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Quantitative distribution of GABA-immunoreactive neurons in the visual cortex (area 17) of the cat

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Summary. Cortical neurons using the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) are known to contribute to the formation of neuronal receptive field properties in the primary visual cortex (area 17) of the cat. In order to determine the cortical location of GABA containing neurons and what proportion of cortical neurons might use GABA as their transmitter, we analysed their distribution quantitatively using a post-embedding GABA immunohistochemical method on semithin sections in conjunction with stereological procedures. The mean total numerical density of neurons in the medial bank of the lateral gyrus (area 17) of five adult cats was 54,210 ± 634 per mm^3 ($\bar{x}$ ± SD). An average of 20.60 ± 0.48% ($\bar{x}$ ± SEM) of the neurons were immunoreactive for GABA. The density of GABA-immunoreactive neurons was somewhat higher in layers II, III and upper VI, compared with layers I, IV, V and lower VI, with the lowest density being in layer V. The proportion of GABA-immunopositive cells relative to immunonegative neurons gradually decreased from the pia to the white matter. Layer I was different from other layers in that approximately 95% of its neurons were GABA-immunoreactive. The results allowed the calculation of the absolute numbers of GABAergic neurons in each layer under a given cortical surface area and could provide the basis for the quantitative treatment of cortical circuits.

Key words: Visual cortex – GABA – Immunocytochemistry – Inhibition – Neuronal density – Stereology

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Introduction

Neurotransmission mediated by gamma-aminobutyric acid (GABA) plays a significant role in the shaping of the neuronal receptive field properties in the mammalian visual cortex (Sillito 1984). In accordance with a transmitter role, both GABA (Somogyi et al. 1984, 1985) and its biosynthetic enzyme, glutamate decarboxylase (GAD) (Ribak 1978, Freund et al. 1983, see rev. Houser et al. 1984) have been localised immunocytochemically in the somata and axon terminals of cortical neurons. The types of neurons containing the markers for GABAergic neurotransmission are just beginning to emerge through procedures that allow not only the identification of the markers but also the characterisation of their three dimensional organisation and the tracing of their synaptic connections (see Somogyi and Hodgson 1985). However, virtually nothing is known about the density and absolute number of GABAergic neurons, or about their quantitative laminar or depth-distribution within the mammalian visual cortex. This is due to the limitations of the procedures that have been available for their direct visualisation. Previous estimates for the distribution of GABAergic neurons in the mammalian cerebral cortex have been derived from studies using [^{3}H]-GABA uptake, or GAD, to localise GABA-ergic neurons (Fitzpatrick et al. 1983; Houser et al. 1983; Solnick et al. 1984). The development of the post-embedding GABA-immunocytochemical technique overcomes many limitations inherent in the use of the above markers and provides a more accurate method for the localisation of GABA-ergic neurons.

The relative proportion that GABAergic neurons constitute of the total neuron population is a first measure of their significance, therefore the present

proportion of [^{3}H]-GABA accumulating neurons in a small sample of layer IV and arrived at a proportion of about 8.2%. This is substantially lower than the approximately 20% GABA-immunopositive cells obtained in our study. However, the comparison of [^{3}H]-GABA accumulating neurons in the study of Solnick et al. (1983) and the GABA-immunoreactive ones in the present study is difficult for several reasons. Firstly, it is not clear if all the neurons accumulating [^{3}H]-GABA also contain endogenous immunoreactive GABA, because in the monkey visual cortex GABA-immunonegative cells were found to become strongly labelled following [^{3}H]-GABA injections (Kisvárday et al. 1984). If a similar situation existed in the cat, and all neurons had an equal chance to take up [^{3}H]-GABA, this would result in a higher number of [^{3}H]-GABA labelled cells than that obtained by GABA-immunocytochemistry. While the low numbers obtained by Solnick et al. (1983) do not exclude the possibility that not all [^{3}H]-GABA cells contain immunoreactive GABA, it raises the question that perhaps not all cells containing GABA take up selectively [^{3}H]-GABA as seems to be the case for cerebellar Purkinje cells (Storm-Mathisen 1976, see Cuenod et al. 1982). Alternatively, the labeling of a cell by exogenous [^{3}H]-GABA may depend on its position relative to the injection site which seems to be the case in the monkey visual cortex (Somogyi et al. 1983a). Thus it may be difficult to provide the same conditions for all the cells in a given cortical lamina following intracortical [^{3}H]-GABA injections.

It is more difficult to explain the discrepancy between our results and those given in a preliminary report by Fitzpatrick et al. (1983). Using an antiserum against GAD they found that an average of 8–15% of neurons were immunopositive in the visual cortex of the cat. Unfortunately, before a full description of their sampling procedure and quantitative methodology becomes available, the possible reasons for the discrepancy between the two results are impossible to ascertain.

