Effects of Early Monocular Eyelid Suture upon Development of Relay Cell Classes in the Cat's Lateral Geniculate Nucleus

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ABSTRACT Horseradish peroxidase (HRP) was injected into visual cortex of four normal cats and five cats raised with monocular lid suture, and retrograde labelling was assessed in cells of the lateral geniculate nucleus. In all but one of the sutured cats (noted below) focal injections were carefully limited to area 17 or 18 and analysis of labelling focused on laminae A and A1. The effects of deprivation were indistinguishable whether lamina A or A1 was deprived, and in all cases, the nondeprived laminae had labelling essentially identical to that seen in normal cats.

After area 17 injections (bilateral in one normal cat and unilateral in 3 deprived cats), roughly 77% of the cells in nondeprived laminae were labelled and they were mostly small to medium in size. Deprived laminae, when compared to nondeprived laminae, had two abnormalities: (1) cells, both labelled and unlabelled, were smaller; and (2) roughly 11% fewer cells (i.e., 66%) were labelled, and this represents a small but statistically significant difference for each cat. After area 18 injections (bilateral in one normal cat plus unilateral in 3 other normal and 3 deprived cats), roughly 15% of the cells in nondeprived laminae were labelled, and they tended to be large in size. Deprived laminae, when compared to nondeprived laminae, had three abnormalities: (1) only 5-6% of the cells were labelled, and these tended to be quite faintly labelled; (2) the volume occupied by labelled cells was small; and (3) both labelled and unlabelled cells were reduced in size. Finally, large bilateral injections were made throughout occipitotemporal cortex in one lid sutured cat in an effort to label completely the terminal zones of cells in the medial interlaminar nucleus (MIN), a division of the lateral geniculate nucleus; this cat also had a prior intraocular injection of tritiated proline to provide through subsequent autoradiography a delineation of deprived and nondeprived portions of MIN. Roughly 78% of the cells in nondeprived portions of MIN were labelled in this cat. In the deprived portions, only about 51% of the cells were labelled, and these tended to be faintly labelled. Also, labelled cells were smaller, and unlabelled cells were larger in deprived than they were in nondeprived portions.

Since prior studies have shown that, within the A laminae, X-cells project exclusively to area 17 whereas the Y-cell population projects to areas 17 and 18, these data are taken as further support of the conclusion that geniculate Y-cells are more seriously affected by the early deprivation than are geniculate X-cells. That is, these data are consistent with the suggestion that a similar population of Y-cells in deprived laminae (roughly 10% of the overall cell total) fail to transport HRP from area 17 or area 18 injections. This can be extended to the MIN, which seems to be comprised nearly exclusively of Y-cells. However, these conclusions must be considered tentative, since interpretation of HRP data can be difficult as evidenced by discrepancies in the literature.

It has been known since the classical studies of Wiesel and Hubel ('63b, '65; see also Hubel and Wiesel, '70) that cats raised with monocular eyelid closure suffer severe physiological abnormalities in their geniculocortical pathways. In the striate cortex of these cats, cells can usually be influenced only by the nondeprived eye instead of the normal pattern of binocular activation (Wiesel and Hubel, '63b; Wilson and Sherman, '77). Physiological abnormalities have also been described for the lateral geniculate nucleus. In normal cats, laminae A and A1 are comprised chiefly of X-cells and Y-cells.[1] These represent parallel geniculocortical relays, respectively, of retinal X- and Y-cell input (Enroth-Cugell and Robson, '66; Cleland et al., '71; Hoffmann et al., '72). Y-cells differ from X-cells physiologically along a number of dimensions: they tend to demonstrate less linear summation within the receptive field; to have larger receptive fields; to respond to faster stimulus velocities; to respond more transiently to appropriate standing contrasts; and to have faster conducting axons (see also, Kratz et al., '78a). Sherman et al. ('72) have shown that Y-cells in the lateral geniculate nucleus appear to be affected rather selectively by the visual deprivation. They found that, in the deprived laminae, few normal Y-cells could be located electrophysiologically whereas the X-cells seemed unaffected. Since retinal ganglion cells are not detectably affected by lid suture, these changes presumably occur central to the optic tract (Sherman and Stone, '73). This effect of lid suture has been extended in the cat to the medial interlaminar nucleus (MIN), a subdivision of the lateral geniculate nucleus comprised nearly exclusively of Y-cells (Kratz et al., '78b,c). It has also been extended to the lateral geniculate nucleus of the tree shrew *(Tupaia glis)* which normally has X- and Y-cells in its lateral geniculate nucleus (Norton et al., '77).

However, until recently in the lid sutured cats, there have been few anatomical deficits described which could be correlated specifically with the above physiological deficits. The striate cortex in these animals seems grossly normal in histological appearance (Wiesel and Hubel, '63b; Wilson and Sherman, '77; see however, Shatz et al., '77), and whereas cells in the deprived geniculate laminae were described as being 20-40% smaller than their nondeprived counterparts (Wiesel and Hubel, '63a; Guillery and Stelzner, '70), it had not been possible to relate this specifically to Y-cell deficits (Sherman et al., '72; Hickey et al., '77).

Two recent studies have identified anatomical abnormalities in deprived geniculate laminae which can be rather specifically linked to the physiological deficits reported for Y-cells. LeVay and Ferster ('77) have suggested that X- and Y-cells can be identified by the presence (X-cell) or absence (Y-cell) of a cytoplasmic structure referred to as a "cytoplasmic laminar body" (CLB). Based on this, they reported that Y-cells in deprived geniculate laminae were fewer in number and much smaller in size than Y-cells in nondeprived laminae, and that deprivation effects on the X-cells were considerably less severe. Garey and Blakemore ('77) made use of a different method of identifying these cells based on evidence that X-cells project only to area 17 while the Y-cell population projects both to areas 17 and 18 (Stone and Dreher, '73; LeVay and Ferster, '77). It is not clear if the Y-cell projection pattern is due to individual cells sending branching axons to both areas, or to different subpopulations projecting to one or the other area. Nevertheless, Garey and Blakemore ('77) isolated a Y-cell population by marking it with horseradish peroxidase (HRP) retrogradely transported from area 18. Based on this identification and subsequent cell size measurements, they also concluded that Y-cells were more affected by the lid suture than were X-cells.

Independently of the above two studies, we also sought anatomical correlates for the geniculate Y-cell deficits in monocularly sutured cats. Our approach was nearly identical to that of Garey and Blakemore ('77). That is, we injected HRP into various cortical loci in monocularly deprived cats and observed the retrograde labelling in the lateral geniculate nucleus. As did the above mentioned authors, we also obtained data which support the relatively selective physiological consequences of deprivation upon Y-cells. Unlike our conclusions however, our data are not in complete agreement with those of Garey and Blakemore ('77), and this raises questions, which are discussed below, concerning interpretation of HRP results. A preliminary report of these findings has appeared (Lin and Sherman, '77).

[1] W-cells have also been described in the most ventral C laminae (Wilson et al., '76), but since little is known of the effects of visual deprivation upon W-cells, and since the present report does not analyze the C laminae, W-cells are not further considered (see also Rowe and Stone, '76).

MATERIALS AND METHODS

Subjects

Data from nine adult cats were analyzed in this study. Four normal cats (N1, N2, N3, N4), purchased as adults, provided control data. The remaining five were born and raised in the laboratory. Each of these five had the lids of one eye sutured at eight to ten days of age (i.e., approximately the time of natural eye opening), and the eye was kept closed until the terminal experiments were performed by which time the cats were at least 12 months of age. Of these, four had the left eye sutured (LMD42, LMD48, LMD53, and LMD57), and one had the right eye sutured (RMD51). Daily inspections ensured that no lid holes exposing the pupil existed for any of the deprived eyes (Loop and Sherman, '77).

Histological procedures

Our general procedures have been previously described (Lin et al., '77) and will be briefly outlined here. The cats were anesthetized with barbiturate, placed in a stereotaxic headholder, and the exposure of cortex was done with routine surgical procedures. In every cat except LMD57, a single 0.2-0.3 μl injection of a 30% solution of Sigma VI HRP was made into either area 17 or 18 of each side. We slowly injected each aliquot via a Hamilton syringe over a 30-minute period. The injections in each area were aimed at the center of layer IV away from the 17/18 border (see also RESULTS). For area 17, these injections were placed at a depth of 2-3 mm about 1.0 mm lateral to the sagittal sinus near the zero coronal plane; for area 18, these injections were placed at a depth of 2-3 mm roughly 1 mm medial to the lateral sulcus and also near the zero coronal plane (Otsuka and Hassler, '62). For reasons given in RESULTS, many injections in these and other cats were unsatisfactory and were not analyzed in detail. Injections limited to area 17 or 18 were successfully made in 13 hemispheres of the eight cats excluding LMD57 (see below), and these data were analyzed in detail. In cat LMD57, a much larger bilateral injection was made: on each side, ten separate injections of 0.3 μl each were placed near the zero coronal plane along a mediolateral strip including areas 18, 19 and the lateral suprasylvian area. This was done in order to label extensively the projection zone of MIN (Rosenquist et al., '74; Lin et al., '77). Following a 48-hour survival period, the cats were anesthetized, perfused intracardially with 10% saline followed by a phosphate buffered 1% paraformaldehyde and 1% glutaraldehyde solution. The visual cortices and lateral geniculate nuclei were then stereotaxically blocked, cut coronally into 40-μm sections, and treated for HRP reaction product according to the procedures described by LaVail and LaVail ('74). All cats in this series had their deprived eyes opened one to seven days before the HRP injections, and both eyes were opened throughout the 48-hour survival period. This ensured that any interlaminar asymmetry in HRP staining was not due to lack of activity in some laminae caused by eye closure during the survival period (Nauta et al., '74; Strick et al., '76; Singer et al., '77).

An additional procedure was used for cat LMD57 in order to visualize the "hidden lamination" of MIN (Guillery, '70). An injection of 100 μCi of tritiated proline was placed into the vitreous of its nondeprived eye eight days prior to the HRP injections. Following the HRP procedures described above, autoradiography was performed on the same sections through the lateral geniculate nucleus according to the methods described by Cowan et al. ('72).

Cell cross-sectional areas were determined by tracing soma outlines with a drawing tube attachment on a microscope at 1,000 $\times$.

Sampling procedures

Only cells with clearly visible nucleoli were considered. Occasionally ($<2\%$), a cell was so heavily labelled that a potentially present nucleolus was obscured (Lin et al., '77), and such cells were ignored.

For cell cross-sectional measurements, we employed our previously described (Lin et al., '77) sampling procedure. That is, matched zones were selected in laminae A and A1 (or in the hidden laminae of MIN), and every cell within each zone was measured. The zones were centered within the region of densest HRP labelling.

