the eye.

The two kittens killed at two days of age had been injected 24 hours previously. The intention here was to demonstrate the retinogeniculate projection only. All other animals were allowed to survive 7-8 days, in order to permit time for transneuronal labelling of the geniculocortical afferents.

The animals were perfused with 0.1 M phosphate buffer, pH 7.2, followed by 4% paraformaldehyde in the same buffer. After hardening in the fixative for a few days, the visual cortex on both sides was removed, placed in 30% sucrose in the same fixative for two days, and cut as frozen sections at 20 μ m. Sections were cut in a plane tilted back slightly from the horizontal, so that they were roughly parallel with the suprasplenic sulcus. They were mounted on subbed slides and processed for autoradiography as described previously (Wiesel et al., '74). Exposure time for slides intended for photography varied from two weeks to two months, the shortest times being sufficient for the youngest animals.

For the measurement of grain density in the fourth layer, exposure times were reduced, particularly in the young animals, in order to facilitate counting and in order to remain within the linear response range of the emulsion (Rogers, '73). The emulsion was sufficiently thick to capture all β -particles entering it, regardless of surface irregularities in the section. Exposure times varied from two days, for the youngest (8-day) kitten, to two months, for the adult.

A region of area 17 was selected for grain-counting which lay in the suprasplenic sulcus, roughly at the point where the lateral gyri begin to diverge. It corresponded approx-

imately to a visual field position on the horizontal meridian and 10° from the vertical meridian. In most cases the intention was to measure the periodic variation in grain density in the fourth layer in a direction parallel to the cortical surface. The unit area counted was a $100 \times 12.5 \mu\text{m}$ rectangle oriented perpendicular to the layer and confined entirely to its lower part, layer IVc, as determined by inspection of an adjacent section processed for autoradiography and counter-stained with thionin. This measurement was repeated for a total of about 150 consecutive unit areas, thereby traversing slightly less than 2 mm along the layer. The grain counts were smoothed by averaging the count for each unit area with those of its four nearest neighbors, and the resulting values were plotted as a function of distance along layer IV. Background grain density was determined by counting a region of non-visual cortex on the same slide.

In a few animals the laminar distribution of label was determined in a similar manner, but with the direction of counting perpendicular to the layers.

In the lateral geniculate nucleus a different procedure was used, which permitted us to determine the amount of radioactivity present in neuronal nuclei in the various laminae. This measure was required to gain an estimate of the extent to which the cortical labelling pattern was influenced by leakage ("spillover") of radioactivity into the inappropriate laminae of the LGN, i.e., those laminae not receiving innervation from the eye which had been injected. (The rationale behind this procedure is discussed further in the RESULTS section.) The LGN was sectioned coronally on a Vibratome at $100 \mu\text{m}$ in phosphate buffer. Under the dissecting microscope small ($1 \times 2 \text{ mm}$) tissue blocks containing adjacent portions of laminae A and A1 were cut out of the thick sections. The position of these blocks was chosen to be in approximate retinotopic correspondence with the cortical region studied. The blocks were osmicated, embedded in Epon, sectioned at $1 \mu\text{m}$ and processed for autoradiography. After exposures of one week to three months the sections were developed and stained with toluidine blue. Sections from the right and left LGNs, as well as control sections from the LGN of a non-injected animal, were mounted together on the same slide and thus were processed identically.

For each lamina, the outlines of 50 neuronal

nuclei, taken from the entire dorso-ventral extent of the lamina, were drawn using a $100\times$ oil immersion objective and a camera lucida attachment. The number of grains over each nucleus was noted. The total nuclear area was measured using a PDP-11 computer equipped with a graphic tablet, and the labelling density was expressed as grains per $1,000 \mu\text{m}^2$ for each lamina. Background, as measured from the control sections, was subtracted from the counts. This background was, however, very close to zero.

For the physiological recordings, eight animals were used. Their ages were 10, 13, 13, 17, 22, 35, and 92 days, as well as one adult. Two of the animals (22 and 92 days of age) were also used in the autoradiographic study.

The animals 35 days of age and younger were initially anesthetized with a halothane- N_2O mixture, to facilitate the insertion of venous and tracheal cannulae. Subsequent surgery was performed under pentothal anesthesia (20 mg/kg i.v.). Atropine sulfate (0.04 mg/kg) and dexamethasone sulfate (0.4 mg/kg) were administered intramuscularly. A hole was drilled in the skull and the dura reflected. The skull was then attached to a gantry with dental cement. For the duration of the experiment, anesthesia was maintained with N_2O , and the animal was paralyzed with Flaxedil (10 mg/kg/hr), thereby necessitating artificial respiration. Expired CO_2 and rectal temperature were monitored. The 92-day-old and adult animals were prepared as described previously (Shatz and Stryker, '78).

The nictitating membranes were retracted with phenylephrine and the pupils dilated with atropine sulfate. Appropriately-sized contact lenses were used to protect the corneas. The poor quality of the optics in the four youngest animals precluded the accurate refraction of the eyes or the plotting of retinal landmarks.

Techniques for stimulation and for recording with tungsten microelectrodes were similar to those previously described (Hubel and Wiesel, '62; Shatz and Stryker, '78). The main intent of the experiments was to record from many neurons in layer IV and assess their eye preference. Penetrations were made in either a parasagittal or coronal plane, and we attempted to isolate a single unit about every 50-100 μm . Electrolytic lesions (2-3 μA for 2-3 seconds) were made at sites of particular interest. At the completion of the experiment the animal was perfused; the electrode

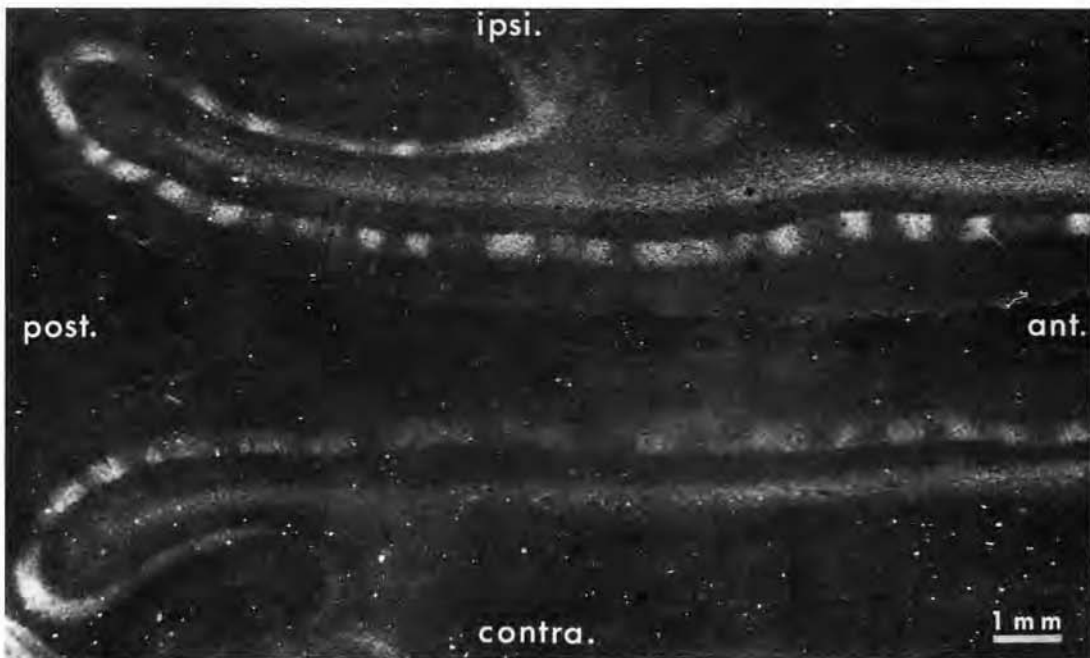


Fig. 1 Darkfield autoradiograph of the visual cortex of a 92-day-old normal cat, ipsilateral and contralateral to the eye which had been injected with ^3H -proline eight days previously. The section plane is tilted back somewhat from the horizontal, and follows approximately the line of the suprasplenic sulcus. Note the patchy distribution of label in the fourth layer, corresponding to ocular dominance columns for the injected eye, and the greater clarity of the columnar pattern on the ipsilateral side.

tracks and lesions were reconstructed histologically from cresylviolet-stained sections.

RESULTS

The distribution of afferents in the mature animal

The autoradiographic labelling pattern seen in the cat's visual cortex following eye injections has already been described in coronal and parasagittal sections (Shatz et al., '77). It was demonstrated in that study that the ocular dominance columns in area 17 are organized into a system of irregular bands running from the 17-18 border down the medial surface of the hemisphere. Since we wished to have sections orthogonal to these bands (as far as the variability in their detailed arrangements would permit) and at the same time orthogonal to the cortical layers, we sectioned the cortex horizontally (METHODS). Such sections are more favorable than are coronal sections for showing the periodic waxing and waning in grain density which corresponds to the ocular dominance columns for the injected and non-injected eyes.

Figure 1 is such a section from a 92-day-old cat. It shows that the patches of label, though visible on both sides of the brain, are more distinct on the side ipsilateral to the injected eye. On the contralateral side a considerable amount of label is present between the heavily labelled patches. This difference is always seen, and has been interpreted by Shatz et al. ('77) to mean that there may be a real difference between the two sets of columns: that those dominated by the ipsilateral eye also receive some contralateral input, while contralaterally dominated columns are essentially free of ipsilateral-eye input. The possibility was noted in that study, however, that the labelling pattern may not be an entirely faithful reflection of the distribution of afferents serving the injected eye.

