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Effects of dark rearing on the development of visual callosal connections

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Summary. It is now well established that during normal postnatal development there is a partial elimination of the callosal projections of cortical areas 17 and 18 in the cat and that visual experience early in life can modulate this process. In the present experiments, we quantitatively studied the influence of light, per se, by rearing cats in total darkness. Dark rearing exaggerates the normally occurring partial elimination of immature callosal projections: it causes a significant reduction in the total number of neurons in both the supra- and infragranular layers that send an axon through the corpus callosum and slightly narrows the distribution of these neurons across areas 17 and 18. These data demonstrate that visual stimulation is not necessary either to initiate the partial elimination of immature callosal projections or to stabilize a large fraction of the callosal projections present at birth. However, normal visual stimulation is necessary for the stabilization of the normal complement of callosal projections.

Key words: Corpus callosum – Development – Vision – Dark rearing – Cat

Introduction

Visual experience affects the development of visual callosal connections. In kittens raised with convergent or divergent strabismus, monocular enucleation, monocular eyelid suture or alternating monocular occlusion, callosally projecting neurons (callosal neurons) in areas 17 and 18 have a more widespread distribution than in normal kittens (Innocenti and Frost 1979; Berman and Payne 1983; Frost et al. 1988); in kittens raised with strabismus, callosal axon terminals are also abnormally widespread (Lund et al. 1978).

These experience-dependent modifications of callosal connectivity may depend on processes similar to those proposed to explain the modifications of ocular dominance column width in area 17 induced by monocular deprivation (Rakic 1976; Hubel et al. 1977). Geniculocortical axons representing the two eyes are largely segregated in adult animals but overlap earlier in life; visual experience exerts its effect on ocular dominance column width by affecting the degree to which geniculocortical axons representing one or the other eye withdraw from territories they occupy early in development. Similarly, vision may exert its effect on the distribution of callosal neurons by modulating the elimination of transitory callosal axons (Innocenti 1981; Innocenti et al. 1986), which in normal development leads to a characteristically restricted tangential distribution of callosal neurons (Innocenti et al. 1977; Innocenti and Caminiti 1980). The data of some recent studies suggest that dark rearing (DR) can at least partially stabilize immature, widespread distributions of geniculocortical axons in area 17 (Swindale 1981, 1988; Kalil 1982; Mower et al. 1985), although this result has not been universally obtained (Stryker and Harris 1986). Therefore, we wondered whether DR would increase the number or distribution of callosal neurons in mature cats by stabilizing some of the normally transient callosal axons present earlier in life.

It would also be instructive to know the effects of light, per se and of retinal ganglion cell activity, per se on the development of callosal connectivity. Comparison of the effects of rearing with bilateral eyelid suture and with bilateral enucleation (Innocenti and Frost 1980) yields two surprising differences: i) although both manipulations result in a

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subnormal number of callosal neurons in areas 17 and 18, the effect is greater for lid suture than for enucleation, even though enucleation would seem to be the more severe form of deprivation; ii) enucleation produces an abnormally wide distribution of callosal neurons in areas 17 and 18, while lid suture produces a slightly narrower than normal distribution. These differences might be explained by two obvious differences between enucleated and eyelid-sutured cats: i) light can evoke responses in retinal ganglion cells and influence the brain in lid-sutured- but not enucleated cats; ii) spontaneous and light-evoked retinal ganglion cell activity reach the brain in lid-sutured- but not enucleated cats. We thought that DR would be a good way to determine the effects of light on the development of callosal connectivity since for DR light is eliminated, while unlike for enucleation, the retina is intact.

Here we report that rearing kittens in the dark accentuates the normally occuring partial elimination of immature callosal connections in areas 17 and 18.

Methods

Nine cats from 4 litters were born and reared in total darkness. Strips of Kodak Tri-X film were attached to the walls of the rearing chamber and periodically developed to confirm that the film was unexposed and that the cats had not been exposed to light. When the cats were 94–209 days old, they were removed from the chamber for pathway tracing experiments. (The weights of the cats fell within the normal ranges for their ages). Four normal, adult cats were subjects in identical experiments.

Cats were initially anesthetized with Ketalar (20 mg/kg, IM), then intubated and maintained on Halothane, ca. 1.5% in a mixture of 2 parts N_2O and 1 part O_2. They also received atropine (0.25 mg, IM), Dexamethasone (2 mg/kg, IV) and 5% dextrose in Ringer solution (60 ml/kg, IV, administered continuously during surgery). Surgery was performed aseptically; EKG and temperature were monitored continuously. A craniotomy was made to expose the lateral and postlateral gyri. We made 10–12 0.5 µl injections of 40% horseradish peroxidase (HRP; Sigma type VI) in H_2O in these gyri using a Hamilton syringe with a 27 g needle inserted into the cortex through small holes in the dura. Injections were spaced ca. 1.5 mm along the anterior-posterior axis and alternated between the medial and lateral sides of the gyri. This procedure completely fills areas 17 and 18 with HRP (Innocenti and Frost 1980). At the completion of surgery, the craniotomy was closed with Silastic polymer and the cats received Dexamethasone (2 mg/kg, IV) and Flocillin antibiotic (45,000 units/kg, IM). After reanimation, the cats were returned to their cages.

