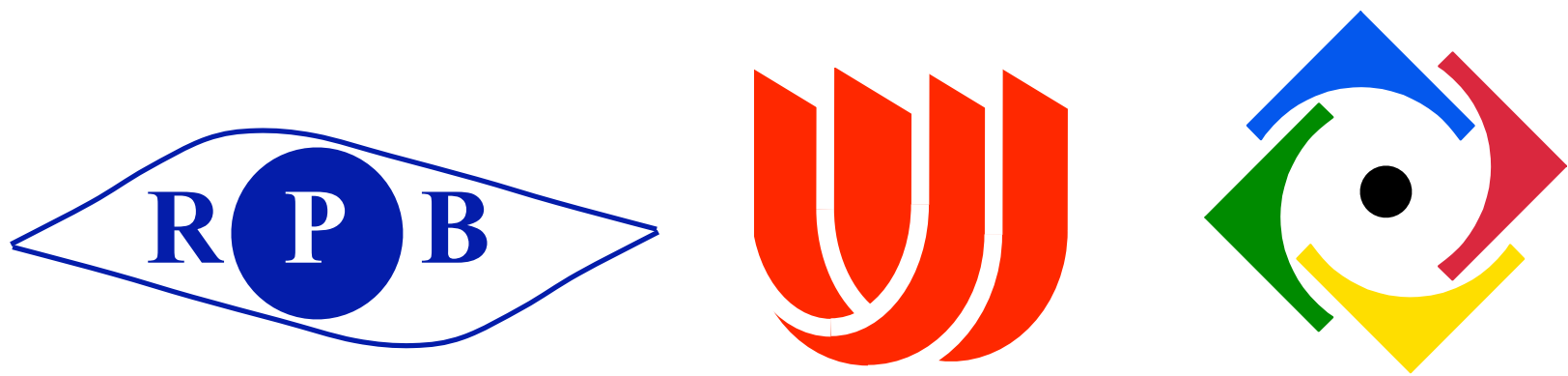


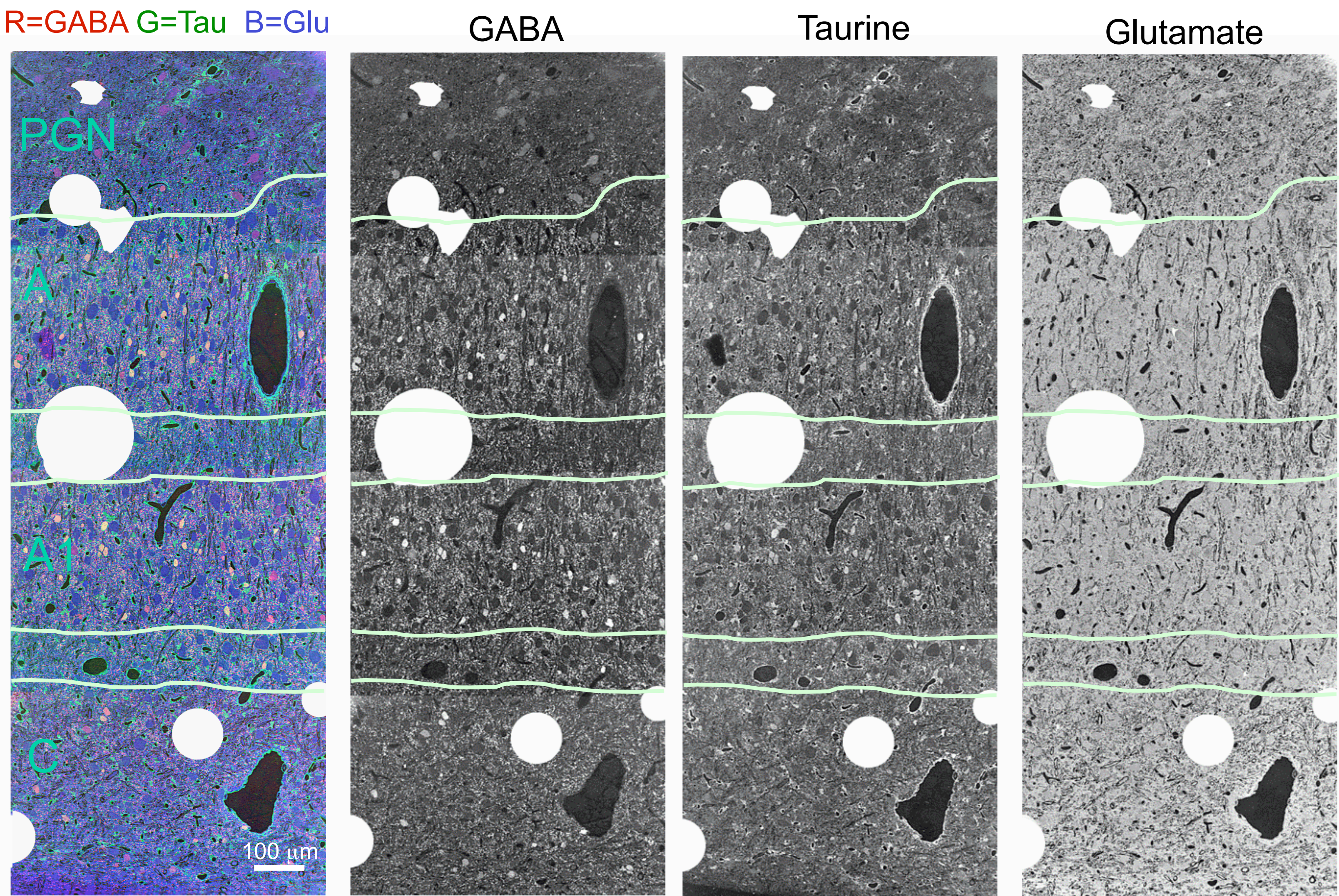
# NEUROCHEMICAL CLASSES OF NEURONS IN THE CAT LGNd AND PGN 3384 B242

M.T. SCHMOLESKY<sup>1</sup>, B.W. JONES<sup>2</sup>, Y-C. WANG<sup>1</sup>, A.G. LEVENTHAL<sup>1</sup> AND R.E. MARC<sup>2</sup>. DEPT OF NEUROBIOLOGY AND ANATOMY<sup>1</sup> AND MORAN EYE CENTER<sup>2</sup>, UNIVERSITY OF UTAH    Support: NEI EY04951, EY02576 and Research to Prevent Blindness

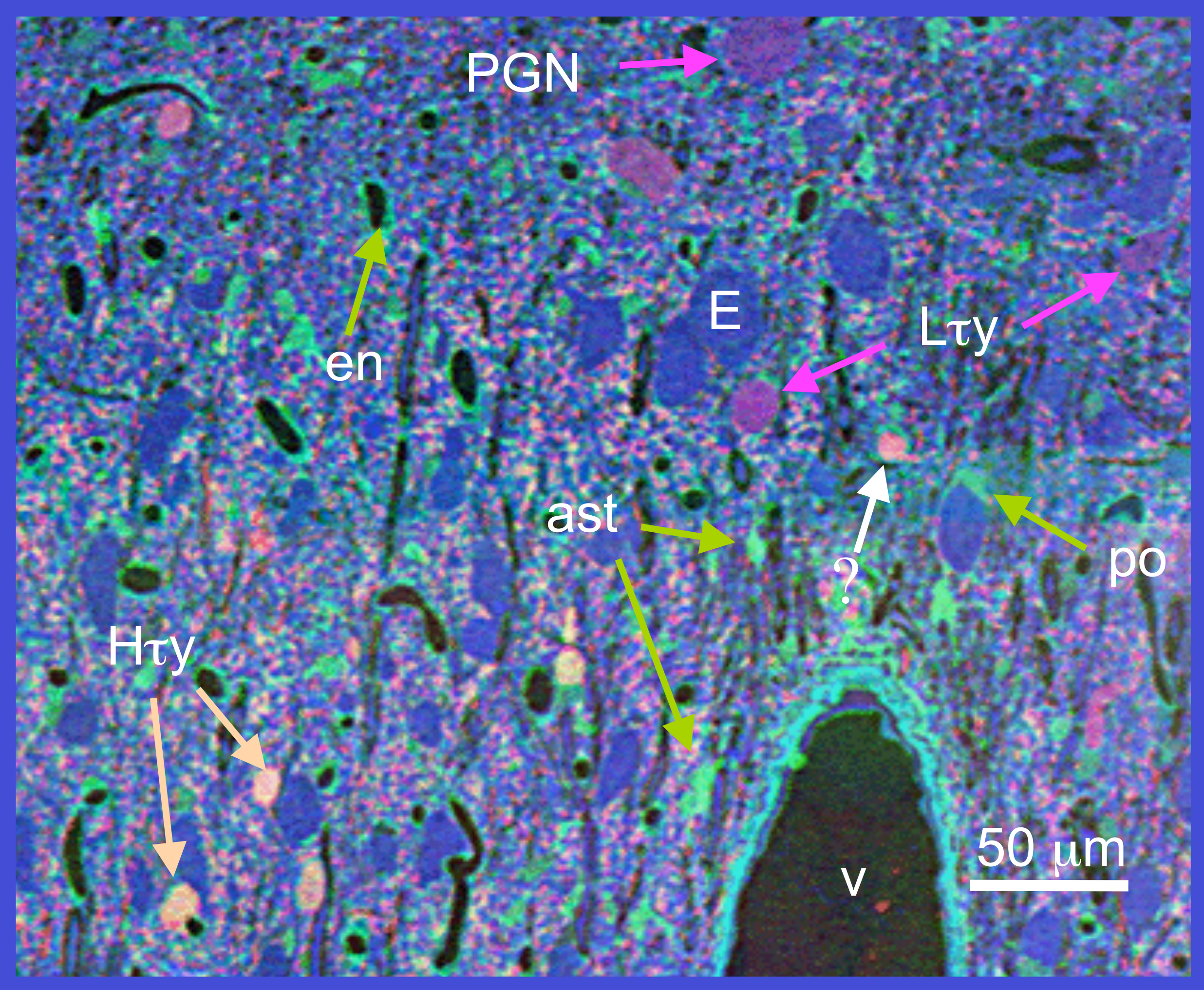


**Purpose:** Physiological and anatomical experiments have shown that the A and A1 laminae of the cat lateral geniculate nucleus (LGNd) contain two distinct types of glutamatergic and GABAergic cells, the X (sustained response, medium diameter) and Y (transient response, large diameter) cells. The C laminae contain Y-like cells in the most dorsal region and W-like cells (sluggish, small-medium diameter) in the remainder. The perigeniculate