Percentage of relay cells in the cat's lateral geniculate nucleus

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Guillery⁶ used the Golgi method to study 4 classes of cells in the cat's lateral geniculate nucleus. His 3 largest classes (classes 1, 2, and 4) of cells were identified as relay cells projecting to visual cortex, and cells in his smallest class (class 3) were identified as interneurons. Since Golgi methods selectively and somewhat capriciously stain less than 2% of the available neurons²¹, it is almost impossible to derive a clear estimate from such data of the relative ratio of relay cells versus interneurons. The recent development of the horseradish peroxidase (HRP) marking technique offers a much less selective means of identifying lateral geniculate relay cells. That is, HRP injected into visual cortex is picked up by geniculocortical axons and/or terminals and transported back to the cell body where it is visualized by a reaction product¹⁰. Norden and Kaas^{19,20} made use of this technique in three primate species (*M. mulatta*, *A. trivirgatus*, and *G. senegalensis*) to determine that lateral geniculate relay cells comprise at least 94–98% of the neurons in that nucleus.

However, previous estimates from the cat suggest perhaps a lower percentage of lateral geniculate relay neurons. Chow and Dewson³ concluded from retrograde degeneration following cortical lesions that only 65% of these neurons projected to cortex, but because of the possibility of sustaining geniculocortical collaterals, this should be viewed as a minimum estimate. HRP injected into single cortical areas^{5,7,14} never produced more than 70% labeled neurons in the lateral geniculate nucleus. However, these experiments were not designed to study the percentage of relay cells since injections were limited to small cortical loci, and it appears that different populations of lateral geniculate neurons project to different cortical regions^{4,7,14,24,25}. Also, estimates based on the HRP technique must be viewed as a minimum estimate because not all cells with axons projecting to an area of HRP injection (i.e. lateral geniculate relay cells) will transport the enzyme¹⁷. This raises the question as to whether the lower estimates of lateral geniculate relay cells in the cat compared to the monkey represent a true species difference or an artifact due both to less efficient labeling of cat relay cells for unknown reasons and also to a failure to label completely the geniculocortical projection. We sought to answer this question by applying a potentially more sensitive HRP technique and by extensive HRP injections throughout all known geniculocortical areas in the cat.



Fig. 1. HRP/DMSO-labeled neurons with dark- and bright-field optics. A: dark-field view of a labeled pyramidal cell in layer III of the Clare-Bishop area after an HRP/DMSO injection limited to area 17. The apical dendrite is directed upwards towards the pial surface. B: bright-field view of the same neuron as shown in A. The arrow points to a branch from the apical dendrite. The scale is 30 μ m and also applies to A and C. C: dark-field view of a similar neuron (layer III pyramidal cell) from the same hemisphere as shown in A and B. D: MIN neuron labeled after extensive cortical injection of HRP/DMSO. The intense labeling would obscure the nucleolus if it occurred in this section. Fine processes of the cell are delineated (arrow). The scale is 15 μ m.

TABLE I

Percentage of labeled neurons in the dorsal lateral geniculate nucleus of the cat

Percentage of labeled neurons in the dorsal lateral geniculate nucleus following HRP/DMSO cortical injection. Two different estimates of relay cell ratios are offered in the bottom line. The first numbers are estimates calculated by including larger labeled cells (>150 sq.µm) without visible nucleoli due to the intense HRP reaction product. The numbers in parentheses are a more conservative estimate based only on cells (labeled or not) with clearly visible nucleoli.

	Lamina A	Lamina A_1	C Laminae	MIN
Total cells sampled	71	47	97	67
Labeled cells with clear nucleolus	29	36	50	34
Labeled cells without clear nucleolus	35	9	34	24
Unlabeled cells	7	2	13	9
Percentage of labeling	91.1 (80.6)	95.9 (95)	86.6 (79.4)	86.6 (79.1)

Recently, Keefer⁸ has demonstrated that dimethylsulfoxide (DMSO) can be used with HRP to enhance neuronal labeling, perhaps by increasing HRP uptake and/or transport efficiency. We have made use of this modification of the HRP technique to estimate the ratio of relay cells in various portions of the cat's lateral geniculate nucleus. We injected a 30% solution of HRP (Sigma VI or Miles Lab.) alone or in combination with 2% DMSO bilaterally into the occipitotemporal cortices of two cats. The cats were anesthetized with sodium pentobarbital and exposure of cortex was achieved with routine surgical procedures. Sixteen separate 1-µl injections of the HRP solution were placed in each hemisphere via a 5- μ l Hamilton syringe, and the injection sites ranged from area 17 medially to Clare-Bishop Area laterally along a 2-mm wide lateral strip. Each injection was slowly delivered over a 10-min period. The areas injected included all known cortical projection zones of the lateral geniculate nucleus, including the laminar portion (A, A₁, and the C laminae) plus the medial interlaminar nucleus (MIN)^{2,5,9,13,15,16,22}. Following a 48-h survival period, the cats were perfused intracardially with saline followed by buffered 1% paraformaldehyde and 1% glutaraldehyde. The visual cortices and lateral geniculate nuclei were blocked, cut coronally into 40- μ m sections, and treated according to procedures described by LaVail and LaVail^{11,12}. Areas of the most intense HRP labeling were chosen and within these areas all neurons, labeled or not labeled, were counted. For all considerations of unlabeled cells below, only those with clearly visible nucleoli were studied. Because intense HRP reaction products frequently obscured possible nucleoli (see Fig. 1D), two separate estimates of the percentage of relay cells were used. The first estimate was based on the ratio of all labeled cells (over approximately 150 sq. μ m) versus total number of cells counted within a labeled area. A second, more conservative estimate was based on the ratio of labeled cells with a clear nucleolus to the total number of cells within a labeled area (see Table I). Cell cross-sectional areas were determined by tracing some outlines with a drawing-tube attachment on the microscope at $1000 \times$, and only the population of cells with visible nucleoli were measured.