We used slightly different sampling procedures for estimating the percentage of labelled cells found within the laminae. These procedures differed slightly depending upon the specific injection site and/or region to be analyzed, and they are briefly reiterated in RESULTS. Our primary objective was to derive a reliable estimate of any relative differences between laminae in terms of the percentage of

labelled cells. Throughout this paper, the *relative* difference (R%) in labelling between deprived and nondeprived laminae is defined as 100% minus the ratio (expressed as a percentage) of the percentage of labelled cells in the deprived lamina (D%) divided by this percentage in the nondeprived laminae [N%; i.e., R% = 100%(1 − D/N)]. In order to obtain a sufficiently large sample to detect subtle interlaminar differences, we chose larger zones than used for soma cross-sectional measurements and counted every labelled and unlabelled cell within these zones. Within each cat these zones were carefully matched between laminae and were centered upon the densest region of HRP labelling. With the exception of cases with area 18 injections, these sampling zones did not extend beyond the region where the density of HRP labelled cells dropped discernibly.

However, after area 18 injections, possibly due to smaller injections and an overall lower percentage of labelled cells, the sampling zones clearly included regions of less dense labelling (i.e., towards the boundaries of the zones). We have argued elsewhere (Lin et al., '77) that the most reliable estimate of the actual percentage of geniculate cells projecting to a given injection site is derived from a sample limited to the densest zone of HRP labelling. This follows, because further from this geniculate region may be found cells which project axons further from the center of the injection site where available HRP for uptake and transport is less; these cells, then, may not display detectable label. Throughout this paper the *absolute* difference (A%) in labelling between deprived and nondeprived laminae is defined as the percentage of labelled cells in the nondeprived lamina (N%) minus that in the deprived lamina (D%; i.e., A% = N% − D%). Such an estimate of absolute differences in the percentage of relay cells depends on the most reliable estimate within each laminae. Therefore, for area 18 injections, considerations of *absolute* interlaminar differences in the percentage of labelling are based on these smaller samples confined to the densest zone of labelling, whereas we continued to use the larger samples to provide the most reliable estimate of *relative* interlaminar differences in the percentage of labelled neurons.

RESULTS

The HRP results reported here are consistent with the notion that geniculate Y-cells are more seriously affected by rearing with lid suture than are geniculate X-cells. That is, compared to nondeprived laminae, deprived laminae were more deficient in labelling after area 18 HRP injections than after area 17 injections, and X-cells project only to area 17 whereas the Y-cell population projects both to areas 17 and 18 (Stone and Dreher, '73; LeVay and Ferster, '77). Furthermore, deprived MIN regions displayed less labelling than did nondeprived regions following large occipitotemporal cortical injections of HRP, and practically all MIN neurons are Y-cells (Kratz et al., '78b). However, the conclusion that Y-cells are more affected by deprivation than are X-cells required a number of assumptions, and an attempt will be made to clarify these as the data are described below.

Laminae A and A1

Determination of injection sites

Since the rationale for much of this experiment stems from observations that X-cell projections are limited to area 17 while Y-cell projections extend as well to area 18 (Stone and Dreher, '73; LeVay and Ferster, '77; see also Garey and Powell, '67; Garey and Blakemore, '77), it is crucial to clarify our criteria for ensuring that injections were limited

Fig. 1 Photomicrographs of cortical HRP injection sites and labelling in the lateral geniculate nucleus. a. Low power brightfield view of injection centered in area 17 of case LMD48(R). The borders of area 17 are indicated by black lines running perpendicular to the cortical layers. b. Brightfield view of lateral geniculate nucleus of LMD48(R). The black rectangle outlines the zone of labelled neurons, which are seen more clearly in c. This rectangle also outlines the view seen in higher power in figure 2b. Note that the labelling is limited in mediolateral extent and well away from the medial border of laminae A and A1. c. Darkfield view of same region as shown in b. The labelled neurons are more clearly seen here. d. Low power of brightfield view of injection centered in area 18 of case RMD51(R). The borders of area 18 are indicated by black lines perpendicular to the cortical layers. e. Brightfield view of lateral geniculate nucleus of RMD51(R). As in b, the black rectangle outlines the zone of labelling which is limited in mediolateral extent and well away from the medial edge of laminae A and A1. The area within the rectangle is shown in higher power in figure 2f. f. Darkfield view of same region as shown in e. g. Low-power brightfield view of cortical injection which seems mostly limited to right area 18 in cat RMD44. The borders of area 18 are indicated as in d. h. Brightfield view of right lateral geniculate nucleus of cat RMD44. The black rectangle, which shows the zone of labelling, extends to the medial edge of laminae A and A1. i. Darkfield view of same region as in h. The bars in a, d, and g are 2 mm, and the 1 mm bar in b applies as well to c, e, f, h, and i.

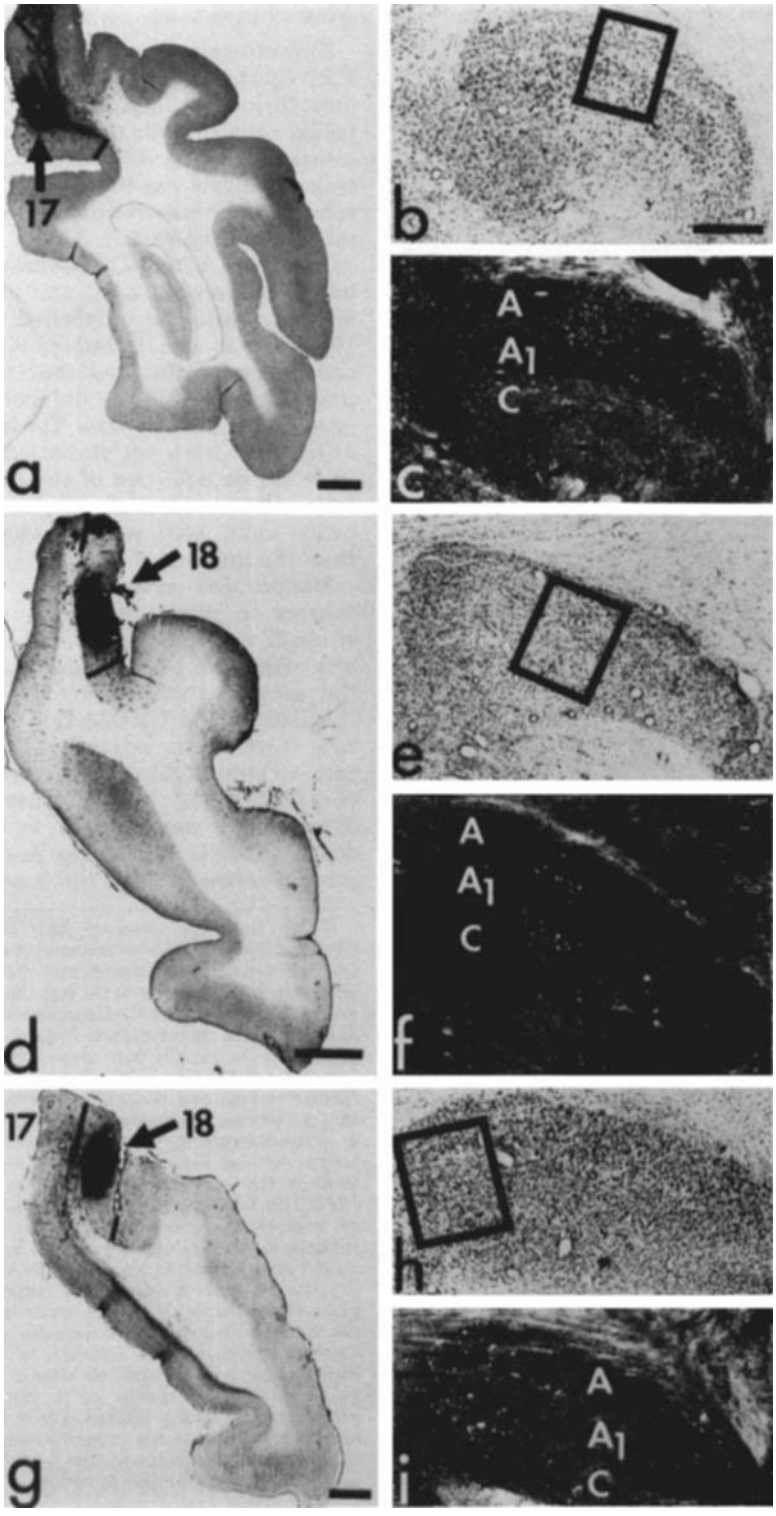

Figure 1

to one or the other area and avoided the 17/18 border as planned (MATERIALS AND METHODS). Two criteria must be fulfilled. First, the appearance of the injection site must be essentially confined to and centered within the *gray* matter of one or the other of these areas with minimal involvement of the underlying white matter. However, since the apparent size of the injection site can vary with survival time (Vanegas et al., '78), this condition alone is insufficient, and we relied chiefly upon our second criteria. That is, the mediolateral extent of labelling within the lateral geniculate nucleus must be confined and occur well away from the medial edge of laminae A and A1. Since the vertical meridian of the visual field is mapped onto both this medial edge of laminae A and A1 (Sanderson, '71) as well as the 17/18 cortical border (Tusa et al., '78), it follows that labelled cells in this geniculate region imply HRP spread to the 17/18 border. Consequently, if an HRP injection designed for area 17 or 18 resulted in labelled cells located medially in lamina A or A1, it was considered to have spread to the other area, perhaps through white matter (Lund et al., '75), even if inspection of cortical histology suggested an injection confined to one area. Such cases were not infrequent and are not further considered here except to illustrate this point in figure 1. This problem arose in our experience only with injections aimed at area 18, presumably because the white matter below area 18 carries geniculate fibers headed for area 17, whereas below our injection sites in area 17, few if any geniculate fibers heading for area 18 would be expected. Only those few cases (13 hemispheres in 8 cats; see table 1) which passed both of our criteria were analyzed in detail and discussed below. Figure 1 offers examples based on these criteria of an injection limited to the right area 17 of LMD48 (fig. 1a-c), an injection limited to the right area 18 of RMD51 (figs. 1d-f), and an injection aimed for the right area 18 of RMD44 but which presumably spread to area 17 (figs. 1g-i). Both cortical injections shown in figures 1d,g appear to be essentially contained within area 18. Only in the former is geniculate labelling clearly away from the medial edge of the A laminae (figs. 1e,f), since the labelling in the latter extends to this medial edge (figs. 1h,i). This illustrates our reliance on the appearance of geniculate labelling to determine containment of injection sites.

Area 17 injections

Normal cat. Area 17 was injected with HRP bilaterally in cat N1 without spread to area 18, and laminae A and A1 had heavily labelled cells in both hemispheres. No obvious interhemispheric difference was seen, so detailed analysis was limited to the right hemisphere, and these results are summarized in table 1 and figure 3.

