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Monocular deprivation affects X- and Y-cell retinogeniculate terminations in cats

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The X- and Y-cell pathways in cats form two functionally distinct, parallel systems from the retina through the lateral geniculate nucleus (LGN) to the visual cortex[1–4]. We recently used the technique of intraaxonal injection of horseradish peroxidase (HRP) to demonstrate major differences between X- and Y-cells in their retinogeniculate termination patterns[5] (compare Figs 1*a* and 2*a*). Normally, axons of X-cells innervate geniculate lamina A or A1 (depending on the eye of origin) in narrow zones oriented perpendicular to the lamination. Some X-cells also terminate sparsely (that is, with few boutons) in the medial interlaminar nucleus (MIN), a subdivision of the LGN. Y-cell axons terminate either in laminae C and A (from the contralateral retina) or in lamina A1 (from the ipsilateral retina) in broad zones[5,6], and most also terminate densely (with many boutons) in the MIN. We now report that cats raised with monocular lid suture develop abnormal retinogeniculate termination patterns. Many X-cell axons arising from the deprived eye have unusually broad terminal fields in lamina A or A1, and some also densely innervate the MIN. Many Y-cell axons from the deprived eye have dramatically shrunken or absent terminal fields in the A laminae and MIN. These changes constitute the most peripheral effects of monocular deprivation discovered so far, are consistent with previous reports of functional abnormalities among deprived geniculate neurones[4,7,8] and suggest possible mechanisms by which the visual environment influences neuronal development.

Previously described methods[5,9,10] were used to obtain results from nine adult cats (>8 months old) raised with continuous monocular deprivation from the first postnatal week. Briefly, recording micropipettes filled with 3% HRP, 0.2 M KCl and 0.05 M Tris were bevelled to 90–120 MΩ. An optic tract axon was first recorded extracellularly and identified as X or Y by standard criteria[10,11], including axonal conduction velocity (derived from response latency to electrical stimulation of the optic chiasm) and linearity of spatial summation to visual stimuli (measured by responses to counterphased, sine-wave gratings). Each axon was then impaled, its identity as X or Y was verified intracellularly, and HRP was iontophoresed into it[5,9]. After sufficient survival time, the brain was sectioned coronally at 100 μm and reacted with 3-3′-diaminobenzidine plus cobalt chloride[12]. The retinas in four animals were dissected free, flat-mounted on slides and reacted with *o*-dianisidine[13].

We successfully injected and recovered 9 X- and 11 Y-cell axons from deprived retinas and 11 X- and 7 Y-cell axons from nondeprived retinas. Axonal morphology in lid-sutured animals was also compared with that of 16 X- and 11 Y-cell axons recovered from normal, adult cats[5]. All axons from the nondeprived eye appeared identical in their termination patterns to axons in normal cats. Among the deprived axons, however, 4 X-cells and 8 Y-cells developed abnormal morphology. Nonetheless, we found no obvious physiological differences between deprived and nondeprived axons.

Monocular deprivation increases the density (number of boutons) and extent of many X-cell terminal fields. Figure 1 illustrates two X-cell axons from the deprived eye. The terminal field in Fig. 1*a* has apparently normal morphology, being restricted to a narrow zone in lamina A1. As in other normal axons, a thin medialward branch enters the brachium of the superior colliculus but could not be traced to any terminal zone. In contrast, a representative example of a morphologically abnormal X-cell terminal arbor is shown in Fig. 1*b*. The axon exhibits an abnormally wide terminal field in lamina A1, and it also has many more terminals in the MIN than any normal

