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Recovery from monocular deprivation in the monkey. III. Reversal of anatomical effects in the visual cortex

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[Plates 1-4]

Transneuronal autoradiography was used to study the effects of visual deprivation on the ocular dominance stripes in layer IVc of the striate cortex of *Erythrocebus patas* (Old World) monkeys. The animals were studied after: (a) 21-28 days of monocular deprivation starting at, or within, a few days of birth; (b) the same treatment followed by a further 3, 6, 15 or 126 days of monocular vision through the originally closed eye (reverse suturing), or followed by 15 or 96 days of vision through both eyes (reopening). One other monkey was monocularly deprived from birth to 189 days. In most cases the behaviour of the ocular dominance stripes formed by the initially closed eye was studied.

After 24 days of monocular deprivation from birth, the input from the normal eye was distributed uniformly within layer IVc, with no periodicity evident. After 21 days of deprivation, the deprived eye's input formed narrow stripes occupying about 38% of layer IVc in the operculum. Seven months of monocular deprivation reduced this to about 29%. Opening the closed eye after the deprivation produced no change in the area innervated: when periods of 15 or 96 days of binocular vision followed the deprivation, the areas innervated by the initially deprived eye were 26 and 30% respectively. However, in both cases the deprived eye's input formed blobs and spots, rather than uniformly narrow stripes.

In contrast to reopening, reverse suturing increased the fraction of layer IVc occupied by input from the initially deprived eye. In the operculum, the effects of reverse suturing appeared to be fully developed after only 6 days of reversal: the initially deprived eye's stripes having expanded to occupy about 50% of layer IVc. A further 9 days' reversal produced little change in this.

In the visual cortex in the calcarine fissure, the effect of the initial deprivation was more severe, and the expansion induced by reverse suturing more pronounced. The initial deprivation caused the stripes to shrink to occupy 24% of layer IVc; after 6 days of reverse suture the proportion increased to 52%, while after 15 days of reverse suture about 88% of IVc was occupied.

These results show that reverse suturing can cause fresh growth of afferent axons in regions of layer IVc from which they had been at least partially removed, either by the normal process of segregation, or as a consequence of monocular deprivation. Taken in conjunction with the

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findings of the accompanying two papers (Blakemore *et al.* 1981; Garey & Vital-Durand 1981) they also suggest (a) that there is a close relationship between the extent of layer IVc innervated and cell size in the lateral geniculate nucleus, and (b) that many of the physiological consequences of deprivation experiments are secondary to anatomical changes in the distribution of afferents from the lateral geniculate nucleus.

INTRODUCTION

When one eye of a young monkey or kitten is kept closed for a period of days or weeks, most neurons in the primary visual cortex lose their normal binocular responsiveness, and can be driven only by stimuli delivered *via* the non-deprived eye (see, for example: Wiesel & Hubel 1963; Hubel & Wiesel 1970; Baker *et al.* 1974; Crawford *et al.* 1975). This result can be explained in two ways: either cortical afferent synapses driven by the deprived eye become less numerous, or they remain present but become 'suppressed' and physiologically inactive (see Blakemore *et al.* 1978). There is good evidence that a long period of monocular deprivation results in a loss of synapses driven by the deprived eye. This can be shown because layer IVc of the cortex is divided into striped regions separately innervated by the two eyes (Hubel & Wiesel 1972; Wiesel *et al.* 1974; LeVay *et al.* 1975; Shatz *et al.* 1977). Normally, left and right eye stripes are of equal width, about 350 μm . However, after 3 or more weeks of monocular deprivation, the deprived eye stripes are about half their normal width, while those from the normal eye become broader (Hubel *et al.* 1977; LeVay *et al.* 1980). For long periods of deprivation it is possible therefore to account for the physiological changes in responsiveness of cortical neurons as the result of anatomical changes, without needing to invoke the extra hypothesis of synaptic suppression. However, as little as one day of monocular deprivation in the kitten (Olson & Freeman 1975; Movshon & Dürsteler 1977) may alter a neuron's responsiveness (although the effects are only just detectable) and it is not known whether synaptic numbers could change rapidly enough to account for this.

Further questions about the mechanism of monocular deprivation are raised by a consideration of the way in which the pattern of eye dominance stripes develops. At, or before birth, in both cat and monkey, inputs from both eyes are spread throughout layer IVc (Rakic 1976, 1977; LeVay *et al.* 1978). In the following weeks, inputs gradually disappear from some areas, and remain in others, so that by 10–12 weeks in the cat, and possibly somewhat earlier in the monkey, the adult pattern of alternating stripes is formed. The process of segregation has been explained by a mechanism involving competition, where inputs from one eye, if locally more numerous, cause rejection of inputs from the other eye (Hubel *et al.* 1977). This hypothesis makes it possible to explain the effect of monocular deprivation in terms of a perturbation of a developmental process that would otherwise result in the formation of stripes of equal width for the two eyes (Swindale 1980). The simplest way to explain changes in stripe width on this basis would be in terms of an increased rejection of deprived eye terminals at the edges of a presumptive stripe. However, experiments involving reverse suturing (opening the originally closed eye, and closing the other one) show that eye dominance

stripes, which appear on the basis of physiological recording to be shrunken, can re-expand after a period of reverse suturing to a normal, or even greater than normal, width (Blakemore & Van Sluyters 1974; Movshon & Blakemore 1974; Movshon 1976; Blakemore *et al.* 1978; Blakemore *et al.* 1981). Unless the initial deprivation has simply left regions of cortex with functionally inactive terminals, which can be reactivated by reverse suturing, these results can only be explained by supposing that afferents from the initially deprived laminae of the lateral geniculate nucleus may regenerate synapses in regions of cortex from which they had previously been removed by the initial deprivation. This hypothesis would therefore imply that the competitive process at work in the cortex allows the formation of new connections, as well as the removal of pre-existing ones.

To see whether short periods of deprivation can cause anatomical shrinkage of ocular dominance stripes, and whether reverse suturing can subsequently induce their expansion, we used the technique of transneuronal autoradiography (Wiesel *et al.* 1974) to study the stripes in young monkeys given an initial 21–28 days of monocular deprivation, starting shortly after birth, and followed by various periods of reverse suturing, or control periods of reopening of both eyes. The results show that columns are shrunken after as little as 3 weeks of deprivation, and can expand significantly within a week of starting reverse suturing. This does not take place if the closed eye is simply reopened. Corresponding physiological changes in ocular dominance also occur over the same time scale (Blakemore *et al.* 1981). These findings are in broad agreement with those recently reported by LeVay *et al.* (1980).

