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Monoclonal Antibody Cat-301 Identifies Y-Cells in the Dorsal Lateral Geniculate Nucleus of the Cat

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ABSTRACT

In mammalian visual pathways, information is carried in parallel channels from the retina through the visual thalamus to visual cortex. The cat's visual pathway comprises at least three major channels that begin with the X, Y, and W ganglion cells in the retina. In the dorsal lateral geniculate nucleus (LGN) of the thalamus, neurons in the X, Y, and W channels receive input from their retinal counterparts and can be discriminated from one another on the basis of their anatomical and physiological properties.

The search for molecular properties that might correlate with anatomically or physiologically defined classes of neuron has been a major area of research in recent years. Monoclonal antibody Cat-301 recognizes a neuronal surface-associated proteoglycan in many areas of the mammalian central nervous system. In the cat LGN Cat-301 immunoreactivity is restricted to a subset of neurons. We show here that the distribution, size, morphology, and cortical projection pattern of Cat-301-positive LGN neurons match those previously described for Y-cells. Taken together with our previous studies of the development of immunoreactivity and the sensitivity of Cat-301 staining to visual deprivation, these studies suggest that Cat-301 specifically recognizes Y-cells in the cat LGN. These results indicate that neurons within a physiologically and anatomically defined cell class share a molecular property. They further suggest that differences in molecular traits may reflect, and possibly subserve, differences in anatomical and physiological characteristics.

Key words: visual system, parallel pathways, molecular specificity, surface protein, proteoglycan

In mammalian visual pathways, information is carried in parallel channels from the retina through the visual thalamus to visual cortex. Each channel carries a relatively distinct component of visual information. The cat visual pathway is comprised of three major channels, X, Y, and W. Neurons in each channel have been characterized anatomically and physiologically in the retina and dorsal lateral geniculate nucleus (LGN) of the thalamus. In the cat LGN, Y-cells have many features that distinguish them from X-cells and W-cells, including physiological response properties, cell morphology, and synaptic connections (for reviews, see Rodieck, '79; Sherman and Spear, '82; Wilson et al., '84).

As our general understanding of the genetic basis of cellular phenotype increases, a major question in neurobiology is whether physiological and anatomical properties of neurons might be reflected by, and possibly derive from, specific molecular properties. If this were the case, distinct classes of neuron might express molecular species that, by qualitative or quantitative differences, would differentiate them from other classes of neuron.

Monoclonal antibody Cat-301 recognizes a surface-associated antigen on neurons in many areas of the cat central nervous system (CNS) (Hockfield et al., '83). In each area of the CNS, only a subset of neurons expresses the Cat-301 antigen. In our previous studies we reported that the cat LGN contains Cat-301-positive neurons (Hockfield et al., '83, Hendry et al., '84; Sur et al., '88). In the present study we examine in detail the laminar distribution, cell body size, projection pattern, and morphology of Cat-301-positive neurons in the cat LGN. The data presented here suggest that Cat-301 specifically recognizes LGN Y-cells. They further indicate that neurons within a physiologically

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and anatomically defined cell class can share molecular properties.

MATERIALS AND METHODS

Normal animals. Tissue was obtained from normal, adult cats. Animals were anesthetized deeply with sodium pentobarbital and perfused transcardially with phosphate-buffered saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH7.4 (PB). Brains were dissected from the animals, equilibrated in 30% sucrose, and sectioned in the coronal plane at 50 μm on a freezing sliding microtome. Sections were collected in PB with 0.2% sodium azide (to retard bacterial growth).

Immunohistochemical procedures. A set of sections through the LGN were processed for Cat-301 immunoreactivity. Free-floating sections were incubated in monoclonal antibody Cat-301 (as a full-strength tissue culture supernatant) with a final concentration of 2% triton-X 100 overnight on a shaker. Sections were rinsed in PB and then incubated in HRP-conjugated antimouse secondary antibody (Cappel) diluted 1:100 in tissue culture medium (Dulbecco's modified Eagle's medium with 10% fetal calf serum) with 2% triton-X 100 for 2 hours at room temperature. Following several rinses in PB, the bound HRP was visualized by incubation in 3,3′-diaminobenzidine (0.03% in PB) with hydrogen peroxide (0.003%). The HRP reaction was intensified by either cobalt chloride (Adams, '77) or by the addition of 0.03% nickel ammonium sulfate to the reaction mixture. Control sections were processed identically to those for Cat-301, except that either tissue culture medium alone or a different monoclonal antibody was substituted for Cat-301.

Cell measurements. A series of coronal sections stained for Cat-301 was counterstained with cresyl violet. In two cats, neurons with well-defined nucleoli were counted, their somata drawn, and their diameters measured with the aid of a 20× objective. The purpose of the measurements was twofold. First, we wished to determine the proportion of neurons in laminae A, A1, and C and in the medial interlaminar nucleus (MIN) that were Cat-301-positive, along with their sizes relative to other neurons. We therefore measured the sizes of all neurons (both antibody-positive and antibody-negative) in 200-μm-wide dorsoventral swaths through the middle of the various LGN subdivisions until a sample size of 85–100 neurons was reached. Second, we wished to determine accurately the size distribution of Cat-301-positive neurons in each division of the LGN. To obtain a larger sample of Cat-301-positive neurons, we measured soma sizes of these neurons in three fields of view across the mediolateral extent of the LGN in a section from the anterior-posterior middle of the LGN.

Retrograde labelling. In order to determine the proportion of Cat-301-positive neurons projecting to cortical areas 17 and 18, injections of the retrograde tracers HRP (20% in saline) or fluorogold (4% in distilled water) were made in area 17 or 18. Injections were made with a Hamilton syringe; one injection of 50–100 nl was made in each hemisphere. Survival times ranged from 2 days (for HRP) to 5 days (for fluorogold). Injected, anesthetized animals were perfused with 4% paraformaldehyde and the LGN was sectioned as described above. Sections containing retrogradely transported fluorogold were stained for Cat-301 with a secondary antibody conjugated to either fluorescein isothiocyanate or Texas red and observed with fluorescent optics.