nucleus (PGN) lies dorsal to the LGNd and contains solely large GABAergic cells and glia. This project was designed to examine possible neuronal classes in the cat LGNd and PGN based upon aspartate, GABA, glutamine, glutamate, and taurine concentrations.

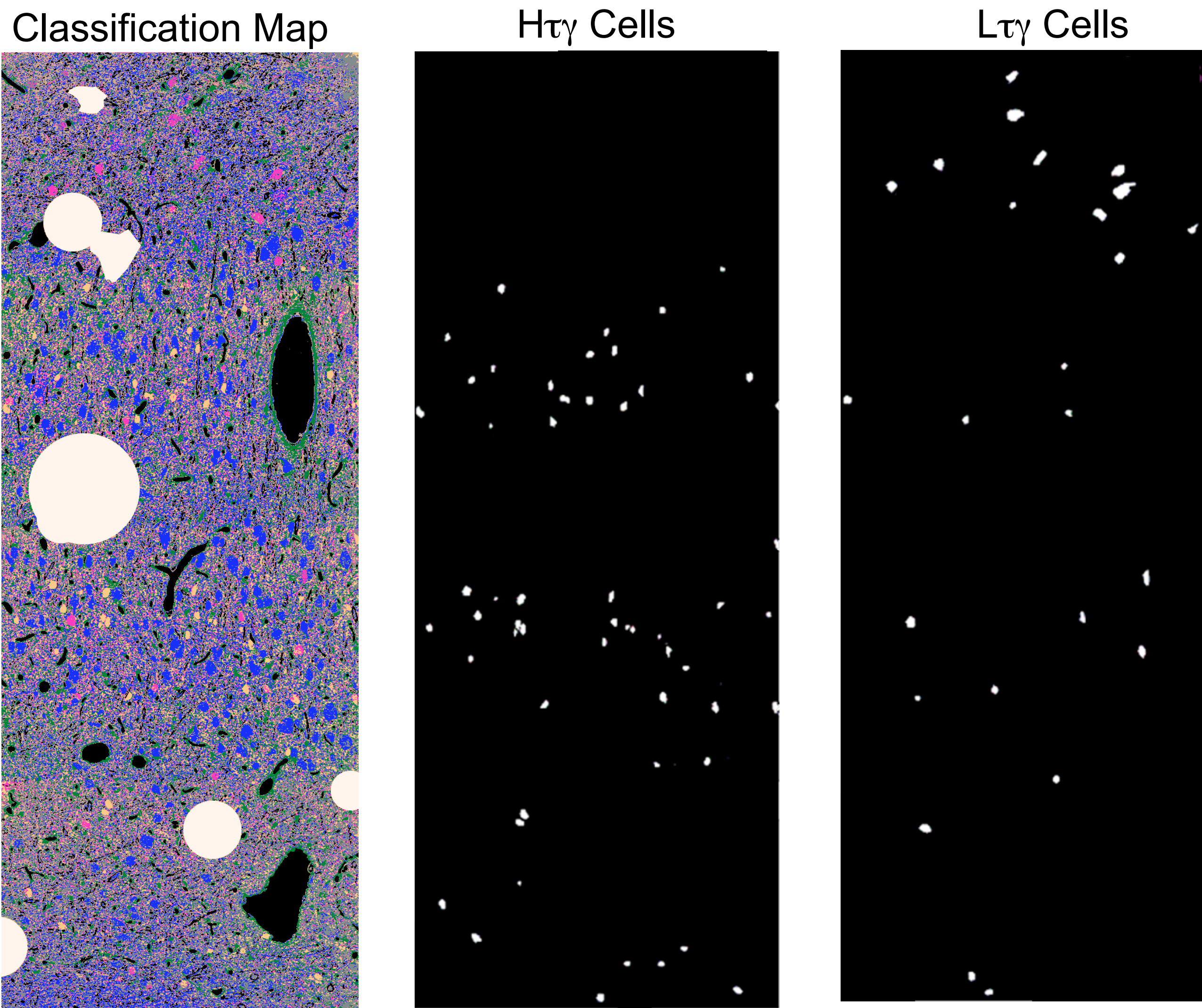
**Methods:** Three adult cats were intra-cardially perfused with 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer. The LGNd region was blocked and vibratome sectioned coronally at 200  $\mu\text{m}$ . Tissue was processed for conventional epoxy resin embedding, serially sectioned at 250 nm in the coronal plane and serially probed with IgGs selective for aspartate (D),  $\gamma$ -aminobutyrate (GABA or  $\gamma$ ), glutamate (E), glutamine (Q), and taurine ( $\tau$ ). Signals were visualized with silver-intensified gold reagents (1 nm gold 2° IgG). Identical regions of LGNd tissue were image captured for each signal with a calibrated