METHODS

Nine juvenile *Erythrocebus patas* (Old World) monkeys of both sexes were used. Details of the experimental procedures are summarized in figure 1. Eight of the monkeys had their right eyelids sutured together on the day of birth, or shortly afterwards, for periods of 21–28 days. Two animals were killed at this stage. In the remaining six, the right eye was then opened; in four of these the left eye was sutured shut for periods of 3, 6, 15 and 126 days; the other two animals were allowed normal vision through both eyes for periods of 15 and 96 days before electrophysiological recording and sacrifice. One other monkey was monocularly deprived from birth until 189 days of age. At times that varied from 11 to 19 days before death (figure 1), one eye (usually the right eye) was injected with a mixture of 1–1.5 mCi of [³H]proline (specific activity 117 Ci/mmol) and 1–1.5 mCi of [³H]fucose (specific activity 26 Ci/mmol) in 100 µl of 0.9% saline.

At the termination of electrophysiological recording experiments that lasted 1–2 days (see Blakemore *et al.* (1981) for details) the monkeys were perfused through the heart with buffered saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and left in the fixative for a further 12–24 hours, and then stored in 0.1 M phosphate buffer, sometimes for several weeks, before further processing. Blocks for autoradiography were placed in 30% sucrose until they sank, and frozen sections tangential to the flat surface of the operculum were cut at 30 µm. Sections were mounted on chrome-gelatin-coated slides, defatted in xylene, and coated with Ilford K5 emulsion, by means of the loop technique (Jenkins 1972). After exposure periods of 2–3½ months, slides were

developed in D19 (Kodak) for 3 min, fixed in hypo, and mounted in DPX, normally without counterstaining.

Photomontage reconstructions of the pattern of label in IVc were made from series of photographs taken with dark field illumination. Measurements of the area of IVc occupied by the labelled afferents were made by tracing round the outlines of labelled regions on tracing paper, and then cutting out the areas and weighing them. The results were expressed as a percentage of the total area (also measured by weighing) of layer IVc included in the measurements. To gain some idea of the accuracy of the results, a second set of tracings was made after an interval, and these measurements differed, on average, by less than 5% of the original values. The average of both sets of measurements was used for analysis.

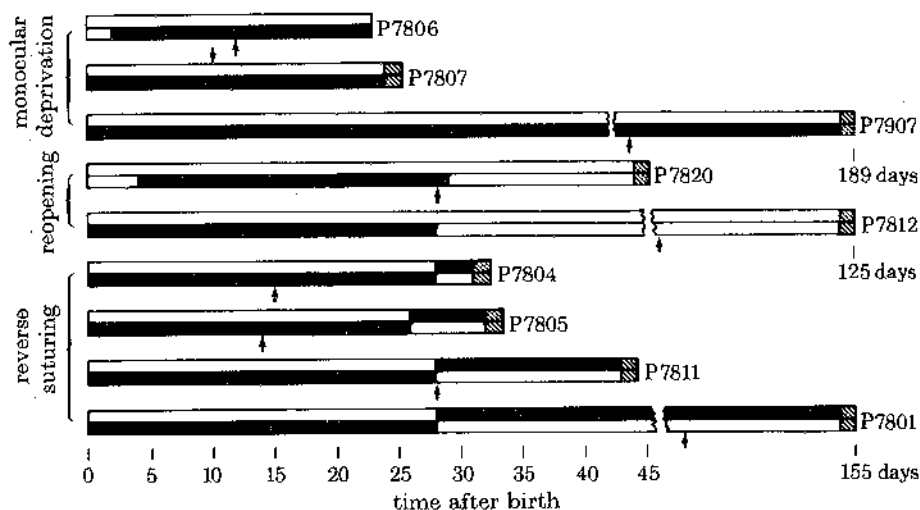


FIGURE 1. The periods of eye closure received by the animals used in this study. Each animal is represented by a pair of horizontal bars, the upper for the left, the lower for the right eye, which are shaded black when the eyes are closed. As most of the animals were recorded from electrophysiologically, there was a period of about a day of binocular visual stimulation at the end of each treatment, indicated by hatching. The times of the eye injections are also indicated by arrows placed next to the appropriate bars.

RESULTS

Satisfactory autoradiographs were obtained from all but one of the monkeys (P7801, reverse sutured for 126 days). The best results were generally obtained from the animals allowed more than 15 days' survival after injection. In three animals, good results were obtained from only one hemisphere (P7804, P7806 and P7811); but in the others no significant differences in the behaviour of the left and right hemispheres were noted.

In some animals, autoradiographs were made of sections of tissue from the part of striate cortex buried in the calcarine fissure underneath the operculum. In this region, labelling was more intense than in the operculum, where the central few degrees of visual field are represented. A similar observation has been made by

others (see, for example: Kaas *et al.* 1976; Hendrickson *et al.* 1978; Spatz 1979).

The pattern of labelling in striate cortex produced by eye injections in normal *Erythrocebus* monkeys has been described by Hendrickson *et al.* (1978) and is similar in all important respects to that in *Macaca* and other Old World monkeys: label is mainly present in layer IVc in the form of stripes with a periodicity of about 800 μm , and in lesser amounts in layers IVa and VI. The present results are in agreement with this.

Monocular deprivation

Two monkeys were deprived of vision in one eye between the ages of 2 and 23 days (P7806), and from birth until 24 days (P7807). The former was injected in the deprived eye and the latter in the experienced eye. There was a marked difference in the pattern of input from the deprived and the normal eyes (figures 2, 3, plate 1). Input from the normal eye was uniformly heavy throughout layer IVc, with hardly any evidence of periodicity, or of gaps in the label. The deprived eye's input however was clearly periodic, and formed narrow stripes which were estimated to occupy about 38% of IVc in the operculum, and about 24% of IVc in the calcarine cortex (figure 8, plate 3). Though no data from normal animals of similar age are available for comparison, it seems clear that 3-4 weeks of monocular deprivation has had a substantial effect on the distribution of both normal and deprived inputs to layer IVc. In the monkey deprived from birth until 189 days of age (P7907), the shrinkage was more marked, the stripes occupying about 29% of IVc in the operculum (figure 6, plate 2). No data were obtained from the calcarine cortex in this animal.

Reopening

Two monkeys were allowed 15 and 96 days of vision through both eyes, following monocular deprivation from 4 to 29 days of age, and from birth to 28 days respectively. In both cases the deprived eye was injected. Figures 4, 5 and 7 show the results obtained: in both animals the period of binocular vision has done little or nothing to reverse the effects of the initial deprivation; the proportions of layer IVc occupied by patches of label being about 26 and 30% for 15 and 96 days of reopening respectively. The results suggest, however, that reopening may have changed the pattern of labelling from one of stripes to blobs. This difference might reflect individual variation between monkeys, although both monkeys show the same sort of change, and it is more severe after the longer periods of reopening. In figures 4 and 5, plate 2, the label forms spots, many of which have the same width as that expected of a normal stripe, and which are connected by narrow weak streaks of label. After 96 days of reopening (figure 7, plate 2) stripes are almost absent, and the label forms irregularly shaped blobs with a poorly defined overall periodicity. This may be the result of the elimination, during continued binocular vision, of the weaker streaks of label between the blobs, which have enlarged, perhaps to compensate for this loss.