Sections containing retrogradely transported HRP were processed for retrograde label and Cat-301 using a double-label immunohistochemical strategy. Fixation in 4% paraformaldehyde does not sufficiently preserve HRP enzyme activity. Therefore, instead of histochemical visualization procedures, retrogradely transported HRP was visualized immunologically. Sections were incubated in goat anti-HRP (Cappel) (diluted 1:100 in tissue culture medium with 2% triton-X 100) overnight, followed by HRP-conjugated rabbit-antigoat secondary antibody (diluted 1:100 in tissue culture medium with 2% triton-X 100) for 2 hours. The bound HRP was visualized with DAB without intensification. This procedure allowed for optimal detection of retrogradely transported HRP. The sections were washed extensively and then stained for Cat-301 as described above. The secondary antibody for Cat-301 was an HRP-conjugated antimouse antibody and the HRP was visualized with DAB with nickel sulfate. This produced a brown, intracellular HRP signal from the retrogradely transported HRP and a black, surface associated signal from the Cat-301. Control sections showed that there was no cross-reactivity between the secondary antibodies and the inappropriate primary antibodies.

Immunostaining intracellularly filled cells. Vibratome sections, 400 μm thick, of the LGN containing neurons intracellularly filled with Lucifer yellow were generously provided by David McCormick (McCormick and Pape, '88). The sections were fixed in 4% paraformaldehyde, equilibrated in 30% sucrose, and then resectioned at 50 μm on a freezing sliding microtome. Sections were stained for Cat-301 immunohistochemistry by incubating in Cat-301 (as described above) followed by visualization with Texas red-conjugated antimouse secondary antibody (Fisher) diluted 1:100 in tissue culture medium. Sections were collected on slides and observed under fluorescent illumination.

RESULTS

Monoclonal antibody Cat-301 recognizes a surface-associated antigen on neurons in many areas of the cat CNS (Hockfield et al., '83, Hendry et al., '88). In each area, only a subset of neurons expresses the Cat-301 antigen. Here we describe the pattern of Cat-301 immunoreactivity in the cat LGN and show, by several criteria, that Cat-301 selectively recognizes Y-cells.

Distribution of Cat-301-positive neurons in cat LGN

One characteristic feature of Y-cells is their distribution through the LGN. In our first experiments, we analyzed the distribution of Cat-301-positive neurons and compared it to that reported for Y-cells in the LGN.

Figure 1a shows a coronal section of the cat LGN stained with monoclonal antibody Cat-301. Cat-301-positive cells were found throughout the LGN complex, including the laminated LGN, the medial interlaminar nucleus, and the perigeniculate nucleus. Comparison with cresyl violet stained sections (Fig. 1B, C) showed that within the laminated LGN, layers A, A1, and C as well as the interlaminar zones contained a large number of Cat-301-positive cells. In layers A, A1, and C only a subset of neurons were

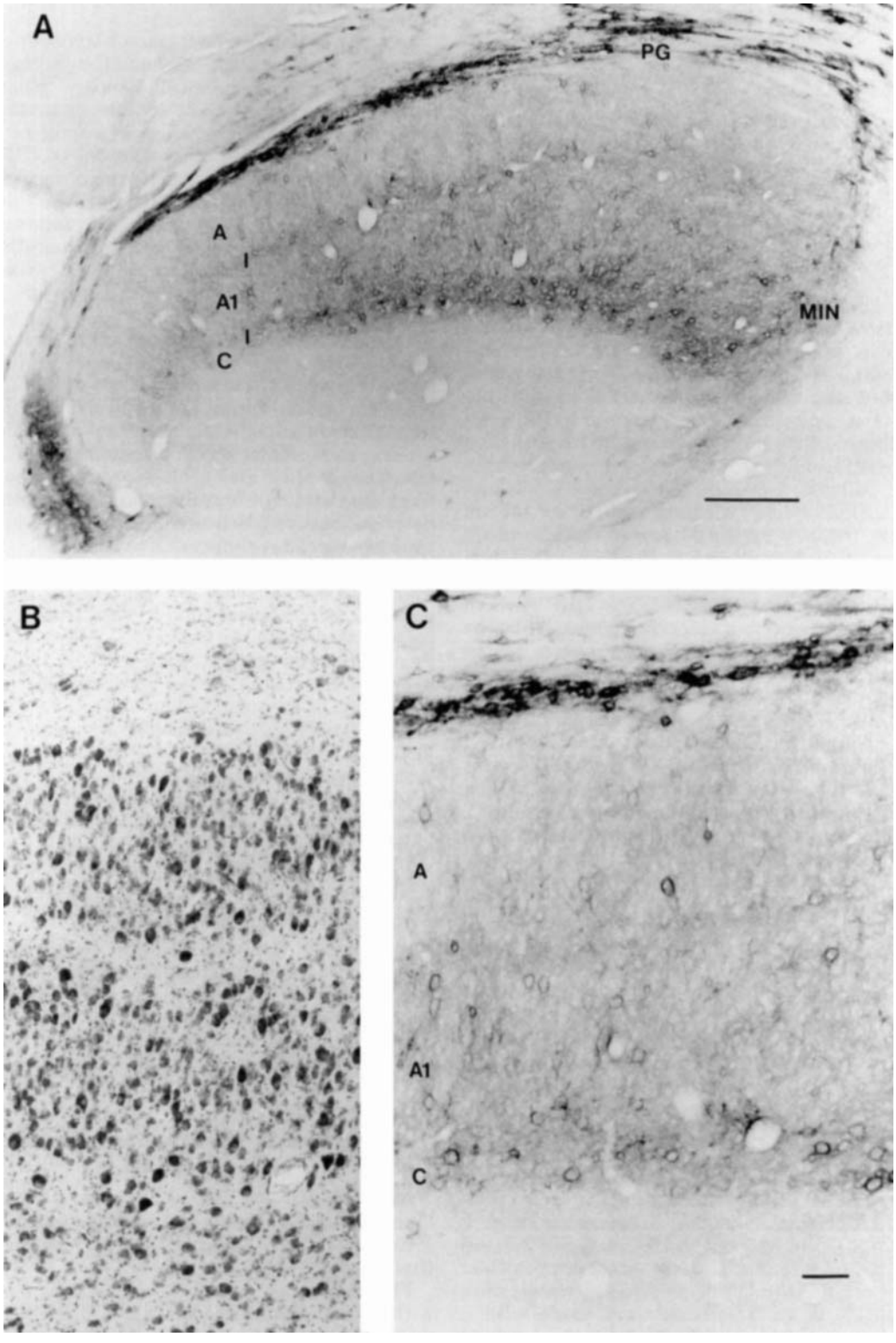

Fig. 1. Monoclonal antibody Cat-301 recognizes subsets of neurons in the cat LGN. **A.** Coronal section through the LGN of a normal cat stained with Cat-301 and visualized with an HRP-conjugated secondary antibody. Antibody-positive cells are found in the laminated LGN in layers A, A1, and C and in the interlaminar zones (I). The medial interlaminar nucleus (MIN) and the perigeniculate nucleus (PG) also contain antibody-positive cells. Note the relatively lower level of staining of the monocular segment. Bar = 200 μm. **B** and **C.** Comparisons between cresyl violet (B) and Cat-301 (C) stained sections show the relative density and distribution of Cat-301-stained neurons in the laminated LGN. Bar = 100 μm.