CCD camera at 100X magnification (resolutions of 1.04 pixels/ $\mu\text{m}$ ). Image strips 400-600  $\mu\text{m}$  wide were created from the dorsal to ventral extent of the LGNd for each signal by digitally mosaicking/registering smaller images with GCPWorks (PCI Remote Sensing, Arlington VA). Signal images were analyzed with Image Pro Plus (Media Cybernetics, Silver Spring, MD), and displayed using PhotoShop® 5.0 (Adobe Systems Inc., San Jose, CA).



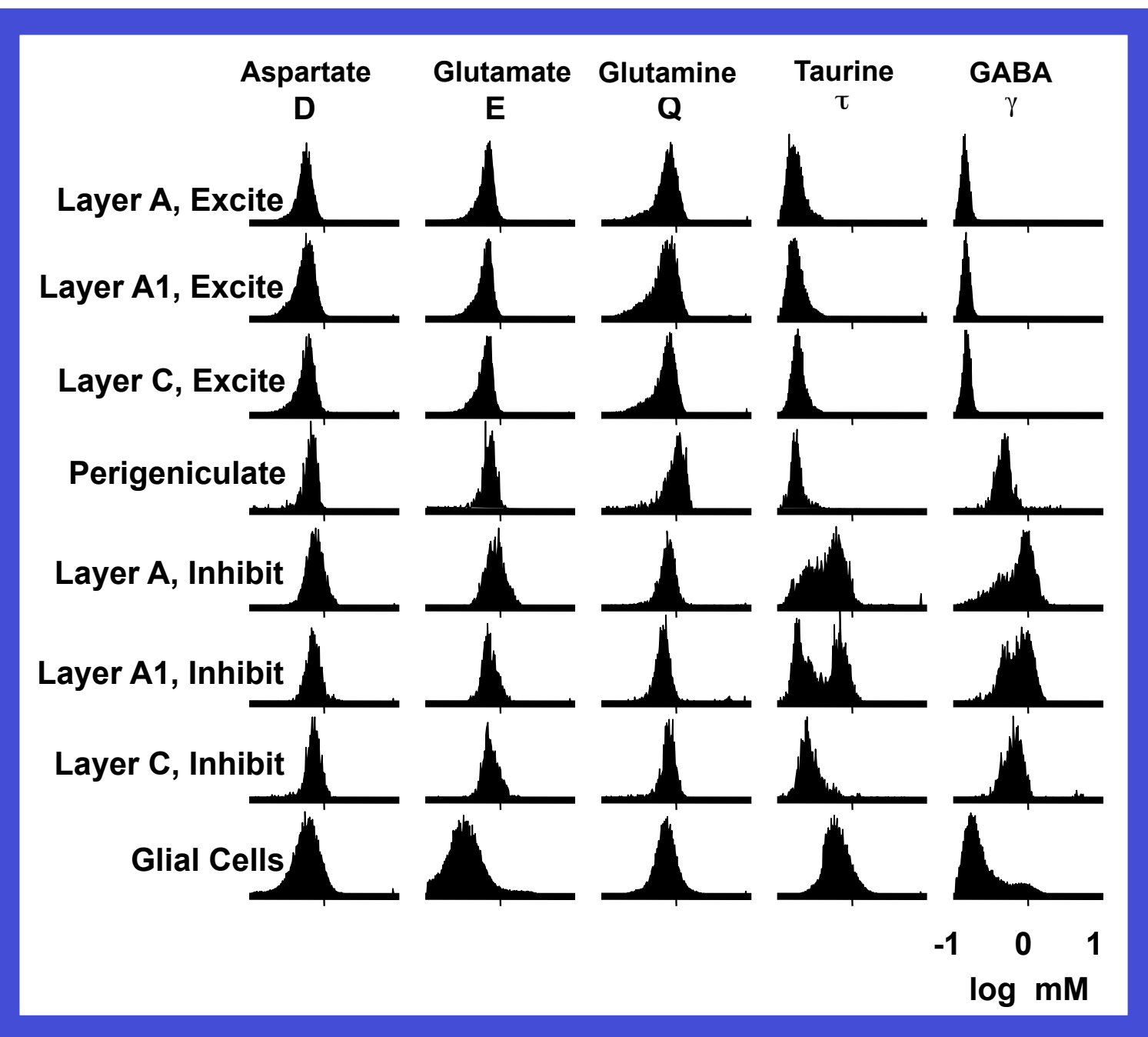
**Results [1]:** The three grayscale intensity-mapped images shown above were combined to form the single RGB image. Careful visual inspection suggests that there may be two neurochemical types of GABAergic cells in the A and C laminae, one with high [taurine] (tan) and one several degrees less so (violet). Only violet cells appear in the perigeniculate. Glutamate cells appear uniformly blue. White objects are image sections deleted due to microbubbles or impurities. Laminar separations (light green) were estimated based on cell and fiber profiles.