Reverse suturing

After periods of 26–28 days of deprivation beginning on the day of birth, four monkeys received 3, 6, 15 or 126 days of monocular visual experience through the originally deprived eye. For unknown reasons the level of label in the cortex of the animal that received 126 days of reversal was exceptionally low, and this animal is thus omitted from most of the presentation of results. All the animals received injections in the originally deprived eye, since the intention was to see whether shrunken stripes could expand.

In calcarine cortex, where the level of label was high, and the pattern of stripes distinct, the effects of reverse suturing were pronounced (figures 9, 10, plate 3). After 6 days of reversal the area of layer IVc occupied by the originally deprived eye had increased from 24 to 52%, while a further 9 days of reversal increased this figure to around 88%. Figures 8 and 10 also show regions that from their shape and position in the cortex are presumed to correspond with the location in the visual field of the blind spot in the uninjected (contralateral) eye (figure 8) and the location of the blind spot in the injected (ipsilateral) eye (figure 10). These are

DESCRIPTION OF PLATE 1

FIGURE 2. P7806: montage of dark field autoradiographs made from sections cut tangential to the operculum of the right hemisphere of a 23 day old monkey that was monocularly deprived for 21 days. The deprived eye was injected. The label forms narrow stripes which were estimated to occupy at most 38% of layer IVc. (Compare this with figure 8, which shows the distribution of label in the underlying calcarine cortex of the same animal.) In this and subsequent figures the scale bar represents 1 mm.

FIGURE 3. P7807: a single section through the operculum of the right hemisphere of a 25 day old monkey monocularly deprived since birth, and whose open eye was injected. Label is present throughout layer IVc, which appears in this section as two connected rings because of the uneven curvature of the cortex. The two small regions free of label near the centre and to the right of the picture are artefacts.

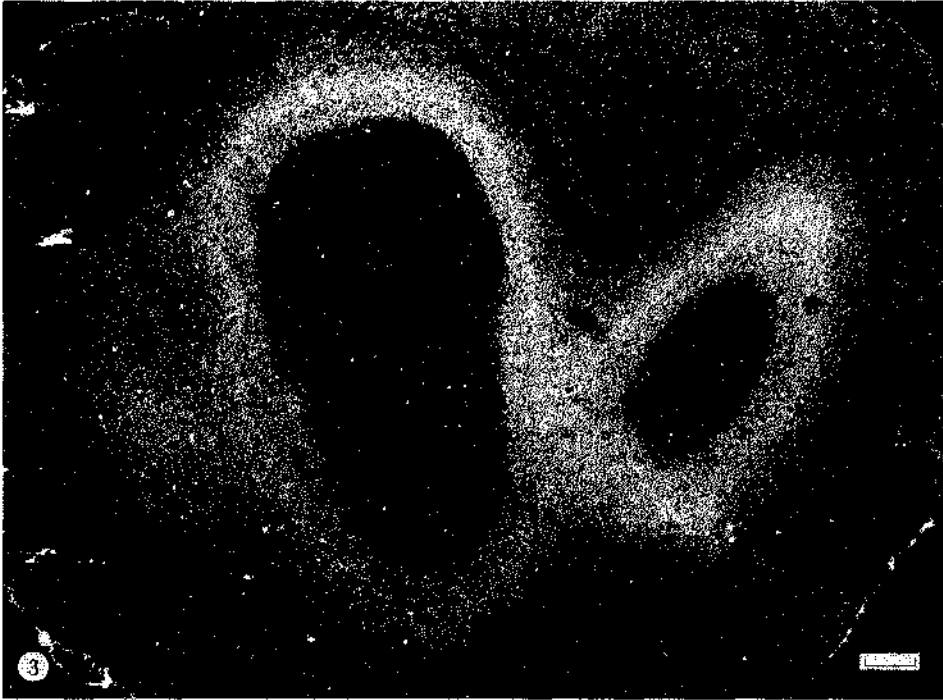
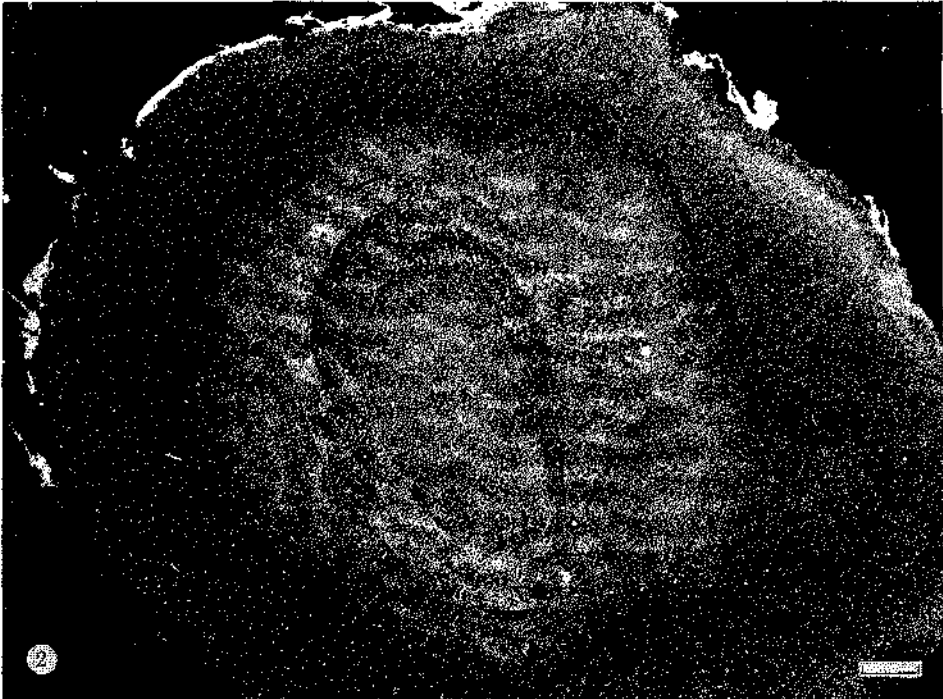
DESCRIPTION OF PLATE 2

FIGURE 4. P7820, left hemisphere: montage of autoradiographs from the operculum of an animal whose right eye was closed for 25 days, and then reopened for 15 days. The right eye was injected. The label forms elongated blobs connected by narrower weak streaks of label.