There are several reasons for hesitating to accept the autoradiographic picture as a quantitative representation of the geniculocortical projection. For one thing, the maximum labelling density was generally, though not invariably, greater on the ipsilateral than on the contralateral side, though there is no

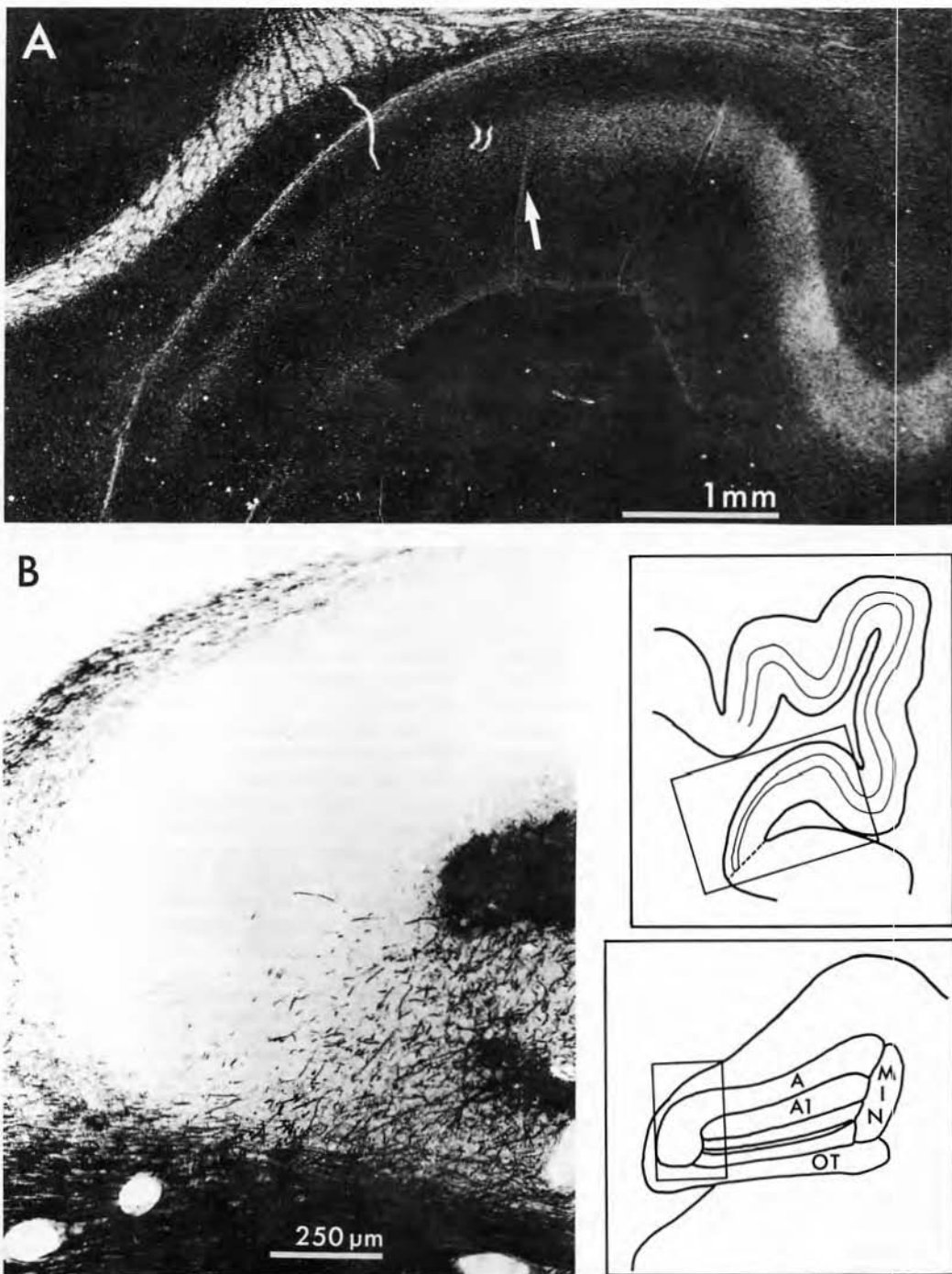


Fig. 2 Autoradiographs of the representations of the temporal crescent (monocular segment) in visual cortex (fig. 2A) and LGN (fig. 2B), ipsilateral to an eye injection in an adult cat.

A Visual cortex. Coronal section through upper bank of splenial sulcus (see sketch). Arrow marks the end of the binocular segment. More laterally (to left in figure) the labelling density in layer IV decreases, but remains well above background levels. Darkfield.

B Monocular segment of LGN (see sketch at right). Labelled fibers from the injected eye traverse the extreme margin of lamina A on their way to laminae A1 and Cl (visible at right side of figure). MIN, medial interlaminar nucleus; OT, optic tract. Brightfield.

reason to believe that the density of terminals is greater for afferents representing the ipsilateral eye. Also, labelling density on both sides tended to be lower near the representation of the area centralis than peripherally; this too was not expected from physiological studies. These appearances are most likely explained by uneven labelling of the retinal ganglion cells (the injections were made through the lateral margin of the eye) and by a greater magnification of the area centralis representation over and above that expected from the retinal ganglion cell density (shown for the monkey by Malpeli and Baker, '75).

It is clear, however, that *some* part of the cortical label is not situated in the appropriate afferents at all, for in the representation of the temporal crescent (monocular segment) ipsilateral to the injected eye, where no radioactive afferents should be present, a level of labelling was seen which, though low, was still considerably above background (the presence of this label was not noted in the study of Shatz et al., '77). The labelling of this area is illustrated in figure 2A, which is a coronal section through the upper bank of the splenic sulcus in an adult cat. The grain density fell off abruptly at the edge of the binocular segment (arrow in fig. 2A), but gradually increased again in the more lateral part of the monocular segment, which represents the furthest periphery of the visual field. The corresponding region of the lateral geniculate nucleus is shown in figure 2B. Here, heavily labelled fibers on route to lamina A1 traversed the extreme ventrolateral end of lamina A, which also represents the far periphery of the visual field. From this comparison, it seems likely that geniculate neurons not themselves innervated by the injected eye may nevertheless pick up radioactive label which has leaked from fibers of passage or diffused from neighboring laminae. Enough of this label must be transported to the cortex for the projections of geniculate neurons in the monocular segment to be easily visible in autoradiographs. Because the labelling of the monocular segment was not uniform, we did not attempt to quantify the relationship between the labelling of geniculate cell bodies and that of their terminals in the cortex.

Leakage of radioactive label may pose an even greater problem in the binocular segment of the LGN. In thick sections viewed at low magnification (figs. 3A,F) the label appeared to be rather well restricted to the ap-

propriate laminae: to lamina A1 ipsilateral to the injection and lamina A on the contralateral side (the C laminae are not considered in detail in this paper because, as shown by LeVay and Gilbert, '76, their projection to area 17 largely avoids the fourth layer, the main object of our study). Fibers from the injected eye did, however, traverse lamina A1 on route to their sites of termination in lamina A on the contralateral side (fig. 3F), while on the ipsilateral side (fig. 3A) lamina A was free of labelled fibers of passage except, as noted above, at its lateral extremity. Furthermore, contralateral lamina A1 was sandwiched between two radioactive laminae (A and C) whereas lamina A on the ipsilateral side was apposed to a radioactive lamina only on its ventral aspect. Both these features suggested that leakage of radioactive material might well be more severe contralateral than ipsilateral to the injected eye, thus offering a partial explanation of the difference between the cortical labelling pattern on the two sides of the brain.

A more detailed picture of the distribution of radioactivity within the binocular segments of the LGN was obtained by examination of autoradiographs of 1 μ m thick Epon sections (figs. 3B-E). In contralateral lamina A (fig. 3D) and ipsilateral A1 (fig. 3C) most silver grains occurred in clumps in the neuropil, presumably overlying optic nerve terminals. Grains were also found over neuronal cell bodies. In ipsilateral lamina A (fig. 3B) there were altogether few grains. In contralateral A1, on the other hand (fig. 3E), there was heavy labelling of myelinated fibers and appreciable numbers of grains over cell bodies.

In order to quantify the neuronal labelling,

Fig. 3 Brightfield autoradiographs of the lateral geniculate nucleus of a 92-day-old cat, ipsilateral (figs. 3A-C) and contralateral (figs. 3D-F) to the injected eye.

A, F Thick, unstained sections including adjacent portions of laminae A (above) and A1 (below). The dense clumps of grains, probably corresponding to optic nerve terminals, are restricted to the appropriate laminae (ipsilateral A1 and contralateral A), but on the contralateral side labelled fibers of passage traverse the non-innervated lamina A1. $\times 150$.

B-E One-micrometer Epon sections, counterstained with toluidine blue, of ipsilateral lamina A (fig. 3B), ipsilateral A1 (fig. 3C), contralateral A (fig. 3D) and contralateral A1 (fig. 3E). In the laminae innervated by the injected eye (figs. 3C,D) grains occur in clumps over the neuropil and more sparsely over cell bodies. In the ipsilateral lamina A (fig. 3B) few grains are found over either neuropil or cell bodies, while in contralateral lamina A1 (fig. 3E) grains overlie cell bodies as well as some myelinated axons. $\times 720$.