In lamina A, 72.5% of the cells were labelled and they averaged 284.5 μm^2 in cross-sectional area, while the unlabelled cells averaged 172.2 μm^2 in size. In lamina A1, 72.1% of the cells were labelled and they averaged 296.7 μm^2 in size, while the unlabelled cells averaged 180.8 μm^2 in size. These interlaminar differences were not statistically significant ($p > 0.1$ on a X^2-test of the ratio of labelled cells and on a t-test of cell sizes). Also, the labelled cells were roughly two-thirds larger than the unlabelled cells.

Monocularly sutured cats. Three hemispheres in monocularly sutured cats were studied with restricted area 17 injections away from the 17/18 border as determined by the aforementioned criteria. In two cases [LMD42(L) and LMD53(L)], the left area 17 was injected in left monocularly deprived cats, so lamina A1 was deprived. In the other case (LMD48[R]), area 17 contralateral to the deprived eye was injected, so lamina A was deprived (table 1). Figures 2a-d show a short coronal series through the zone of geniculate

Fig. 2 Darkfield views of HRP labelled geniculate cells. a-d. coronal series (a is rostral, d is caudal) through the right lateral geniculate nucleus of case LMD48(R) following an HRP injection to the right cortical area 17. The arrows indicate the interlaminar zone between laminae A and A1. Since the projection lines in the cat's lateral geniculate nucleus tilt with respect to the coronal plane (*see text and Sanderson, '71*), the labelled zone dorsally is further rostral, and ventrally, posterior. Note, however, that no obvious qualitative difference exists in labelling intensity between the deprived lamina A and nondeprived lamina A1. e-g. Coronal series (e is rostral, g is caudal) through the right lateral geniculate nucleus of case RMD51(R) following an HRP injection to the right cortical area 18. The arrow in e indicates the zone between laminae A and A1, and the arrows in f, g indicate the zones between laminae A and A1 plus A1 and C. As in a-d, the labelled zone is tilted with respect to the coronal plane of sectioning. Note that many more labelled cells can be seen in nondeprived laminae A and C than in deprived lamina A1. The rectangle in f is reproduced at higher power in h. h. Darkfield view of nondeprived lamina C and deprived lamina A1 in case RMD51(R). The arrows point to faintly labelled cells in the deprived lamina which contrast to the intensely labelled neurons seen in the nondeprived laminae. The bar in a is 100 μm and applies to a-g. The bar in h is 30 μm

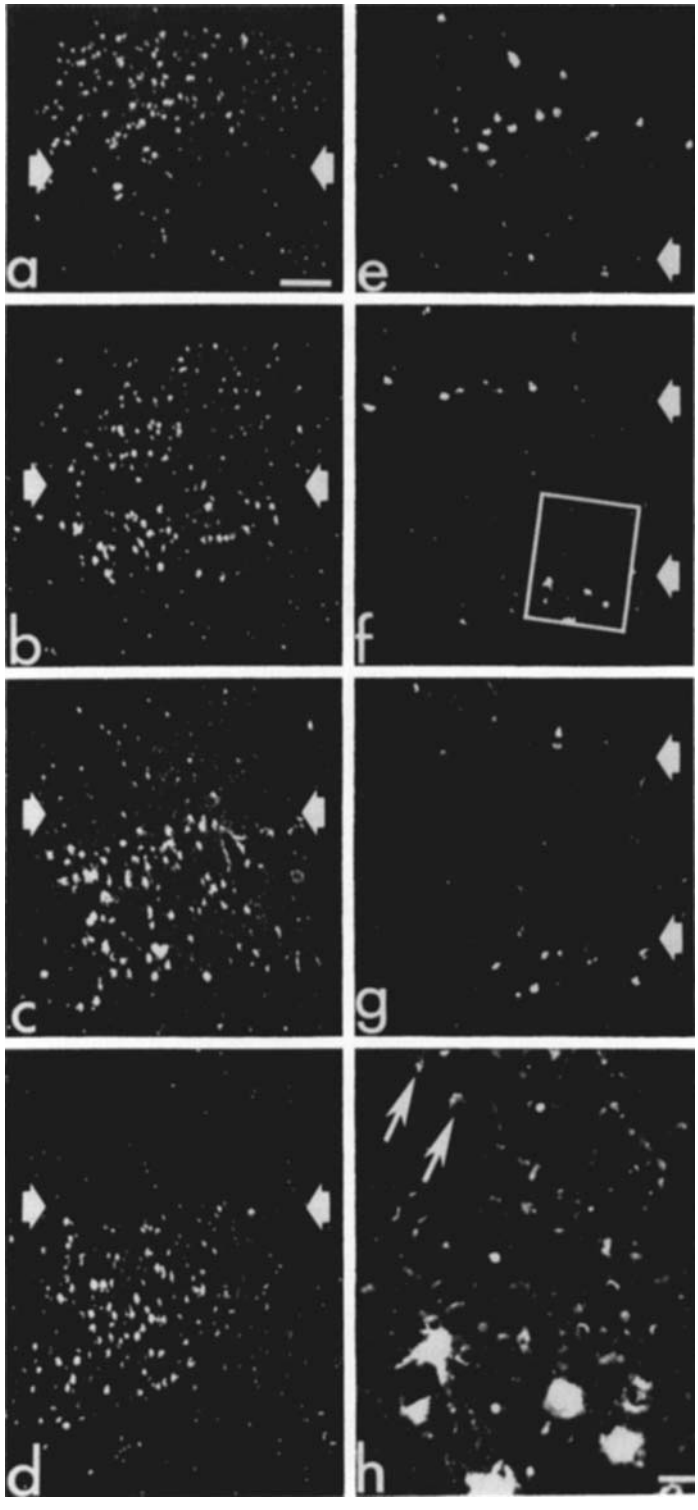

Figure 2

TABLE 1

LGN neuronal labelling percentages and cell size measurements after area 17 injections of HRP

(A) case	(B) laminae	(C) total cell no. sampled	(D) % of labelling	(E) relative % of reduction	(F) absolute % of reduction	(G) labelled cell mean area	(H) normalized % of labelled cell size decrease	(I) unlabelled cell mean area	(J) normalized % of unlabelled cell size decrease
N1	A	215	72.5	—	—	284.5	—	172.2	—
	A_1	491	72.1	—	—	296.7	—	180.8	—
LMD48(R)	*A (Dep)*	*271*	*66.4*	*16.1* [1]	*12.7*	*271.7*	*19.6* [3]	*215.4*	*21.6* [4]
	A_1	283	79.1	—	—	352.4	—	288.5	—
LMD53(L)	A	228	75.4	—	—	388.3	—	314.7	—
	A_1 (Dep)	*219*	*66.7*	*11.5* [2]	*8.7*	*239.5*	*40.8* [3]	*202.0*	*38.8* [4]
LMD42(L)	A	232	76.7	—	—	306.5	—	275.0	—
	A_1 (Dep)	*202*	*65.8*	*14.5* [2]	*10.9*	*199.7*	*37.5* [3]	*156.7*	*45.7* [4]

Shown are the percentages of labelled cells and cross-sectional areas for labelled and unlabelled cells. See MATERIALS AND METHODS for a description of how the values in columns E and F were obtained. Statistical significance levels are also shown for interlaminar differences. Data for deprived (Dep) laminae are italicized. Since, in normal cats, cells in lamina A1 are slightly larger than those in lamina A (Guillery, '73; Hickey et al., '77) the cell sizes for cases LMD48(R), LMD53(L), and LMD42(L) were normalized from the data for cat N1 before the interlaminar comparisons in columns H and J. That is, for lamina A, the cross-sectional areas of all labelled cells were multiplied by 296.7/284.5, and of all unlabelled cells by 180.8/172.2, before statistical comparisons with data from lamina A1. It should be emphasized that the normalization applied here and in table 2 is so small that overall conclusions regarding cell sizes are in no way affected by the normalization. A better determination of deprivation effects upon cell size would be to compare directly the cell sizes between deprived and nondeprived lamina A or A1; but this would require matched HRP injections in both hemispheres, and these were unavailable to us. We chose to normalize cell sizes in comparisons between laminae A and A1 with the justification that this provided the best approximation available to us of actual cell size changes in deprived laminae.

[1] $p < 0.001$ on a X^2-test.
[2] $p < 0.01$ on a X^2-test.
[3] $p < 0.001$ on a t-test.
[4] $p < 0.01$ on a t-test.

neuronal labelling for case LMD48(R). In the cat's lateral geniculate nucleus, the projection lines are tilted so that the dorsal tip lies anterior to the ventral tip (Sanderson, '71). Coronal sections thus indicate more labelling anteriorly in lamina A and posteriorly in the C laminae. This is seen in the series of figures 2a-d, which also shows no obvious qualitative difference between deprived lamina A and nondeprived lamina A1 in terms of the intensity of labelling, the percentage of labelling, or the laminar volume occupied by labelled neurons. However, neurons (both labelled and unlabelled) tended to be smaller in deprived than in nondeprived laminae. These observations also obtained for cases LMD42(L) and LMD53(L), and quantitative analyses of the data are shown in table 1 and figure 3.

Generally, nondeprived laminae had 75.4% to 79.1% labelled cells, in good agreement with the labelling seen in cat N1, and the deprived laminae had 65.8% to 66.7% labelled cells. It is interesting that, in each cat, the deprived lamina had fewer labelled cells than did the nondeprived lamina. Although in absolute terms (MATERIALS AND METHODS), this difference was only 8.7%-12.7% (mean: 10.8%) it was reliable and statistically significant (table 1).

A more obvious difference between deprived and nondeprived laminae was seen in terms of the cross-sectional area. Compared to cells in nondeprived laminae, the deprived cells were smaller by values ranging from 19.6% to 45.7% (table 1). These interlaminar differences were roughly equal for labelled and unlabelled cells within each cat, although the overall difference varied from about 20% to 40% among the three cats.

Area 18 injections

Normal cats. In three normal cats (N2, N3, and N4), bilateral injections were made into area 18. Two injections failed to meet the criteria for sites limited to area 18, and thus four hemispheres were analyzed. Among these four cases, results were indistinguishable, and only one hemisphere of cat N2 will be described in detail. Table 2 and figure 4 summarize these results.