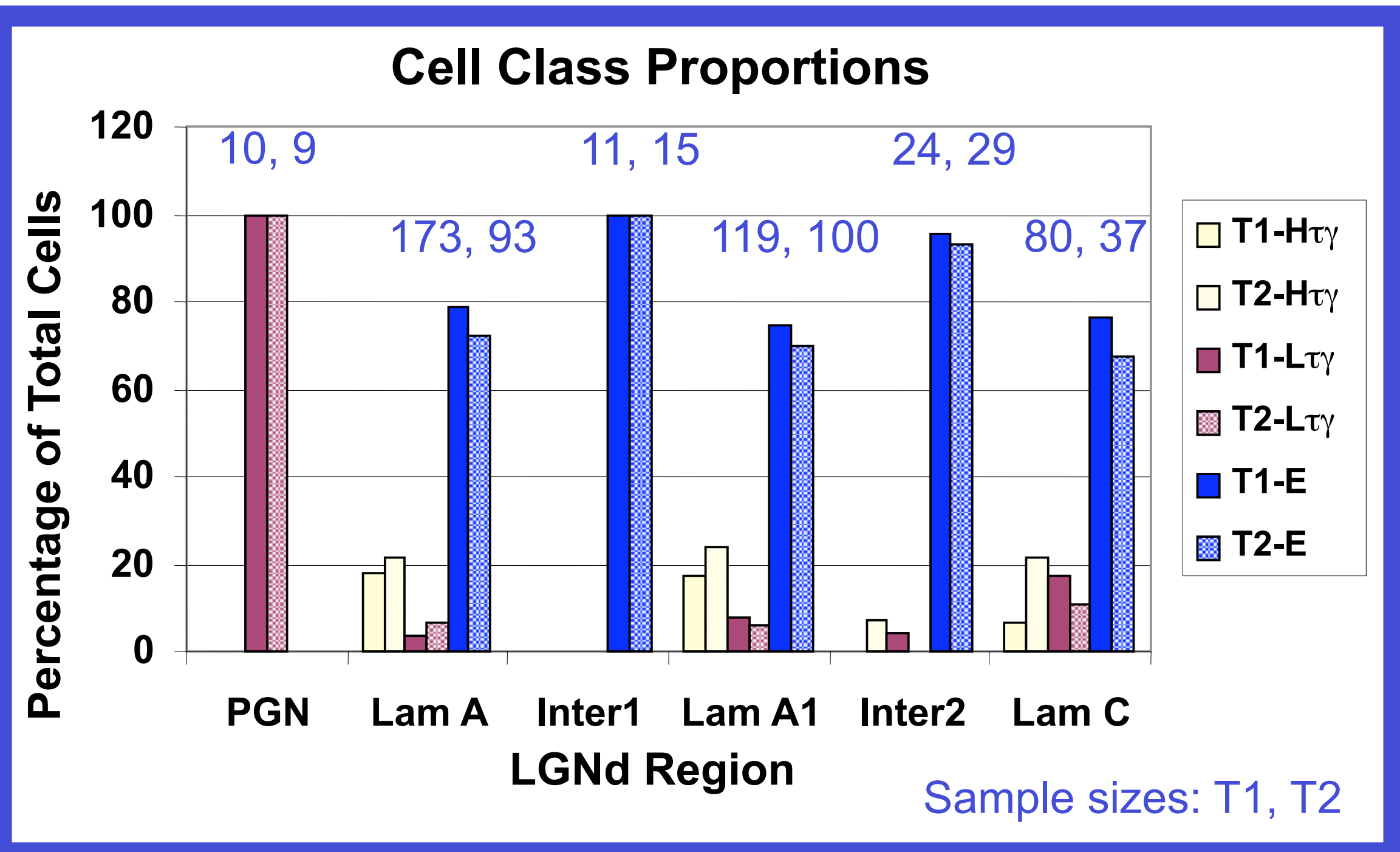
**Results [2]:** High and low [taurine] GABA cells are shown (H $\tau\gamma$  and L $\tau\gamma$ ). Glutamate cells (E) predominate throughout the LGNd but are absent within the PGN. Astrocytes (ast), presumptive oligodendrocytes (po) and endothelial cells (en) are marked by their high concentration of taurine and lack of GABA and glutamate. Not all cells can be easily categorized using visualization of RGB images.



**Results [3]:** K-means classification of all five signals provides a quantitative means of determining statistically distinct cell classes in the tissue of interest. Every pixel in the image is individually assigned to a statistical class, each class is designated an arbitrary color to and the entire image is then redrawn to form a Classification