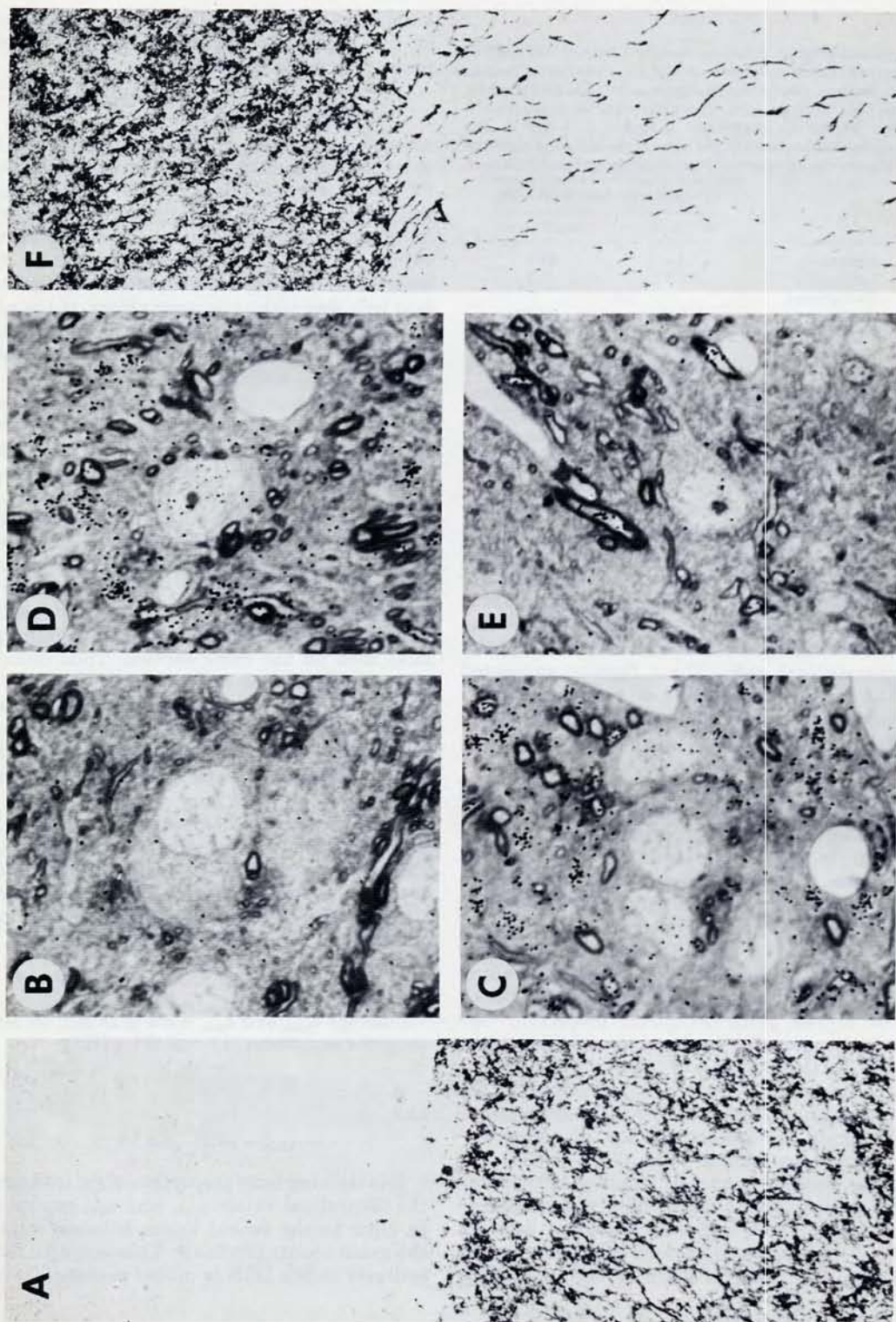


Figure 3

TABLE 1

Autoradiographic labelling density (grains/1,000 μm^2) of neuronal nuclei in laminae A and A1 of the lateral geniculate nucleus, ipsilateral and contralateral to an eye which had been injected with ^3H -proline eight days previously. Same animal as illustrated in figures 1, 3 and 4. The spillover value shown is the ratio of the labelling densities in the two layers, after subtraction of background from each

	Nuclear grain densities in LGN		
	Ipsilateral	Contralateral	
Lamina A	14.4	41.5	
Lamina A1	102.3	15.7	
Background			1.2
Spillover	13.1%	36.0%	

the grain density over neuronal nuclei in these four laminae was measured, as described in the METHODS section. Nuclear rather than total perikaryal densities were measured in order to avoid including grains derived from radioactivity in optic nerve terminals or fibers closely adjacent to the cell membrane. These grain densities are given for the 92-day-old cat in table 1. The ratio of neuronal labelling in the inappropriate laminae to that of the appropriate laminae (after subtraction of background) was defined as "spillover." This was 13.1% on the ipsilateral side and 36.0% on the contralateral side. These figures suggested that significant leakage of radioactivity between laminae does occur, and is particularly severe on the side contralateral to the eye injection.

It is to be noted that each measure of spillover is a ratio computed separately for the ipsilateral and contralateral sides of the brain. It takes no account of absolute differences in labelling density between the two sides. In most animals the nuclear labelling was heavier on the ipsilateral side (for example, see table 1). This difference, also observed in the cortex, may be due to uneven labelling of the retina. It does not prevent further analysis of the geniculocortical projection (see below), since for each region of the cortex studied, this analysis is based on the nuclear labelling ratio of laminae A and A1 only in the corresponding region of the LGN on that side.

Making the assumption that the geniculocortical terminals in area 17 are labelled in proportion to the nuclear labelling of their cell bodies, it follows that the observed grain density in layer IV can only fluctuate between theoretical maximum and minimum values set by the spillover ratio in the LGN. (The

validity of this assumption will be considered in the DISCUSSION.) This assumption is expressed by the equation:

$$\frac{t_{\min} - b}{t_{\max} - b} = s \quad (\text{assumption 1})$$

where t_{\min} and t_{\max} are the theoretical minimum and maximum grain densities in layer IV, b is cortical background, and s the spillover in the LGN as defined above. It may be noted that the maximum and minimum values (t_{\max} and t_{\min}) will be reached only where the afferents are completely segregated into left- and right-eye populations. If the afferents are not completely segregated the amplitude of fluctuation will be less than that defined by t_{\max} and t_{\min} .

Actual measurements of cortical grain density were made as described in the METHODS section. The regions examined were chosen to be in approximate retinotopic correspondence with the part of the LGN used for determination of spillover. Such counts for the cortex ipsilateral and contralateral to the injected eye in the 92-day-old cat are plotted graphically in figure 4. The background grain density, indicated by the dotted lines in figure 4, was estimated by counting grains in a non-visual cortical area on the same section. Thus counts above background reflect geniculocortical transport. As was suggested by simple inspection of the autoradiographs, grain density between labelled patches remained significantly above background, particularly on the contralateral side.

The theoretical maximum and minimum grain densities (t_{\max} and t_{\min}) were added to the graphs (solid lines) by making the further assumption that the observed mean grain density (m) reflects a roughly equal contribution of afferents serving the two eyes. Thus

$$\frac{t_{\max} + t_{\min}}{2} = m \quad (\text{assumption 2})$$

Values for t_{\max} and t_{\min} were obtained by solving the equations (1) and (2), giving

$$t_{\min} = \frac{2s(m-b)}{s+1} + b \quad (3)$$

and

$$t_{\max} = \frac{2(m-b)}{s+1} + b. \quad (4)$$

It is striking from inspection of figure 4 that the theoretical values t_{\max} and t_{\min} appear to be close to the actual limits between which the grain counts fluctuate. This suggests that spillover in the LGN is indeed responsible for

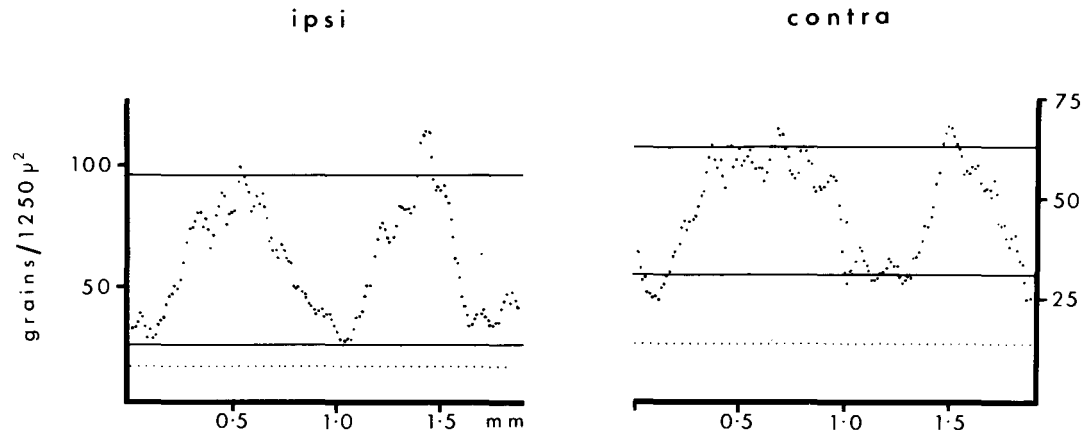


Fig. 4 Grain density in layer IVc of the visual cortex of the 92-day-old cat of figures 1 and 3, ipsilateral and contralateral to the injected eye. The ordinate represents grain counts in successive $12.5 \times 100 \mu\text{m}$ rectangles oriented normal to the layer; abscissa represents distance along the layer. Each point plots the average of its own and the four surrounding bins. The dotted line shows the background grain density. The two solid lines show the theoretical maximum and minimum grain densities, t_{max} and t_{min} , calculated from grain counts made in the lateral geniculate nucleus (see text). Note that the observed grain counts rise and fall between the theoretical limits on both sides of the brain.

producing the difference in the appearance of the columns on the two sides of the brain. The observed labelling pattern in the fourth layer is completely consistent with the notion that the center of each column—whether belonging to the ipsilateral or the contralateral eye—is innervated almost exclusively by geniculocortical fibers serving that eye, although there must be extensive overlap at the borders. This interpretation is supported by the physiological results presented below.

Development

We next studied the development of this pattern in layer IV, by performing eye injections in a series of kittens ranging from newborn to three months of age. Of the three animals injected at one day of age, two were perfused 24 hours later. The aim with these animals was to demonstrate the retinogeniculate projection only, and in particular to determine whether there was any overlap of left- and right-eye input to the LGN as has been described by Rakic ('76) for early fetal monkeys. Such an overlap, if still persisting at birth, would confuse the autoradiographic picture in the cortex. The labelling pattern closely resembled that seen in the adult, however, in that labelled terminals were entirely restricted to the appropriate laminae (not illustrated). There were, however, signs of immaturity—the LGN had not yet fully rotated into

the adult position, so that the optic tract lay more dorsolaterally than is seen later. Also in Nissl-stained sections the neurons appeared immature and closely-packed.