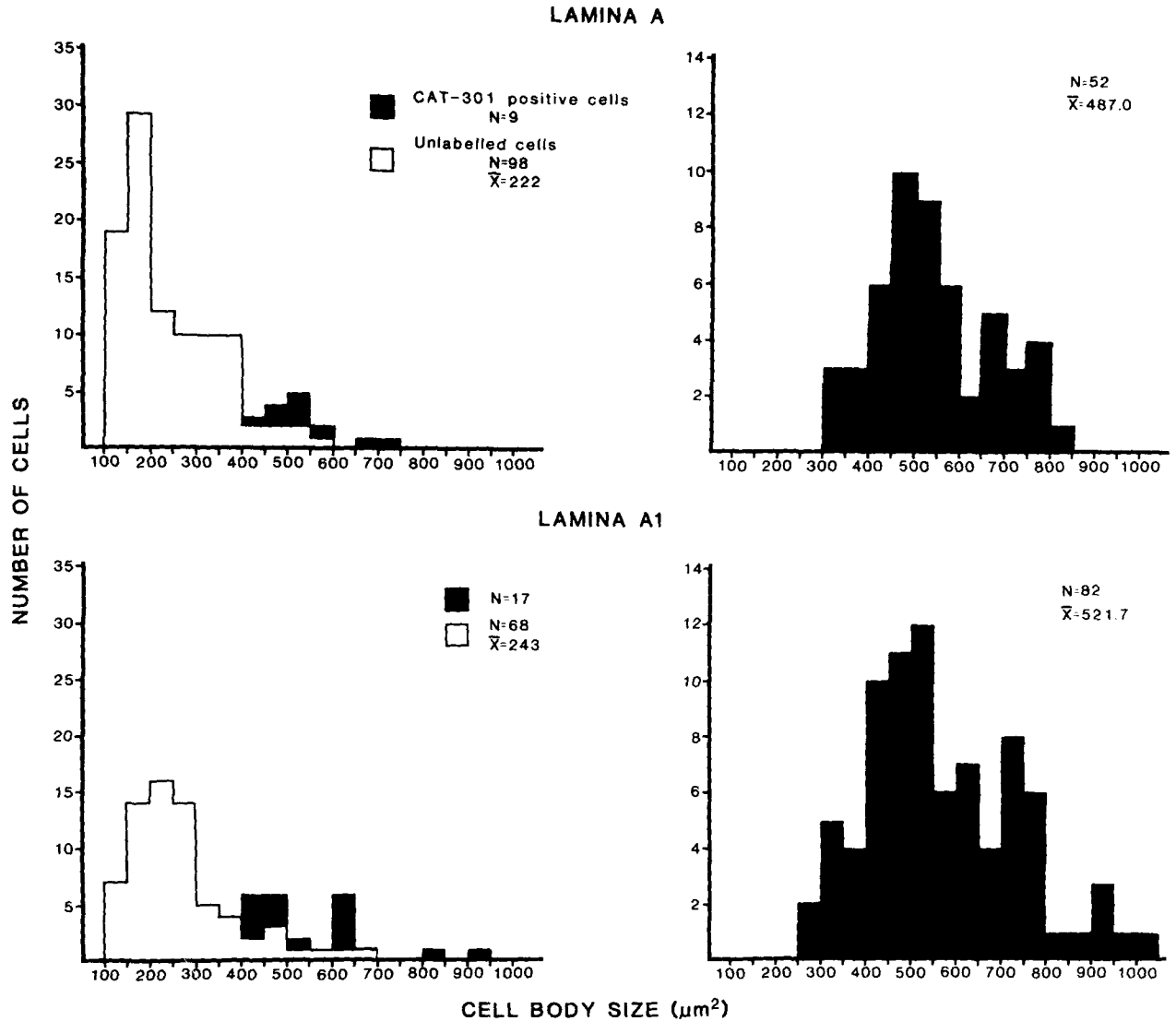

Fig. 2. Histograms illustrating the number and size of Cat-301-positive neurons in laminae A and A1. In lamina A approximately 10% of neurons are antibody positive and their mean size is 487 μm^2. In lamina A1 approximately 20% of neurons are antibody-positive and their mean size is 522 μm^2. This is consistent with the impression given in Figure 1 that lamina A1 contains a greater number of antibody-positive cells than does lamina A. See Methods for experimental details.

Cat-301-positive; in the interlaminar zones the vast majority of neurons were Cat-301-positive (Figs. 2, 3).

Cat-301 staining of layer A1 appeared somewhat more intense than the staining of layer A (Fig. 1A,C). Calculation of the percentage of neurons labelled in each layer showed that layer A1 contained a slightly greater percentage of Cat-301-positive cells than did layer A (Fig. 2). This difference in the relative abundance of Cat-301-positive cells accounted, at least in part, for the apparent difference in staining intensity between these two layers.

In the C complex, Cat-301-positive neurons were restricted to the most dorsal part, corresponding to layer C (magno-C). The more ventral part of the C complex (parvo-C) was largely devoid of Cat-301-positive neurons. The medial interlaminar nucleus also contained a large number of immunoreactive neurons.

Size of Cat-301-positive neurons

Each of the three major classes of LGN neurons has a characteristic distribution of cell sizes (see Sherman and Spear, '82). In order to determine if Cat-301-positive neurons fit into a classification based on cell size, we measured Cat-301-positive and Cat-301-negative neurons in Cat-301 stained, cresyl-violet counterstained sections of the LGN. Measurements from one cat are shown in Figures 2 and 3. In layers A, A1, and C, as well as in the MIN, Cat-301-positive neurons represented the largest cell class. In layer A, the mean size of antibody-positive cells was 487 μm^2, in layer A1 the mean size was 522 μm^2, in layer C the mean size was 498 μm^2, and in the MIN the mean size was 549 μm^2. Overall, the size of Cat-301-positive neurons was around 500 μm, and these neurons were among the largest cells in the cat LGN.