48 h postoperatively, the cats were deeply anesthetized by a lethal dose of Nembutal and transcardially perfused with the following sequence of solutions (all in 0.1 M PO_4 buffer, pH 7.4): i) 0.9% NaCl (ca. 1000 ml), ii) 1% paraformaldehyde, 2% glutaraldehyde (ca. 2000 ml), iii) 10% sucrose (ca. 2000 ml), iv) 20% sucrose (ca. 2000 ml). Solutions i and ii were at ambient temperature, iii and iv were at 4° C. The brains were then dissected, stored over night in 30% sucrose in PO_4 buffer at 4° C and sectioned frozen at 80 µm. A 1 in 2 series of sections was then reacted for HRP histochemistry using Mesulam's ('78) tetramethyl benzidine (TMB) technique and mounted on chrome-alum treated slides (preincubation and incubation were at ambient temperature in the dark; all other steps were at 4° C). After drying for 1–2 h at ambient temperature, then over night at 4° C, the sections were dehydrated rapidly in graded alcohols at 4° C, coverslipped with Permount containing 1% BHT and stored at 4° C.

All of the sites of HRP injection were similar in size, location and density, and resembled those obtained previously using similar techniques (Innocenti and Frost 1980; Innocenti et al. 1985). The HRP reaction product fills most of the grey and white matter of the lateral and postlateral gyri; occasionally a lighter precipitate extends into the suprasylvian gyrus. The completeness of the injections in areas 17 and 18 was confirmed by retrograde and anterograde transport of HRP to all of the dorsal nucleus of the lateral geniculate body (LGd; except, in some cases, the caudolateral extremity).

In all 4 normal cat brains and 6 of 9 DR cat brains, HRP-labeled callosal neurons in a series of sections spaced 320 µm apart and encompassing the caudal 13–15 mm of the cortex were charted at 250 × using a computer-microscope. Additional sections between those used for the reconstructions were inspected. Criteria for the identification of labeled neurons were similar to those previously described (Innocenti and Frost 1980). The significance of differences in the mean number of callosal neurons per section in differently reared animals was determined using a 2-tailed Mann-Whitney U-test.

Using the computer, we made flattened reconstructions of the distributions of labeled callosal neurons (Innocenti et al. 1985). Briefly, in each coronal section, the labeled neurons were projected onto a contour running 400 µm below the pial surface. The contour was straightened and divided into 100 µm segments and the number of neurons projected onto each segment was indicated by vertical lines whose lengths were proportional to the number of neurons. The rows of lines representing the sections were aligned using the convexity of the lateral gyrus as a landmark. The cytoarchitectonic border between areas 17 and 18 was determined on selected sections counterstained with toluidine blue, using the criteria of Otsuka and Hassler (1962). In the remaining 3 DR cat brains, we obtained partial reconstructions confirmatory of the results from the fully reconstructed ones.

In each of the fully reconstructed brains, we measured the mediolateral width of the cortical volume containing callosal neurons (callosal efferent zone, CZ) at the 3 rostrocaudal levels that divide area 17/18 into 4 equal parts. The mean of these 3 widths was taken as a measure of the width of the callosal zone in each animal. We then used the Mann-Whitney U-test to determine the significance of differences in the width of the callosal zone in normal and DR cats.

Results

Number of callosal neurons in areas 17 and 18 of normal cats

The distributions of labeled callosal neurons in normal, adult cats were as previously reported using TMB and diaminobenzidine (Innocenti 1980; Innocenti and Frost 1980; Innocenti et al. 1985; Seagraves and Rosenquist 1982). They are summarized here for purposes of comparison with the distributions in DR cats.