The density and numbers of GAD-immunoreactive neurons was also lower in the somatosensory cortex of the monkey (Houser et al. 1983) than in the present study. Houser et al. (1983) used pre-embedding conditions and colchicine treatment to enhance somatic staining, conditions that could result in lower estimates. Alternatively species and areal differences may also be responsible. Nevertheless, the use of thick sections in a combined immunocytochemical and morphometric study has problems concerning the limited depth of penetration of the immunoreagents as well as section thickness effects on cell counting procedures (Weibel 1979).

Comparison with intracellularly marked populations

Recent advances in intracellular recording techniques and the subsequent iontophoretic injection of the marker horseradish peroxidase (HRP) to visualise the morphology of cortical neurons have raised the hope that a comprehensive picture of the patterns of cortical connectivity will be achieved in the foreseeable future (see Wiesel and Gilbert 1983; Martin 1984). The available samples are relatively small and before interpreting the connectivity of particular layers it would be useful to know whether the intracellularly filled neurons in a cortical area or within a given cortical layer provided a representative sample of all the neurons present. Intracellular filling with HRP in the cat has revealed (Gilbert and Wiesel 1979; Martin et al. 1983; Freund et al. 1983; Peters and Saint Marie 1984; Kisvárday et al. 1985) smooth dendritic neurons with axonal and dendritic arborisations differing just as widely as in Golgi studies (Szentágothai 1973; Tömböl 1978; Meyer 1983). Although there are smooth dendritic cells which are for example cholinergic (Houser et al. 1985) the great majority are probably GABAergic. Certainly all GAD- or GABA-immunoreactive neurons whose dendritic arborisations have been revealed so far, have possessed smooth or sparsely spiny dendrites (Freund et al. 1983; Somogyi et al. 1983b; Somogyi and Hodgson 1985). Thus a first approximation to the problem of representative sampling could be the comparison of the proportions of smooth dendritic cells in the intracellularly filled sample with the proportion of GABAergic cells in the cortex. The sole anatomical study to provide a quantitative account of the cells filled intracellularly with HRP has been published by Martin and Whitteridge (1984). Out of 115 neurons they found that 15 (12.1%) had smooth dendrites. This figure is somewhat lower than the proportion of GABA-immunopositive cells determined by the present study for the same cortical area. This could indicate that some distinct GABAergic neuronal categories have not been electrophysiologically or morphologically identified, or alternatively some types are less frequently sampled by intracellular recording.

Laminar distribution of GABAergic neurons

The data obtained here allows the calculation of the absolute number of cells in the different laminae. Beaulieu and Colonnier (1983) have previously determined the laminar density of neurons in the monocular and binocular regions of the cat visual cortex (area 17). Taken in conjunction with the data

study was undertaken to estimate quantitatively their cortical density and laminar distribution.

Material and methods

The material used in this investigation was derived from five normally reared adult cats. Following an overdose of anaesthetic (Sagatal), the animals were perfused through the heart with a fixative solution containing 1% paraformaldehyde (TAAB) and 2.5% glutaraldehyde (TAAB) dissolved in 0.1 M phosphate buffer (PB, pH 7.2–7.4). The brains were then removed and placed in fresh cold fixative (4° C) for up to 12 hours. From both brain hemispheres, tissue blocks containing the lateral gyrus of the visual cortex were dissected out and further processed as either 80–100 μm thick sections or as small slabs of tissue. Some 80 μm thick sections were stained with cresyl violet. Most of the other sections and the blocks were post-fixed for 1–1.5 h in 1% OsO_4 dissolved in 0.1 M PB, dehydrated in an ascending series of alcohols, and resin-embedded (DURCUPAN ACM). Vibratome sections were flat-embedded on clean glass slides, whereas tissue slabs were embedded in plastic capsules. From each animal, at least ten semithin sections (0.5 μm thick) were cut at regular intervals (20 μm apart) perpendicular to the pial surface of the lateral gyrus in area 17. These sections were then dried onto chrome gelatin coated glass slides and stored at 56° C overnight.

Post-embedding GABA immunocytochemistry

To demonstrate GABA-immunoreactive cells in the visual cortex, the slides and the adhering semithin sections were incubated with rabbit anti-GABA serum (Code No GABA-7) using a post-embedding immunocytochemical procedure with the same reagents as described previously (Somogyi and Hodgson 1985). The only notable deviation in the procedure was that following the immunocytochemical reactions the slides were stained with 1% toluidine blue prior to being dehydrated and covered with a neutral mounting medium. The immunological and biochemical specificity of the anti-GABA serum have been described elsewhere (Hodgson et al. 1985; Somogyi et al. 1985). The terms 'immunopositive' and 'immunonegative' are used respectively to describe neuronal profiles displaying specific GABA-like immunoreactivity and profiles in which GABA-like immunoreactivity was absent.