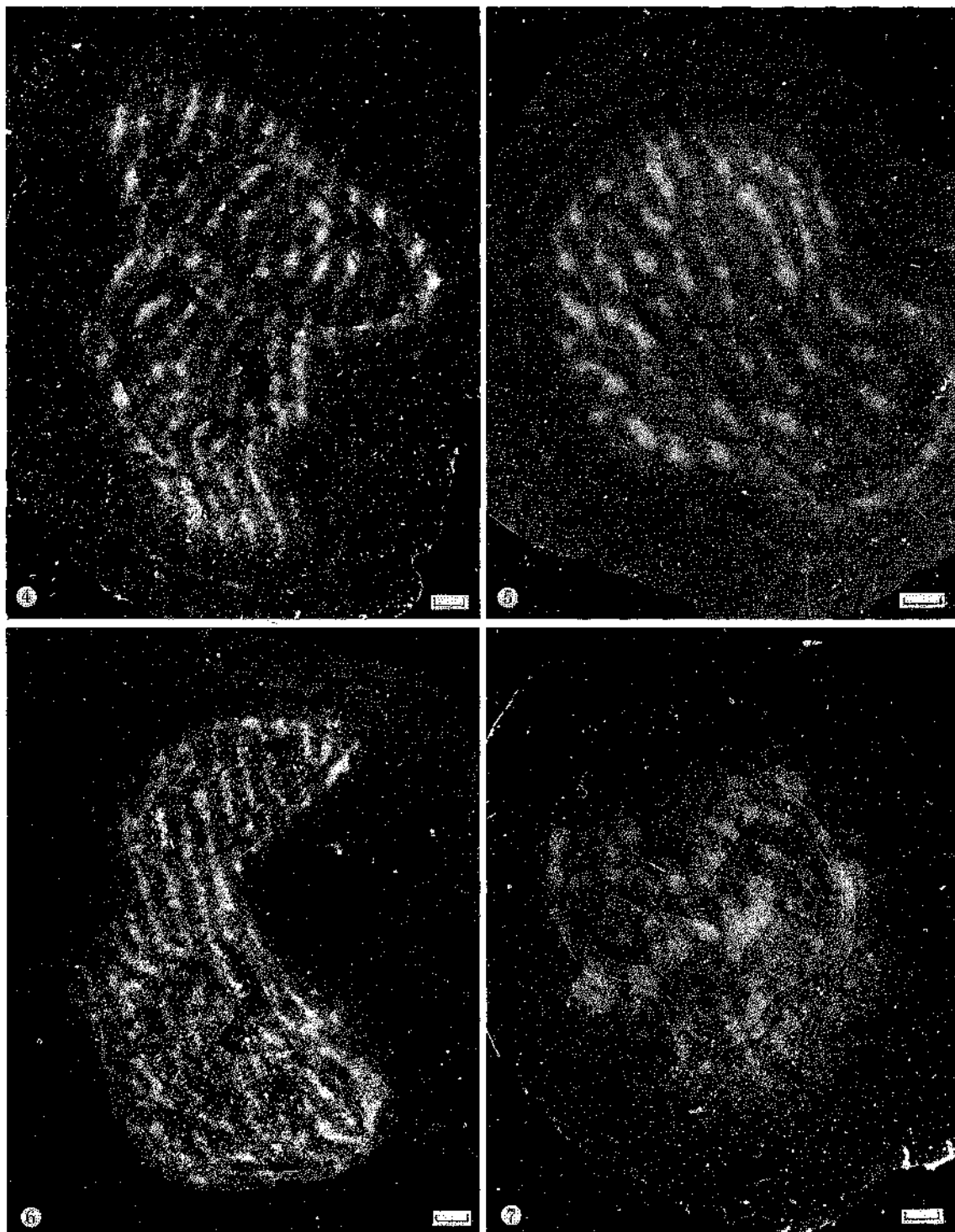
FIGURE 5. P7820, right hemisphere, showing a pattern of labelling similar to that observed in the left hemisphere. About halfway between the edge of the label in layer IVc and the edge of the section is a thin streak of label in layer IVa. The label in this layer was particularly strong in this animal, and in other sections the 'honeycomb' pattern described by Hedrickson *et al.* (1978) could be seen.

FIGURE 6. P7907, right hemisphere: reconstruction from serial sections through the operculum of a monkey monocularly deprived from birth until 189 days of age. The deprived eye was injected. The obscurity of the labelling in the lower part of the figure is probably artefactual.

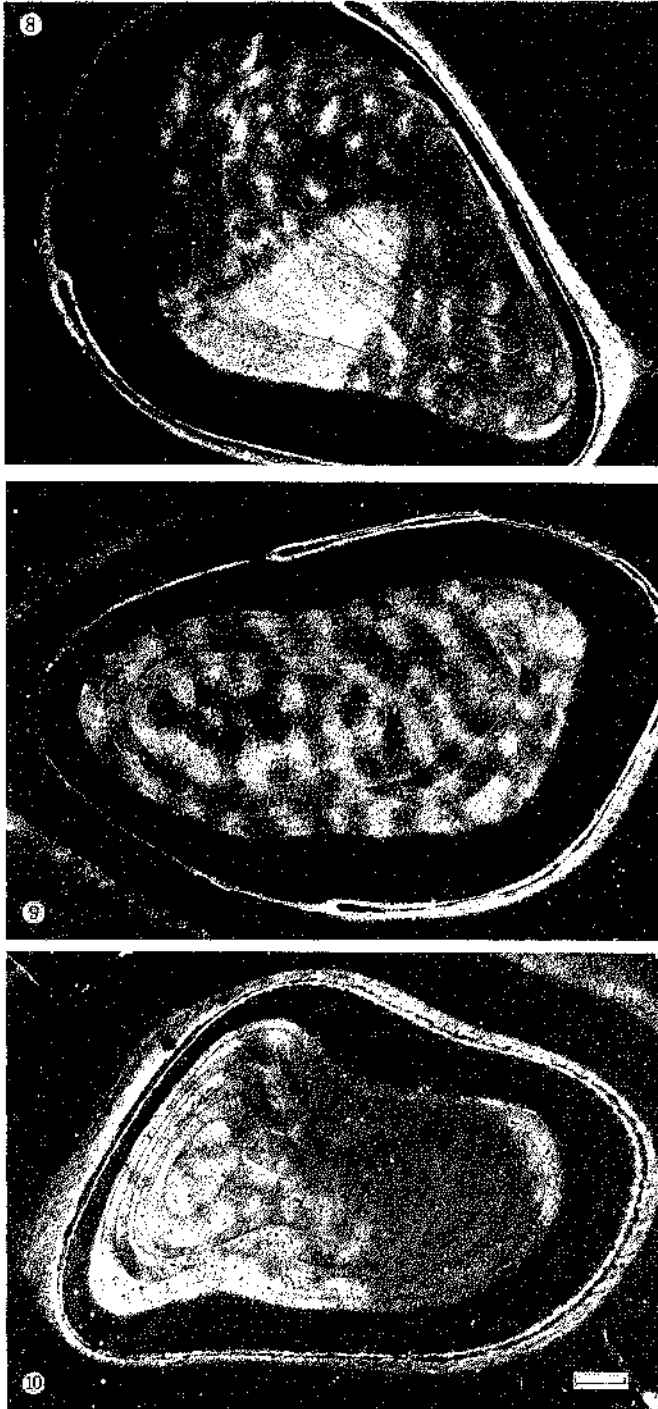
FIGURE 7. P7812, right hemisphere: this animal was monocularly deprived from birth until 4 weeks of age, and then allowed vision through both eyes for a further 96 days. The previously closed eye was injected. The distribution of label is quite abnormal, and takes the form of blobs and spots of varying size. Many of these have a diameter greater than that expected of a normal stripe. Overall, the proportion of layer IVc occupied remains less than normal.