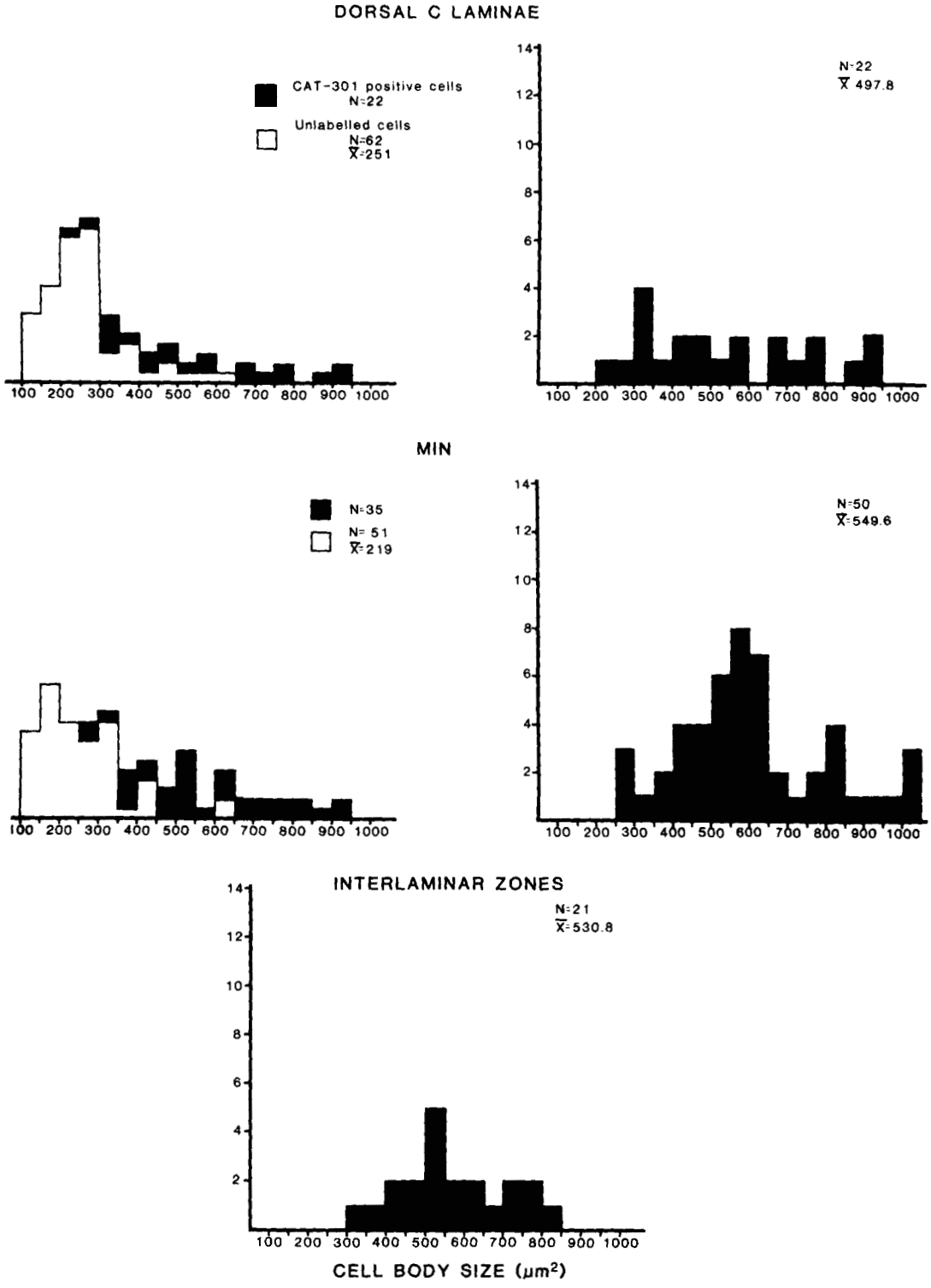

Fig. 3. Histograms illustrating the number and size of Cat-301-positive neurons in the C laminae and in the MIN and interlaminar zones. In the dorsal C laminae approximately 26% of neurons are antibody-positive and their mean size is 498 μm^2. In the MIN over half of the neurons are antibody-positive and their mean size is 550 μm^2. Virtually all of the neurons in the interlaminar zones are antibody-positive. See Methods for experimental details.

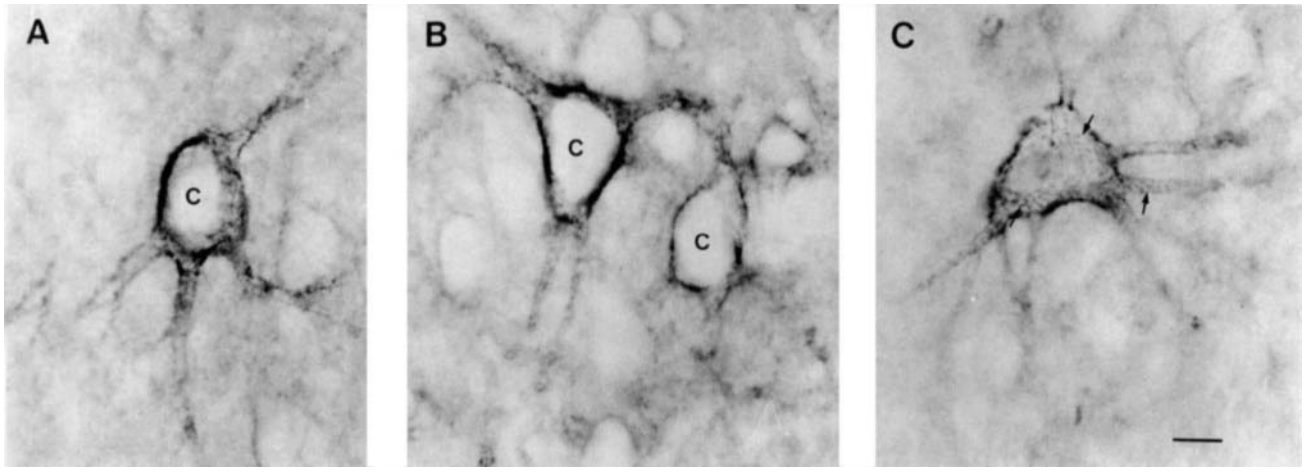

Fig. 4. Cat-301-positive neurons have morphological traits matching those of Y-cells. Antibody-positive cells have large, round cell bodies (c) with stout, radiating dendrites. At this magnification the association of Cat-301 immunoreactivity with the surface of cell bodies can be appreciated. Where the surface of the cell body or dendrite is within the plane of focus, the inhomogeneous distribution of antigen over the cell surface is revealed (arrows in C). Bar = 10 μm.