In both cats, the HRP reaction product covered nearly all of the occipito-





Fig. 3. Histograms of cell sizes within various portions of the lateral geniculate nucleus following HRP/DMSO injection of cortex. Labeled and unlabeled cells are separately represented, and only the 180 cells with clearly visible nucleoli were considered. Thus many intensely labeled cells were not considered for these histograms (see Fig. 1D and text).

temporal cortices. The injections of HRP plus DMSO provided more intense labeling of neurons than injections of HRP alone, and frequently dendrites and/or axons were extensively labeled (see Fig. 1). We reasoned that, since the HRP method may not label all possible relay cells¹⁷, the most accurate estimate of relay cell ratios would be that offering the highest ratio. Therefore, the following analysis is based on the hemisphere (HRP plus DMSO) providing the highest percentage of labeled lateral geniculate neurons, although the other injections (HRP alone) yielded labeled neuron ratios which were not significantly lower. The analysis was done separately in lamina A, lamina A₁, the C laminae, and MIN. Although other thalamic regions were heavily

Fig. 2. Labeling among lateral geniculate neurons after HRP/DMSO injection of cortex. The 4 columns indicate intensely labeled zones for lamina A (1), lamina A₁ (2), the C laminae (3), and MIN (4). The top row shows low power, bright-field views of the nucleus at the 4 appropriate rostro-caudal levels. (The scale in 1 represents 1 mm and applies to the remaining photomicrographs in this row.) The middle and bottom rows represent identical, higher power views (in bright- and dark-field, respectively) of regions indicated by the arrowhead in the top photomicrographs. (The scale for all photomicrographs in the lower two rows is indicated for the middle picture of the first column and represents 50 μ m.) In row 4, the lower two photomicrographs have been rotated 60° counterclockwise whereas the orientation in the remaining columns was unchanged.

labeled, particularly the inferior, lateral and medial division of the pulvinar complex (see Niimi et al.¹⁸ for nomenclature), these are not considered further here.

Fig. 2 shows the labeled neurons in the various regions of the lateral geniculate nucleus. Because the cortical injections were along a limited lateral strip, the most intensely labeled zones of lamina A and A₁, the C laminae and MIN were found in separate coronal sections. Table I summarizes the ratios of labeled neurons from each of these areas and shows that roughly 85-95% of the cells are relay cells. A more conservative estimate (i.e. considering only those labeled cells with visible nucleoli) yields a slightly lower ratio, and a less conservative estimate (i.e. also counting larger heavily labeled neurons whose nucleoli would be obscured by the reaction product) provided a slightly higher ratio. This suggests that the cat's lateral geniculate nucleus probably has no more than about 10% interneurons. Even this value may be an overestimate since some relay cells may not transport sufficient HRP during the survival period to be clearly labeled.

Fig. 3 shows the size distribution of the labeled and unlabeled cells in these areas. Only the 180 cells with clear nucleoli were considered. Note that in lamina A, A₁, the C laminae and MIN practically all of the neurons larger than 150 sq. μ m are labeled. This suggests that in these portions of the lateral geniculate nucleus, interneurons are among the smallest in the nucleus^{6,14}. In the C laminae it appears that some cells smaller than 150 sq. μ m are labeled, and this is in accordance with the results of Holländer and Vanegas⁷. Since the C laminae is the only segment of the lateral geniculate nucleus with electrophysiologically defined W-cells²⁵, and since W-cells in the retina are thought to be the smallest of ganglion cells¹, these small labeled cells in the C laminae may correspond to a portion of this unique relay cell class.

Since many relay cells may have escaped HRP labeling for unknown reasons, this suggests that probably fewer than 10% of cells in the nucleus are interneurons. However, these interneurons appear to have an extensive ramification of processes⁶ so that few may be needed to innervate most or all of the relay cells²³. Finally, as others have suggested^{6,14}, the interneurons have the smallest somata in the lateral geniculate nucleus.

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