Map. This map can be used to select cells of each class for further analysis (e.g. [amino acid], proportions of cells, and cell sizes in each class). The black and white images above show our selection of the H $\tau\gamma$  and L $\tau\gamma$  cells.



**Results [4]:** Image masking techniques were used to select glutamatergic cells ("Excite"), GABAergic cells ("Inhibit") and glia. The ranges of amino acid concentrations found within each cell type were then plotted. Histogram abscissas indicate concentrations while the ordinates indicate the probability of encountering a particular [amino acid] in that cell type. Notice that all neurons contain the same relative [E]. Thus, only the content of significant [ $\gamma$ ] and/or [ $\tau$ ] can be used as a marker of the excitatory or inhibitory nature of neurons. Notice also the bimodal and complex [ $\tau$ ] and [ $\gamma$ ] histograms found in the A and A1 laminae.



**Results [5]:** The proportions of each neuron class were determined for two tissue sections (T1 and T2) 400  $\mu\text{m}$  removed in LGNd. T2 corresponds to all tissue images shown. As previously reported,  $\gamma$  cells make up 20-30% of LGNd neurons. We find that H $\tau\gamma$  cells far outnumber L $\tau\gamma$  cells in the A laminae, just as X  $\gamma$  cells outnumber Y  $\gamma$  cells. We also find that H $\tau\gamma$  cells are structurally smaller than L $\tau\gamma$  cells, again as X  $\gamma$  cells are smaller than Y. Thus, the results indicate that [ $\tau$ ] and [ $\gamma$ ] are molecular markers for two neurochemical classes of GABA cells that appear to correspond to X and Y GABA cells in the cat LGNd. In contrast to the A laminae, L $\tau\gamma$  cell counts are larger (T1) or nearly equal to (T2) H $\tau\gamma$  counts in the C laminae, suggesting that W-like  $\gamma$  cells are neurochemically diverse.