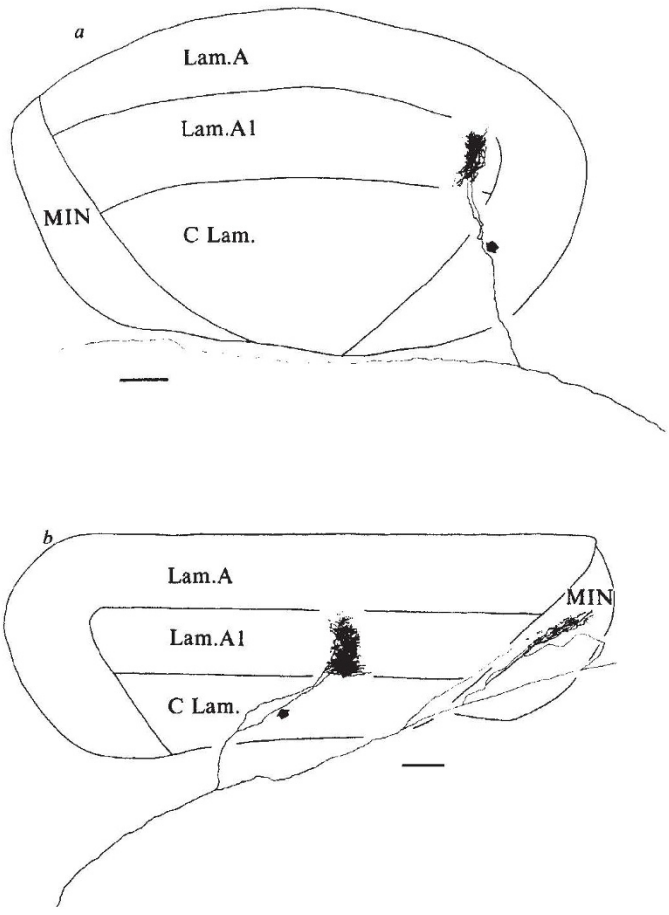

Fig. 1 Geniculate terminal zones of X-cell axons from the deprived eye. Terminal zones of these X- and Y-cell axons were circularly symmetrical in the horizontal plane, and did not show obvious variations in size that could be related to eccentricity or type of centre response (that is, ON or OFF). *a*, Axon from the ipsilateral eye with apparently normal terminal pattern for an X-cell. This axon has 788 terminal boutons in lamina A1, which is within the range of 500–900 boutons for normal X-cell axons in lamina A or A1. Its receptive field was ON centre, had a centre diameter of 1.0°, and was located 44° from the vertical meridian and 23° below the zero horizontal parallel. The axon's conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.8 ms. The axon and its terminal field were reconstructed from 21 serial, 100-μm thick, coronal sections. Scale bar, 250 μm. *b*, Axon from the ipsilateral eye with abnormal terminal pattern. The extent of terminal field in either lamina A1 or the MIN exceeds any seen for normal or nondeprived X-cells. Thus, this axon has 1,077 terminal boutons in lamina A1 and 175 boutons in the MIN (compared with 7–31 boutons for normal X-cell axons that innervate the MIN). This axon's receptive field was OFF centre, had a centre diameter of 0.6°, and was located 11° from the vertical meridian and 3° below the horizontal zero parallel. Its conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.7 ms. The axon and its terminal field were reconstructed from 17 serial, 100-μm thick, coronal sections. Scale bar, 250 μm.

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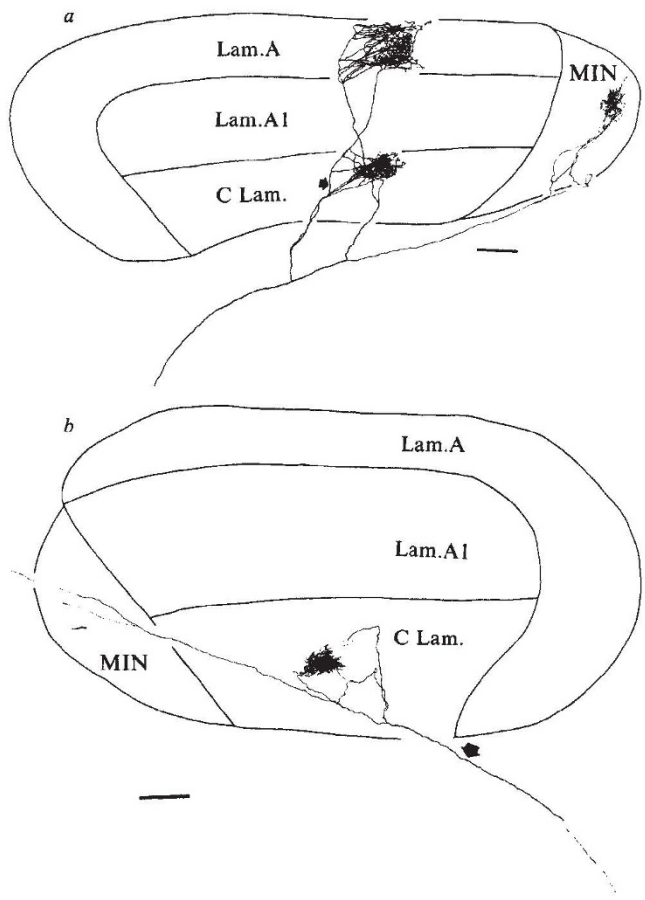