Morphometric analysis

The morphometric analyses were performed within the medial bank of the lateral gyrus (area 17) – a region relatively unaffected by the curvature of the cortex where the pial surface runs approximately linear and parallel to the white matter (Fig. 1). GABA immunoreacted sections were viewed using a ×10 ocular and a ×100 oil-immersion objective lens. With the aid of a drawing tube, a sampling strip (100 μm wide) was positioned randomly over the medial bank extending vertically from the pial surface to the white matter (Fig. 1). The strip was divided into equally sized test-areas (quadrats) 100 × 100 μm. The size of the strip and its division into quadrats had been adjusted beforehand to accomodate an assumed linear shrinkage of 15.0% (Beaulieu and Colonnier 1983) which was considered to be isotropic in all directions.

The cellular profiles of neurons and glia that occured within each quadrat were distinguished on the basis of their perikaryal morphology and Nissl staining characteristics (Ling et al. 1973; Ling and Leblond 1973). Neuronal profiles were further subdivided according to their GABA-immonoreactivity, since the cytoplasm and nuclei of immunopositive neurons were dark-grey, whilst immunonegative neurons were stained solely with toluidine blue (Fig. 2). Because cell nuclei provided the primary distinguishing features between neuronal and glial cells they were used to define 'test' objects. The nuclear and somatic profiles of each

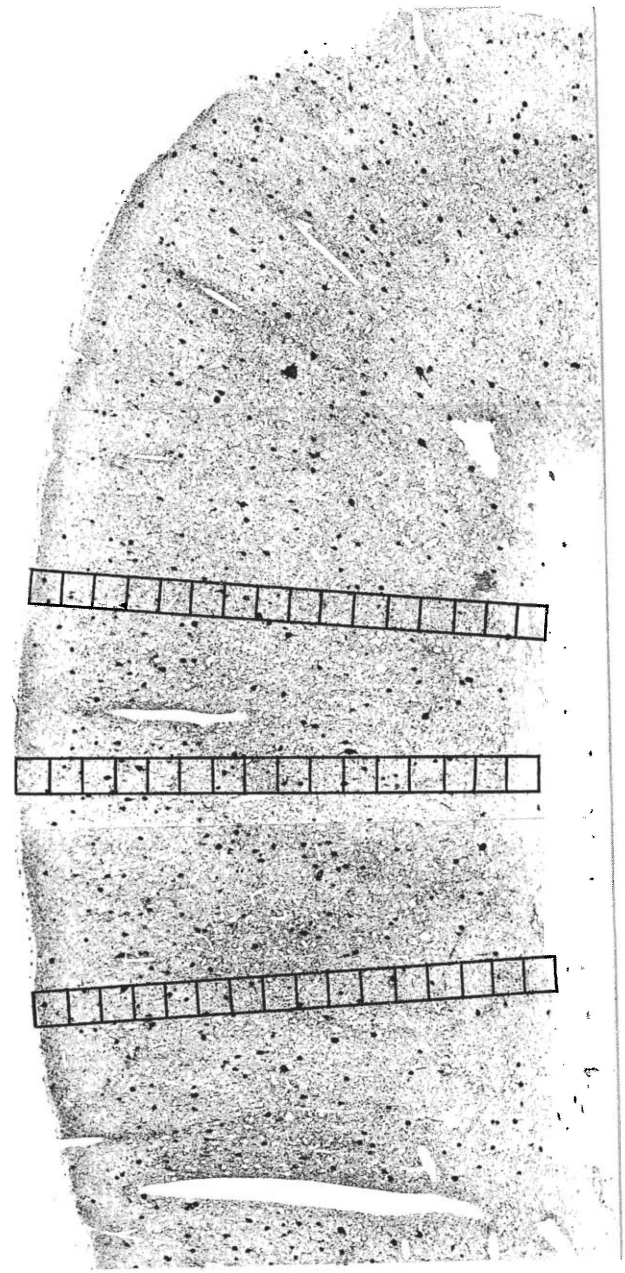

Fig. 1. Semithin section (0.5 μm thick) of the cat's primary visual cortex showing the medial bank of the lateral gyrus. The section was treated to reveal GABA-immunoreactivity and was counterstained weakly with toluidine blue. The position of the sampling strips is indicated. GABA-positive cells appear as dark dots. The width of the strips corresponds to 100 μm