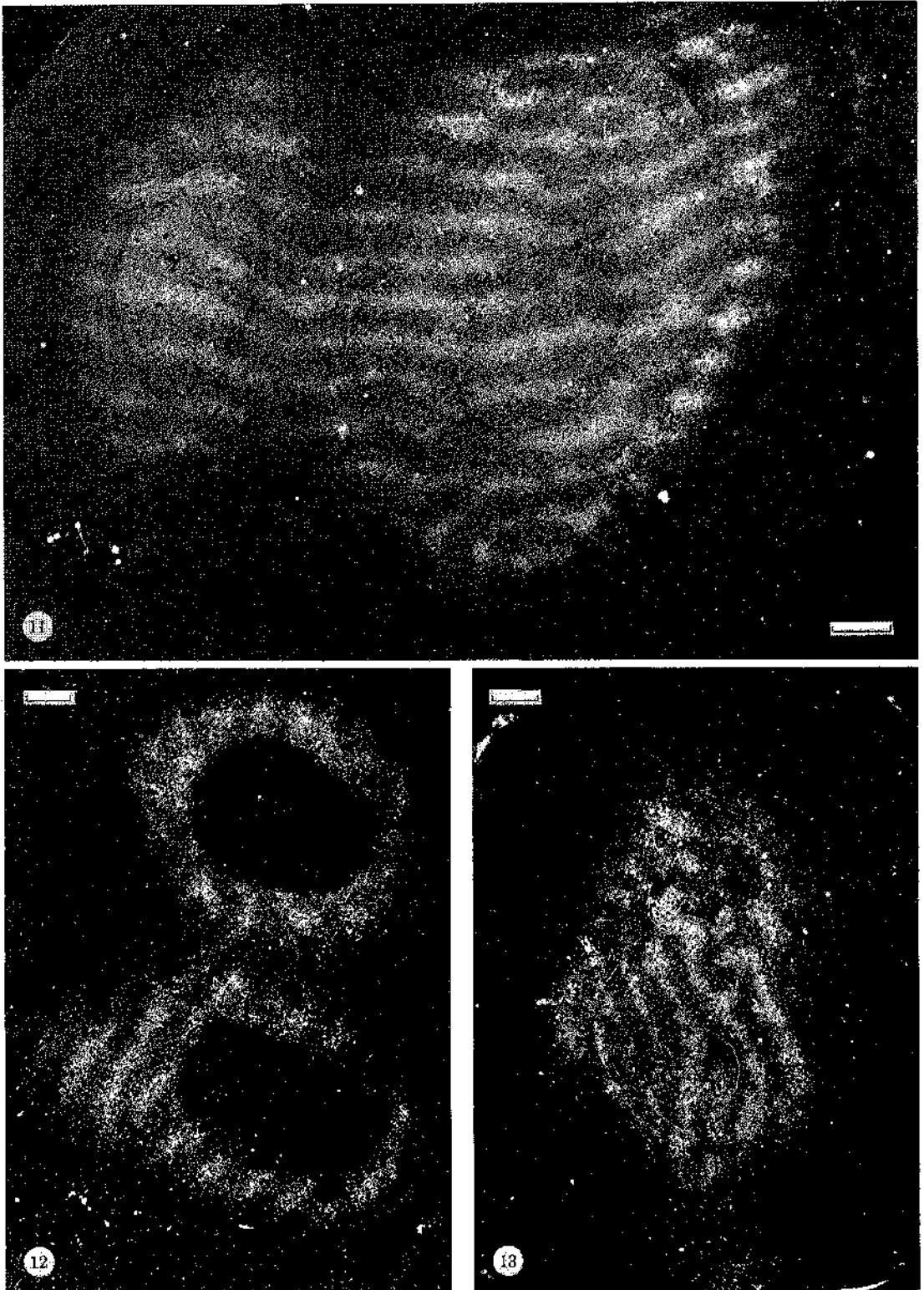
FIGURES 2 AND 3. For description see opposite.



FIGURES 4-7. For description see page 440.



FIGURES 8-10. For description see page 441.



FIGURES 11-13. For description see opposite.

characterized, as one might expect, by a continuous distribution of label in the former case, and a low level of label in the latter. Probably all of this low level can be ascribed to spill over in the lateral geniculate nucleus (i.e. leakage of label into geniculate laminae not innervated by the injected eye). Because the level is so low it seems unlikely that variations in spillover induced by visual stimulation could provide an explanation for the changes in stripe width observed in this animal, or the others.

Measurements of stripe area in the operculum (from the photographs reproduced in figures 11–13, plate 4), though made from only small areas of tissue, also showed that reverse suturing led to the occupation of an increasing portion of layer IVc by the initially deprived eye. However, both the rate and extent of the reversal were less than that in the calcarine cortex. After 6 days of reversal the stripes appeared normal in width and morphology, occupying about 50% of layer IVc. A further 9 days of reversal produced little change in this. What little could be seen in the autoradiographs from the animal with 126 days reversal also suggested that stripes of about normal width and periodicity were present in the operculum. Thus, in the region of cortex where central visual field is represented, it appears

DESCRIPTION OF PLATE 3

FIGURE 8. P7806, right hemisphere, showing the effect of 21 days' monocular deprivation on the distribution of input from the deprived eye in the calcarine cortex of the animal shown in figure 2. The label forms narrow short stripes and spots. The large uniformly labelled area is presumed to correspond to the blind spot in the non-injected eye. Because the calcarine cortex is folded immediately underneath the convexly curved operculum, its curvature is concave, and as a result white matter appears as a heavily labelled ring outside the reconstructed portion of layer IVc.

FIGURE 9. P7805, the right calcarine cortex. This monkey was monocularly deprived for a slightly longer period than the animal shown in the preceding figure, and this was followed by 6 days of monocular vision through the originally closed eye. The input from the initially deprived eye forms a pattern of stripes similar to that expected had neither eye been closed.