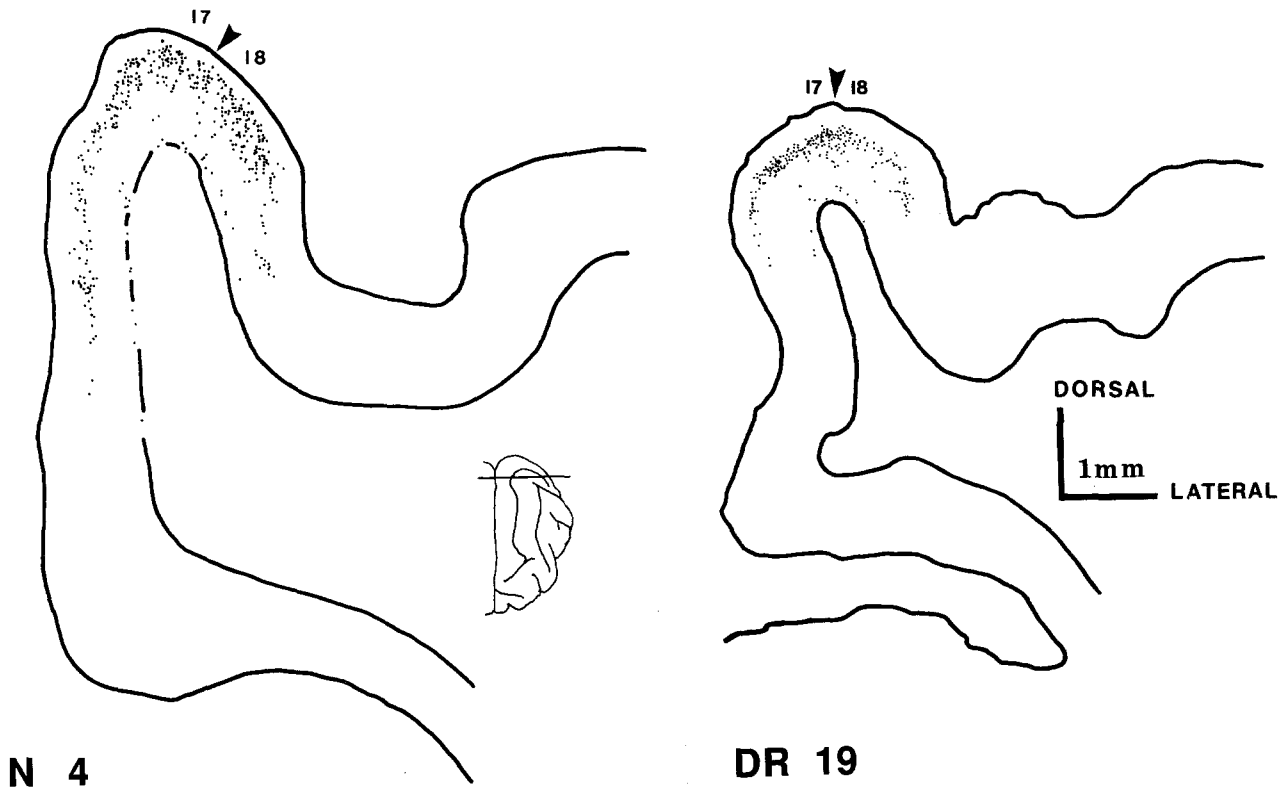

Fig. 1. Computer-microscope plots of the distributions of HRP-labeled callosal neurons in coronal sections through corresponding levels of areas 17 and 18 of one normal (N4) and one DR (DR19) cat. Dorsal is up, lateral is to the right. Arrowheads indicate the 17/18 border as determined by cytoarchitectonic criteria after Nissl counterstaining. Inset between brains shows dorsal view of the left hemisphere of N4 (caudal is up, lateral is to the right); the line across the hemisphere indicates the coronal level from which these sections were taken

In normal cats, callosal neurons lie in a band running anteroposteriorly along the border between areas 17 and 18 and extending 1–3 mm either side of the border (Figs. 1, 4). This callosal zone is flanked by unlabeled (acallosal) regions corresponding to most of area 17 and the lateral part of area 18. Thus, the band of callosal neurons spanning the 17/18 border is distinct from the callosal zones in area 19 or in the splenial sulcus (Innocenti 1980; Seagraves and Rosenquist 1982). However, at different rostrocaudal levels in different animals, 1–3 bridges of callosal neurons stretch across the full mediolateral extent of area 18 to join the callosal zone in area 19. For counting purposes, the lateral border of the callosal zone in areas 17 and 18 was extrapolated across these bridges (Fig. 4).

In areas 17 and 18, callosal neurons are distributed in two radially separated, superposed laminae in layers III and IV (subzone a) and layer VI (subzone c) (Fig. 1). Neurons in the two subzones were easily distinguished; the few callosal neurons in layer II were attributed to subzone a and the few callosal neurons in layer V to the nearest subzone. There are many more callosal neurons in subzone a than in subzone c (Innocenti 1980).

The boundary between areas 17 and 18 is notoriously difficult to determine precisely in the cat; therefore, separate counts of the callosal neurons in each area were not attempted. The number of labeled callosal neurons distributed around the area 17/18 border in each section decreases from caudal to rostral (Figs. 2–4). Superimposed on this trend are one or more peaks that correspond approximately to the area centralis representation and, less reliably, to the bridges crossing area 18. The average number of neurons per section in subzones a and c of our normal cats was higher than we obtained previously using the TMB technique working elsewhere (Innocenti et al. 1985) but the difference was not statistically significant (263.7 vs 157.4 neurons/section, $p=0.28$ and 39.1 vs 26.4 neurons/section, $p=0.34$, in subzones a and c, respectively). Multiple factors may have accounted for this difference, including the current use of cold

Table 1. Summary of data used in statistical comparisons of normal and DR cats. From left to right, columns indicate case, mean number of callosal neurons per section in subzone a of areas 17/18, mean number of callosal neurons per section in subzone c of areas 17/18, and mean width of subzone a at the rostrocaudal levels that divide areas 17/18 into 4 equal parts (see methods)

Case	Subzone a neurons	Subzone c neurons	CZ width (mm)
N 3	269.7	39.2	7.39
N 4	333.8	44.4	6.94
N 5	218.8	40.4	4.78
N 6	232.3	32.4	4.68
Mean +/− sd	263.7 +/−51.5	39.1 +/−5.0	5.95 +/− 1.42
DR 8	204.8	26.9	5.18
DR 12	185.6	30.7	5.13
DR 13	202.5	14.3	4.32
DR 14	262.5	37.1	5.96
DR 19	154.9	15.2	2.93
DR 20	164.0	23.1	2.67
Mean +/− sd	195.7 +/− 38.4	24.6 +/− 8.9	4.37 +/− 1.32

rinsing and dehydration solutions to reduce the loss of reaction product, a practice that was not followed in our prior study, and the use of different fixatives (here, 1% paraformaldehyde, 2% glutaraldehyde; previously, 3% paraformaldehyde). Furthermore, in the present study, the number of callosal neurons per section was averaged for a region extending to within 880 μm of the caudal edge of the cortex, while previously, the region analyzed extended only to within 1800–3000 μm of the caudal limit of the cortex. As we previously observed (Innocenti et al. 1985) there was significant, true interanimal variation in the mean number of callosal neurons per section (Table 1).

Number of callosal neurons in areas 17 and 18 of dark-reared cats

DR does not alter the radial distribution of callosal neurons. In DR as in normal cats, callosal neurons in areas 17 and 18 are distributed in two distinct, radially separated, superposed laminae in layers III/IV (subzone a) and layer VI (subzone c) (Fig. 1).

The callosal efferent zone in areas 17/18 of DR cats contains 74.3% of the normal number of labeled callosal neurons in subzone a and 62.8% of the normal number in subzone c (Table 1). In DR as in normal cats, there is interanimal variability in the number of labeled callosal neurons per section. Therefore, we tested the significance of our results statistically using the Mann-Whitney U-test applied to the mean number of neurons per section in each animal, with n expressing the number of animals. This test showed that DR causes a significant reduction in the mean number of labeled callosal neurons per section in both subzones ($p=0.038$ for subzone a, $p=0.02$ for subzone c).