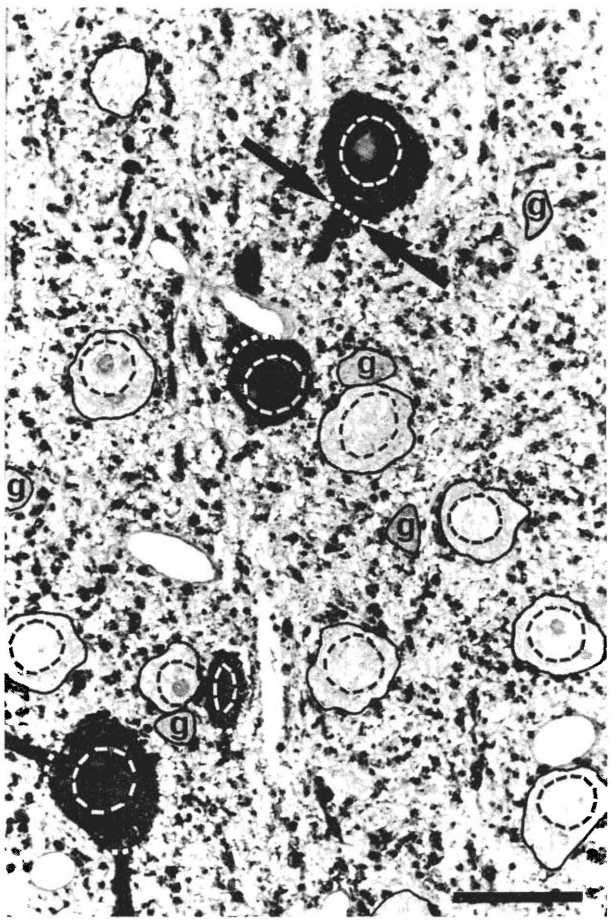

Fig. 2. Light micrograph of an area in Fig. 1 corresponding to layer IV. The magnification of this photomicrograph is similar to that at which data was collected. GABA-immunopositive profiles are darkly stained. The somata and nuclei of neurons have been delineated by solid and broken lines respectively. The white dots indicate where the perikaryal boundaries were rounded off (one example is arrowed). Glial cells (g) are indicated. Scale bar: 20 μm

'test' immunopositive and immunonegative neuron were drawn onto the sampling strip via the drawing tube. The curvature of neuronal perikaryal profiles was maintained where dendrites emerged from the cell body, and in the rare instances where extremely infolded neuronal nuclei were bisected by the plane of the section, both outlines were combined and drawn as one profile and considered representative of that nucleus. In addition to all the neuronal nuclear profiles occuring within the quadrat, only those profiles intersecting the left and upper borders which satisfied the 'forbidden line' rule of Gundersen (1977) were counted and included for morphometric analysis. For each quadrat within a sampling strip, the number of immunopositive and immunonegative neuronal profiles occuring per unit quadrat area (N_Ap. and N_An. respectively) were determined. Additionally, the area-equivalent circle diameter (d.circ) of each 'test' nuclear and perikaryal profile were measured using a computerised planimeter (Reichert Videoplan). Data were stored separately for immunopositive and immunonegative neurons.

Using the procedure described above which conforms to a 'systematic stratified random sampling procedure' (Weibel 1979), the medial bank was sampled on average three times in each of the ten sections per animal. The absolute perpendicular depth of the cortex was measured within each sampling strip, and the quadrat in which the transition between cortical layer VI and the underlying white matter was most prominent represented the deepest quadrat in the cortex used for the morphometric analysis.

Volume numerical density (N_V) estimation of neurons

It was assumed that the visual cortex contained a heterogeneously dispersed population of unequally sized neuronal cells, each possessing a single spherical nucleus of varying diameter. Estimates of the volume numerical density of nuclei will therefore yield direct estimates of the volume numerical density of neurons (i.e. the number of immunopositive and immunonegative neurons per unit volume of tissue). Measurements of the mean observed nuclear diameter will give an underestimate of their 'true' mean nuclear diameters, because in a population of cells sectioned randomly a proportion of nuclei will be cut non-equatorily (Weibel 1979). In each animal, the planar morphometric data were combined from sampling quadrats at corresponding depths (quadratic tiers) below the pial surface. For each quadratic tier, size-frequency distributions (using N_A and d.circ data) were constructed separately for immunopositive and immunonegative neurons. Depending on the sample size, these distributions were divided into between 10–15 size classes, with the mid-point of each size-class being set according to Cruz-Orive (1978). The distributions were then corrected for missing small profiles and finite section thickness effects (Weibel 1979). The resulting corrected size-frequency distributions of the nuclear profiles were then 'unfolded' mathematically (Saltykov 1958; Weibel 1979) to give the underlying nuclear size-frequency distributions for immunopositive and immunonegative neurons. From these nuclear size-frequency distributions the volume numerical densities of GABA-immunopositive (N_Vp) and GABA-immunonegative (N_Vn) neurons were determined (Cruz-Orive 1978). Estimates of N_Vp and N_Vn were calculated separately for each tier of quadrats throughout the cortex of each animal. The total volume numerical density (TN_V) at each quadratic tier was calculated ($TN_V = N_V p + N_V n$). Additionally the proportional volume numerical density of immunopositive neurons was determined as a percentage of TN_V for each quadratic tier ($= 100 \times [N_V p/TN_V]$).