The third newborn kitten was killed at eight days of age, and autoradiographs of the visual cortex on both sides showed heavy labelling in layer IV, both in area 17 and 18 (not illustrated). The labelling pattern was strikingly different from that seen in adults, in that it was continuous and uniform along the layer, with no trace of periodic variations. In the cortex of the animal perfused at 15 days of age a very similar labelling pattern was seen. This is illustrated in figure 5A. Besides the continuity of labelling, the laminar distribution of label was also different from the adult pattern. This is analyzed further below.

By 22 days of age (fig. 5B) periodic fluctuations in the labelling density could be seen in the fourth layer on the side ipsilateral to the eye injection. On the contralateral side the label was almost uniform, but very slight fluctuations could be discerned on close inspection of the sections. At 33 days of age the patchiness on the ipsilateral side was clearer, but on the contralateral side the labelling was still very nearly uniform (not illustrated).

In the next animal, perfused at 39 days of age (fig. 5C), the patches of label in layer IV on the ipsilateral side were as well-defined as those of the adult. Patches were now also

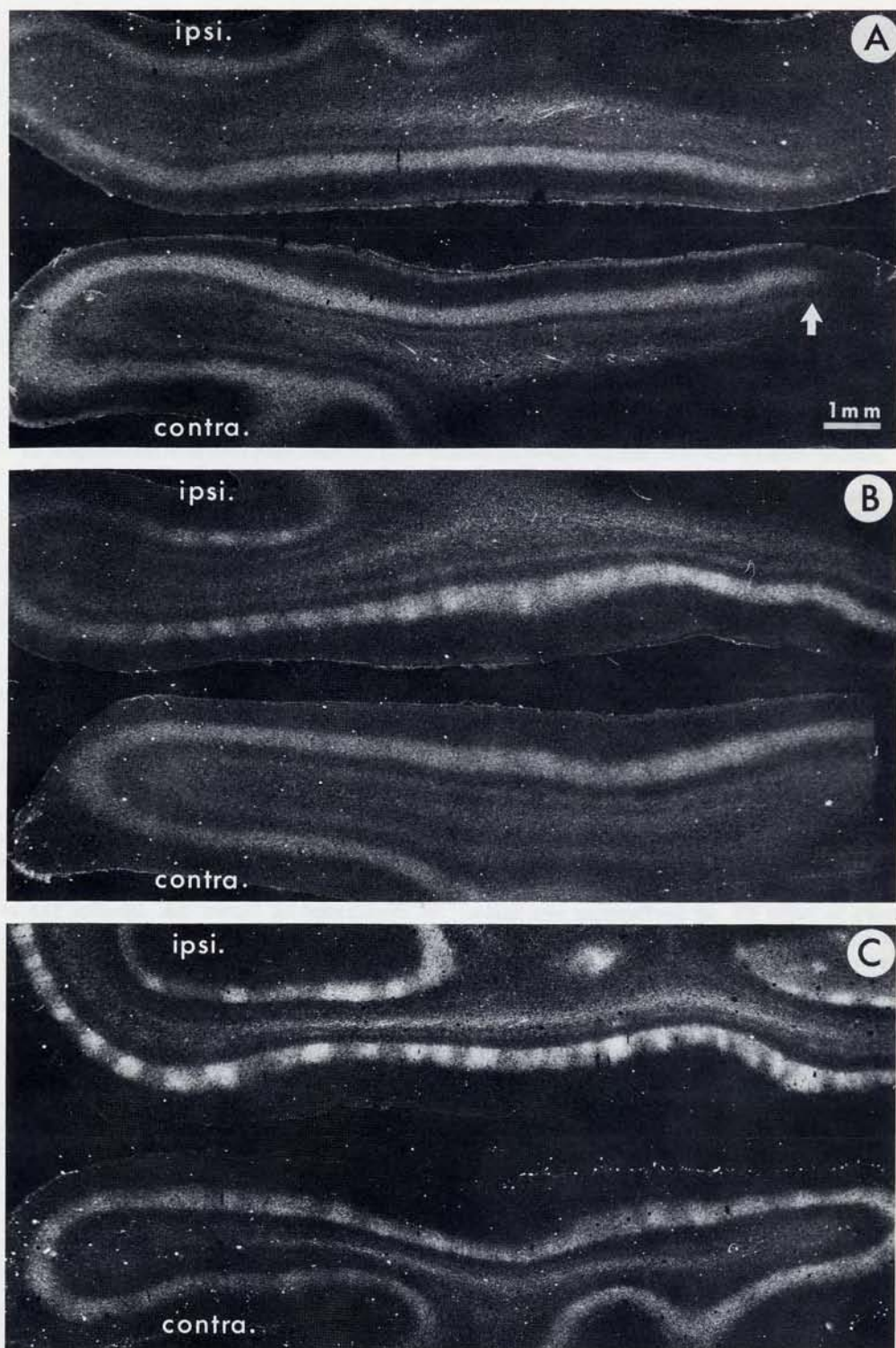


Figure 5

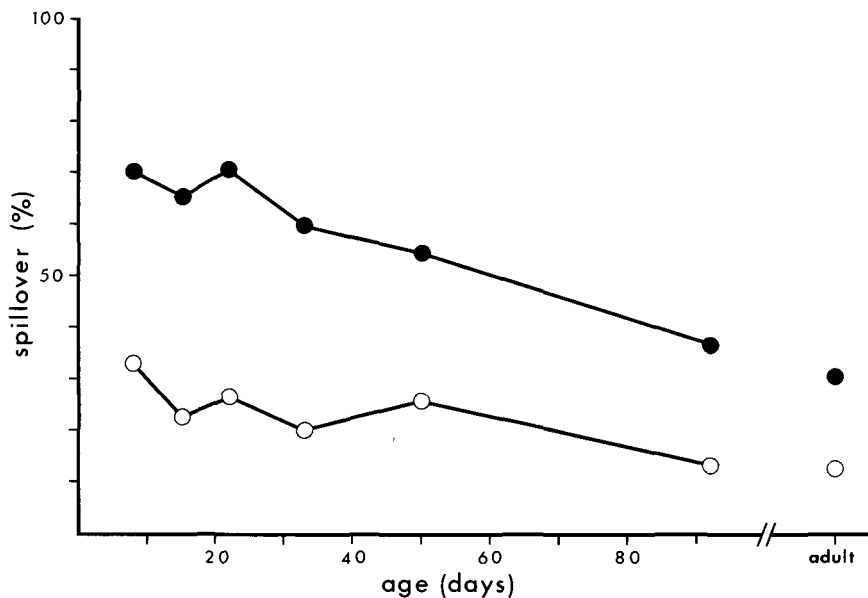


Fig. 6 Spillover of radioactivity into the inappropriate laminae of the lateral geniculate nucleus in kittens, as a function of age. Open circles: ipsilateral to the injected eye. Filled circles: contralateral to eye injection. Each point gives the labelling density over neuronal nuclei in the inappropriate lamina (A or A1) as a percentage of that measured in the lamina innervated by the injected eye. Spillover is greatest in the youngest animals, and is more severe on the contralateral than the ipsilateral side.

clearly visible on the contralateral side, but they were not yet quite as clear as seen contralaterally in the adult.

In view of the evidence, discussed above, that spillover of radioactivity in the LGN may obscure the actual extent of segregation in the cortex, we felt it necessary to determine spillover levels for each of the kittens. The method used to determine spillover was identical to that described above for the older animal. The spillover for the ipsilateral and contralateral LGNs of each kitten is plotted as a function of age in figure 6. From the figure it may be seen that spillover is considerably greater in the

Fig. 5 Darkfield autoradiographs of visual cortex ipsilateral and contralateral to the injected eye in three kittens, aged 15, 22 and 39 days. Plane of section as in figure 1.

A Fifteen-day-old kitten (injected at 8 days). The label is continuous and uniform in layer IV on both sides of the brain. Note also the heavy projection to layer I, which stops at the anterior border of area 17 (arrow).

B Twenty-two-day-old kitten (injected at 15 days). A mild, periodic fluctuation in the density of labelling is visible on the ipsilateral side. On the contralateral side such a fluctuation is barely discernible.

C Thirty-nine-day-old kitten (injected at 31 days). On the ipsilateral side the labelling pattern is similar to that seen in the adult. On the contralateral side the pattern is still a little less distinct than is seen in the adult (compare fig. 1).

younger animals, and is always more severe on the contralateral side. In the youngest kittens spillover on the contralateral side reached 70%, meaning that the eye injection had resulted in almost equal neuronal labelling in the appropriate and inappropriate laminae! The spillover did not decline fully to adult levels until about three months of age. Superimposed on this gradual decline were slight variations from animal to animal.

Cortical grain counts for the complete series of animals are shown in figure 7, along with the background levels and t_{\min} and t_{\max} calculated from the spillover. The grain counts confirmed our visual impression that the labelling along layer IV was essentially uniform in the youngest two animals, and that fluctuation in labelling density occurred earliest on the ipsilateral side.