Although the reductions in the number of callosal neurons occur at all rostrocaudal levels, we have the impression that the reduction in subzone a may be somewhat greater in the caudal 3 mm or so of areas 17/18 (which represent the contralateral upper quadrant; Tusa et al. 1979) than elsewhere. Data on the rostrocaudal distribution of callosal neurons are summarized in Figs. 2 and 3, where we have compared the distributions in 4 pairs of normal and DR cats: a) the normal and DR cats (N-4/DR-19) with, respectively, the most and least callosal neurons per section in subzone a of their groups; b) a normal/DR pair (N-5/DR-8) matched within 6.9% for the mean number of callosal neurons per section in subzone a; c) the normal and DR cats (N-4/DR-14) which in their respective groups had the most callosal neurons per section in subzone a; d) the normal and DR cats (N-5/DR-19) which in their respective groups had the least callosal neurons per section in subzone a. In all 4 normal cats, the number of subzone a callosal neurons per section rises rapidly passing rostrally from the caudal tip of the cortex, hits a relatively high peak, then plateaus, or declines slowly (Fig. 2); in all the DR cats except DR-14, the number of subzone a callosal neurons per section rises more slowly to peak at about 3 mm, then plateaus, or declines slowly (Fig. 2). We have previously observed a similar effect in cats reared with binocular eyelid suture (Innocenti and Frost 1980). Extreme cases N-4 and DR-19 clearly differ most in the caudal 3 mm of the cortex. The data from cat DR-14 suggests that when a DR cat has many callosal neurons, their distribution resembles that in normal cats. The rostrocaudal distributions of callosal neurons in subzone c of both normal and DR cats resemble the corresponding distributions in subzone a (Fig. 3). We have not tested the possibility that, within either subzone, specific depths or neuronal types may be preferentially affected.

Tangential distribution of callosal neurons in normal and dark-reared cats

In normal, adult cats, both the mediolateral width of subzone a and its position with respect to the crown of the lateral and postlateral gyri show significant individual variations (Table 1; Fig. 4). The position of subzone a reflects that of the border between areas 17 and 18 as determined cytoarchitectonically. Few callosal neurons extend medially

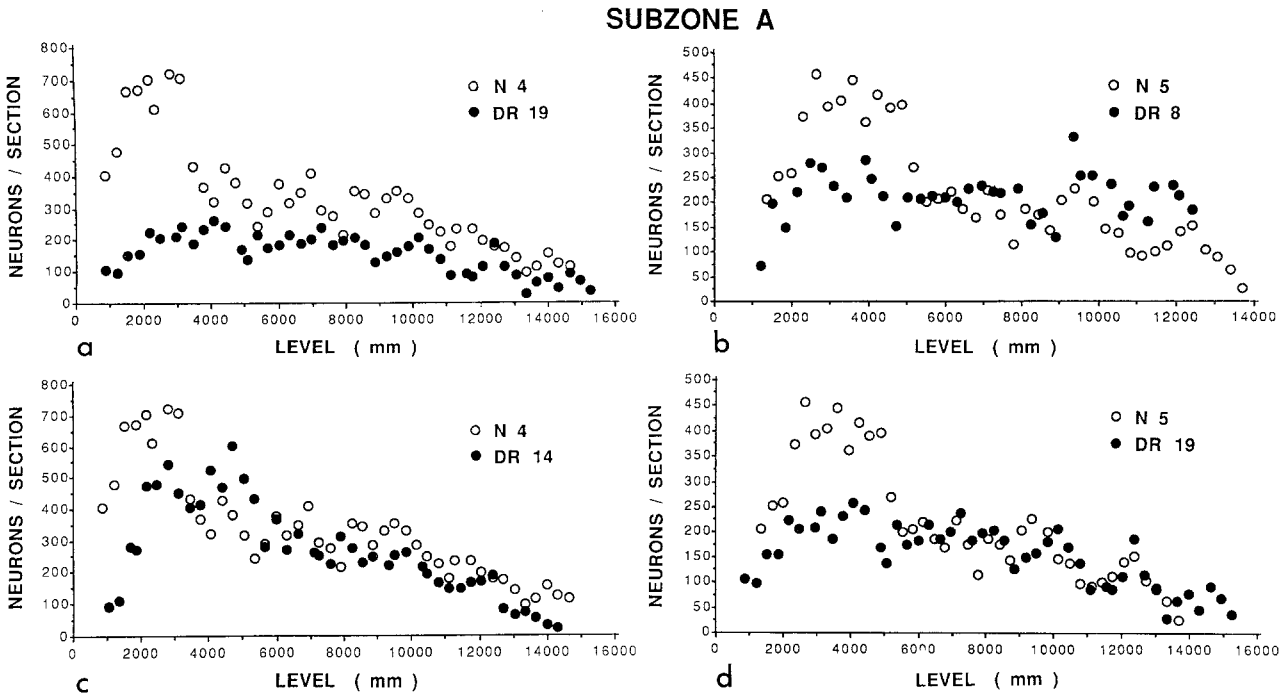

Fig. 2a–d. Rostrocaudal distributions of labeled callosal neurons in subzone a in two normal- (N 4, N 5) and three DR cats (DR8, DR14, DR19). Instructive pairings of normal and DR cases as described in text. The horizontal axis indicates positions of coronal sections in millimeters rostral to the caudal extremity of the neocortex; the vertical axis indicates the number of HRP-labeled callosal neurons in subzone **a**. ○ represents data points from normal cats while ● represents data points from DR cats. The distributions represent: **a** the normal and DR cats (N 4/DR 19) with the most and least callosal neurons, respectively, of their groups; **b** a normal/DR pair (N 5/DR 8) matched within 6.9% for the mean number of callosal neurons per section; **c** the normal and DR cats (N 4/DR 14) which in their respective groups had the most callosal neurons per section; **d** the normal and DR cats (N 5/DR 19) which in their respective groups had the least callosal neurons per section