Using the morphometric procedure described above the mean diameters ($\overline{D}$) of immunopositive and immunonegative neuronal somata and nuclei were calculated from the mean profile diameters ($\overline{d}$) derived from the corrected nuclear and somatic profile size-frequency distributions ($\overline{D} = (4/\pi) \times \overline{d}$; Weibel 1979).

Statistical analysis. The overall interanimal variation was determined using a one-way analysis of variance (ANOVA).

Results

Qualitative light microscopical observations

Immunopositive somata, processes and punctate structures, as well as the somata and processes of immunonegative neurons were present throughout the entire depth of the cortex and also within the white matter (Figs. 1 and 3).

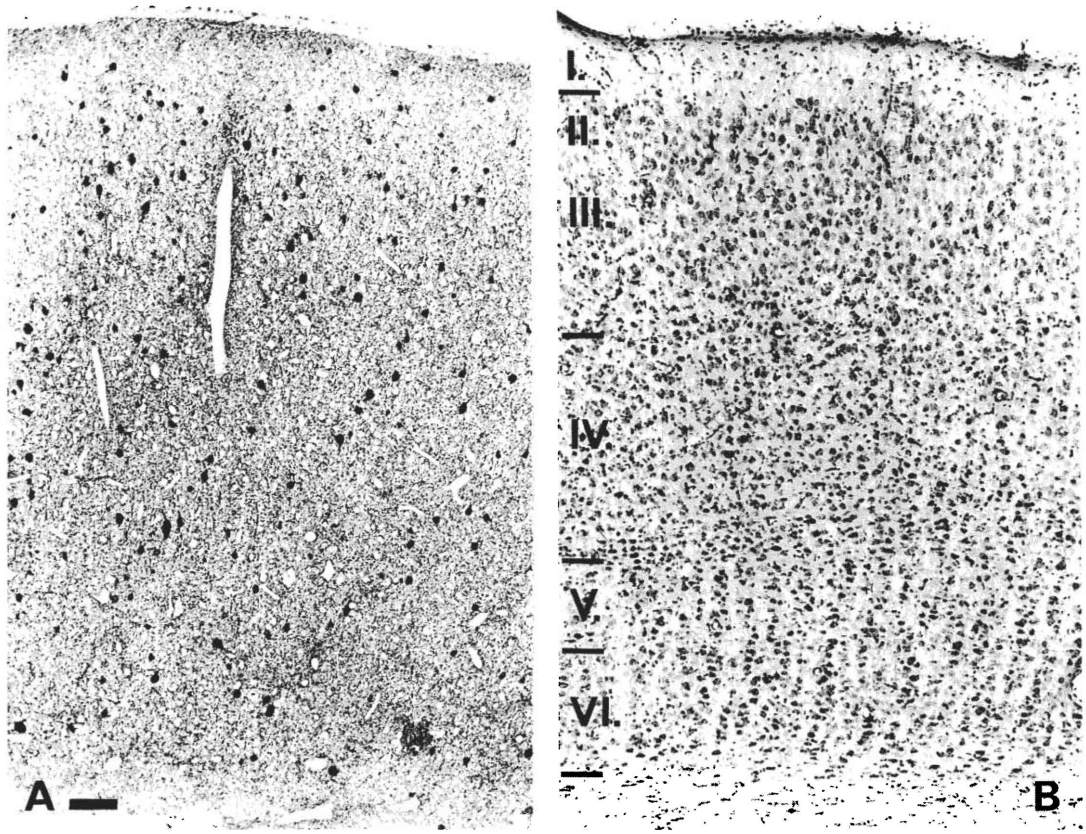

Fig. 3. A Laminar distribution of GABA-immunoreactive neurons (black) in the medial bank of the lateral gyrus, as demonstrated in a semithin (0.5 μm thick) section. **B** Cresyl violet stained 80 μm thick section of the same area shown for the delineation of laminar boundaries. Scale: 100 μm