Labelling was much sparser after area 18 injections than after area 17 injections, and we could not obtain a reasonable sample of la-

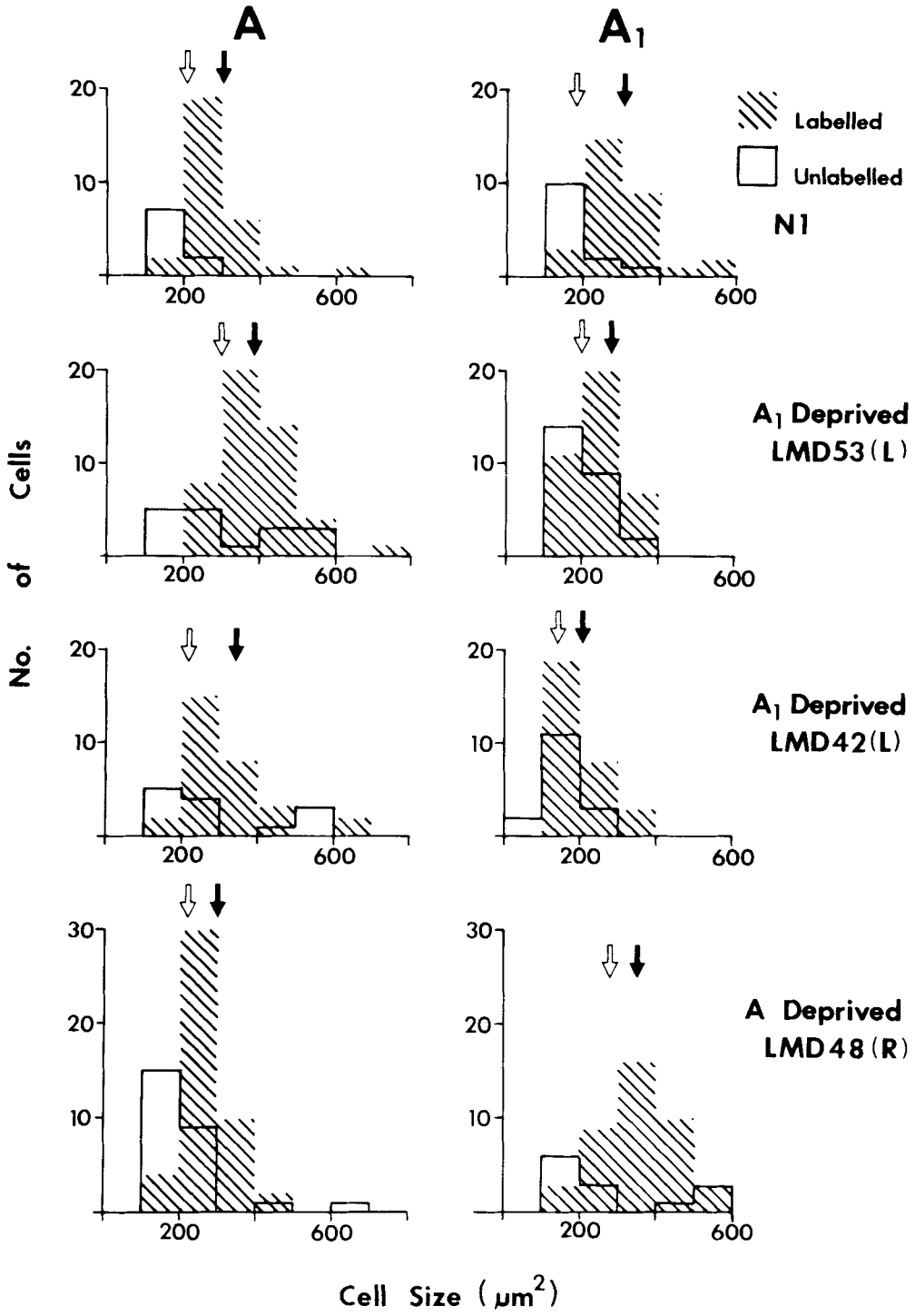

Fig. 3 Frequency histograms of labelled and unlabelled geniculate cell sizes after HRP injections into area 17. Shown are the distributions in laminae A and A1 for a normal cat (N1) and three monocularly sutured cats [LMD53(L), LMD42(L), and LMD48(R)]. The open arrows signify the mean size for unlabelled cells; the filled arrows, for labelled cells. The total number of cells measured, respectively, for laminae A and A1 are: N1, 38 and 43; LMD53(L), 64 and 63; LMD42(L), 43 and 46; LMD48(R), 72 and 54 (see also table 1).

belled cells without including regions of less dense labelling. Instead, the larger sample in parentheses was taken by counting every labelled cell in several sections and then counting every unlabelled cell bounded on each side by a line drawn normal to the laminae through the nucleolus of the most medial labelled cell and the most lateral labelled cell. The actual volume of sampled tissue was practically identical in each lamina. Because of the reduction in labelling away from the center of the labelled zone (where a smaller sample was also taken), this probably underestimates the absolute percentage of labelling while it provides a more certain relative assessment of labelling between laminae (MATERIALS AND METHODS). Thus the smaller samples (no parentheses) are used for absolute estimates of labelling percentages, and the larger samples (parentheses), for interlaminar comparisons. In lamina A, 14.0% of the cells were labelled (or 11.8% of the larger sample) and they averaged 566.7 μm^2 in size; unlabelled cells were only 318.4 μm^2 in size. Practically identical results were seen in lamina A1. That is, here 14.7% (or 12.0%) of the cells were labelled and they averaged 638.9 μm^2 in size; unlabelled cells averaged only 328.6 μm^2 in size. Similar results obtained in cats N3 and N4. No interlaminar differences in the percentage of labelled cells were evident (range: 12.1% to 14.3% for samples limited to the densest region of labelling), and lamina A1 cells were slightly larger than those in lamina A (see also table 1 and Hickey et al., '77).

Monocularly sutured cats. Three monocularly sutured cats (LMD48[L], LMD53[R] and RMD51[R]) had unilateral area 18 injections which satisfied the criterion for injection sites limited to this area. The injection was ipsilateral to the deprived eye in cases LMD48(L) and RMD51(R), so that lamina A1 was deprived; it was contralateral to the deprived eye in LMD53(R), so that lamina A was deprived. The results were quite consistent among cats and the deprivation effects were indistinguishable whether lamina A or A1 was deprived. Qualitative differences between deprived and nondeprived laminae were immediately obvious. The labelled cells had much less reaction product and thus tended to be much lighter in label, fewer cells were labelled, and the volume occupied by labelled cells within the lamina was considerably smaller. Figures 2e-g illustrate a short coronal series which shows the difference in labelling between deprived lamina A1 and nondeprived lamina A in case RMD51(R). The very light labelling of deprived cells made it difficult to assess whether or not they were indeed labelled (fig. 2h). Thus, fields were scanned at 1,000 × and care was taken to identify all labelled cells. We were possibly less careful in the nondeprived laminae and may have missed a few very lightly labelled cells there, so our estimates of reduced numbers of labelled cells in deprived laminae are probably conservative.[2] Also, since we could not quantify the intracellular intensity of label, we ignored this difference and, in our subsequent analysis, considered deprived, lightly labelled cells equivalent to nondeprived, heavily labelled cells. With this in mind, figures 4-6 further illustrate the relative labelling (numbers of cells plus laminar space) in the deprived and nondeprived laminae of cases LMD48(L), RMD51(R) and LMD53(R) by indicating each labelled cell in a short, coronal series of sections. Compared to nondeprived laminae, deprived laminae had both fewer labelled cells as well as a smaller volume of neuronal labelling in mediolateral and rostrocaudal extent. Quantitative analysis of these data are summarized in table 2 and figure 7, and particular sampling strategies for these analyses are indicated below.

In the above sampling procedures for normal cats or after area 17 injections in visually deprived cats, equal and matched areas of laminae A and A1 were chosen for study. Since the volume occupied by the labelled neurons seemed equal between laminae, this was justified. However, after area 18 injections in the lid sutured cats, the volume of labelled neurons was considerably less in deprived than in nondeprived laminae. If equal volumes of these laminae were sampled, relatively more of the labelled zone would be included in the deprived than in the nondeprived laminae. To control partially for this and to obtain a

[2] It seems reasonable to assume that a continuum of labelling among cells occurs. That is, one should find very lightly labelled cells with very few HRP granules, very heavily labelled cells with densely packed HRP granules, and all intermediate ranges of labelling. However, heavily labelled cells were rare in deprived laminae following area 18 injections whereas nearly all labelled cells in nondeprived laminae were relatively heavily labelled. This creates a problem related to the adoption of a consistent criterion for determination of whether or not a cell was labelled, since it is likely that some cells had label too light to detect. This is further complicated by our inability to quantify the intracellular density of labelling. However, since we found so many more lightly labelled cells in deprived than nondeprived laminae it is likely that any differences in our criterion for identifying a labelled cell favored such cells in deprived laminae.

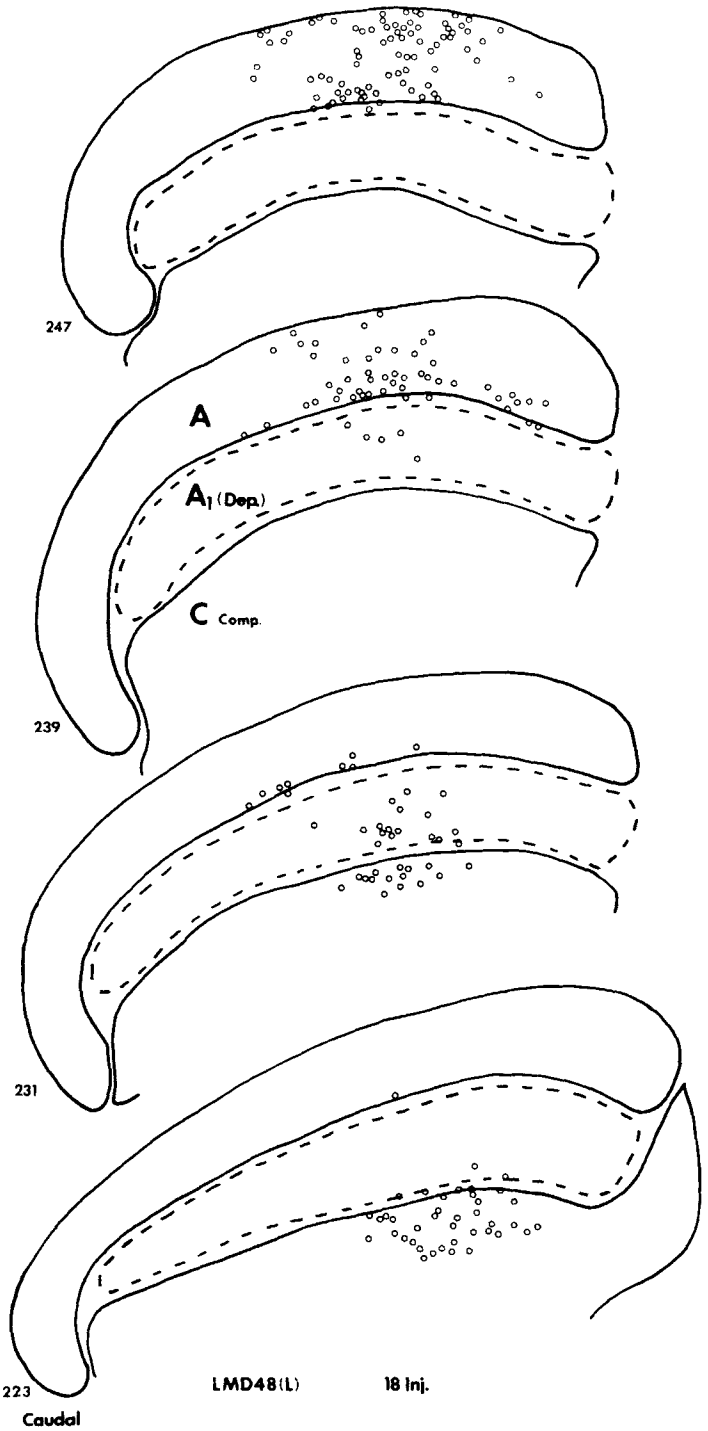

Fig. 4 Drawing showing labelled geniculate cells in coronal series for case LMD48(L) after an HRP injection into the left cortical area 18. The labelled cells are shown as small circles, and the deprived lamina A1 is outlined by a dashed line. Although most deprived cells here and in figures 5 and 6 are labelled much more faintly than nondeprived cells (fig. 2h), this difference in label intensity is not considered in this figure (see text). Compared to the nondeprived lamina A, the deprived lamina A1 not only has fewer labelled cells, but the laminar extent of labelling is reduced both mediolaterally and rostrocaudally.