In the ipsilateral cortex of the 8- and 15-day-old animals, the theoretical limits t_{\min} and t_{\max} were well clear of the actual grain counts, indicating that spillover did not account for the lack of periodic fluctuation in grain density. Thus we conclude that afferents serving the ipsilateral eye are uniformly distributed along the fourth layer at these

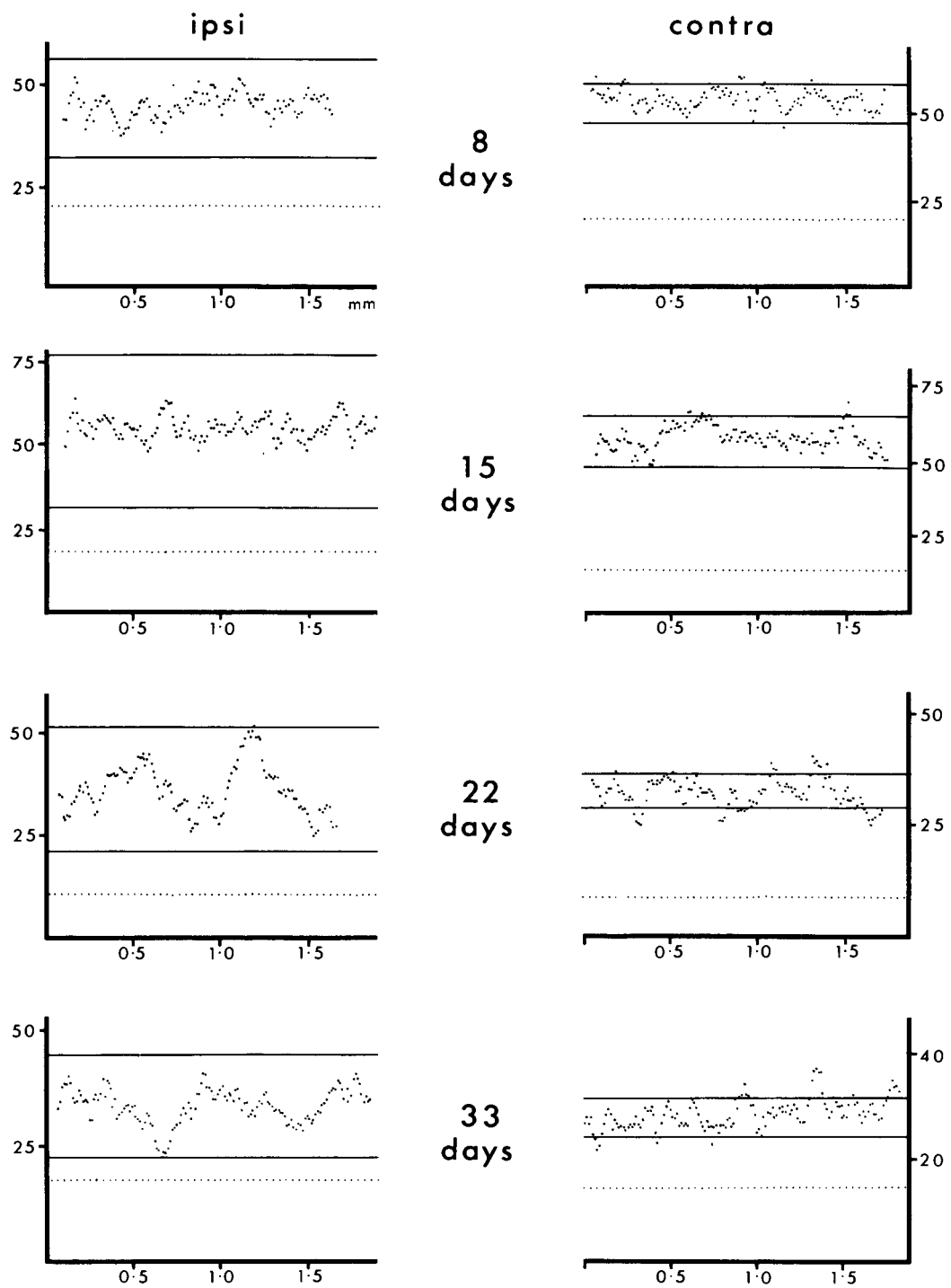


Figure 7

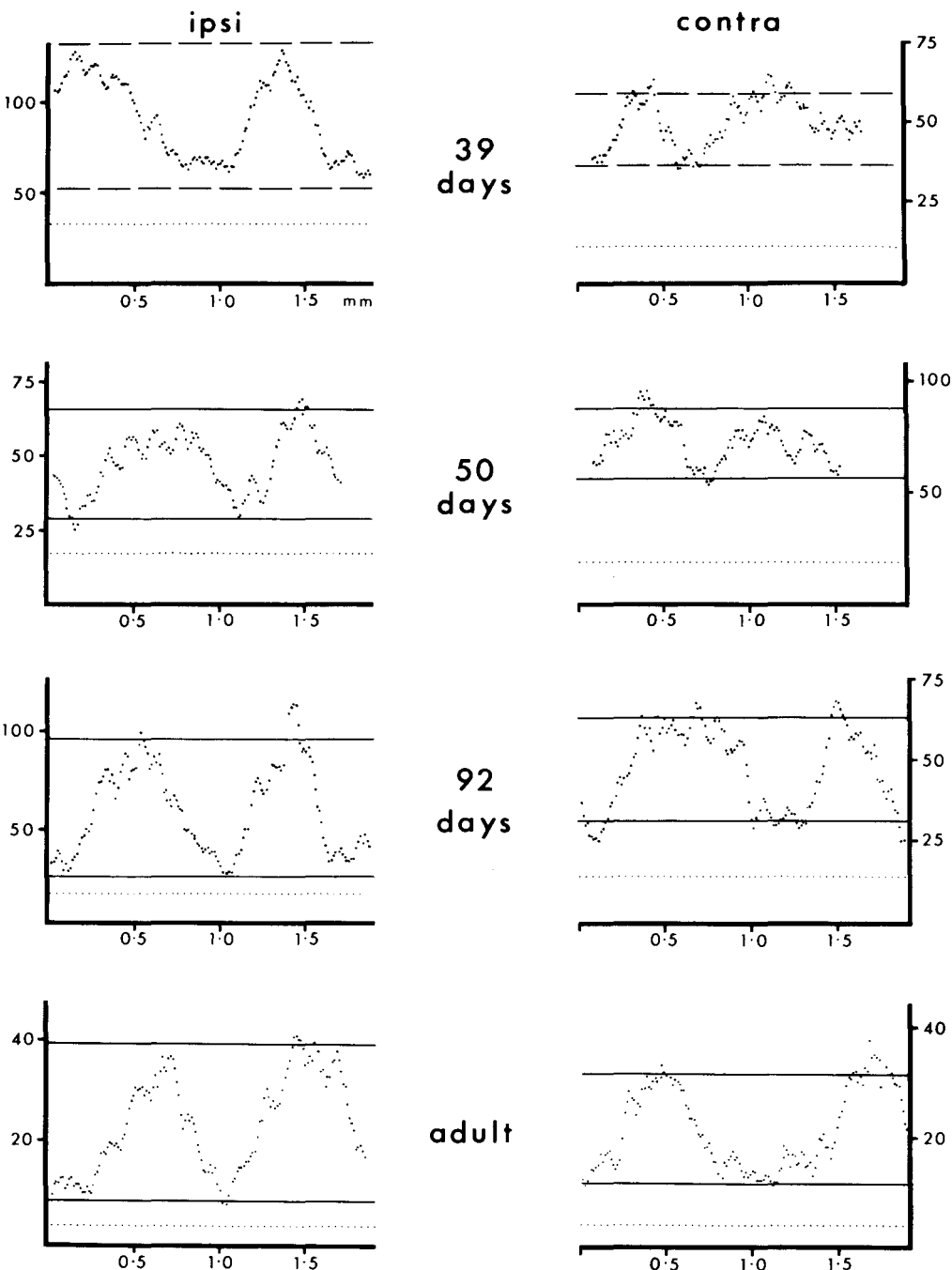


Fig. 7 Grain counts from layer IVC of the visual cortex on both sides of the brain, for the complete series of eight animals. The stated age is the age at death; eye injections were performed eight days previously. The theoretical limits t_{max} and t_{min} (solid lines) were calculated from spillover as described in the text, except that for one animal (39 days old—interrupted lines) they are derived from an estimate of spillover obtained by interpolation from the graph of figure 6. Background grain density is given by the dotted lines. Exposure times were not always identical for the two sides of the same brain. For further description see text.

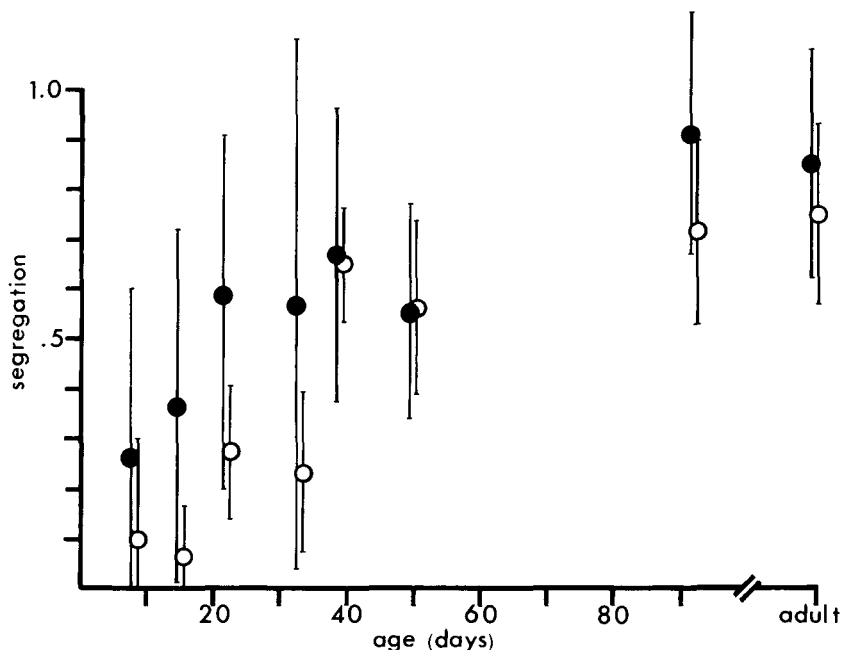


Fig. 8 Segregation index for geniculocortical afferents serving the ipsilateral (open circles) and contralateral (filled circles) eyes, as a function of age. The vertical bars indicate the 68% confidence limits (1 standard deviation). The large measure of uncertainty for the contralateral afferents in the younger animals is a reflection of the very great spillover of radioactivity in the lateral geniculate nucleus. For further explanation, see text.

early ages. On the contralateral side, on the other hand, t_{\min} and t_{\max} were very close to each other and to the actual grain counts, indicating that fluctuations in innervation density of the contralateral afferents, even if they had been present, would have been obscured by spillover. We consider it likely that the afferents serving the contralateral eye are in fact uniformly distributed at these ages, like the ipsilateral fibers (DISCUSSION), but the grain counts on this side provide no evidence one way or the other.