Table 1. Somatic diameters of GABA-immunopositive and GABA-immunonegative neurons in cat number 5. Mean values ± SD (calculated from the corrected size-frequency distributions of somatic profiles)

Depth below pia in μm	Approx. Lamina	GABA-immunoreactivity	
		Immunopositive	Immunonegative
0–100	I	12.4 ± 3.0 μm	14.2 ± 3.2 μm
100–300	II	12.7 ± 2.7 μm	14.9 ± 2.3 μm
300–400	IIIa	13.9 ± 3.0 μm	15.4 ± 3.0 μm
400–600	IIIb	13.3 ± 3.2 μm	15.9 ± 3.0 μm
600–800	IVa	15.5 ± 3.2 μm	14.8 ± 2.1 μm
800–1100	IVb	15.9 ± 2.7 μm	15.0 ± 3.2 μm
1100–1300	V	16.2 ± 3.0 μm	20.1 ± 3.9 μm
1300–1600	VI	17.8 ± 2.2 μm	16.2 ± 2.7 μm

GABA-immunopositive neurons. In non-serial semithin sections, immunopositive neuronal somata, processes and puncta appeared uniformly distributed throughout area 17. Immunopositive perikaryal profiles possessed non-pyramidal neuronal morphology ranging in shape from fusiform to circular with radiate dendritic processes. The mean size of immunopositive neurons varied according to cortical position – the larger somatic sizes (15–25 μm) being more frequent in layers IV, V, and VI (Table 1).

GABA-immunonegative neurons. The size, perikaryal morphology, and cortical distribution of immunonegative neuronal profiles varied with respect to cortical depth. In layers II, III, and V the vast majority of immunonegative neuronal profiles were ovoid or distinctly pyramidal in shape, with the largest somatic profiles (25–35 μm in diameter) occuring in a region corresponding to lower layer V (Table 1).

GABA-immunoreactivity in the neuropile. Dark immunopositive punctate profiles, 1–3 μm in diameter, were distributed homogeneously throughout the cortex and to a much lesser extent in the underlying white matter. The density of these profiles in the cortex was relatively uniform, showing no discernable clustered distribution. However, the im-

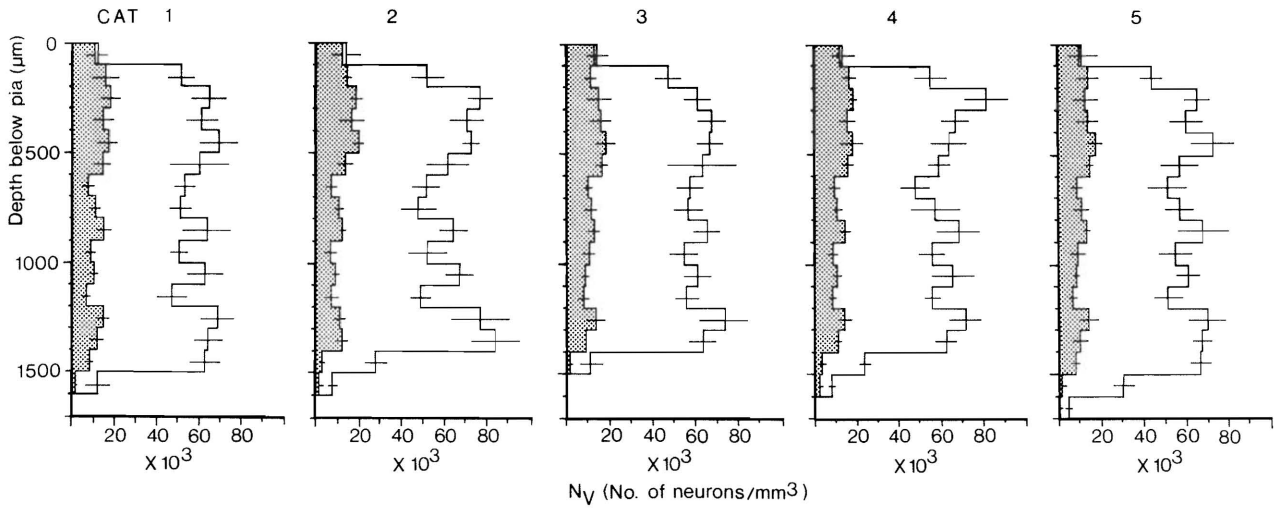

Fig. 4. Cumulative volume numerical densities (N_V) of GABA-immunopositive (dotted) and immunonegative (empty) neurons in 100 μm sampling tiers of cortical area 17 in five cats. The pattern is similar despite the differences in cortical thickness between the animals