Morphology of Cat-301-positive neurons

Intracellular recording and filling experiments have shown that Y-cells generally have morphological features that distinguish them from X-cells (see Sherman and Spear, '82). We used two approaches to determine whether the morphological features of Cat-301-positive LGN neurons matched those of Y-cells. First, we examined the morphology of Cat-301 stained neurons in sections from intact LGN. In these sections, Cat-301 staining extended over the cell body and a short distance along the proximal dendrites of LGN neurons (Fig. 4). The truncated distribution of Cat-301 immunoreactivity prevented the visualization of the complete dendritic arbor; however, the morphology of cell bodies and proximal dendrites stained with Cat-301 could provide some insight into the morphological class of neuron identified by the antibody. Antibody-positive cells (Fig. 4) had round cell bodies with dendrites that emerged from around the entire circumference of the cell body and coursed radially. The proximal parts of the dendritic arbor visualized with Cat-301 generally were stout.

Further morphological characterization was obtained by studying LGN neurons filled with Lucifer yellow in tissue slices that were then counterstained with Cat-301 (Fig. 5). The slice preparation prohibited definitive assignment of a neuron to a physiological class; however, we used this approach simply to visualize the entire dendritic arbor of Cat-301-positive neurons. Four large intracellularly filled neurons were counterstained for Cat-301 and all four demonstrated Cat-301 immunoreactivity. These neurons had large cell bodies with radially oriented dendrites, which, in some cases, crossed laminar borders.

Cortical projection of Cat-301-positive neurons

Previous electrophysiological and anatomical studies suggest that from the A laminae of the LGN, Y-cells project to visual cortical areas 17 and 18, while X-cells project only to area 17 (Humphrey et al., '85; see Sherman and Spear, '82 for review). In order to determine if the projection pattern of Cat-301-positive neurons matches that described for Y-cells, we next examined neurons retrogradely labelled from areas 17 and 18 for Cat-301 immunoreactivity. Following injections of HRP or fluorogold restricted to area 17 or area 18, sections of the LGN were processed to visualize both the retrograde marker and Cat-301 immunoreactivity.

HRP or fluorogold injections in area 18 (Fig. 6) produced retrograde labelling in neurons in LGN layers A, A1, and C. Retrogradely labelled neurons in the A laminae had large diameters and demonstrated the characteristic, surface-associated Cat-301 immunoreactivity (Fig. 7). In two animals in which there was good retrograde transport from an injection restricted to area 18, every cell filled with either HRP or fluorogold was also labelled with Cat-301.

Injections of HRP or fluorogold into area 17 labelled many more cells in the A and C laminae than did injections into area 18. These cells also had a wider spectrum of soma sizes (cf. Geisert, '80). Only a small subset of the retrogradely labelled cells in the A laminae was Cat-301-positive: in one case with good retrograde transport from area 17, the proportion of retrogradely labelled cells that were Cat-301-positive was 10% in lamina A and 15% in lamina A1. In the dorsal C laminae, all large retrogradely labelled cells were also labelled with Cat-301, whereas small retrogradely labelled cells were not.

DISCUSSION

Neurons in the cat LGN can be divided into three major cell classes based on morphological and physiological criteria (Rodieck, '79, Lennie, '80; Sherman and Spear, '82). In our previous work we showed that monoclonal antibody Cat-301 recognizes a subset of neurons in the cat LGN and visual cortex (Hockfield et al., '83; Hendry et al., '84). The experiments reported here support and extend our earlier observations by demonstrating that the morphological characteristics of Cat-301-positive neurons in the LGN match those previously described for Y-cells.

Distribution of Cat-301-positive neurons matches the distribution of Y-cells in the LGN

We show that Cat-301-positive neurons are found in layers A, A1, and C and in the interlaminar zones of the laminated LGN. Antibody-positive neurons are also found