Fig. 2 Geniculate terminal zones of Y-cell axons from the deprived eye. *a*, Axon from the contralateral eye with apparently normal termination pattern for a Y-cell. This axon has 683 terminal boutons in lamina A (the range for normal Y-cell axons in lamina A or A1 is 650–1,400 boutons) and 121 terminal boutons in the MIN (normal range 90–170 boutons). Its receptive field was OFF centre, had a centre diameter of 1.3°, and was located 4° from the vertical meridian and 7° below the zero horizontal parallel. The axonal conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.5 ms. The axon and its terminal field were reconstructed from 19 serial, 100-μm thick, coronal sections. Scale bar, 250 μm. *b*, Axon from the contralateral eye with abnormal terminal pattern. The axon terminates densely only in the C-laminae, has no terminal boutons in the A-laminae and sparsely innervates the MIN with only 6 boutons. This contrasts sharply with the normal pattern described in the text. This axon's receptive field was OFF centre, had a centre diameter of 1.6°, and was located 7° from the vertical meridian and 40° below the zero horizontal parallel. Its axonal conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.4 ms. The axon and terminal field were reconstructed from 18 serial, 100-μm thick, coronal sections. Scale bar, 250 μm.

X-cell axon seen to date. Figure 3 summarizes some of these phenomena for arbors in the A-laminae.

Monocular deprivation affects Y-cells even more dramatically than it does X-cells, but in a different way. Figure 2*a* shows a normal Y-cell terminal field from the deprived retina. The axon terminates densely in laminae A and C, plus the MIN. In contrast, Fig. 2*b* illustrates an example of a deprived Y-cell axon that terminates densely in the C-laminae, sparsely in the MIN and not at all in the A-laminae. In normal cats, every Y-cell axon found terminates densely in lamina A or A1, and the majority that terminate in the MIN do so densely there[5,6]. Of six deprived Y-cell axons from the contralateral eye, four innervate only the C-laminae and do not terminate in lamina A. Four additional deprived Y-cell axons (one contralateral, three ipsilateral) have terminal fields in the A-laminae that are narrower and with fewer boutons than normal. Thus, deprived Y-cell terminals are most affected in the binocular segment of the A-laminae (Fig. 3), less so in the MIN, and seem normal in the C-laminae. The clearest effect of monocular lid suture on retinogeniculate Y-cell terminations, however, is that many contralateral Y-cell axons innervate only the C-laminae and do not innervate lamina A. If these four axons that fail to innervate lamina A are removed from the comparison in Fig. 3, the remaining Y-cell terminal fields are only marginally smaller than those of the normal and nondeprived Y-cells. More data are needed to determine whether the effects of deprivation are continuous such that some Y-cell terminal zones in the A-laminae are missing, some smaller than normal, and some normal, or whether the effects cause complete elimination of some Y-cell terminal fields with no effect on others.

We are confident that the abnormally small terminal fields seen in many deprived Y-cell axons are not due to incomplete or poor staining. These axons were darkly stained, as were the nondeprived and normal axons, and each axonal branch could be traced to a terminal bouton. Also, the same tissue often included axons with normal and abnormal morphology.

Figure 4 shows the retrograde labelling obtained for the somata of two identified axons that were filled with HRP. Such labelling was obtained for four X- and four Y-cells in deprived retinas and three X- and five Y-cells in nondeprived retinas. X-cell somata were consistently smaller than Y-cell somata[14], but no obvious size differences could be seen between the deprived and nondeprived somata despite large changes in the terminal field extents of the former. However, such comparisons are complicated by differences in retinal eccentricity[15,16].