The 22- and 33-day animals are similar to each other in showing periodic fluctuations in labelling density on the ipsilateral side. For the most part, however, these fluctuations do not reach t_{\min} and t_{\max} , indicating that the ipsilateral afferents have begun to aggregate into patches, but have not reached the state of segregation seen in the adult. On the contralateral side the level of spillover is still very high, making the grain counts difficult to interpret.

In the animals 39 days and older, the counts on both sides indicate that the mature degree

of segregation has been reached. Grain counts fluctuate between levels at or close to t_{\min} and t_{\max} , and the apparent sharpening of the columns on the contralateral side at ages later than 39 days is mainly due to the progressive decline in spillover.

Segregation index

To facilitate comparisons of the extent of segregation among the different animals, an index of segregation was computed from the cortical grain counts. This index was computed by (1) smoothing the grain counts using a linear phase digital filter (McClellan et al., '63) and (2) measuring the area between the smoothed grain counts and the mean level, and expressing it as a fraction of the area of a smoothed square wave with upper border t_{\max} and lower border t_{\min} . For perfectly segregated columns, the grain density plot of which would be a square wave, the value of this segregation index would be 1; while completely intermingled afferents would give a segregation index of zero. The 68% confidence limits (± 1 standard deviation) were assessed by a Monte-

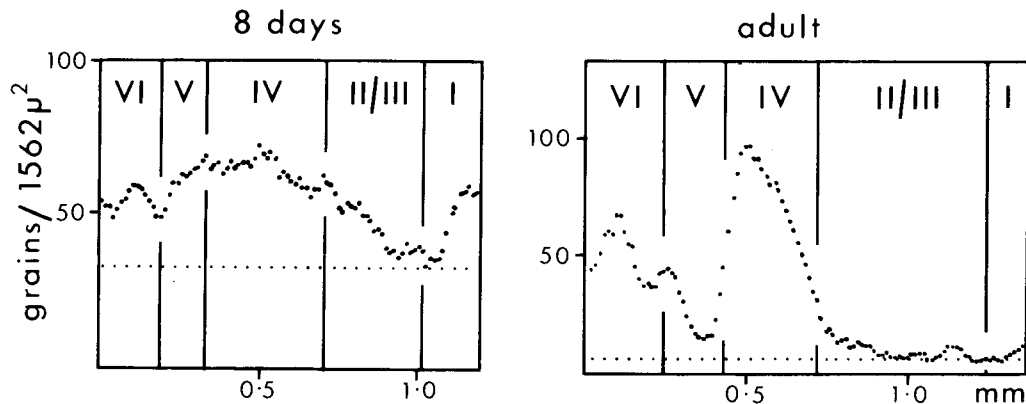


Fig. 9 Laminar distribution of transneuronally transported label in visual cortex after an eye injection in an adult cat and in an 8-day-old kitten. Grain counts obtained and smoothed as for figure 4. In the adult the label is concentrated in layers IV and VI, while in the young kitten the main peak extends from layer IV into layers V and III, and there is an additional peak in the upper part of layer I. The adult labelling distribution resembles the projection pattern of afferents from the A laminae of the LGN only, while the distribution seen in the kitten resembles a combination of the projection patterns from the A and the C laminae (LeVay and Gilbert, '76). Dotted line indicates background.

Carlo method: the mean change in area produced by random noise whose variance was equal to the overall variance of the raw minus smoothed grain counts.

The values of the segregation index are plotted as a function of age in figure 8. No significant segregation is apparent in the first two animals, 8 and 15 days old, although on the contralateral side the large range of the confidence limits allows the possibility of partial but not complete segregation. In the 22-day-old animal the beginning of segregation is seen, and it appears largely completed by 39 days. Some degree of further segregation may occur after 39 days, but it would require a larger series of animals than the present one to be sure on this point. The adult values of the segregation index are lower than 1; this is due to overlap at the borders of columns, as is evident from the shape of the curves in figure 7. At the centers of each column, the segregation is essentially complete.

Laminar distribution of afferents

Besides the progressive change in the distribution of label along layer IV, there are also differences between the laminar labelling pattern seen in young and adult animals. Grain counts made across the cortical laminae for the youngest (8-day) kitten and for an adult are plotted in figure 9. In the adult most of the label is restricted to layer IV, with a subsidiary component in layer VI. In the 8-day-

old kitten label is distributed more widely through the layers. The main band centered on layer IV extends well into layers V and III, and besides the subsidiary band in layer VI another very prominent band is situated in the upper part of layer I. This band is also evident in the 15-day-old animal, and as may be seen in figure 4A it ends abruptly at the anterior border of area 17. This band is much less prominent in the 22-day-old animal and almost invisible in older animals. This difference between young and older animals is interpreted as reflecting a change in the balance between the inputs from the A and C laminae of the LGN (DISCUSSION).

Physiology

An electrode penetration tangential to the fourth layer in an adult cat, and the ocular dominance distribution of the single units encountered is reconstructed in figure 10A. With one exception, all units were identified as cortical neurons on the basis of their receptive field properties. Eye preference varied periodically as the electrode traversed the layer. These variations have been shown to correspond to patches of left- and right-eye afferents, as demonstrated by the transneuronal autoradiographic method (Shatz and Stryker, '78).

The example of figure 10A shows the most abrupt transitions we have observed. More commonly binocular cells are interposed be-

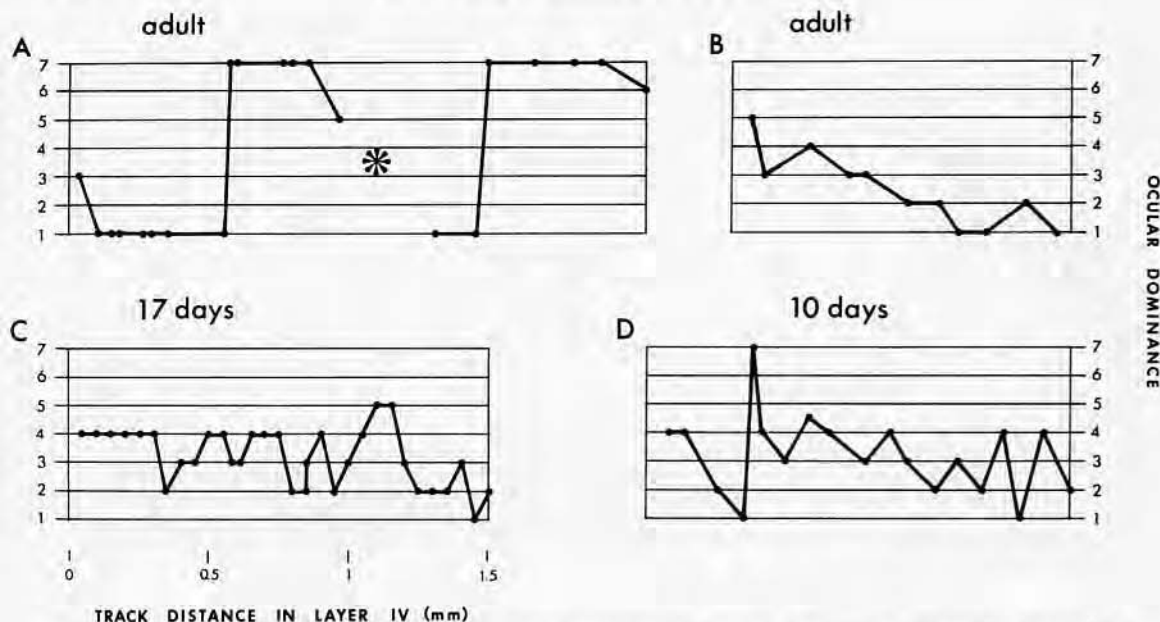


Fig. 10 Ocular dominance distribution of cells encountered during the passage of an electrode tangentially through the fourth layer in four cats: two adults (figs. 10A,B) and two kittens (figs. 10C,D). Each point represents an isolated single unit, whose ocular dominance (on the 7-point scale of Hubel and Wiesel, '62) is plotted against its position along the electrode track. In the kittens (figs. 10C,D) most cells could be driven from either eye, with on the whole a slight preference for the contralateral eye. In one adult (fig. 10A), 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. In figure 10B, an example is shown of a slower and more progressive shift in ocular dominance.

tween the patches of monocularly-driven cells, so that a gradual, progressive shift in ocular dominance may be observed, as shown in the example of figure 10B. It is not known whether this variability reflects differences in the alignments of the penetrations with respect to the columnar borders, possible differences in ocular dominance distribution between the sublaminae of layer IV, or a genuine variability in the sharpness of columnar borders.

The results of two penetrations made in the fourth layer of a 10- and a 17-day-old kitten are shown in figures 10C and 10D. Most cells were strongly binocular (ocular dominance groups 3-5) and there were no periodic variations in eye preference.

We obtained similar results in two other animals, both 13 days old. The pooled results from all four kittens are presented in histogram form in figure 11. Each histogram gives the ocular dominance distribution of cells recorded in a particular cortical layer, and

equivalent data for adult animals (from Shatz and Stryker, '78) are included for comparison.

Cells in all cortical layers, including layer IV, were markedly binocular as compared with the adult. As in the adult, there was an overall dominance by the contralateral eye. This dominance was much more marked in the more peripheral parts of the visual field, but the difficulty in plotting retinal landmarks in these young animals precluded our determining exact receptive field eccentricities.