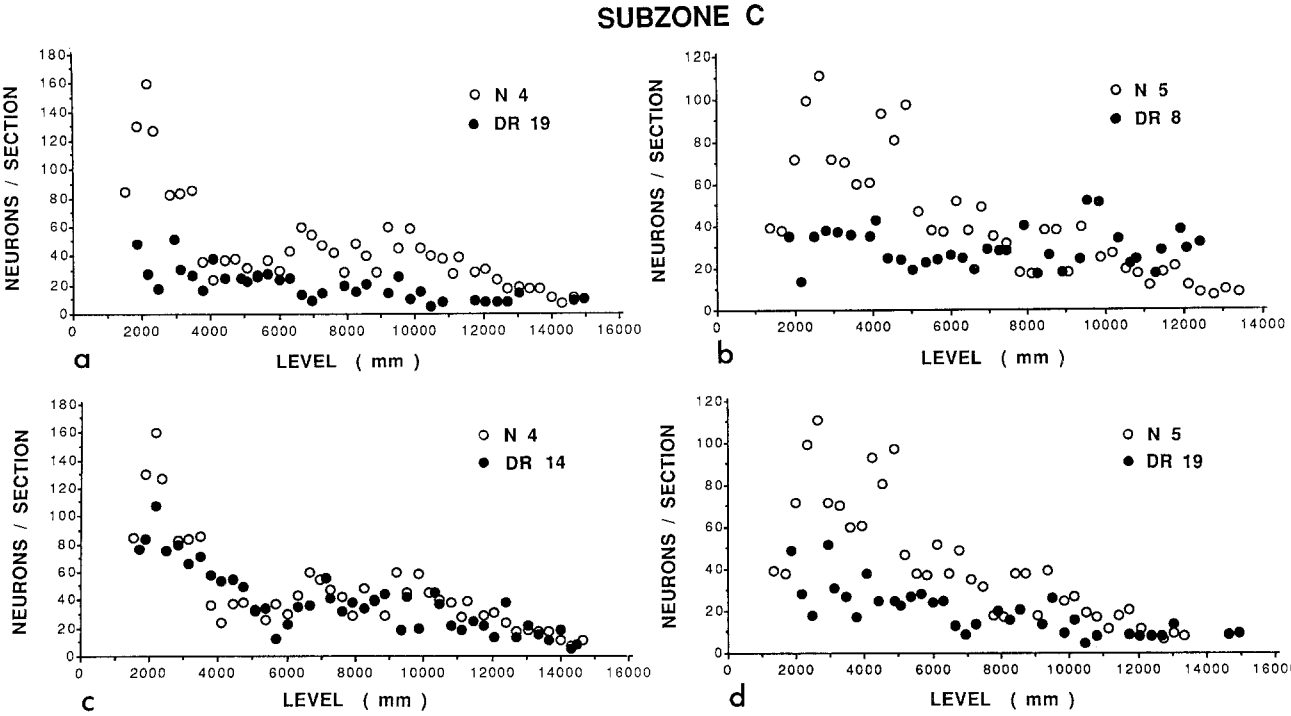

Fig. 3a–d. Rostrocaudal distributions of labeled callosal neurons in subzone c for the same cats as illustrated in Fig. 2. Same instructive pairings of normal and DR cases as described in text and illustrated in Fig. 2. Same conventions as in Fig. 2 except that vertical axis represents the number of labeled callosal neurons in subzone c