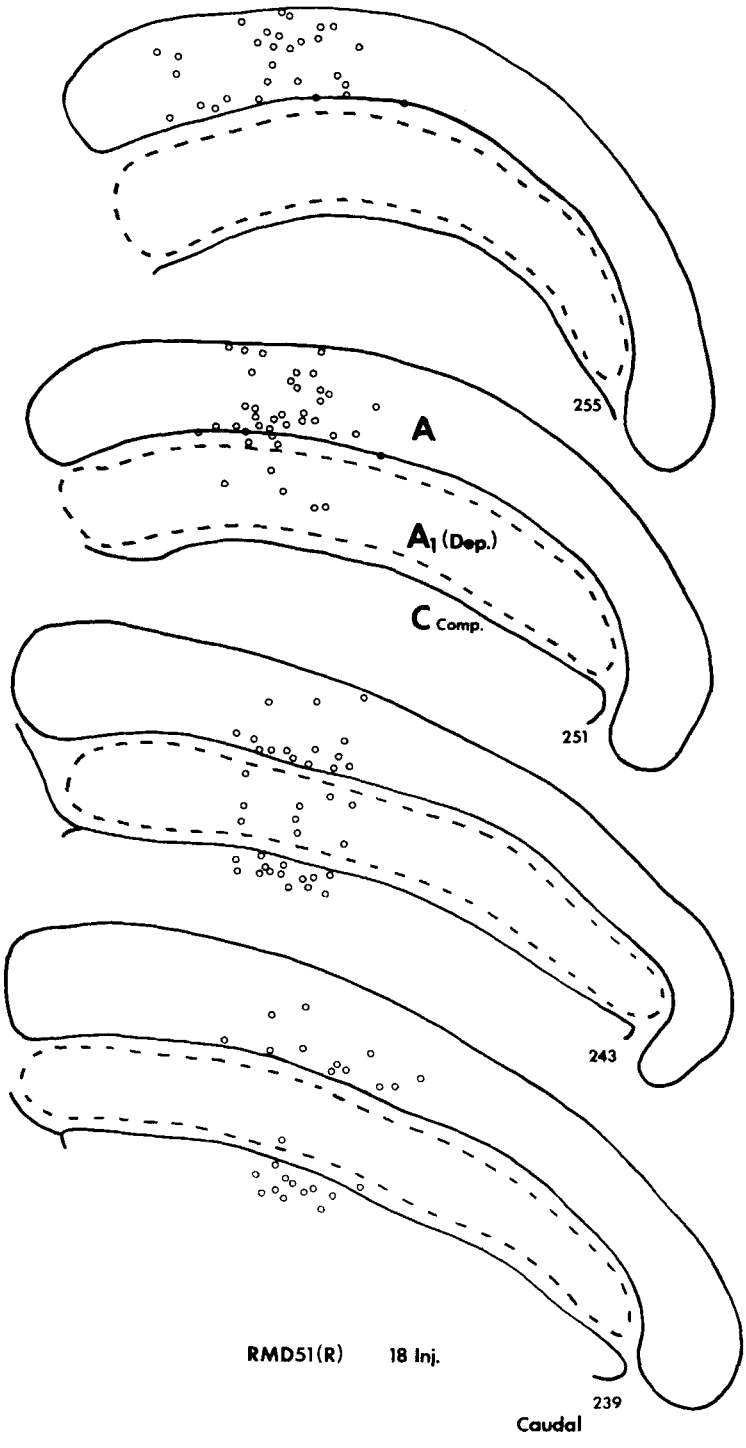

Fig. 5 Drawing showing labelled geniculate cells in coronal series for case RMD51(R) after an HRP injection into the right cortical area 18; conventions as in figure 4.

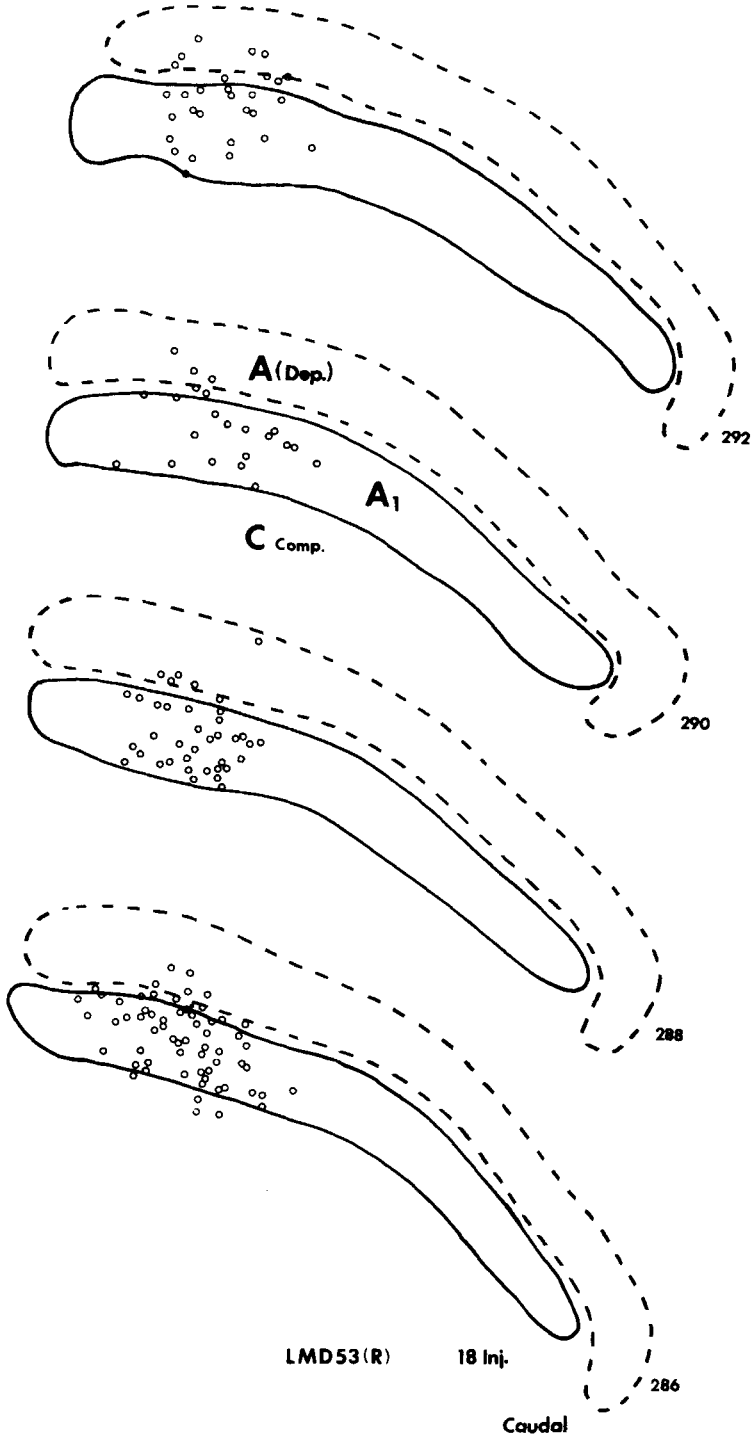

Fig. 6 Drawing showing labelled geniculate cells in coronal series for case LMD53(R) after an HRP injection into the right cortical area 18; conventions as in figure 4.

TABLE 2

LGN neuronal labelling percentages and cell size measurements after area 18 injections of HRP

(A) Case	(B) laminae	(C) total cell no. sampled	(D) % of labelling	(E) relative % of reduction	(F) absolute % of reduction (estimated)	(G) labelled cell mean area	(H) normalized % of labelled cell size decrease	(I) unlabelled cell mean area	(J) normalized % of unlabelled cell size decrease
N2	A	50 (228)	14.0 (11.8)	—	—	566.7	—	318.4	—
	A_1	61 (234)	14.7 (12.0)	—	—	638.8	—	328.6	—
LMD53(R)	A(Dep)	— *(627)*	*4.0 (4.0)*	*72.8* [1]	*10.9*	*400.0*	*21.4* [2]	*233.7*	*24.5* [3]
	A_1	54 (865)	14.8 (14.7)	—	—	573.8	—	319.6	—
RMD51(R)	A	46 (1,075)	15.2 (6.3)	—	—	610.5	—	296.2	—
	A_1(Dep)	— *(980)*	*5.1 (2.1)*	*66.7* [1]	*10.1*	*583.3*	*15.2* [1]	*238.1*	*22.1* [3]
LMD48(L)	A	40 (1,295)	15.0 (6.6)	—	—	563.3	—	373.5	—
	A_1(Dep)	*—(1,094)*	*8.6 (3.7)*	*43.9* [1]	*6.4*	*473.1*	*25.5* [1]	*280.5*	*27.2* [3]

For the normal cat (N2) and nondeprived laminae in the lid sutured cats, two sample sizes were used to derive the percentage of labelled cells (see text). The smaller sample, limited to the densest region of labelling and indicated in columns C and D without parentheses, was used for estimates of *absolute* labelling percentages; whereas the larger sample, which extended beyond the densest labelling region and indicated in columns C and D with parentheses, was used for *relative* estimates of within subject, interlaminar differences in labelling percentages. Only the larger sample was used for deprived laminae. The absolute percentage of reduction in labelling (column F) was estimated by multiplying the absolute value of percent labelled cells in nondeprived laminae by the relative percent of reduced labelling. Other conventions are as in table 1.

[1] $p < 0.001$ on a X^2-test.

[2] $p < 0.01$ on a t-test.

