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Targets and Quantitative Distribution of GABAergic Synapses in the Visual Cortex of the Cat

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Abstract

The morphology and postsynaptic targets of GABA-containing boutons were determined in the striate cortex of cat, using a postembedding immunocytochemical technique at the electron microscopic level. Two types of terminals, both making symmetrical synaptic contacts, were GABA-positive. The first type (95% of all GABA-positive boutons) contained small pleomorphic vesicles, the second type (5%) contained larger ovoid vesicles. Furthermore, 99% of all cortical boutons containing pleomorphic vesicles were GABA positive, and all boutons with pleomorphic vesicles made symmetrical synaptic contacts. These results together with previously published stereological data (Beaulieu and Colonnier, 1985, 1987) were used to estimate the density of GABA-containing synapses, which is about 48 million/mm^3 in the striate cortex. The postsynaptic targets of GABA positive boutons were also identified and the distribution was calculated to be as follows: 58% dendritic shafts, 26.4% dendritic spines, 13.1% somata and 2.5% axon initial segments. A total of 11% of the postsynaptic targets were GABA immunoreactive and therefore originated from GABAergic neurons. The results demonstrate that the majority of GABAergic synapses exert their action on the membrane of dendrites and spines rather than on the somata and axons of neurons.

Introduction

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the cerebral cortex (Krnjevic, 1984). GABA-mediated neurotransmission contributes to several specific response properties of cortical neurons (for review see Sillito, 1984) and it is also important in preventing the development of abnormal activity as found for example in focal epilepsy (Bernasconi, 1984; Ribak *et al.*, 1982). Most of the GABA in the cortex is synthesized and released by intrinsic cortical neurons, which show great variation in several characteristics, including the nature of their postsynaptic targets (for review see Somogyi, 1989). While the physiological properties and synaptic connections of identified GABAergic neurons is under intensive investigation (see for example Kisvarday *et al.*, 1985; Martin, 1988; Martin *et al.*, 1989), there is no information on the quantitative distribution of GABAergic synapses in any cortical area. One reason for this is that previous immunohistochemical studies for markers such as glutamate decarboxylase or GABA could only demonstrate the overall pattern of GABAergic innervation. Technical limitations have prevented the determination of the number, density and proportion of GABAergic synapses. The recently introduced immunogold method for the detection of GABA overcomes some of these limitations and makes quantitative studies possible (Somogyi and Hodgson, 1985).

Immunocytochemistry at the electron microscopic level has shown that most GABAergic boutons make so called symmetrical synapses and contain pleomorphic or ellipsoid synaptic vesicles (Ribak, 1978; Freund *et al.*, 1983; Wolff *et al.*, 1984). The quantitative distribution, density, and postsynaptic targets of terminals forming this type of synapse, identified on the basis of structural criteria alone, is known in the visual cortex of the cat from stereological investigations (Beaulieu and Colonnier, 1985, 1987). However, it is not clear to what extent such quantitative data can be equated with GABAergic synapses, as some terminals making symmetrical contacts synthesize acetylcholine (Wainer *et al.*, 1984; Houser *et al.*, 1985; de Lima and Singer, 1986), noradrenaline (Papadopoulos *et al.*, 1989), serotonin (Tork *et al.*, 1988), dopamine (Seguela *et al.*, 1988), or contain neuroactive peptides (Hendry *et al.*, 1984a; Freund *et al.*, 1986; Peters *et al.*, 1987; Jones *et al.*, 1988). The present study was undertaken to determine to what degree the previously obtained quantitative data (Beaulieu and Colonnier, 1985, 1987) on the number, distribution, and targets of

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symmetrical synapses established by boutons containing pleomorphic vesicles, can be used as a reflection of the immunocytochemically characterized GABAergic bouton populations. The visual cortex of the cat is one of the best areas for such a study as a great deal of information is available about the properties, role and development of GABAergic neurons and synapses in this area.