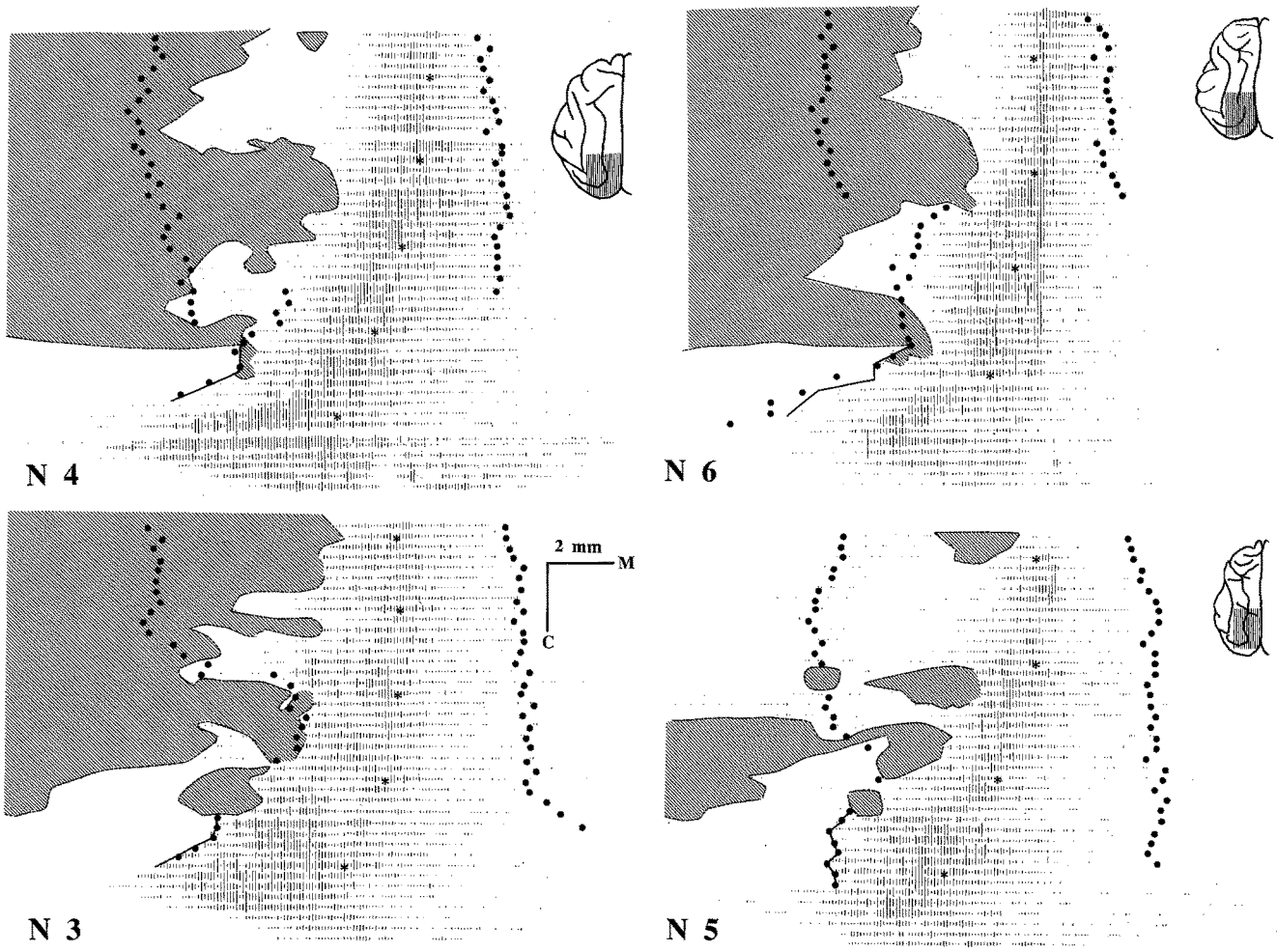

Fig. 4. Dorsal view computer reconstructions of a part of callosal subzone **a** in 4 normal adult cats. Flattened representations of postlateral and lateral gyri represent hatched areas in corresponding insets of dorsal views of brains (traced from photographs). Dotted lines represent, from lateral to medial, the fundi of the lateral, postlateral and suprasplenial sulci. The asterisks mark the boundary between areas 17 (medially) and 18 (laterally). The neurons in subzone **a** of each section were projected onto a line running parallel to the pial surface and 400 μm deep; the line was divided into bins of 100 μm and the number of neurons in each bin was represented by a line segment whose length is proportional to the number of neurons in the bin; the number of labeled neurons represented by a unit of line segment height is the same in each case. Each row of line segments represents one section. Stippling indicates regions of areas 18 and 19 that are continuous with the reconstructed parts of the callosal zone and within which there is a high density of labeled callosal neurons; parts of these regions form "bridges" described in the text and show a great deal of individual variability in both normal and deprived cats. Neuronal counts used for histograms and statistics exclude the stippled regions and correspond to the parts of subzone a reconstructed with line segments. Solid lines caudolaterally in each reconstruction indicate that at the levels where the lines are present the reconstruction could not be extended further laterally since the cortex lateral to the lines was sectioned obliquely. Scale lines represent 2 mm. M, medial; C, caudal

into area 17 as far as the suprasplenial sulcus, and this happens mainly rostrally.

In DR as in normal cats, the density of callosal neurons peaks near the 17/18 border and diminishes progressively with increasing distance from the border. In DR cats, the mediolateral width of subzone a appears slightly decreased, compared to that in normal cats (cf. Figs. 4 and 5). This narrowing of subzone a could be the consequence of the reduction in the number of callosal neurons (Innocenti and Frost 1980). In DR cats, the mean of our measure of the width of the callosal zone was 73.4% of normal (Table 1); the narrowing was not statistically significant (Mann-Whitney U-test; $p = 0.258$) but this negative result may be due to the small numbers of normal and DR cats.