FIGURE 10. P7811, the left calcarine cortex. An initial 28 days of monocular deprivation was followed by 15 days of monocular vision through the originally closed eye. The input from this eye is now spread through nearly the entire extent of layer IVc, with the exception of a large lightly labelled region, which is presumed to correspond to the blind spot in the injected (contralateral) eye.

DESCRIPTION OF PLATE 4

FIGURE 11. P7804: reconstruction from serial sections through layer IVc of the right operculum of an animal monocularly deprived for 28 days and subsequently allowed 3 days' monocular vision through the other eye. The initially closed eye was injected with label. Compare with the results shown in figure 2 from an animal monocularly deprived for 21 days.

FIGURE 12. P7805: a single section through the left operculum of a monkey reverse sutured for 6 days following 26 days of monocular deprivation, showing the distribution of inputs from the originally closed eye to layer IVc. As in figure 3, this layer appears in two rings because of the uneven curvature of the cortex.

FIGURE 13. P7811: reconstruction from serial sections through layer IVc of the left operculum of a monkey reverse sutured for 15 days following 28 days of monocular deprivation. Compare this with figure 10, which shows the distribution of label from the originally closed eye in the calcarine cortex from the same hemisphere of this animal.

that reverse suturing can do no more than restore approximate equality to the two eyes' inputs.

No differences in the response of layers IV α and IV β to reverse suturing were observed, in contrast to the observations of LeVay *et al.* (1980) on macaque monkeys reverse sutured at 4 weeks of age.

DISCUSSION

Interpretation of autoradiographs

A number of problems can complicate the interpretation of autoradiographs, and these are likely to be particularly severe in young monkeys. They include: spillover of label into inappropriate geniculate laminae; the fact that segregation of terminals in the cortex may not be complete, so that the boundaries of the stripes may not be sharply defined; and the effect of both the initial exposure time of the autoradiographs and the use of photographic techniques which may introduce a nonlinear relation between the amount of label in the tissue and the resulting intensity in a photographic image.

It has been shown that spillover is more severe in young kittens than in adult cats (LeVay *et al.* 1978), and the same may be true of the young monkeys used in the present study. Normally, spillover results in a uniform and light distribution of label in the gaps between the injected eye's stripes. Where the boundaries of the stripes are well defined, as they are in normal macaques of 6 or more weeks of age (LeVay *et al.* 1980), the presence of even a high level of spillover should not influence estimates of stripe area. If stripe boundaries are less well defined, as they might be in younger monkeys, then a high level of spillover might cause an overestimation of stripe area. Two observations suggest this was not the case in the present study. First, when the level of labelling was high, the boundaries of the stripes were usually well defined, which suggests that segregation is normally well advanced in *Erythrocebus* monkeys at 4 or more weeks of age. Secondly, examination of the blind spot representation in one animal (figure 10), where most of the label present could be ascribed to spillover, showed that the level was low. With the exception of the monkeys that were reverse sutured for 3 and 6 days the level of label in the gaps between the stripes was also relatively low.

The exposure times of the autoradiographs, which varied from 2 to 3½ months, were such that the response of the emulsion should have been a nearly linear function of the amount of radioactivity present (Rogers 1973). The subsequent photographic procedures were chosen to maximize contrast by using high contrast film, developer, and photographic paper. With such procedures misjudged exposures easily lead to loss of detail through over- or under-saturation of the image, and particular care was taken therefore to ensure that visual impressions of the final photographs always closely corresponded with the appearance of the autoradiographs themselves.

Monocular deprivation

The results from the animal monocularly deprived from birth until 24 days (P7807, figure 3), and whose normal eye was injected, showed an almost uniform

distribution of label in layer IV c, with no convincing indication of any periodicity. Despite the uniformity of label, electrophysiological recording (Blakemore *et al.* 1981) from the same animal, as well as the evidence from the monkey whose deprived eye was injected (figure 2), both suggested that input from the deprived eye was still present, in the form of narrow stripes. This suggests that the two eyes' inputs were not distributed in an exactly complementary fashion. However, there might be fluctuations in intensity sufficient to accommodate a complementary input from the deprived eye, but not large enough to be apparent as small depressions in an otherwise high overall level of label.

A greater sensitivity to deprivation in calcarine as opposed to opercular cortex has not been reported before in monkeys. This suggests either that the calcarine cortex is simply more sensitive to the effects of deprivation, or that the critical period for the operculum is already nearing its end in the fourth week of age. If the timing of the critical period is linked to the process of segregation as has been suggested (Hubel *et al.* 1977; Swindale 1980) then the greater susceptibility of the calcarine cortex to deprivation would imply that segregation is less advanced in this region at 3 weeks than in the operculum. The autoradiography gives no indication of this, but comparisons are difficult to make given the substantial differences in the overall level of label in the two areas.

Reopening

During the period of 15 days of binocular vision following 4 weeks of monocular deprivation that P7820 received, the pattern of labelling appeared to change from one of narrow stripes to shorter spots of nearly normal width, with the total proportion of IV c innervated remaining about the same. A still more exaggerated change of the same sort seems to have occurred after 96 days of reopening (P7812, figure 7). The result is surprising, since one would not expect a period of binocular vision to cause an apparent deterioration in columnar structure. One possible explanation for this is that the monkeys were strabismic after the deprivation. This possibility has been suggested for cats in similar circumstances (Olson & Freeman 1978), and is also a frequent secondary complication of occlusion amblyopia in humans. The effect of strabismus on columnar structure in monkeys is unknown, but in otherwise normal cats it seems to have little effect beyond sharpening column boundaries (Shatz *et al.* 1977). The effect of strabismus following deprivation, however, might well be different.

Reverse suturing

The main results of the reverse suturing experiments are summarized in figure 14, which plots the percentage of layer IV c occupied by afferents from the initially deprived eye as a function of time after the beginning of reversal. The figure shows that in all three monkeys that underwent deprivation alone (in two followed by binocular vision) the percentage area of IV c occupied was low (23–30%) and substantially less than the normal value of around 50%. The area occupied however was greater than 40% in all three animals that underwent reverse suturing. Although the length of the initial period of deprivation varied from 22

to 28 days (see figure 1 for details), this cannot account for the differences between the reverse sutured animals and the others. All of the reverse sutured animals were initially deprived for 5–7 days longer than the animal (P7806) that underwent deprivation alone: so the effects of the initial deprivation should have been more severe in these animals.