Besides the unusual degree of binocularity, the responses of cortical neurons differed in other respects from those seen in the adult. As noted by Hubel and Wiesel ('63), cells responded with few spikes, the spikes were of longer duration, and the response had a longer latency to visual stimulation. Cells also fatigued easily, making receptive fields hard to study. The fields tended to be large, and often could not be categorized into the familiar classes of simple, complex, and hypercomplex. Since the main intention was to determine the

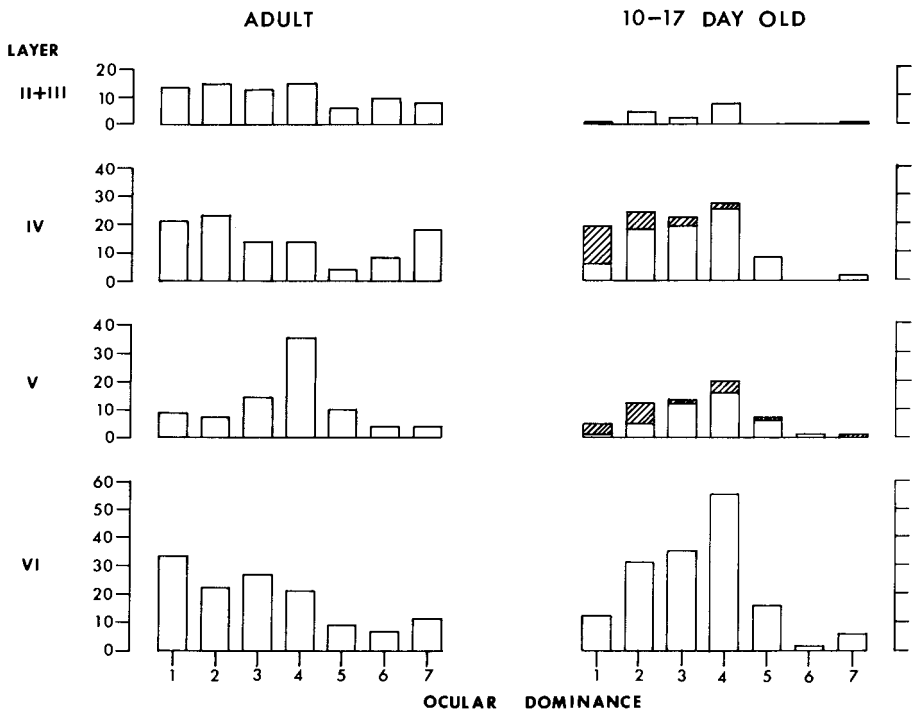


Fig. 11 Ocular dominance histograms for cells recorded in individual cortical laminae of adult cats (data from Shatz and Stryker, '78) and of young kittens (pooled results from 4 kittens aged 10-17 days). In all cases the laminar assignments are made from histological reconstructions of electrode tracks with marking lesions. For the kittens, cells encountered at a visual field eccentricity beyond about 30° are indicated by hatching: these cells were strongly dominated by the contralateral eye. Recordings from the adults were restricted to the central 15° of the visual field.

The histograms show that for all cortical layers the cells are on the average more binocular in young kittens than in adults. This difference is most marked for layers IV and VI.

ocular dominance of a large sample of cells, no attempt was made to study the response properties in detail.

We have not recorded extensively from animals at intermediate ages. In one 22-day-old animal, whose autoradiographic labelling pattern is shown in figure 5B, recordings clearly showed the beginnings of segregation according to eye preference. During one 200- μ m stretch of the traverse through the fourth layer, the influence of the contralateral eye was weak or absent, suggesting that some aggregation of the contralateral afferents had occurred, even though it was not detectable in the autoradiographs on account of spillover (fig. 7).

Another animal was recorded from at 35 days of age. Most cells in the fourth layer were nearly equally influenced by the two eyes. From the autoradiographic results for the 33- and 39-day-old kittens (fig. 7) we would have

expected a more advanced degree of segregation to be evident. This result suggests that there may be more variation in the time course of segregation than indicated by the data in figures 7 and 8. Alternatively the electrode may have travelled along the border between two existing columns; because we did not inject an eye in this animal we cannot resolve this issue.

The ocular dominance distribution of cells in layer IV of the 92-day-old cat was indistinguishable from that of adults, as might be expected from the autoradiographic labelling pattern in this animal (fig. 1) and the grain counts (fig. 7).

DISCUSSION

This study has shown that the geniculocortical afferents serving the two eyes are intermixed in the fourth layer of the newborn kitten's cortex, and separate out from each other

during the period between three and six weeks of age. Before segregation begins, cells in layer IV are more nearly equally influenced by the two eyes than in the adult, and they are not clustered into groups of common eye preference. These findings suggest that a functional reorganization of the cortex occurs during postnatal development. This developmental sequence is similar to that described for the monkey (Rakic, '76; Hubel et al., '77), the main difference being that in the monkey the process of segregation begins *in utero*, while in the cat its onset is delayed until the third postnatal week.

A major problem with the interpretation of the cortical autoradiographs has been posed by the phenomenon of spillover—the leakage of radioactivity from labelled retinal fibers and terminals, and its reuptake by the wrong set of geniculate neurons. We have concluded that spillover degrades the cortical labelling pattern by causing transport of significant amounts of radioactive material to the ocular dominance columns serving the non-injected eye.

This conclusion is based on the assumption that the radioactivity transported axonally by the two sets of geniculate neurons is in the same ratio as the labelling density of their cell nuclei. This would not be the case if a substantial fraction of the axonally transported material were to consist of large molecules specifically transferred at the retinogeniculate synapse, while the nuclear labelling reflected reutilization of breakdown products for the synthesis of new but sedentary proteins. There is no evidence at present, however, to suggest that a specific transsynaptic transfer of large molecules occurs, let alone contributes significantly to the axonally transported label (Specht and Grafstein, '73; Droz and Koenig, '73). It is more likely that the bulk of the radioactivity is transferred in the form of labelled metabolites, especially proline itself, which enter a common pool used for synthesis of both axonal and nuclear proteins.

We were reassured in this view by the results of the calculation relating spillover in the LGN to the cortical labelling pattern. It was striking, for example, that the actual cortical grain counts never significantly exceeded the limits (t_{\min} and t_{\max}) set by this assumption, as might well have been so if our measure of spillover had been irrelevant. On the contrary, where spillover was greater, the

fluctuations in grain density were correspondingly smaller.

Our analysis of the cortical labelling pattern takes no account of the possibility that label may be released from the geniculocortical terminals and move substantial distances in the cortex. Two observations make it seem unlikely that this is an important phenomenon for interpreting cortical grain counts. First, the very sharp fall-off in grain density at the border between layers IV and V in the adult animal (fig. 9) argues against widespread migration of label from the afferent terminals. Second, the examination of autoradiographs of the LGN shows that by far the largest portion of the radioactive label remains confined to the optic nerve fibers and their terminals (figs. 3A,F). This is true both for kittens and adults. If the release and movement of label occurs in the cortex to the same degree as in the LGN, it would produce so few silver grains as to have an insignificant effect on the measurements.

Another possible objection to the analysis presented here is that the measurements of spillover in the LGN were performed at a single time, namely eight days after eye injection. Since transport from the LGN to the cortex presumably occurs over a period of several days before sacrifice, only a series of measurements made during this period could form the basis for a theoretically rigorous determination of spillover. We base our belief that the use of a single survival time is satisfactory on our observation, both in cats and monkeys, that varying the survival time after eye injection affects only the intensity of the cortical labelling, not its pattern of distribution.

Adult organization

In adult cats, the difference in spillover between the two sides of the brain appeared to account quantitatively for the greater continuity of labelling in the contralateral cortex. Spillover in the LGN was consistently worse on the side contralateral to the eye injection, probably because of differences in the anatomical arrangement and innervation pattern of laminae A and A1, as described in the RESULTS. It seems now that the two sets of afferents are equally and virtually completely segregated at the centers of columns. In this respect our findings for the cat appear similar to those previously described for the monkey (Wiesel et al., '74). A possible difference between the species lies in the degree of overlap at the bor-