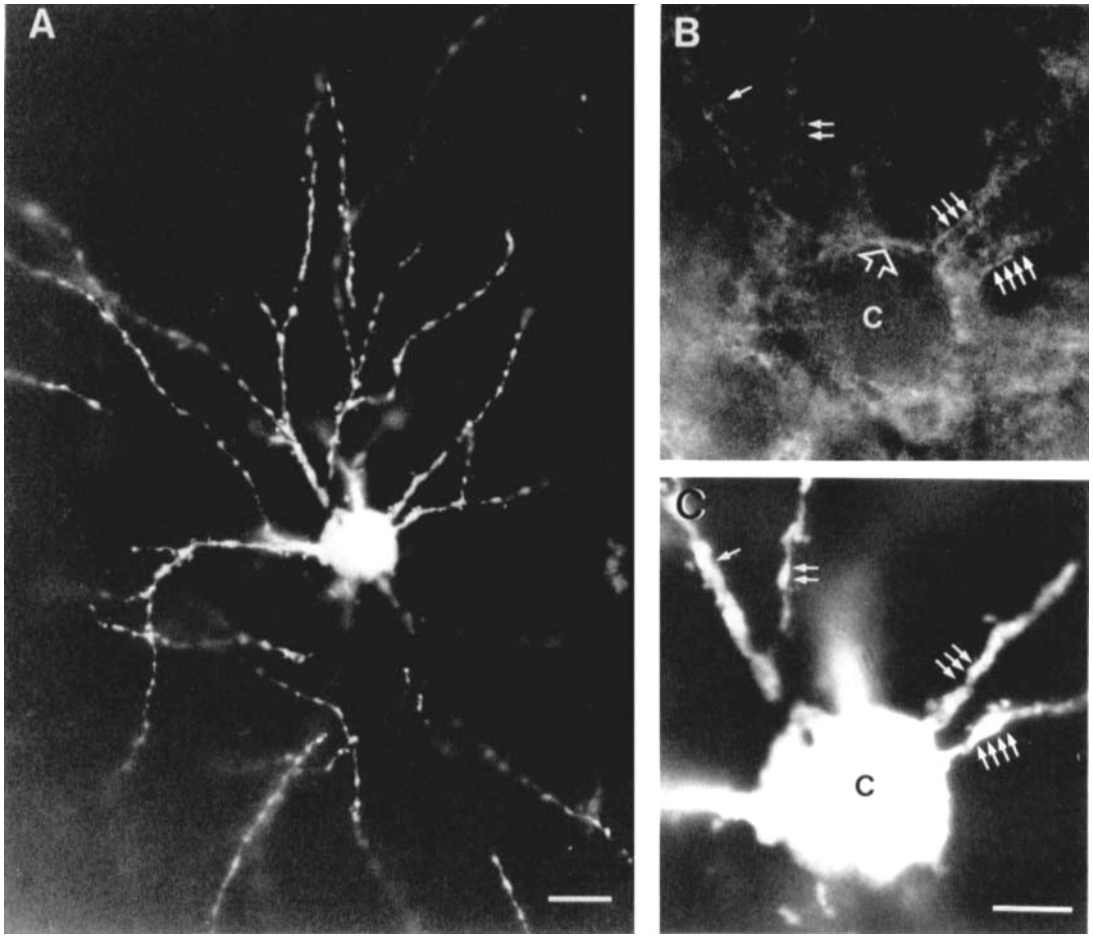

Fig. 5. Intracellularly filled LGN neurons with Y-cell morphology are Cat-301-positive. **A.** Lucifer-yellow filled neuron from a slice preparation of cat LGN demonstrates Y-cell morphology. The round cell body has radiating dendrites with few appendages. Bar = 20 μm. **B.** The same cell as in (A) stained with Cat-301 and visualized with a Texas Red-conjugated secondary antibody. Cat-301 immunoreactivity is distributed over the surface (open arrow) of the cell body (c) and the proximal dendrites. **C.** Higher magnification of the same cell illustrating the morphology shown with lucifer yellow. Each of the dendrites indicated in panel (B) is indicated in the micrograph in (C) by the same number of arrows. Bar C = 10 μm also applies to B.

in the medial interlaminar and perigeniculate nuclei. Each layer of the laminated LGN contains a characteristic population of relay cells (see Sherman and Spear, '82). Layers A and A1 contain both X- and Y-cells; layer C contains Y- and W-cells; and layers C1-3 contain W-cells. Analyses of retinogeniculate input suggest that retinal input to each layer derives from physiologically distinct ganglion cell classes, such that retinal X-cells project to layers A and A1 and Y-cells to layers A, A1, and C (Sur and Sherman, '82; Bowling and Michael, '84). Physiological studies indicate that the medial interlaminar nucleus also contains a substantial population of Y-cells (Kratz et al., '78; Dreher and Sefton, '79). The medial interlaminar nucleus receives collaterals from many retinal Y-axons (Sur et al., '87). In sum, the distribution of LGN neurons with Y-like electrophysiological properties matches the distribution of Cat-301-positive neurons reported here.

Fewer studies have addressed the distribution of Y-like cells in the interlaminar zones and in the perigeniculate nucleus. Retinogeniculate Y axons, but not X axons, often have terminals that extend into the interlaminar zones (Bowling and Michael, '84; Sur et al., '87), suggesting that cells in the interlaminar zones are likely to receive Y input. The perigeniculate nucleus does not receive direct input from retinal ganglion cell axons. However, LGN relay cell axons often give off collaterals to the perigeniculate nucleus on their way to the cortex. Interestingly, intracellular HRP studies suggest that many more Y-cells than X-cells have collaterals in the perigeniculate nucleus (Friedlander et al., '81; Humphrey and Weller, '85b).

Size, morphology, and projection pattern of Cat-301-positive neurons match those of Y-cells

Neurons in the LGN can be divided into several different morphological classes. Early studies correlating physiological with anatomical classes of LGN neurons suggested that the class of neurons with the largest cell diameters, the class 1 cells, corresponds to the Y-cell class and that the classes of neuron with smaller diameters (classes 2–4) correspond to X-cells, W-cells, and interneurons (LeVay and Ferster, '77; see Sherman and Spear, '82). The identification of the largest class of LGN neurons as Y-cells has been in large measure (although see below) upheld by more recent studies using intracellular methods to physiologically identify and then label single neurons (Friedlander et

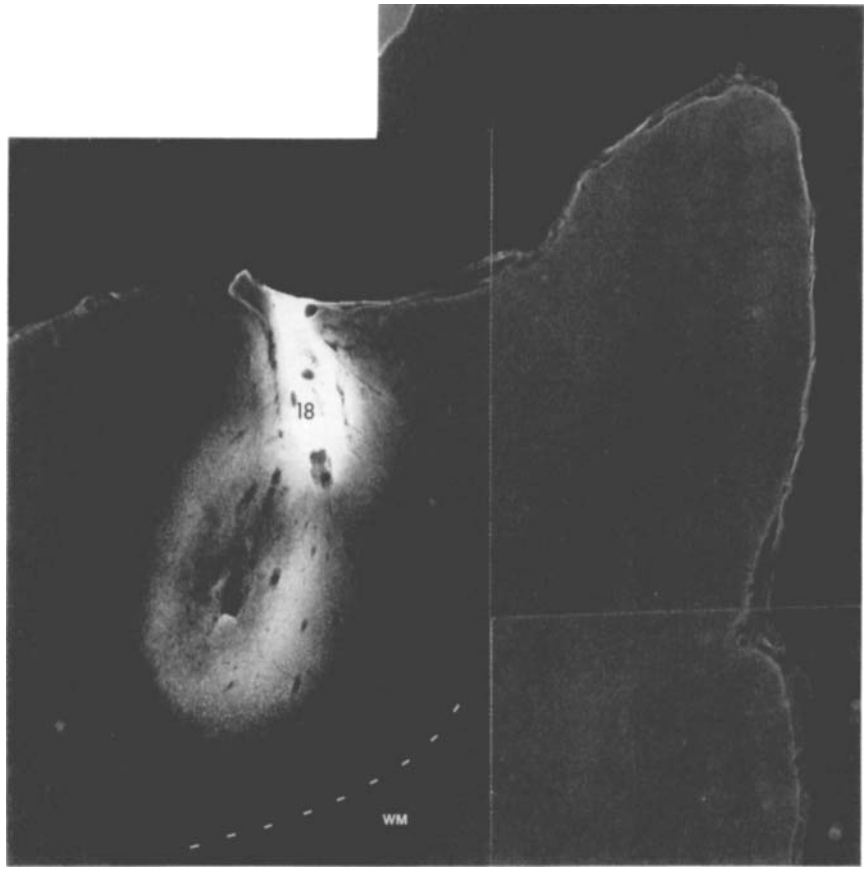

Fig. 6. An injection of fluorogold in area 18. This injection site was restricted to area 18 and did not impinge upon the underlying white matter (WM; dotted line represents the border between the cortical grey matter and the underlying white matter.). Neurons in the LGN retrogradely labelled from this injection are illustrated in Figure 7.

al., '81). These studies have provided detailed information about Y-cell morphology: Y-cells have cell bodies that range in size from just over 200 to over 900 μm^2 and have thick, radially oriented dendrites that often cross laminar borders.

Here we show that the morphological features of LGN neurons recognized by monoclonal antibody Cat-301 match those described for Y-cells. Cat-301-positive neurons represent the largest cell class in the LGN. The proximal dendrites of antibody-stained cells are thick and emerge radially from the cell body. Furthermore, we show here that intracellularly filled, Cat-301-positive neurons have thick, long dendrites with few branches or appendages. These results show clearly that Cat-301 identifies class 1 cells in the LGN as defined by Guillery ('66).