[3] $p < 0.001$ on a t-test.

more accurate estimate of the relative difference in the percentage of labelled cells between these laminae (MATERIALS AND METHODS), samples were taken in coronal sections that involved counting every labelled and unlabelled cell throughout the mediolateral extent of neuronal labelling as described above for cat N2. Sections were chosen at different levels if necessary to ensure that these samples included the zone of densest labelling in the lamina under study. Based on this analysis (table 2), we conclude that, in relative terms, the deprived laminae compared to nondeprived laminae have from 43.9% to 72.8% (average 64.5%) fewer labelled cells. In other words, deprived laminae have only about one-third the expected proportion of labelled neurons. These should be treated strictly as relative estimates. The actual percentage of labelled cells was estimated for the nondeprived laminae by considering only the smaller samples in the densest zones of labelling in order to estimate the absolute difference in the percentage of labelled neurons between laminae (MATERIALS AND METHODS).

Labelling was so sparse in deprived laminae that we could not confidently select an appropriate zone for this. Instead, an estimate of this value for the deprived laminae was made by multiplying the estimates for the nondeprived laminae by the relative labelling ratios calculated above. Thus, nondeprived laminae had 14.8%, 15.2% and 15.0% of labelled cells, whereas the respective deprived laminae had 8.6%, 5.1% and 4.0% of labelled cells. In *absolute* terms, therefore, we estimate that deprived laminae have 6.4%-10.9% (mean: 9.1%) fewer labelled cells than do nondeprived laminae.

Although we found no evidence in cats N2, N3, and N4 that a significant difference exists in the percentage of labelled neurons between laminae A and A1 following an area 18 injection of HRP, Hollander and Vanegas ('77) suggested that lamina A1 includes more cells projecting to area 18 than does lamina A. For this reason, two of the three lid sutured cats, RMD51(R) and LMD48(L), had HRP injected into area 18 ipsilateral to the deprived eye. Lamina A1 was thus deprived, and our observation in these cats, that many fewer labelled cells were found in deprived lamina A1 than in nondeprived lamina A, would underestimate the effect of deprivation if the suggestion of Hollander and Vanegas ('77) were correct.

Neuronal cross-sectional areas were measured in these cats as described above for normal cats. Labelled cells were only 15.2% to 25.5% smaller in deprived than in nondeprived laminae, and likewise, the unlabelled cells were 22.1% to 27.2% smaller in these laminae (table 2 and fig. 7). However, because of the small sample size of labelled cells in deprived laminae, these data are subject to serious

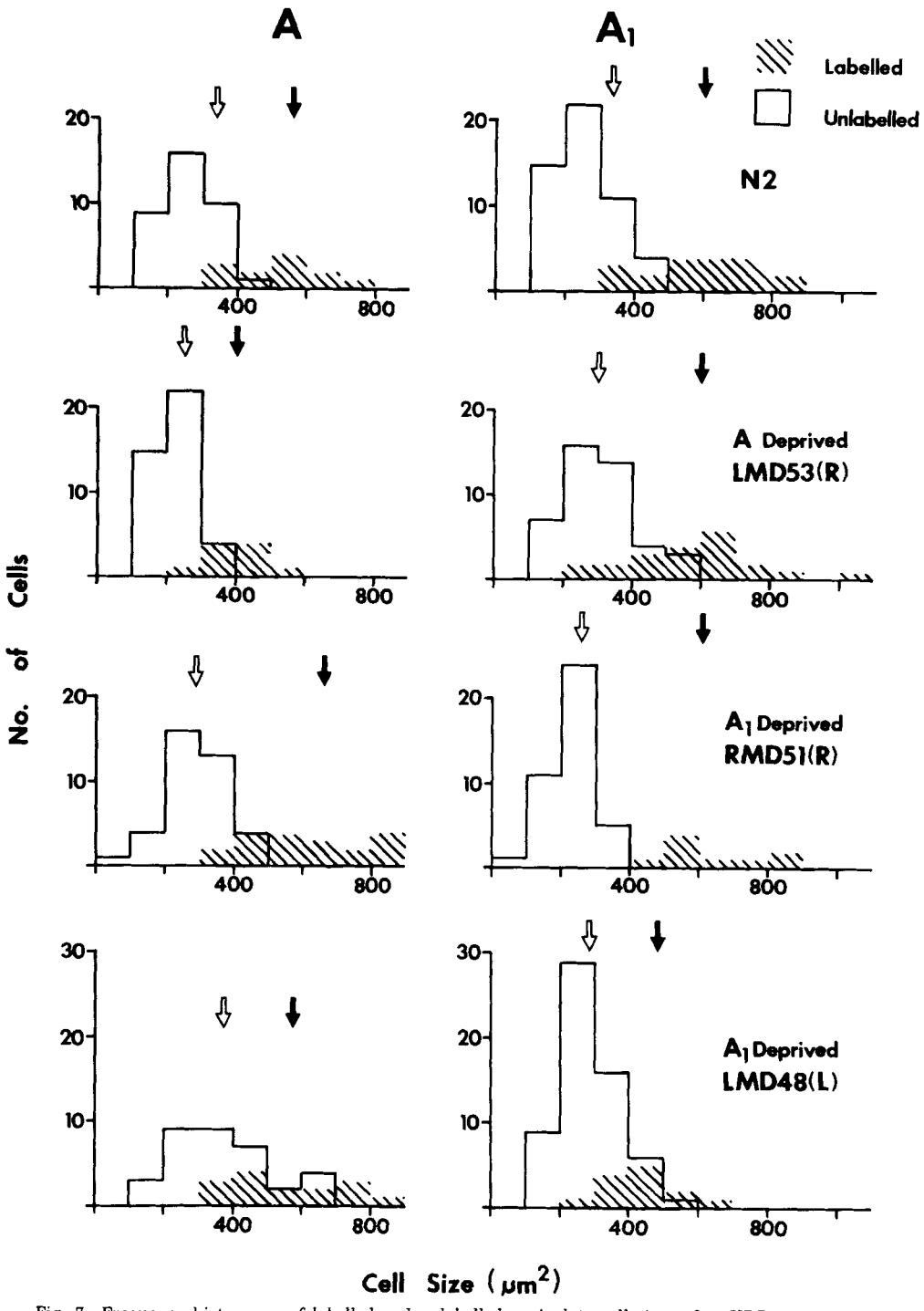

Fig. 7 Frequency histograms of labelled and unlabelled geniculate cell sizes after HRP injections into area 18; conventions as in figure 3. Shown are the distributions in laminae A and A1 for a normal cat (N2) and cases from three monocularly sutured cats [LMD48(L), RMD51(R), and LMD53(R)]. The total number of cells measured, respectively, for laminae A and A1 are: N2, 50 and 61; LMD53(R), 45 and 54; RMD51(R), 46 and 44; LMD48(L), 40 and 67 (see also table 2).

sampling problems and should be treated with skepticism.

Medial interlaminar nucleus

A more direct study of retrograde HRP transport in deprived and nondeprived Y-cells was made in cat LMD57. Since MIN is comprised almost entirely of Y-cells which are affected by deprivation quite like their counterparts in laminae A and A1 (Kratz et al., '78c), and since few interneurons are found here (Lin et al., '77), we could study a nearly pure Y-cell population. The bilateral HRP injections were extensive and covered essentially all of the cortical projection of these cells, and the intraocular injection of tritiated proline followed by autoradiography permitted a determination of whether HRP labelled cells were in the deprived or nondeprived portion of MIN. Figure 8 shows both the injection sites plus autoradiographs and labelled neurons within MIN. Note that unlike the smaller HRP injections described above, here HRP spread extensively into the underlying cortical white matter.

As was seen in laminae A and A1 after area 18 injections, cells in deprived portions of MIN tended to be faintly labelled compared to those in nondeprived areas. Table 3 and figure 9 summarize much of these data. Due to the relative sparseness of cells in MIN, a slightly different sampling procedure was employed. Again, only cells with clearly visible nucleoli were considered, but every such cell was sampled throughout the extent of the medial interlaminar nucleus in the two sections used for study. One section was chosen for each hemisphere, and each section had the densest collection of labelled cells in deprived and nondeprived zones of the coronal series. In the hemisphere contralateral to the deprived eye, percentages of labelled cells were 38.5% (deprived) and 75.0% (nondeprived); and in the other hemisphere, they were 59.5% (deprived) and 81.8% (nondeprived). The nondeprived values were only slightly below our highest percentage of labelled cells seen in a normal hemisphere (Lin et al., '77). If the data from both hemispheres are pooled for this cat, statistically fewer cells are labelled in deprived than in nondeprived regions (51.5% vs. 78.3%; $p < 0.01$ on a X^2-test). Also, compared to their nondeprived counterparts, labelled, deprived cells were 38.3% *smaller* and unlabelled, deprived cells were 69.0% *larger*. In normal cats (Lin et al., '77), the few unlabelled cells are typically much smaller than the labelled cells (as they are in nondeprived portions of cat LMD57), and this suggests that the relay cells are normally larger than the interneurons. The data from deprived regions of MIN suggest that those cells which fail to transport HRP are larger than the normal interneurons, presumably because most of these cells would have developed into normal relay cells without the deprivation.

DISCUSSION

These results indicate abnormalities in deprived laminae as revealed by retrograde transport of HRP from cortical injection sites. Injections in area 17 produce fairly little interlaminar asymmetry, although in deprived laminae, the cells are smaller and about 10% fewer cells in absolute terms are labelled than in nondeprived laminae. After area 18 injections, a variety of clear interlaminar differences are noted, including: (1) many cells in deprived laminae are very lightly labelled compared to those in nondeprived laminae; (2) fewer cells are labelled in deprived than in nondeprived laminae, and the interlaminar difference is nearly 3:1 in relative terms but still represents roughly a 10% absolute difference; (3) the volume occupied by labelled cells is smaller in deprived than in nondeprived laminae; and (4) labelled cells in deprived laminae are somewhat smaller than those in nondeprived laminae. Similar changes are seen in the deprived region of

Fig. 8 Photomicrographs showing cortical HRP injection site and geniculate labelling in the medial interlaminar nucleus (MIN) for cat LMD57. Only the right hemisphere is illustrated, but the left hemisphere illustrates the same points. Autoradiography was also performed following an intravitreal injection of tritiated proline into the right (nondeprived) eye in order to reveal lamination in MIN (see text). a. Low power brightfield view of large cortical injection site that includes all known cortical terminal zones of MIN. The lateral geniculate nucleus (LGN) can also be seen here. b. Brightfield view of lateral geniculate nucleus. MIN is indicated, and the rectangle indicates the zone shown in higher power in d, e. c. Darkfield view of same region as b. This autoradiograph shows the lamination clearly. The ipsilateral (nondeprived) eye's input to MIN stands out, and the deprived portion of MIN is found dorsal, medial and ventral to this. d, e. Bright- and darkfield views of MIN region indicated by rectangle in b. HRP labelled neurons can be clearly seen at this magnification. f, g. Bright- and darkfield views of MIN region indicated by rectangle in d. Neurons marked by HRP are more heavily labelled and more numerous in nondeprived (labelled by autoradiography) than in deprived (unlabelled by autoradiography) MIN regions. The bar in a is 5 mm. The bar in b is 1 mm and applies as well to c. The bar in d is 200 μm and applies as well to e. The bar in f is 100 μm and applies as well to g.