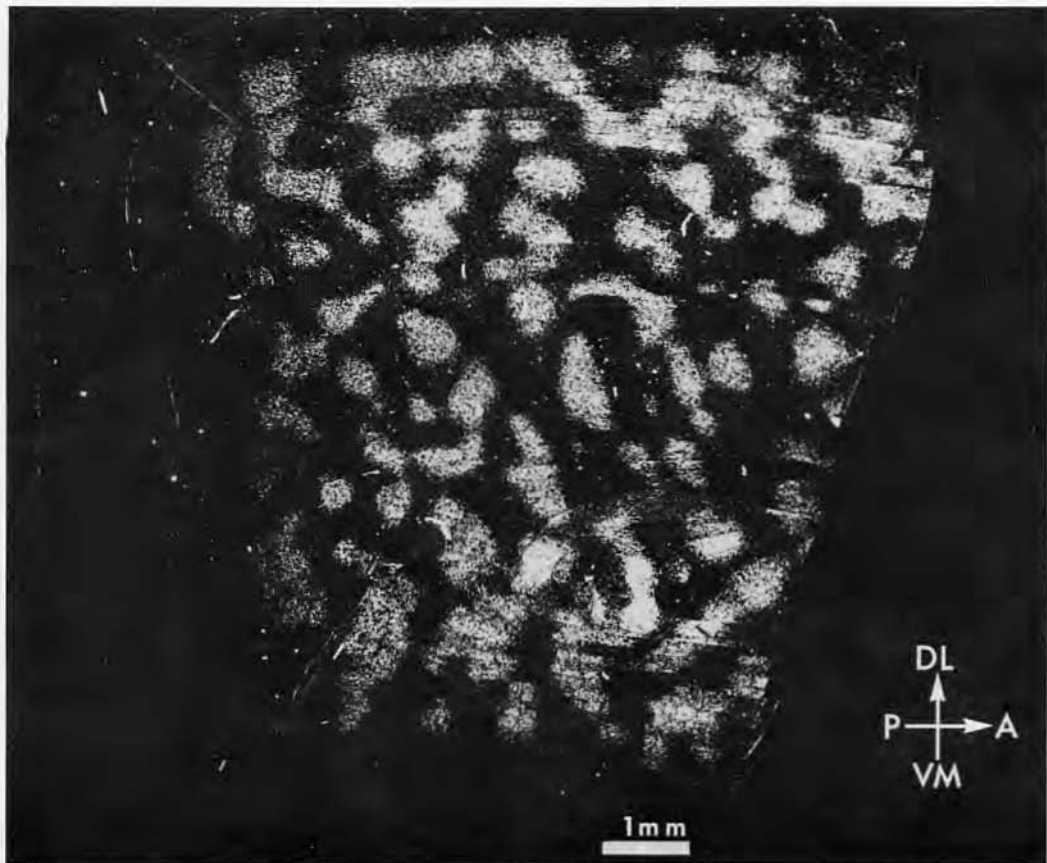


Fig. 12 Surface view of the pattern of ipsilateral-eye columns in layer IV of a normal adult cat. Montage prepared from autoradiographs of serial tangential (parasagittal) sections of area 17. The intersecting arrows indicate the antero-posterior and dorsolateral-ventromedial axes of the reconstruction. The upper edge of the figure is close to the border between areas 17 and 18; the lower edge is in the splenial sulcus.

ders of columns. In the cat the overlap must be extensive, since the grain counts have more the appearance of a sine wave than a square wave. This conclusion is supported by the physiological results from the fourth layer, since binocularly-activated cells are commonly encountered at the borders of columns (Shatz and Stryker, '78), while the transitions are more abrupt in the monkey (Hubel and Wiesel, '74; LeVay et al., '75).

If both sets of afferents are similar in their degree of segregation in the fourth layer, one is left with the question as to the anatomical basis for the reported physiological dominance of the contralateral eye. One possibility is that the columns for the contralateral eye are somewhat wider, on average, than those for the ipsilateral eye. Inspection of large num-

bers of horizontal and coronal sections has suggested that this is likely to be true, although we have not attempted to quantify this. Reconstruction of the columnar pattern from tangential (parasagittal) sections (Shatz et al., '77; Shatz and Stryker, '78) also suggest that the ocular dominance columns for the contralateral eye form a more confluent system, while those for the ipsilateral eye take the form of more isolated segments. This is particularly well shown in the reconstruction of figure 12, which is from the hemisphere ipsilateral to an eye injection in a normal cat. Here the ipsilateral eye columns clearly occupy less than half the available territory in the fourth layer (about 40%), and for the most part take the form of islands rather than bands. Not all animals show such a striking

fragmentation of the ipsilateral eye system, but there is on the whole a definite tendency in this direction.

In the monkey the two sets of columns are topologically similar: they are of the same width and show the same degree of branching and fragmentation (LeVay et al., '75). The rules postulated by those authors to govern columnar development in the monkey could also produce the pattern observed in the cat's cortex, however, if the numbers of geniculocortical afferents serving the two eyes were sufficiently unequal.

The cortex of the infant kitten

Previous studies have shown that the visual cortex of the young kitten is far from fully formed. At two to three weeks of age the average number of synapses per neuron is only about a third of that seen in the adult, and myelin has not yet appeared (Cragg, '72, '75). The responses of many cells to visual stimulation are sluggish and easily fatigued, as if the full complement of synaptic connections had not yet been established (Hubel and Wiesel, '63). In addition to this general immaturity, the present study gives evidence that at least the geniculocortical projection takes a different pattern from that seen in the adult. At this time left- and right-eye afferents appear to be completely intermingled in layer IV.

The measurement of spillover in the LGN has aided our interpretation of the labelling pattern seen in the cortex of the younger animals. Spillover is certainly more severe in the LGN of the younger animals, perhaps on account of the lack of myelination or the poor development of the interlaminar plexuses, but it is clear that on the ipsilateral side at least it is not sufficient to mask any columnar segregation in the cortex that might have been present. The physiological findings suggest that the geniculocortical afferents have formed at least some functional connections at this time and that these too are distributed in a continuous, unsegregated pattern in layer IV. One should, however, point out an alternative, perhaps unlikely, explanation of the physiological results. The greater binocularity of cells in the fourth layer, and the absence of grouping according to eye preference, could be due to intracortical connections which (for unknown reasons) become less significant later in development.

For the contralateral afferents, direct anatomical evidence about their distribution pat-

tern in the young animals is lacking, on account of the severe spillover in the LGN contralateral to the eye injection. For two reasons, however, it is probable that they, like the ipsilateral afferents, are uniformly distributed. First, the physiology in the 10- to 17-day-old kittens supports this notion, in that both eyes influenced cells uniformly throughout the fourth layer. Second, if one set of afferents were to aggregate while the other remained uniformly distributed, there would be periodic variations in total synaptic density, a possibility which seems intuitively unlikely. For similar reasons, we imagine that the segregation of the two sets of afferents proceeds simultaneously, even though spillover prevents our following the initial stages on the contralateral side.

Our physiological findings are not in agreement with those of Blakemore et al. ('75), who described, in a 9-day-old kitten, a columnar organization according to eye preference as marked as that seen in the adult. We do not know the reason for this discrepancy, but our results were consistent inasmuch as all four young animals studied showed the same high degree of binocularity.

The process of segregation

The transneuronal autoradiographic method has permitted us to study the changing distribution of the geniculocortical afferents as an entire population, but in terms of the growth and connections of individual axons a number of alternative possibilities may be considered. One possibility is that segregation in the strict sense does not occur at all; rather the original set of afferents remains intermixed, but is swamped by large numbers of later-arriving axons which form connections in the adult pattern. Such a hypothesis would entail no breakage of synaptic connections. This possibility seems somewhat unlikely, but cannot be excluded without quantitative electron microscopical studies.

We think it more likely that the formation of the adult pattern involves the actual breakage of synaptic connections in the inappropriate columns. If the arborization of each afferent axon were sufficiently large, such a process might not necessitate major movements of the parent trunk. Rather, the axon might maintain or increase its total complement of synaptic connections by replacing those lost in one column with new ones in another.

There is an intriguing parallel in the de-

velopment of the innervation of skeletal muscle. There is good evidence that several motoneurons originally innervate each muscle fiber and make physiologically demonstrable functional connections with it; the adult pattern is reached by a process of elimination (Redfern, '70; Brown et al., '76; Bagust et al., '73). This reduction is thought to take place principally by a retraction of many of the terminal branches of each motoneuron's axon (Korneliusson and Jansen, '76; Riley, '76).

Another example of segregation of originally overlapping projections is in the development of the retinogeniculate pathway of the monkey (Rakic, '76). In view of the complete restriction of afferents from each eye to three of the six geniculate laminae in the adult monkey, a loss of afferents from inappropriate regions must occur during development. Whether functional synaptic connections are formed early in these inappropriate regions remains to be determined. The breakage of functional connections has also been postulated to occur during development of the retinotectal pathway in some species (Gaze et al., '74; Scott and Lazar, '76; but see Jacobson, '77), but apparently does not occur in others (Crossland et al., '75).

Laminar organization

The radioactive label visualized in the transneuronal autoradiographs of the cortex of the adult cat seems to have been transported chiefly by afferents arising in laminae A and A1 of the LGN, for the laminar distribution is similar to that seen after injections of proline directly into these laminae (LeVay and Gilbert, '76). In the youngest kittens, on the other hand, the C laminae seem to make a substantial contribution to the labelling pattern, in that their target zones (layer I and the regions immediately above and below layer IV) are also labelled. The finding of a relatively heavy input to layer I in the younger kittens is consistent with results obtained with the Fink-Heimer method (Laemle et al., '72). The decrease in labelling density in this layer in older kittens might reflect an actual loss of connections—perhaps to the Cajal-Retzus neurons of layer I, which are thought to degenerate postnatally (Bradford et al., '77). Just as with the development of the columnar pattern, however, we cannot exclude the possibility that the reduction in labelling density in layer I is only relative; i.e., that this projection is swamped by a more massive and

later-developing projection to layer IV from the A laminae.

For the assessment of columnar segregation, cortical grain counts were performed in the lower part of layer IV (layer IVc). Although the labelling pattern in the sublaminae of layer IV appeared similar on visual inspection, the possibility exists that the extent of columnar segregation in the adult, and the time course of its development, may be slightly different between laminae IVab and IVc.

Relation to plasticity

The "critical period," the time during which cells in the visual cortex are susceptible to the effects of monocular deprivation, begins during the fourth week of life and ends by about three months of age (Wiesel and Hubel, '63; Hubel and Wiesel, '70). The onset of segregation of the cat's geniculocortical afferents during the third week of life thus slightly precedes or coincides with the onset of the critical period. The two eyes' afferents reach approximately the adult extent of segregation by six weeks of age, a time which precedes the end of the critical period by several weeks. Whether the critical period is the same for all cortical layers is unknown, however: it may end earlier in layer IV than elsewhere. In this context it will be useful to study the distribution of geniculocortical afferents in cats monocularly deprived at progressively later ages. This would enable us to examine in the cat the hypothesis which has been put forward for the monkey to explain the critical period in layer IV (Hubel et al., '77). The hypothesis is that monocular deprivation can produce changes in the distribution of the left- and right-eye afferents only in those regions of layer IV where they overlap, perhaps converging on single cells. Although this hypothesis may apply to the cat, it cannot provide the entire explanation for the end of the critical period, because some degree of overlap at the borders of columns, as judged from the presence of binocular cells and from the autoradiographic results, persists into adult life. This suggests the possibility that some other, as yet unknown factor may operate both to stop segregation short of completion and to end the susceptibility to monocular deprivation.

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