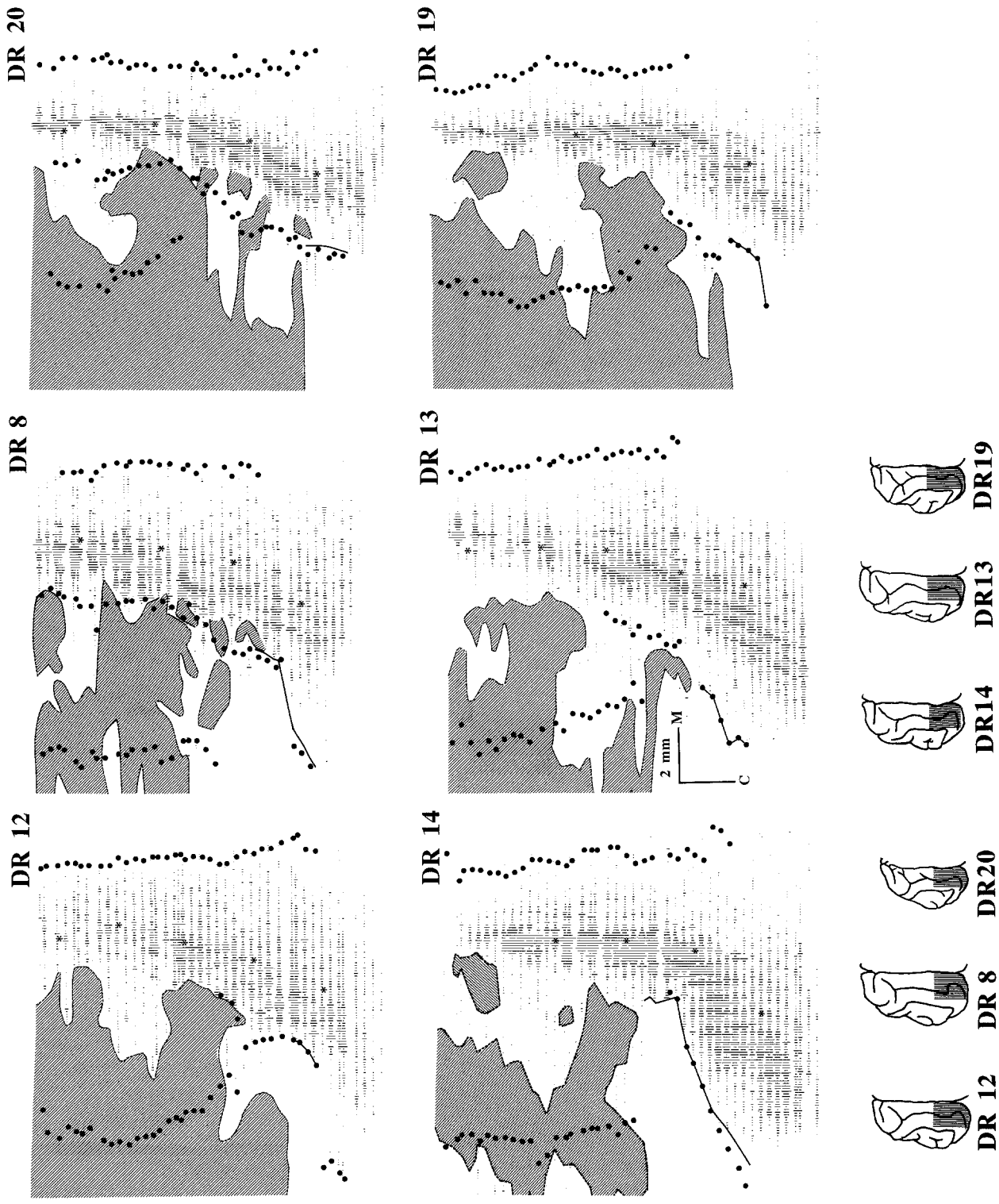

Fig. 5. Dorsal view computer reconstructions of a part of callosal subzone a in 6 adult DR cats. All conventions are as in Fig. 5

Morphology of callosal neurons

Most callosal neurons are pyramidal cells, though some are spiny stellates or spindle-shaped (Innocenti 1980). TMB reaction product permits the visualization only of neuronal somata and proximal dendrites, but still permits the assessment of neuronal size and broad morphological class. Using the TMB technique, we did not notice any abnormalities of neuronal size or form among labeled callosal neurons, although no quantitative measurements were attempted.

Discussion

Reliability of results

The number and tangential distribution of labeled callosal neurons in areas 17/18 of cats reared according to the same paradigm is variable. We have taken multiple measures to reduce the contribution of technical factors to this intragroup variability (Innocenti et al. 1985, p. 264). Much of the variability reflects other factors: Important individual variations in the distribution of callosal neurons have also been reported in normal rats (Cusick and Lund 1981) and monkeys (Van Essen et al. 1982). In the cat, individual variations in retinotopic maps in visual areas (Tusa et al. 1979) may correlate with, and possibly cause, the variations in number and distribution of callosal connections. There are also important individual differences in the total number of callosal axons counted by electron microscopy in both cats (Koppel and Innocenti 1983) and monkeys (LaMantia and Rakic 1984). The standard deviation of the mean number of callosal neurons per section in individual animals is certainly exaggerated by the rostrocaudal variations in the number of callosal neurons per section.

We have presented (Innocenti and Frost 1980; Innocenti et al. 1985) multiple observations that make it unlikely that difficulties in HRP transport or visualization would account for the effects of early binocular eyelid suture on the number and distribution of callosal neurons in areas 17/18; similar arguments apply to the present results in DR cats, although an exact estimate of the DR-induced loss of callosal axons awaits direct axon counts in the corpus callosum.

Effects of dark-rearing on callosal connections

As discussed previously for binocular eyelid suture (Innocenti and Frost 1980; Innocenti et al. 1985), the DR-induced reduction in the number and distribution of callosal neurons in areas 17/18 seems to be related to the natural postnatal reshaping of callosal connections (Innocenti and Caminiti 1980), i.e., the elimination of axons that cortical neurons transiently send through the corpus callosum (Innocenti 1981; Koppel and Innocenti 1983). DR may exaggerate this normal elimination of callosal axons. This interpretation is supported by the fact that DR has its effect during the period when transient connections are eliminated (Innocenti and Caminiti 1980; see also Swindale 1988). We cannot, however, exclude the action of other processes: Postnatally, subzone a is the principal source of transient callosal projections, and undergoes the most severe natural reduction (Innocenti and Caminiti 1980); therefore, subzone a might be expected to be most affected as a consequence of visual deprivation. However, our data indicate that the effect of DR is at least as great in subzone c as it is in subzone a[1] although the postnatal elimination of immature callosal connections is less for subzone c than for subzone a (Innocenti and Caminiti 1980). A deprivation-induced reduction in the number of callosal efferent neurons probably also underlies the reduced density and extent of terminating callosal afferent axons observed with the Fink-Heimer technique in DR kittens (Lund and Mitchell 1979), although we cannot exclude that there is also a reduction in the amount of terminal arbor elaborated by the axon of each callosal neuron.

Nature of the visual control of callosal development

The supernormal reductions in the number of callosal neurons that occur as a consequence of neonatal binocular eyelid suture or binocular enucleation (Innocenti and Frost 1980; Innocenti et al. 1985) suggested that visual experience is necessary for the selective stabilization (Changeux and Danchin 1976) of the normal complement of callosal neurons in areas 17/18. The present results, in which we eliminated the confounding effects of absence of the eyes and of diffuse illumination that penetrates closed eyelids (Loop and Sherman 1977), confirm this inference.