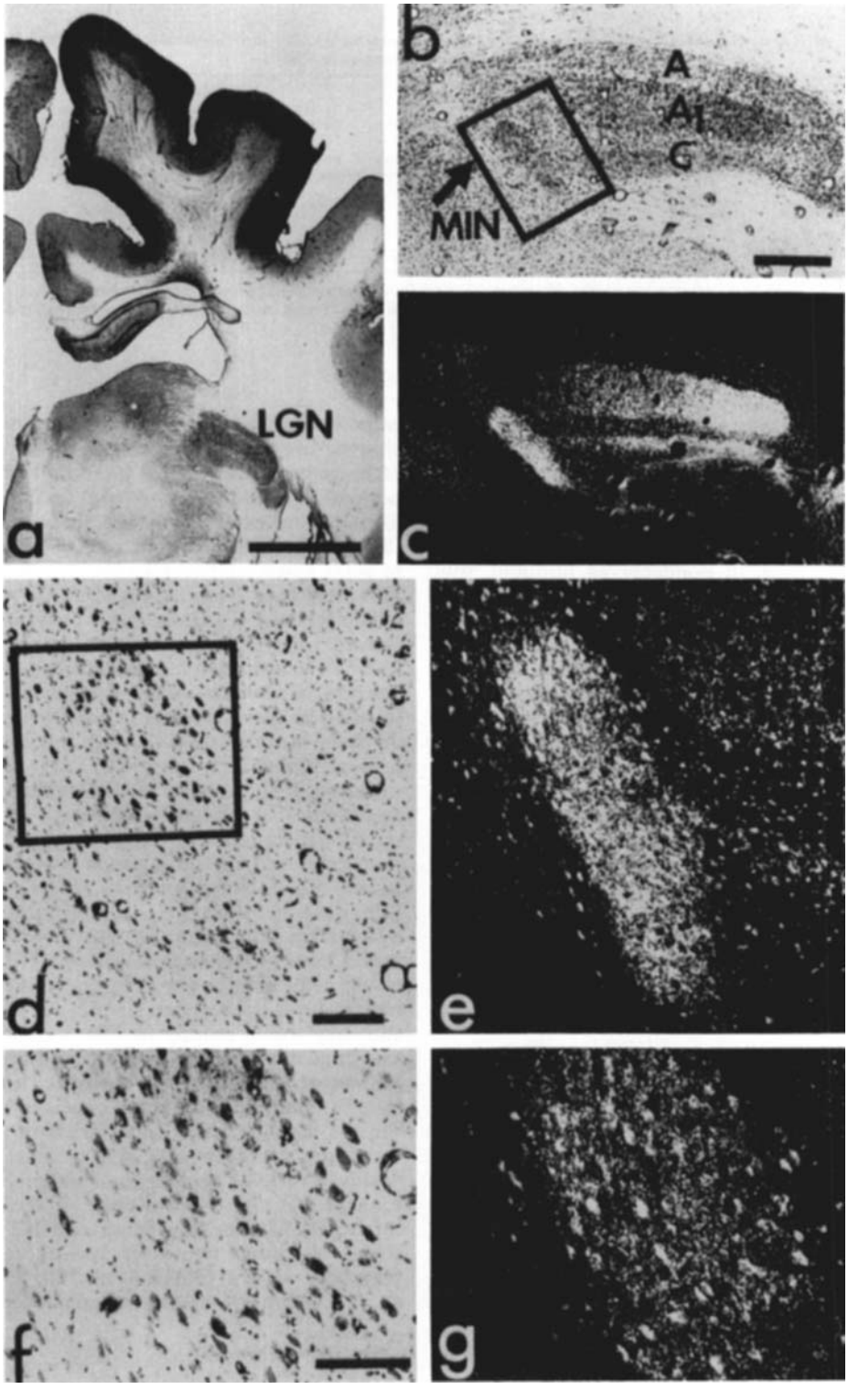

Figure 8

TABLE 3

MIN neuronal labelling percentages and cell size measurements after large cortical injections of HRP in cat LMD57; conventions as in table 1

(A) laminae	(B) total cell no. measured	(C) % of labelling	(D) relative % of reduction	(E) absolute % of reduction	(F) labelled cell mean area	(G) % of labelled cell size decrease	(H) unlabelled cell mean area	(I) % of unlabelled cell size increase
MIN (Nondep.)	46	78.3	—	—	391.7	—	130.0	—
MIN (Dep.)	*68*	*51.5*	*34.2* [1]	*26.8*	*241.5*	*38.3* [2]	*219.7*	*69.0* [2]

Data from both hemispheres are pooled. No normalization for comparisons of cell size measurements was needed since no significant difference exists in normal cats between MIN cell sizes found in the ipsilateral and contralateral ocular dominance laminae (Kratz et al., '78b).

[1] $p < 0.01$ on a X^2-test.

[2] $p < 0.001$ on a t-test.

MIN after large visual cortical injections of HRP. That is, compared to the nondeprived MIN region, the deprived region has less heavily labelled cells which are fewer in number and smaller in cross-sectional area.

Although we detected fewer labelled cells in deprived than in nondeprived laminae, this difference must be qualified by considering our criterion for detecting labelling within cells.[3] We emphasize the distinct possibility that many cells might be labelled with too small an amount of HRP for us to detect in our material. In fact, it may be that no actual difference in the number of labelled cells exists between laminae, but rather the difference is one of a profound decrease in intracellular HRP uptake for deprived neurons. However, since we cannot yet specifically relate HRP uptake to functional parameters (see below), it matters little in the following discussion if the difference between deprived and nondeprived laminae is one of the percentage of labelled cells or the intracellular extent of label. For simplicity below, we shall assume the former.

Interpretation of HRP labelling

Before drawing functional correlations for the above anatomical observations, it is useful to consider why some cells would be more heavily labelled with retrogradely transported HRP than would others. Unfortunately, there are many potential factors which could contribute to this differential labelling, and we cannot yet determine which actually control HRP labelling. Nonetheless, it is of interest to list some of these for brief consideration. (1) If many of the geniculate cells which normally project to a cortical area are physically absent from deprived laminae, one would observe fewer labelled cells in these laminae after appropriate cortical injections. There is no evidence that cells are physically absent, although we cannot rule out this possibility. (2) Some cells in deprived laminae might fail to send axons and/or terminals to a cortical area, and thus have no opportunity to transport injected HRP. Since our injections are essentially limited to gray matter, it is possible in this context that, if axons from LGN cells are limited to white matter, they would not be labelled in our experiments. (3) Some cells in deprived laminae might have a much smaller preterminal arborization with fewer synapses in cortex. If the amount of HRP transported is monotonically related to the extent of preterminal arborization or number of synapses, then cells in deprived laminae would be very lightly labelled. Furthermore, if the arborization were smaller, then cells sending axons away from the center of the injection site would have less chance to take up HRP than would cells with larger arborizations projecting to the same regions. As a consequence of this, one would readily predict that less preterminal arborization for deprived neurons projecting to area 18 should produce a smaller zone of labelling within the deprived lamina. Thus, a smaller preterminal arbor in area 18 could explain both the lighter labelling of deprived cells as well as the smaller labelled zone in the deprived laminae after area 18 injections. (4) Since the extent of HRP uptake seems related to neural or synaptic activity (Nauta et al., '74; Strick et al., '76; Singer et al., '77), it may be that deprived neurons have

[3] See footnote 2 p.818.

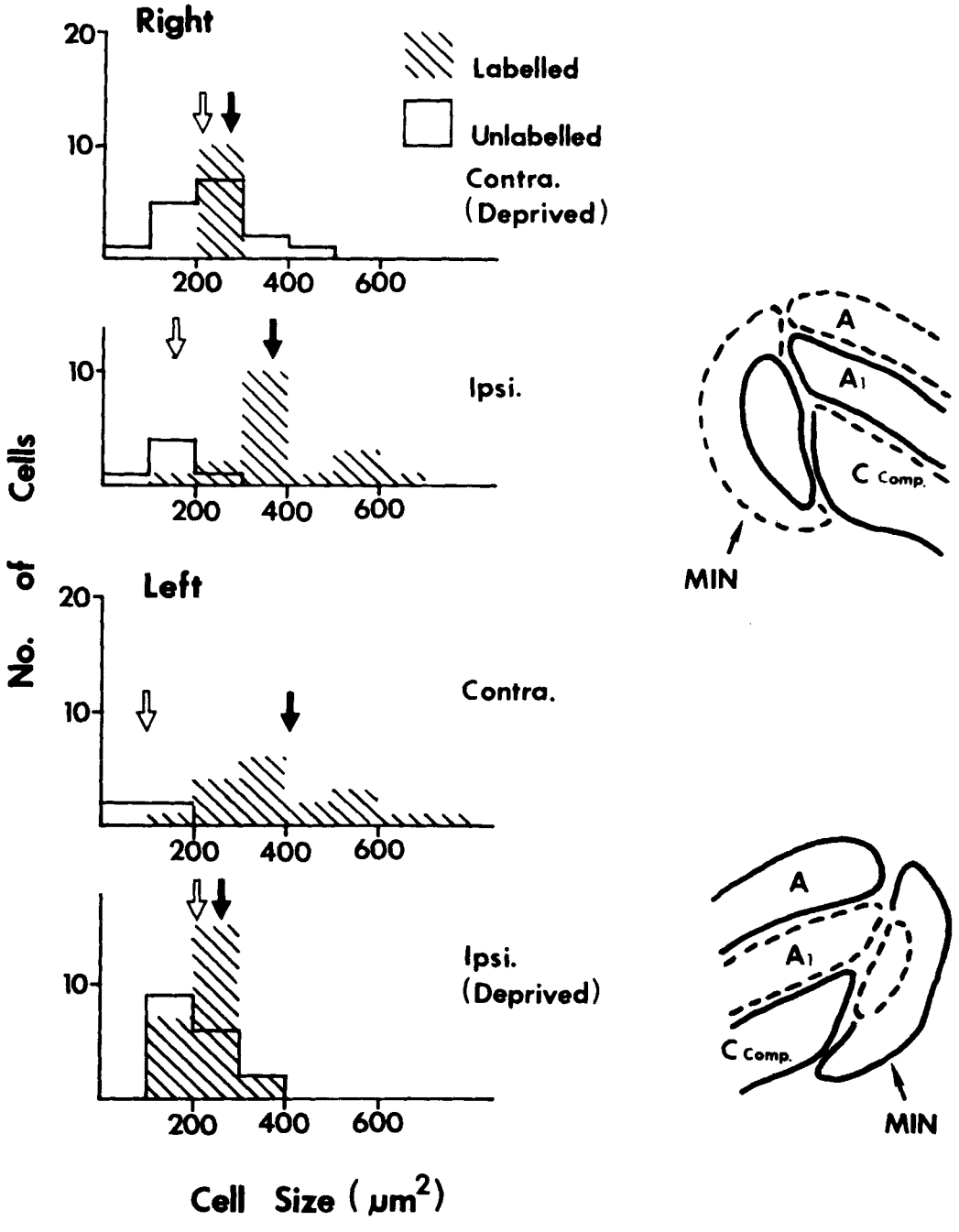

Fig. 9 Frequency histograms (left) of labelled and unlabelled cells in the medial interlaminar nucleus (MIN) of cat LMD57 with outlines indicating deprived and nondeprived regions of MIN (right). Open bars indicate unlabelled cells; and stippled bars, labelled cells. The open arrows indicate the mean cell size of unlabelled cells; and the filled arrows, labelled cells. The total number of cells measured in each histogram, from top to bottom, are: 26, 24, 22, and 42.

fairly normal connections in cortex, but perhaps due to reduced activity, they transport little or no HRP. However, there is no direct evidence that deprived neurons are less active during the survival period after the HRP injections than are nondeprived neurons.