Many studies have established that monocular deprivation causes abnormalities to develop in the cat's central visual pathways[4]. The expanded X-cell and reduced or absent Y-cell retinogeniculate projections described here constitute the most peripheral morphological effects of deprivation seen so far. Furthermore, although our study seems inconsistent with a recent ultrastructural study[17] that reported normal numbers and structure for retinal synapses in the deprived A-laminae,

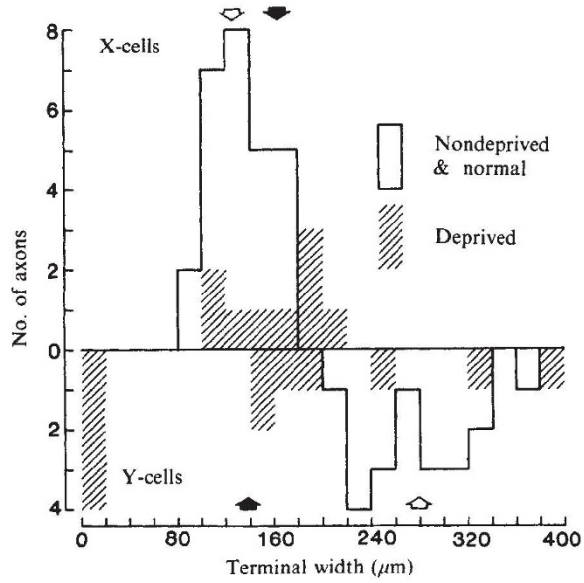

Fig. 3 Maximum widths of terminal zones in the A-laminae for X- and Y-cell axons with receptive fields in the binocular segment. The widths were measured along a plane parallel to the lamination. Because this is the plane of circular symmetry for these terminal fields and there is no effect of eccentricity or centre type on their size (see Fig. 1 legend), it seems unlikely that differences in terminal field widths result from histological artefacts. Data for nondeprived axons and axons from normally reared cats were pooled, as they are morphologically indistinguishable. Open arrows denote mean widths for normal and nondeprived axons; closed arrows, for deprived axons. Terminal zones for deprived X-cell axons are larger than those for nondeprived or normal X-cell axons ($P < 0.05$ on a Mann–Whitney U-test for either comparison). These zones for deprived Y-cell axons are smaller than are those for nondeprived or normal Y-cell axons ($P < 0.01$ on a Mann–Whitney U-test for either comparison). However, if the four contralateral Y-cell axons that do not innervate lamina A are omitted from the comparison, the difference between deprived and nondeprived or normal Y-cell terminal widths in the A laminae is less significant ($0.05 < P < 0.1$ on a Mann–Whitney U-test).

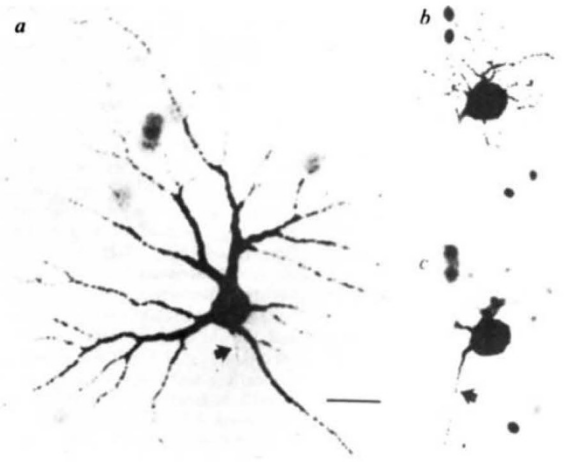

Fig. 4 Photomicrographs of retinal ganglion cells labelled retrogradely following HRP injection into their axons. Each ganglion cell was identified unambiguously by comparing its location relative to retinal blood vessels with plots of the vessels and receptive field made during the recording. *a*, Y-cell in nondeprived retina. The neurone's receptive field was OFF centre, had a centre diameter of 2.6°, and was located 17° from the vertical meridian and 45° below the zero horizontal parallel. The morphology of this Y-cell is characteristic of α cells[14–16]. The arrow points to the axon. Scale bar, 50 μm and applies as well to *b* and *c*. *b*, *c*, Two focal planes of same field of view showing an X-cell in nondeprived retina. The neurone's receptive field was OFF centre, had a centre diameter of 0.9°, and was located 20° from the vertical meridian and 5° below the zero horizontal parallel. The morphology of this X-cell is characteristic of β cells[14–16]. The focus is adjusted for the dendrites in *b* and for the soma and axon (arrow) in *c*.