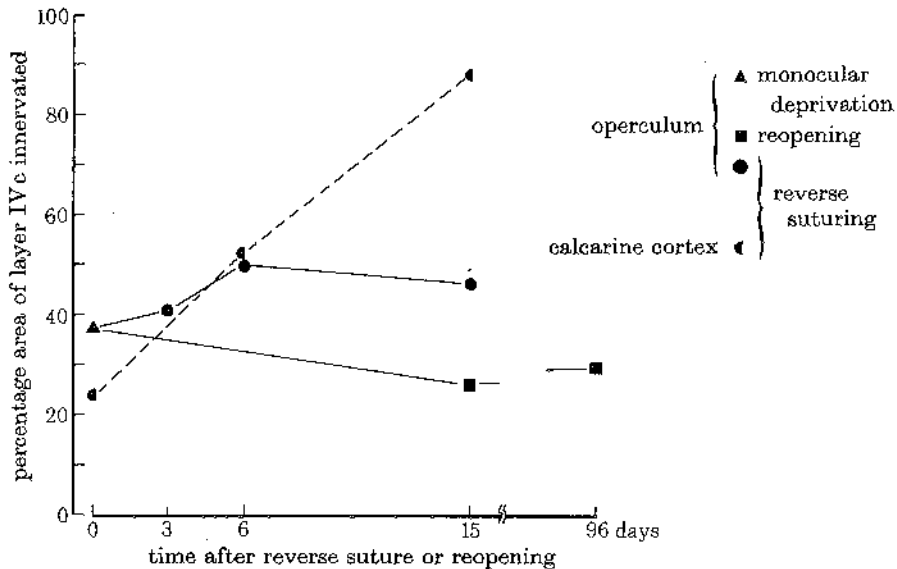


FIGURE 14. The percentage area of layer IVc innervated by the originally closed eye as a function of time after the end of the initial deprivation and the beginning of reverse suturing or reopening.

Two other points are made by figure 14: in the operculum the effects of reversal appeared to be complete by 6 days, and did not do more than restore approximate equality to the two eyes' input, while in the calcarine cortex the rate of change was greater and more prolonged, with input from the initially deprived eye occupying more than two-thirds of the area of IVc after 15 days of reversal. These results therefore, like those of the monocular deprivation experiments, suggest that the operculum is less sensitive to deprivation, either because of a difference in absolute sensitivity, or because of an earlier end to its critical period.

No difference in the response of layers IVc α and IVc β to reverse suturing was observed, although it was noticeable in most of the monkeys used in this study, including the reverse-sutured ones, that the stripes appeared to narrow as label decreased in intensity in the upper parts of layer IVc. (For this reason the uppermost parts of this layer were usually not included in the reconstructions.) However, this slight difference between upper and lower IVc was clearly different from that observed by LeVay *et al.* (1980) in macaque monkeys also reverse sutured at 4 weeks of age (though for longer periods). In these animals the re-expansion produced by reverse suturing was restricted to layer IVc β , producing a marked difference in stripe width in the two sublaminae. One reason for this difference could be that in macaques, but not *Erythrocebus*, the critical period for changes in ocular

dominance column size in layer IV α is already over by 4 weeks. Thus the macaque's visual system may be developmentally more advanced than that of *Erythrocebus* at this age. However, it remains to be seen whether a difference between the two layers would show up in *Erythrocebus* if reverse suturing was begun later.

The finding that shrunken stripes can expand, so that synapses reappear in regions of cortex from which previously they appeared to be absent, may seem to pose problems for theories that link the end of the critical period to the completion of segregation and the final disappearance of left eye inputs from the right eye regions of cortex and vice versa (Hubel *et al.* 1977; Swindale 1980). These theories explain the stability of stripe width in animals more than a few months old in terms of a supposed inability of axons to sprout, or to regenerate synapses in a region from which they have completely disappeared. However, it is reasonable to suppose that the autoradiographic method would not indicate the presence of a low density of synapses, or of fine axon branches lacking synapses, but still capable of forming new ones. (The technique for example gives no indication of the existence of geniculate fibres passing through layer V *en route* to layer IV.) It is perhaps only when these fine branches have disappeared, an event that would pass undetected by autoradiography, that a region of cortex would become immune to reinnervation by a previously rejected input.

The data from the calcarine cortex allow one to put an approximate lower limit on the maximum rate at which cortical synapses can be formed by geniculate afferents in response to reverse suturing. To express this in terms of the rate at which each afferent axon forms synapses, one needs to know (a) the rate of expansion of the stripes, (b) the average density of geniculate synapses per unit area of layer IVc and (c) the number of incoming geniculate afferents per unit area of layer IVc. The measurements made on figures 8 and 9 showed that the area of cortex innervated by one set of afferents can increase from 24 to 52% in 6 days. The density of synapses in monkey visual cortex is $6 \times 10^8/\text{mm}^3$, and shows little variation with depth in the cortex (Cragg 1967). Layer IVc is about 0.2 mm thick, and, as data from the cat suggest (LeVay & Gilbert 1976), geniculate afferents probably form about 30% of the synapses there. The number of geniculate synapses per square millimetre of layer IVc is therefore 3.6×10^7 , and the number of new synapses formed in six days is 28% of this, or $10^7/\text{mm}^2$. The total number of cells in the lateral geniculate nucleus is 1.1×10^6 (Chow *et al.* 1950) and nearly all of these project to the striate cortex (Norden 1974; Winfield *et al.* 1975; Ogren & Hendrickson 1976). The density of geniculate innervation of the cortex is probably not uniform, but increases in more peripheral regions of the visual field (Malpeli & Baker 1975). However, taking the findings of Malpeli & Baker into account, one can calculate that in the region of the cortex close to the blind spot (from which the measurements of stripe area come) there are about 1.4×10^3 incoming axons per square millimetre of cortex. This is close to Clark's (1941) estimate of 1350/ mm^2 based on counts of degenerating geniculate cells following cortical lesions of known area. As only half of these axons will be involved in forming the new synapses (and possibly fewer if not all are involved in the process of re-expansion), the total number formed in 6 days will be at least 1.4×10^4 per

axon, which is a rate of about 100/h per axon. It is difficult to assess the physiological significance of such a rate without knowing both the time required for individual synapse formation and how many new synapses need to be formed before the responsiveness of a cell is altered. However, if the time required is of the order of a few hours, then changes in the numbers of left and right eye synapses contacting individual cells could plausibly explain some of the very rapid changes in binocularity that can follow periods of deprivation lasting only a few hours (Peck & Blakemore 1975; Schechter & Murphy 1976; Olson & Freeman 1975; Movshon & Dürsteler 1977).