Table 2. Summary of the quantitative data derived from the five cats used in this investigation. *Means ± SEM; n = 5. TN_V, total no. of neurons per mm^3; N_Vp, GABA-positive neurons per mm^3; N_Vn, GABA-negative neurons per mm^3. A one-way ANOVA indicated no significant differences between the five animals used in this study at a level of $p \gg 0.05$ for the results of N_Vp (df 4.75; F = 0.13), N_Vn (df 4.75; F = 0.12), and TN_V (df 4.75; F = 0.09)

Neuronal Densities*	
TN_V	54,210 ± 634
N_Vp	11,182 ± 191
N_Vn	43,057 ± 706

munopositive puncta frequently surrounded the somata and processes of both immunopositive and immunonegative neurons. Indeed, the immunonegative somatic profiles of pyramidal cells in layers II, III, and V were strongly delineated by being surrounded by many immunopositive puncta, some of which could be seen to be connected by fine immunopositive fibres (Fig. 2). Additionally, immunopositive strands composed of several puncta, could be clearly seen to surround the descending axon initial segments of pyramidal shaped neuronal profiles in layers III and V.

Quantitative results

Cortical depth measurements. The mean absolute depth of the medial bank in area 17 for the five animals was 1,551 ± 27 μm ($\bar{x}$ ± SEM, n = 5). For the individual animals the absolute depth measurements were ($\bar{x}$ ± SEM, n = 30); cat 1, 1,568 ± 86 μm; cat 2, 1,579 ± 148 μm; cat 3, 1,465 ± 126 μm; cat 4, 1,522 ± 131 μm; and cat 5, 1,620 ± 110 μm.

Because of the variation in the absolute cortical depth of the five animals used in this study, N_Vp, N_Vn, and TN_V results are presented separately for each animal. The quantitative data for each animal are presented as depth-distribution histograms (Fig. 4) with the standard deviation estimates for N_Vp and N_Vn being derived directly from the nuclear size-frequency distributions.

Volume numerical density of neurons (N_V). The mean total numerical density of neurons TN_V in the medial bank of the lateral gyrus in cortical area 17 was 54,210 neurons per mm^3 (Table 2). The depth distribution of TN_V was relatively uniform outside layer I (Fig. 4).

The mean cortical volume numerical density of GABA-immunopositive neurons (N_Vp) for the five animals investigated in this study was 11,181 immunopositive neurons per mm^3 (Table 2). The depth distribution of N_Vp decreased with increasing cortical depth (Fig. 4). For all the animals the overall mean density of immunopositive neurons was much higher in the supragranular layers (0–600 μm below the pia; 15,158 per mm^3) compared with the mean density in the granular and infragranular layers (600 μm – bottom of cortex; 8,766 per mm^3).

The percentage cortical depth distribution of GABA-immunopositive neurons (%[N_Vp/TN_V]) is shown in Fig. 5 which is derived from the five animals used in this study. The proportion that N_Vp constituted of TN_V was calculated for each quadratic

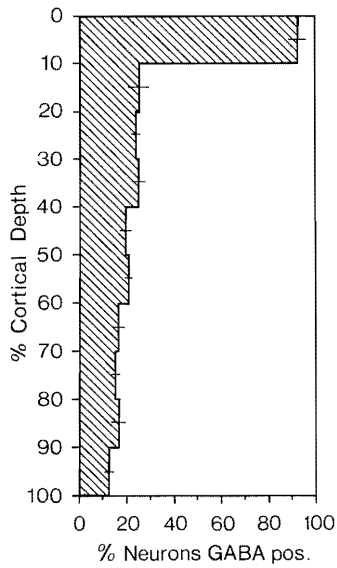

Fig. 5. The proportion of GABA-immunoreactive neurons (hatched) at different depths below the pia as a percentage of all neurons present. The data is derived from Fig. 4 but because of the differences in cortical thickness, it was normalised and expressed as percentage depth below pia

tier down the cortex of each animal. The absolute cortical depth of each animal (see above) was then segmented into cortical depth bins with each bin representing 10% of the overall cortical depth. By calculating the percentage depth at which each 100 μm quadratic tier occurred, the percentage values for immunopositive neurons were then allocated to the appropriate cortical depth bin. This manipulation of the data was undertaken to overcome the problem of interpreting quantitative data from animals with differing cortical thickness.