We cannot as yet choose among the above alternative explanations, and indeed others not considered may be more important. It may be that one or some combination of these factors determines HRP labelling. It is interesting to note that of the factors considered, only a reduction in the extent of preterminal arborization is consistent with a smaller zone of labelled cells.

Functional correlates

Two major conclusions may be drawn concerning the effects of early monocular suture in terms of neuronal retrograde transport of HRP. First, in laminae A and A1, deprived neurons which project to area 18 are relatively more affected by the lid suture than are deprived neurons which project to area 17. Second, neurons in MIN also are seriously affected by early eyelid suture.

Laminae A and A1

X-cells project only to area 17 whereas the Y-cell population projects both to areas 17 and 18 (Stone and Dreher, '73; LeVay and Ferster, '77). Therefore, area 18 HRP injections presumably label only Y-cells, and area 17 injections label both X- and Y-cells. It has been previously demonstrated that X-cells in deprived laminae of monocularly sutured cats develop fairly normally, whereas the deprived Y-cells do not (Sherman et al., '72). The relatively normal response properties of deprived X-cells are consistent with fairly normal projections to area 17, and therefore, both nondeprived and deprived laminae should have many labelled cells after area 17 injections. That is, X- and Y-cells would be labelled in nondeprived laminae, and at least the X-cells would be labelled in deprived laminae. On the other hand, the nondeprived laminae should be much more heavily labelled than deprived laminae after restricted area 18 injections if the lack of normal Y-cell responses in deprived laminae implied abnormal projections to area 18. We found in fact that deprived laminae had only about one-third the number of labelled cells as did the nondeprived laminae following area 18 injections, and most of these deprived cells were quite poorly labelled. These observations correlate closely with the physiological data (Sherman et al., '72).

It is also interesting to note from table 1 that, although deprived laminae have many labelled cells following an area 17 injection of HRP, there still are significantly fewer labelled cells than in nondeprived laminae by about 10%. Because fewer cells are labelled after an area 18 injection in normal cats, the relative absence of labelled cells in deprived laminae is more obvious than after area 17 injections, but table 2 shows that the loss is still about 10% in absolute terms (see MATERIALS AND METHODS for a definition of *relative* and *absolute* differences in the percentage of labelled cells between laminae). In other words, HRP injections of either area 17 or 18 result in a comparable *absolute* absence of labelled neurons. This is consistent with (but by no means proves) the hypothesis that the same group of neurons, presumably Y-cells, fails to develop normal projections to areas 17 and 18 from deprived laminae.

MIN

Interpretation of labelling in MIN is simpler because of observations that this structure is comprised nearly exclusively of Y-cells (Kratz et al., '78b). Furthermore, in monocularly sutured cats, the nondeprived MIN regions have normal cells, and the vast majority of cells in the deprived regions seem abnormal and generally unresponsive (Kratz et al., '78c). The physiological observations from laminae A and A1 (Sherman et al., '72) have been extended to MIN. The poor HRP labelling in deprived compared to nondeprived MIN regions thus correlates well with the physiological observations in much the same way as does the HRP labelling in the A laminae.

Comparison with other studies

Garey and Blakemore ('77) have performed experiments essentially identical to ours. Although they arrived at a similar conclusion (i.e., that in deprived laminae, the cells projecting to area 18 seemed more affected than those projecting to area 17), their results are actually quite different. They reported that, regardless of which area was injected, "... The degree of labelling in the deprived laminae is not vastly less than in the nondeprived laminae (p. 275)." Instead, they reported that after area 17 injections the deprived cells were only 20% smaller than non-

deprived cells, whereas after area 18 injections they were 50-60% smaller. We found a larger size differential after area 17 than area 18 injections, only small interlaminar differences in the percentage of labelling after area 17 injections, and relatively many fewer labelled cells in deprived than nondeprived laminae after area 18 injections. Garey and Blakemore comment neither on the intensity of label within cells nor on the size of the labelled zones.

It is useful to consider some of the different experimental procedures that might contribute to the different results in our studies. First and perhaps most important, the animals used had different deprivation histories. All of ours were lid sutured from days 8-10 until studied as adults at least one year later. Garey and Blakemore performed lid sutures on their kittens at 7, 32, or 40 days of age and studied them at 10 to 12 weeks of age. It is possible that the longer deprivation we used more severely disrupted HRP labelling among cells projecting to area 18. That is, with shorter deprivation, many of the more seriously affected and thus smaller cells projecting to area 18 might maintain their ability to transport HRP, but as deprivation continues, these cells lose that ability for unknown reasons.

Second, our injections were probably smaller than theirs. We attempted to limit our injections in area 18 to gray matter centered upon layer 4 whereas they apparently extended their injections to include some of the underlying white matter (see also below and Thorpe and Blakemore, '75). It is possible that small cells in deprived laminae send axons to area 18 that either do not enter gray matter or perhaps extend only to layer 6 (Rosenquist et al., '74; LeVay and Gilbert, '76). If such cells exist, they would be labelled more completely by their injections than by ours.[4]

The third difference is related to the second. This concerns the possibility that injections of area 18 which extend to white matter will also label geniculate fibers heading for area 17. This would be functionally like an injection of both areas 17 and 18. Normally, the cells projecting to area 18 tend to be substantially larger than those projecting to area 17 (see figs. 3 and 8 and tables 1 and 2; see also LeVay and Ferster, '77; Garey and Blakemore, '77). We suggest both that the smaller (X) cells projecting to area 17 incorporate HRP label roughly equally in deprived and nondeprived laminae, and that the larger (Y) cells normally projecting to areas 17 and 18 generally do not incorporate HRP label in deprived laminae. One would then expect an HRP injection of areas 17 and 18 to label many cells in both laminae, but only the nondeprived laminae would contain substantial numbers of the largest, labelled cells (i.e., adding the results of our separate areas 17 and 18 injections). This is precisely the result we obtained from injections aimed at area 18 which extended into area 17 as determined by labelled cells at the medial edge of laminae A and A1 (i.e., cat LMD44 and others not mentioned in this paper showed such a pattern; see figs. 1g-i). Although Garey and Blakemore ('77) do not discuss in detail their criteria for an injection limited to area 18, they do discount as unlikely the possibility that they have labelled fibers of passage despite damage to the underlying white matter. However, it is clear that the apparent (but not actual) size of the injection site can vary dramatically with survival time, which makes it difficult to delineate the extent of HRP available to axons and terminals by inspection of the injection site alone (Vanegas et al., '78), and that damaged fibers of passage will indeed transport HRP retrogradely (Lund et al., '75). In fact, we conclude that we labelled fibers of passage in some cases without obvious damage to white matter. This seems a difficult and variable problem, and we suggest it only as another explanation for variable results. We emphasize that there is no compelling reason to suggest that Garey and Blakemore ('77) have labelled fibers of passage.

Finally, while our injections were placed near the representation of the horizontal zero parallel, Garey and Blakemore ('77) injected area 18 quite anteriorly. It is conceivable, albeit unlikely, that this portion of the geniculocortical pathways reacts differently to the effects of early eyelid suture than does the region we studied.

We can also compare our data with those of LeVay and Ferster ('77) who studied the differential effects of early eyelid suture upon X- and Y- cells in one monocularly sutured cat. They classified neurons according to whether their soma had "cytoplasmic laminar bodies" (CLBs; X-cells) or not (Y-cells). They con-

[4] This also suggests that a small cortical injection centered upon layer 4 might not label all geniculate cells projecting to that area. Because such small injections were used throughout this study, in contrast to the large injections we used previously (Lin et al., '77), tables 1 and 2 possibly underestimate the percentage of geniculate neurons projecting to area 17 or 18.

cluded that Y-cells were more seriously affected by deprivation than were X-cells because: (1) cells with CLBs in deprived laminae were nearly as large as those in nondeprived laminae but deprived cells without them were considerably smaller on average; and (2) in deprived lamina A1 (but curiously not in deprived lamina A), a shortage of cells without CLBs was obtained. Kalil ('78) also reports a shortage of cells without CLBs in the lateral geniculate nucleus of cats raised in total darkness. In terms of cell size, the data of LeVay and Ferster ('77) correspond fairly well with the conclusions of Garey and Blakemore ('77), and the correlation with our results is considerably more complicated. We found that the few deprived cells labelled from area 18 injections of HRP were not dramatically smaller than labelled cells in nondeprived laminae. It may be that the presumptive Y-cells which failed to label in deprived laminae after a cortical injection were the most seriously affected by deprivation and were thus smaller than the labelled cells. In other words, if Y-cells in deprived laminae, which normally would project to area 17 or 18 but fail in these cats to transport detectable HRP, are dramatically smaller than normal Y-cells, then our results are compatible with those of LeVay and Ferster ('77).

Conclusions

All of the available physiological and anatomical evidence supports the notion that early visual deprivation more seriously affects development of geniculate Y-cells than it does development of X-cells. However, it is not possible to draw specific relationships between the physiological observations and the HRP data described here beyond noting the general correspondence. As mentioned above, it is not at all clear what factors cause poor retrograde HRP labelling of the presumptive Y-cells. The possible factors range from a physical absence of these cells to reduced activity among them, and any of these possibilities is consistent with abnormal or poor physiological responses for these neurons. Although the agreement between the physiological and anatomical data is encouraging, we still cannot describe specific structure/function relationships.

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