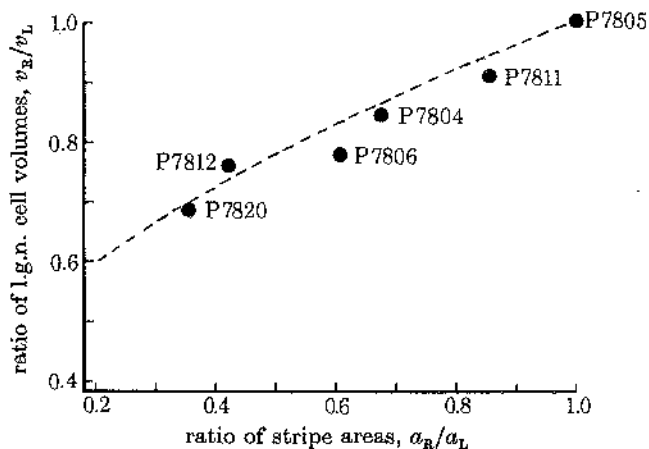


FIGURE 15. The relation between cell size changes in the lateral geniculate nucleus (Garey & Vital-Durand 1981) and the areas of cortex innervated by the cells. The vertical axis shows the ratio between the average volumes, v_R and v_L , of the cells in the geniculate laminae innervated by the right and left eyes respectively, and the horizontal axis shows the estimated ratios between the areas of cortex innervated by the right and left eyes, a complementary distribution of inputs being assumed. The dashed line is a theoretical prediction derived from the assumption that a fixed fraction (about a third) of cell body volume varies in direct proportion to the area of cortex innervated, while the remaining volume remains unchanged.

The relation between l.g.n. cell size and stripe area

The data obtained in this and the accompanying paper (Garey & Vital-Durand 1981) support the hypothesis advanced by Guillery (1972) of a parallel relation between l.g.n. cell size and the extent of geniculate arborization in the cortex. Figure 15 is a graph of the relation between (a) the ratio of l.g.n. cell volumes in right and left eye laminae (obtained by raising the measurements of area to the power $\frac{2}{3}$) and (b) the estimated ratio between the areas of cortex innervated by the right and left eyes. The latter data were obtained from the measurements of stripe area made in the present paper, by assuming a complementary distribution of right and left eye synapses. (Thus if a_R and a_L are the fractional areas of layer IVc innervated by right and left eye l.g.n. laminae respectively, $a_R + a_L = 1$, and $a_R/a_L = a_R/(1 - a_R)$.) The measures of cell volume and stripe area were chosen since it is these two variables that seem most likely to be directly related. Data

from both hemispheres have been pooled (where the data are available) since there has so far been no consistent evidence of any contralateral or ipsilateral differences in l.g.n. cell response to deprivation (Vital-Durand *et al.* 1978; Garey & Vital-Durand 1981), or in the degree of stripe shrinkage, in the monkeys used in this study (although Hubel *et al.* (1977) find differences in macaque monkeys). A possible source of systematic error in the comparison is that the l.g.n. cell size measurements sample regions corresponding to a variety of positions in binocular striate cortex, while the measurements of stripe area used come only from one region in the operculum. These latter measurements, however, are probably representative of the response of the greater part of binocular striate cortex.

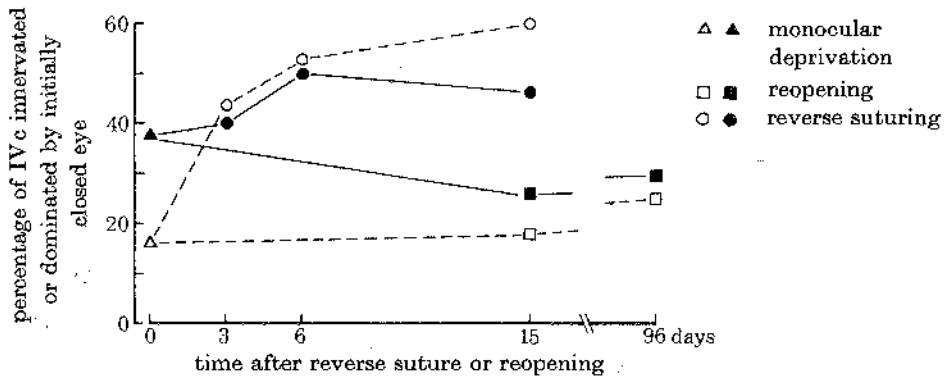


FIGURE 16. A comparison between stripe areas estimated (a) by measurements from autoradiographs (the same data are shown in figure 14) and (b) by estimates derived from physiological measurements of ocular dominance column width in layer IVc of the same animals (data taken from Blakemore *et al.* (1981)). Open symbols show electrophysiological data, closed symbols anatomical data. There is broad agreement between the two sets of data, with the exception of the two points comparing the effects of monocular deprivation, which come from different animals.

Comparison of physiological and anatomical estimates of stripe areas

Figure 16 compares stripe areas estimated (a) from autoradiographs, and (b) from changes in ocular dominance measured by Blakemore *et al.* (1981) from electrode recordings in tangential penetrations through layer IVc of the same monkeys. For the reverse sutured and reopened groups the two types of measurement give similar results, suggesting in agreement with the observations of Hubel *et al.* (1977) and Shatz & Stryker (1978) that the distribution of label in an autoradiograph provides a good indication of the regions of cortex where responses to the injected eye are dominant. However, there are difficulties in relating the anatomical and physiological data from the two animals (P7806 and P7807) that were monocularly deprived for short periods (21 and 25 days respectively). The autoradiographic results from the monkey whose normal eye was injected (P7807, figure 3) showed an almost uniform distribution of label in layer IVc. Despite this, electrophysiological recording from this animal demonstrated the presence of small groups of cells in layer IVc that could still only be driven by the deprived eye. A possible

explanation for this is that synapses in these regions were suppressed and, though anatomically detectable, incapable of producing suprathreshold activity in their postsynaptic cells. However, this interpretation cannot be certain. Autoradiographs give no indication of the absolute density of synapses, and so one cannot draw any conclusions about the relative numbers of normal and deprived eye synapses present in the middle of a deprived eye stripe. Thus it is possible that the density of synapses from the deprived eye exceeded that from the normal eye in those regions where it was capable of dominating cells' responses. One might suppose nevertheless, that the normal eye's input should have remained physiologically detectable. However, electrophysiological experiments can fail to reveal inputs to cells even when these inputs are physiologically active. For example, cells in the visual cortex that would normally be classified as monocular (i.e. ocular dominance groups 1 or 7) can under appropriate circumstances respond to both eyes (Poggio & Fischer 1977). This means that physiologically active inputs from one eye may be present on cells that appear capable of being driven only by the other eye. There is also evidence that, in the cat, cells made monocular by a period of monocular deprivation can retain an active input from the deprived eye, though under normal conditions of recording (which include anaesthesia and paralysis) this input is not revealed (Kratz *et al.* 1976; Hawken & Blakemore 1981). Thus the finding of small numbers of cells still driven monocularly by the deprived eye does not necessarily imply that any remaining synapses from the normal eye must have been functionally inactive.

Electrophysiological recordings were not made from the monkey (P7806) whose deprived eye was injected with label following monocular deprivation. Although the degree of stripe shrinkage in the operculum of this animal was less than might have been expected by comparison with the other monocularly deprived monkey from which recordings were made (figure 16), the difference may not be significant, given the inevitable variability of the physiological measurements, and the fact that the data come from different animals.

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