Recently, physiological and anatomical analyses of LGN cells have suggested the existence of two subpopulations of X-cells. Intracellular labelling suggests that one group of LGN X-cells, "nonlagged cells," can have a morphology typical of class 1 cells (Humphrey and Weller, '88a,b). However, even the nonlagged class 1 cells have soma sizes that are smaller than those of class 1 cells described originally by Guillery ('66), and so are unlikely to be among the neurons identified by Cat-301. Furthermore, nonlagged X-cells may not constitute a cell class uniquely separate from lagged X-cells because brainstem input may interconvert these two seemingly distinct populations (Uhlrich et al., '90). The issue of whether the class 1 cells identified with Cat-301 are all Y-cells therefore demands a more detailed study by intracellularly labelling physiologically characterized cells followed by immunostaining with Cat-301.

LGN X-cells and Y-cells also differ from one another in their cortical projection targets. Both physiological and anatomical studies have demonstrated that Y-cells project to cortical areas 17 and 18, whereas X-cells project only to area 17 (Stone and Dreher, '73; Hollander and Vanegas, '77; LeVay and Forster, '77; Geisert, '80; Humphrey et al., '85). Our experiments show that, following tracer injections limited to area 18, all retrogradely labelled LGN neurons are Cat-301-positive. We regard this result as strong evidence that Cat-301 recognizes Y-cells.

Does Cat-301 recognize all Y-cells?

This kind of study does not permit us to determine definitively whether Cat-301 recognizes all Y-cells. Cat-301 recognizes approximately 10% of neurons in layer A and 20% of neurons in layer A1. Earlier studies on the relative proportion of Y-cells in the LGN suggested that between 10% and 50% of neurons in the A layers are Y-cells (see Sherman and Spear, '82). In some studies, using electrophysiological criteria, Y-cells were found to represent 35% of neurons in the A laminae (LeVay and Ferster, '77; Friedlander et al., '81). The smaller percentage of cells recog-

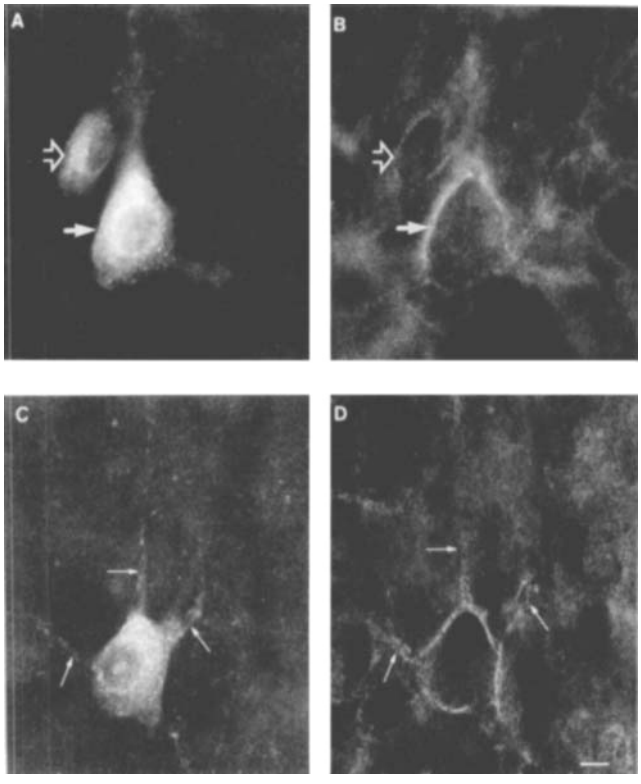

Fig. 7. LGN neurons that project to area 18 are Cat-301-positive. Following fluorogold injections restricted to cortical area 18 (illustrated in Fig. 6), sections of the LGN were stained for Cat-301. Under UV optics, fluorogold labelled neurons had large cell bodies and radiating dendrites (**A** and **C**). These same cells visualized under Texas Red optics (**B** and **D**) show Cat-301 immunoreactivity. Arrows (open and closed) in (A) and (B) indicate two retrogradely labelled, Cat-301-positive neurons. Arrows in (C) and (D) indicate the position of dendrites labelled with both fluorogold and Cat-301. All neurons retrogradely labelled by injections in area 18 were Cat-301-positive. Bar = 10 μm.

nized by Cat-301 can be interpreted in a number of different ways.

One interpretation is that Cat-301 selectively labels a subset of Y-cells. Studies to date (Geisert, '80; Bullier et al., '84; Humphrey et al., '85) indicate that the projection pattern of Y-cells in the A laminae permits their division into three subsets: a subset of Y-cells projecting only to area 18; another projecting to both areas 17 and 18; and a third projecting only to area 17. Following our injections restricted to area 18 all retrogradely labelled cells are Cat-301-positive. By necessity, these injections were small and only a subset of Cat-301-positive neurons are retrogradely labelled. Given that 10–20% of neurons in the A laminae might project to area 18, one possibility is that Cat-301 specifically recognizes the subset of Y-cells that projects to area 18.

The distribution of Cat-301 labelled neurons in the LGN is also consistent with previous descriptions of LGN neurons that project to area 18. HRP injections in area 18 produce a greater number of labelled neurons in layer A1 than in A, and a large number of labelled neurons in the interlaminar zones (Hollander and Vanegas, '77; LeVay and Ferster, '77). This corresponds to the distribution of Cat-301-positive neurons the LGN. Furthermore, LeVay and Ferster ('77) have shown that the very largest cells in the LGN and those that project to area 18 are relatively sparse in the monocular segment, again a pattern that is matched by Cat-301 immunoreactivity (see Fig. 1). Taken together these observations suggest that the very largest class of LGN neurons constitutes a subset of Y-cells, distinct from the remainder of Y-cells with respect to mediolateral distribution, projection to area 18 and Cat-301 immunoreactivity.

A second possibility is that Cat-301 actually labels all A laminae Y-cells. The electrophysiological techniques used to determine the proportion of Y-cells might bias sampling toward large neurons. This would produce an artifactually high percentage of Y-cells; such a bias would be absent from the anatomical techniques used here. A third possibility is that the Cat-301 antigen is present on all Y-cells, but the level of antigen present on some cells may be below the limit of detection of techniques. This possibility seems less likely because increasing the concentration of antibody increases the intensity of the labelling signal and the level of background immunoreactivity, but does not produce changes in the proportion of labelled cells. Our ongoing studies of the functional properties of individual Cat-301-positive neurons may provide a resolution to this issue.