The mean group value for the percentage of cortical neurons that are GABA-immunopositive was derived from the mean N_Vp and TN_V results of each animal (Table 2). For the five animals GABA-immunopositive neurons constituted 20.60 ± 0.48% ($\bar{x}$ ± SEM) of all cortical neurons, with no significant differences between the five animals (df 4.75; F = 0.06) at a level $p \gg 0.05$.

Discussion

Technical considerations, antiserum and immunocytochemistry

Previous work on the characterization of the anti-GABA serum used in the present study showed that among the substances cross-reacting with this serum (Hodgson et al. 1985), only preincubation with GABA conjugated to a solid phase carrier abolished immunoreactivity in visual cortical neurons (Somogyi et al. 1985). This makes it likely that we counted cells that synthesize, store and probably release GABA as their transmitter. The staining of neurons for GABA did not require colchicine treatment to enhance perikaryal antigen concentration as with anti-GAD serum in some cases (Ribak 1978; Houser et al. 1983). This is important, because when colchicine injection is needed it may not reach or affect all neurons equally and it may be difficult to ensure that all neurons reach antigen concentrations at detectable levels. It was reassuring that immunoreactivity for GABA changed in an 'all or none' fashion between immunopositive and immunonegative cells making their identification unequivocal. The use of 0.5 μm thick sections for post-embedding immunocytochemistry overcame penetration problems inherent in pre-embedding methods employing thicker sections especially with glutaraldehyde fixation. Additionally, the use of semithin sections is highly compatible with the morphometric procedures adopted by the present investigation to derive neuronal numerical density estimates since large areas of tissue can be analysed and section-thickness effects are greatly reduced (Weibel 1979). Thus the procedure chosen for this study has some advantages over other possible approaches. At the same time it should be emphasized that as with all immunocytochemistry the negative results, i.e. the lack of staining for GABA in some cells, does not necessarily mean that they do not contain the antigen. Differences in concentrations, different metabolic pathways and differences in the physiological state of the neuron may give false negative results. In this respect it is noteworthy that the perikarya of cerebellar Purkinje cells which are thought to use GABA as transmitter stained only weakly for GABA while neighbouring interneurons were strongly, immunopositive (Somogyi et al. 1985). Weakly staining neurons were extremely rare in our present material and did not seem to comprise a particular sub-population of immunopositive neurons. Nevertheless for the above mentioned theoretical reasons the numbers of GABA-immunoreactive neurons we obtained should be considered as the lower limit of GABA-ergic neurons in the cat visual cortex.

Numerical densities of neurons

Our results on the average density of neurons in the cat cortex agree well with the data obtained by Beaulieu and Colonnier (1983) and with that obtained for cortical layer IV by Solnick et al. (1984). The latter authors also estimated the number and

Table 3. Absolute number of GABA-immunopositive neurons under 1 mm^2 of cortical surface area in given cortical laminae. Neuronal density data for each layer was taken from the work of Beaulieu and Colonnier (1983). Values in parentheses indicate the number of immunopositive neurons within each lamina expressed as a percentage of the total number of immunopositive neurons under any unit of cortical surface area

Lamina	Abs. No of GABA immunopositive neurons
I	1,173 (8.3%)
II	1,755 (12.4%)
IIIa	1,858 (13.1%)
IIIb	2,380 (16.8%)
IVa	2,383 (16.8%)
IVb	2,430 (17.1%)
V	803 (5.7%)
VI	1,396 (9.8%)
Total	14,178 (100.0%)

derived in the present study (Fig. 5) we used the data of Beaulieu and Colonnier to provide mean estimates of the absolute number of GABA immunopositive neurons occuring within given laminae under 1mm^2 of cortical surface (Table 3). These data may be easily transformed into the laminar distribution of the absolute numbers of GABA-immunopositive neurons occuring within an ocular dominance column or in an orientation column. The laminar distribution of cells is highly significant in view of the differences in the 'input' and 'output' relationships of the various layers. Thus it is apparent from Table 3 that more than 50% of all GABA-positive cells are located within layer IV and lower layer III – in laminae where the majority of the geniculo-cortical terminals are located. Layer V, on the other hand, which receives little direct specific thalamic input (LeVay and Gilbert 1976; Bullier and Henry 1979; Martin and Whitteridge 1984; Humphrey et al. 1985; see Martin 1984) has only 5.7% of the GABA-immunopositive cells in the visual cortex. Although the dendrites and axons of GABAergic neurons freely cross laminar boundaries (Somogyi et al. 1983b), the above proportions could indicate that GABA mediated neurotransmission occurs at the early stages of geniculate input into the primary visual cortex. This is supported by the recent demonstration of direct X and Y geniculate input to GABA-immunoreactive neurons in the visual cortex of the cat (Freund et al. 1985).

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