the two sets of data may actually be compatible. Prior evidence suggests that retinal X-cells outnumber Y-cells by roughly 10 to 1 (refs 15, 16). Even with roughly twice as many boutons from Y-cell axons as from X-cell axons[5], retinogeniculate synapses in the A-laminae arising from X-cells probably outnumber substantially those from Y-cells. It is impossible to distinguish between X- and Y-cell retinogeniculate synapses in conventional ultrastructural material[18]. Thus, if one were to study these synapses in deprived laminae without knowledge of X- and Y-cell identity, a fairly substantial loss of terminals from the less numerous Y-cell axons with a slight expansion of terminals from more numerous X-cell axons might result in ultrastructure that appeared fairly normal. On the other hand, our data are consistent with both the loss of recorded geniculate Y-cells from deprived laminae[7] and the finding that many deprived geniculate neurones that normally accept only Y-cell input from the retina accept or retain X-cell input instead[8].

The abnormal terminal arbors we have described suggest that deprivation might cause retinogeniculate X-cell connections to expand at the expense of Y-cell connections by some sort of competitive interaction during development. X-cell axons normally seem to innervate the lateral geniculate nucleus before Y-cell axons do[19,20]. Perhaps, as in many developing pathways[21–23], the immature X-cell pathway is exuberant and is 'pruned back' in competitive interactions with the later-developing Y-cell inputs. The ability of Y-cell axons to dislodge the already-present X-cell axons may be severely reduced during visual deprivation. Consistent with this hypothesis is our observation that deprived Y-cell terminals are most severely affected in the A-laminae, where geniculate X-cells are concentrated. One means of testing this hypothesis would be to study the termination patterns of X- and Y-cell retinogeniculate axons in developing kittens.

Regardless of the developmental mechanisms, our evidence clearly shows that the visual environment can influence the formation of retinogeniculate terminations. Early visual deprivation causes abnormalities in these connections, which most seriously affect the Y-cell pathway. This might well represent the neural basis of amblyopia seen in monocularly deprived cats, as the Y-cell pathway may have a crucial role in normal vision[24].

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Action potential repolarization may involve a transient, Ca^{2+}-sensitive outward current in a vertebrate neurone

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Repolarization of the action potential in squid axon[1] and several types of neurones[2–4] involves a voltage-activated potassium (K^+) current. Voltage clamp analysis has demonstrated that this current has rapid activation kinetics[1,3–5]. In several neuronal types, the same technique has also revealed a slowly activated K^+ current that is calcium (Ca^{2+})-sensitive[3,5–10]. This slow Ca^{2+}-activated K^+ current is the major current underlying the late, slower portion of the after-hyperpolarization following an action potential[11–14]. In several muscle types, fast, transient Ca^{2+}-dependent K^+ currents have been described[15–17] which may contribute to repolarization of the action potential. Rapidly activating, Ca^{2+}-dependent K^+ currents have been observed in sympathetic neurones of the bullfrog and it has been suggested that they contribute to action potential repolarization of those neurones[8,9,18]. We have studied the membrane currents in bullfrog sympathetic neurones using voltage clamp methods and report here a transient outward current that appears to be composed of two separate currents. One of those currents is a transient, Ca^{2+}-sensitive outward current as indicated by a significant reduction of the current by treatments that reduce or block Ca^{2+} entry (Mn^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+} or Ca^{2+}-free Ringer). Such treatments also decreased the rate of action potential repolarization. The results suggest that this current is involved in repolarization of the action potential and consequently may regulate Ca^{2+} entry into the neurone during spike activity.

The IXth and Xth paravertebral sympathetic ganglia of bullfrog (*Rana catesbeiana*) were excised, pinned in a Plexiglass recording chamber and continuously superfused with a Ringer's solution of the following composition (mM): NaCl 117; KCl 2.5; $CaCl_2$ 1.8; HEPES 1 (*p*H 7.3). Most preparations were

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