Cat-301 immunoreactivity is expressed late in development and early visual deprivation reduces antigen expression

The data we present here show that Cat-301-positive cells in the cat LGN have morphological and connectional characteristics consistent with their identification as Y-cells. Two other lines of evidence extend this interpretation. First, the time course of the expression of Cat-301 immunoreactivity follows the time course of the maturation of Y-cell physiological characteristics. The maturation of Y-cells occurs relatively late in development (Daniels et al., '78, Mangel et al., '83). At 30 days postnatal (P30), the LGN contains only a small number of physiologically identifiable Y-cells. The mature complement of physiologically identifiable Y-cells is not reached until P90. X-cells complete their physiological maturation significantly earlier than Y-cells. Cat-301 immunoreactivity is first detected in the cat LGN at P30 and adult levels are not reached until P90 (Sur et al. '88). This late development of the Cat-301 antigen suggests that the antibody selectively marks physiologically mature Y-cells.

The regulation of expression of the Cat-301 antigen in parallel with the maturation of Y-cell physiology is supported by our studies of animals raised under conditions of visual deprivation (Sur et al., '88). The development of normal Y-cell physiology is markedly perturbed by postnatal visual deprivation (see Movshon and VanSluyters, '81; Sherman and Spear, '82). Monocular visual deprivation by lid-suture or binocular deprivation by dark-rearing during the first three months of life severely reduces the proportion of Y-cells that can be physiologically identified in deprived layers of the LGN. We have shown that deprivation of normal visual stimuli early in life also reduces the level of Cat-301 immunoreactivity in the LGN. Following neonatal monocular lid-suture, LGN layers receiving input from the sutured eye show a marked decrease in the number of Cat-301-positive neurons. Following dark-rearing from birth to 90 days, the level of immunoreactivity in the LGN is far below normal (Sur et al., '88; Guimaraes et al., '90). This loss of the Cat-301 antigen following

deprivation correlates with the loss of Y-cells and strengthens the possibility that Cat-301 immunoreactivity is specifically associated with physiologically mature Y-cells. The loss of Y-cells following visual deprivation is far more dramatic in the binocular segment of the LGN than in the monocular segment (Guillery and Stelzner, '70; Sherman et al., '72). As discussed above, Cat-301 may recognize a subset of Y-cells and this subset may be further differentiated from other Y-cells by its selective sensitivity to visual deprivation.

Molecular trait identifies a physiologically defined class of neuron

The demonstration here that monoclonal antibody Cat-301 selectively identifies LGN Y-cells shows that neurons within a physiological class share a common antigenic trait. This supports the view that shared functional properties of neurons might be reflected by shared molecular properties. Our observations in the monkey LGN suggest that molecular properties of neurons also may be phylogenetically conserved in functionally related neurons. While perfect homologies do not exist between the cat and monkey visual systems, the Y-cell channel in the cat, which processes the motion sensitive, low contrast, content of a visual image, is thought to correspond at least functionally to the magnocellular channel in the monkey (e.g., Sherman et al., '76; Schiller and Malpelli, '78). The organizations of the cat and primate LGN with respect to the distribution of Y-cells and magnocellular neurons are quite different. In the monkey LGN, functionally distinct classes of cells occupy different layers, and neurons in the magnocellular pathway are found in the two most ventral LGN layers, the magnocellular layers. Cat-301 recognizes over 90% of the neurons in the magnocellular layers and fewer than 15% of neurons in the parvicellular layers (Hockfield et al., '83; Hendry et al., '84). Cat-301 staining of Y-cells in the cat LGN and magnocellular neurons in the primate LGN suggests that antigens may be phylogenetically conserved by functionally related neurons even when the nuclei containing those neurons have quite distinct cytoarchitectonic features.

We do not yet know what kind of relationship exists between Cat-301-positive neurons in the LGN and in other areas of the CNS. Two issues must be considered in addressing this question. The first issue revolves around the biochemical identity of the Cat-301 antigen: is a single molecule present on all the different Cat-301-positive neurons or is there a family of related molecules all sharing a single epitope? The second issue concerns the identity of Cat-301-positive neurons in other areas of the CNS and the cell biology of the antigen in those areas. Histological studies at the light and electron microscopic levels show that the subcellular localization of the Cat-301 antigen is the same in all areas of the CNS. Cat-301 immunoreactivity is always associated with the surface of antibody-positive cells (McKay and Hockfield, '82; Hockfield and McKay, '83), suggesting that Cat-301 recognizes a single, or a family of related, antigens. Our biochemical studies indicate that the Cat-301 antigen is most likely to represent a family of closely related molecular species. The Cat-301 antigen in the cat, hamster, guinea pig, and human is a 650,000 dalton chondroitin sulfate proteoglycan (Zaremba et al., '89). When isolated from whole brain, which contains a diverse group of Cat-301-positive neurons, the deglycosylated antigen migrates as a relatively sharp band, suggestive of a single protein or a group of proteins with similar biochemical properties. Available gel systems cannot resolve very subtle differences among molecules at this apparent molecular weight, but recent studies have demonstrated the presence of at least two immunologically distinct forms of the high molecular weight Cat-301 proteoglycan (Zaremba et al., '90). Higher resolution techniques may yet reveal further molecular heterogeneity within the Cat-301 antigen.

These histological and biochemical studies suggest that Cat-301 recognizes a family of closely related antigens. This raises the possibility that the shared antigenic properties we observe among diverse classes of neurons may reflect shared functional traits. Two sets of experiments further support this possibility. First, our studies of primate visual system have shown that several different groups of Cat-301-positive neurons are functionally related. From LGN through at least six hierarchically connected visual cortical areas Cat-301-positive neurons are associated with the magnocellular, or motion sensitive, pathway (DeYoe et al., '87, '90; McGuire et al., '89). The other major pathway in the monkey, the parvicellular, or form/color sensitive pathway contains far fewer Cat-301-positive neurons. Second, the activity-dependent regulation of the expression of the Cat-301 antigen appears to be a general feature of Cat-301-positive neurons, as demonstrated for neurons in the cat visual cortex (Guimaraes and Hockfield, '88; Guimaraes et al., '90) and for hamster spinal motor neurons (Kalb and Hockfield, '88, '90a,b). The biochemical and ultrastructural studies described above suggest a role for the Cat-301 proteoglycan in the stabilization of mature synaptic structure (Hockfield et al., '89). Our ongoing studies of the regulation and characterization of the Cat-301 antigen may permit analyses of physiological or anatomical relationships encoded by specific molecular properties.

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