[1] In contrast to DR, rearing with binocular eyelid suture has no significant effect on the number of callosal neurons in subzone c (Innocenti et al. 1985). In Siamese cats, there are more callosal neurons in subzone c than in normal cats, suggesting the stabilization of some normally transient callosal axons originating in subzone c (Wilkes et al. 1986), but it is impossible to say whether this is an effect of the mutation at the albino c locus or an effect of the abnormal vision experienced by most Siamese cats due to their strabismus

In both DR cats and cats reared with binocular eyelid suture, the number of callosal neurons in subzone a is subnormal, although the reduction appears to be greater for lid-sutured cats (56% of normal) than for DR cats (74% of normal). In DR cats the number of callosal neurons in subzone c is also reduced – to about 63% of normal; by contrast, in cats reared with binocular eyelid suture, there is no significant effect on the number of callosal neurons in subzone c (Innocenti et al. 1985).[2] Our estimate of the reduction in the number of callosal neurons in DR cats is probably conservative: Due to a reduction of cortical volume, the density of visual cortical neurons is higher in DR-than in normal cats (Mower et al. 1988). Thus, sections of the same thickness contain a greater fraction of the total volume of the visual cortex in DR cats than in normal cats; using our methods, this would produce an overestimate of the relative number of callosal neurons in DR cats. Both DR and eyelid suture apparently cause small reductions in the width of the callosal efferent zone in areas 17/18.

The presence of a large fraction of the normal complement of callosal neurons in DR cats demonstrates that the stabilization of immature callosal connections is largely independent of vision. The reduced number and distribution of callosal neurons in DR cats demonstrates that the normal partial elimination of immature callosal connections is likewise initiated independent of vision. However, normal vision is necessary for the stabilization of a normal complement of callosal neurons: DR, like binocular lid suture and binocular enucleation, exaggerates the normal developmental reduction in callosal neuron number. These results contrast with those of studies in which DR was reported to completely or partially stabilize immature, widespread distributions of geniculocortical axons in area 17 (Swindale 1981, 1988; Mower et al. 1985; Kalil 1982) and more nearly correspond with those studies in which DR produced no qualitative change in the distribution of geniculocortical axons in area 17 (Stryker and Harris 1986) or area 18 (Mower et al. 1985; Swindale 1988). Even rearing paradigms that maintain callosal efferents which would otherwise be eliminated, do not prevent elimination of most of the original projection (Innocenti and Frost 1979; Frost et al. 1988).

[2] It was not possible to make a similar comparison between DR cats and cats reared with binocular enucleation, since technical factors made it impossible to obtain a quantitative estimate of the effects of neonatal enucleation in our previous study (Frost and Innocenti 1980)

Although DR does not stabilize normally transient callosal connections, it appears that DR is less deleterious than certain forms of abnormal visual experience. Both DR cats and cats reared with binocular enucleation (Innocenti and Frost 1980) have more callosal neurons than cats reared viewing only diffuse illumination as a consequence of binocular eyelid suture (Innocenti and Frost 1980). Similarly, the physiological effects of DR appear to be somewhat less severe than those of binocular lid suture (reviewed in Sherman and Spear 1982; Fregnac and Imbert 1984). These data indicate a possible benefit in the use of opaque occluders in the period preceding correction of early cataract or corneal defects. The data also suggest that the absence of visually-elicited retinal ganglion cell activity contributes to the greater stabilization of immature callosal connections in binocularly enucleated as compared to binocularly eyelid sutured cats.

The present data on the effects of eliminating visual experience contrast with the preliminary results of experiments in which the role of retinal ganglion cell impulse activity on the development of callosal connections was studied by binocularly blocking such activity from birth to 8 weeks of age with intraocularly administered TTX (Frost and Dubin, unpublished data). In TTX-treated cats, as in cats subjected to neonatal binocular enucleation (Innocenti and Frost 1980), callosal neurons persist in peripheral regions of area 17 that are acallosal in normal animals of comparable age (although the relative number of callosal neurons in TTX-treated cats remains to be determined). These data suggest that retinal impulse blockade, unlike DR, results in the stabilization of some normally transient callosal connections.

It is of interest to consider the effects of DR on the callosal connections of areas 17/18 in light of the known physiological effects of DR on other parts of the visual system. In the cat, DR does not affect the response properties or axonal branching patterns of retinal ganglion cells (Garraghty et al. 1987). DR causes a profound reduction in the population of physiologically defined Y-cells in the A-layers of the LGd, while leaving X-cells virtually unaffected (Kratz et al. 1979); there is a parallel reduction in the expression by LGd neurons of a developmentally regulated antigen known to be specific for a subset of Y-cells (Sur et al. 1988). The detailed physiological effects of DR on neurons in areas 17/18 vary among studies (reviewed in Sherman and Spear 1982; Fregnac and Imbert 1984) but it is generally agreed that the percentage of visually responsive neurons

and their maximum response rates are reduced and that their receptive field properties are altered. These effects arise, in part, due to abnormalities in the function of the cortical GABAergic system (reviewed in Mower et al. 1988). These findings have two important implications for the function and development of visual callosal connections: i) Neurons projecting to the corpus callosum show the same range of response properties as other cortical neurons (Berlucchi et al. 1967; Hubel and Wiesel 1967; Innocenti 1980). Therefore, due to the inactivity or elimination of an important class of afferents (geniculate Y-cells) and to the reduced or anomalous visual responses of area 17/18 neurons, there are probably both qualitative and quantitative changes in the information transmitted to the opposite hemisphere by callosal neurons in DR cats. ii) The greater than normal developmental elimination of callosal connections resulting from DR may occur because other neurons that have more spontaneous activity and that share synaptic targets with callosal neurons may have an advantage over the callosal neurons in an activity-dependent competition for the stabilization of their axons (Changeux and Danchin 1976; Bear et al. 1987). Normal vision, binocular eyelid suture, DR, binocular enucleation and retinal impulse blockade could all operate by differential biasing of such competition among the various axonal systems impinging on cortical neurons. Such biasing could be the main morphogenetic action of visual experience.

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