Materials and methods

Specimens from the visual cortex of three adult cats were used in the present study. The material had been embedded and stored in epoxy resin. It was chosen on the basis of the high immunoreactivity for GABA. At the time of perfusion, animals were deeply anaesthetized with an overdose of sodium pentobarbital (Sagatal). They were perfused through the left ventricle first with saline followed by a freshly prepared fixative containing 2% paraformaldehyde and 1% or 2.5% (2 cats) glutaraldehyde dissolved in 0.1 M sodium phosphate buffer (PB, pH 7.4).

After perfusion the cranium was opened, the brain removed, and small cortical slices were kept in fixative for a few hours followed by washes in 0.1 M PB. Slices were sectioned with a Vibratome at 60–80 μm. The sections were washed in PB and postfixed for 1 h in 1% osmium tetroxide dissolved in 0.1 M PB (pH 7.4). They were washed again in PB, then dehydrated in alcohol (1% uranyl acetate was included at the 70% ethanol stage for 40 min) and embedded on glass slides in Durcupan ACM (Fluka) resin. Portions of the supragranular layers (I-II-III), granular (IV), and infragranular laminae (V-VI) were cut out from the slides and re-embedded for further sectioning and electron microscopy. At least three different blocks of tissue were taken from each set of laminae.

Production and characterization of the antiserum to GABA and the postembedding colloidal gold method have been described elsewhere (Somogyi and Hodgson, 1985). Briefly, serial ultrathin sections were mounted on Formvar coated, single slot grids. One to two sections from the series were processed for GABA immunocytochemistry. Sections were treated with 1% periodic acid and 2% sodium periodate for the etching of the resin and the removal of the osmium. After washing, grids were sequentially placed on drops of: (i) 5% ovalbumin, rabbit antiGABA serum (Code no. 9, diluted 1:1000 to 1:3000; Hodgson *et al.*, 1985); (ii) Tris buffer containing 1% bovine serum albumin (BSA) and 0.5% Tween 20 at pH 7.4; and (iii) colloidal gold (15 nm) coated with goat anti-rabbit IgG (Bioclin, diluted 1:20 to 1:40 in the previous solution). Between these steps, grids were washed in Tris (0.05 M) buffered saline (0.9% NaCl). Following the incubation, grids were washed in filtered distilled water and contrasted with a fresh solution of lead citrate.

From each block, an immunolabelled section and the consecutive conventionally stained section were chosen for the quantitative study. In these two adjacent sections, a strip of tissue easily identifiable by a knife mark was photographed at a magnification of ×14 000 and prints were produced at a final magnification of ×35 000. On some occasions, samples were taken around a characteristic blood vessel. In these cases, photographs were centered on small myelinated axons in order to ensure that exactly the same portion was taken in the two consecutive sections. No attempt was made to avoid portions of tissue containing cell bodies with either method of sampling.

All profiles containing a high density of immunogold were identified on the immunoreacted section (see Fig. 1). On the adjacent conventionally stained section, all boutons containing a clear population of the small pleomorphic or ellipsoid synaptic vesicles were marked without our knowing the result of the immunoreactivity. The two populations were then compared in two ways. First, the presence (or absence) of labelling for GABA in boutons containing pleomorphic vesicles was determined. Secondly, the morphology of boutons which were GABA positive was studied. On some occasions, the identification of the vesicle shape and/or the type of synaptic contact, or the degree of immunoreactivity had to be verified on other adjacent sections. Boutons in which determination of a property was equivocal were placed in an undetermined category. No difference was found between the animals treated with fixatives containing 1% or 2.5% glutaraldehyde, therefore the results were pooled.

Two populations of GABA positive boutons could be recognized on the basis of their content of synaptic vesicles. To determine whether the difference was significant the area and the shape factor (which is a fraction for estimating the amount by which a structure varies from a circle; the circle being 1.00 and a line being 0.00) of the synaptic vesicles were measured in 15 GABA positive boutons of both types and in 15 GABA negative terminals containing round vesicles. The analysed boutons were approximately the same size and spatially close to each other in the same section. All terminals were reprinted from the original negative to a magnification of ×190 000. All synaptic vesicles contained in each terminal with a clear membrane structure were measured on an electromagnetic tablet. A total of 978 vesicles in three types of boutons (313 ovoid, 384 round, and 281 pleomorphic vesicles) were computed. An estimation of the mean area and shape factor of vesicles for each terminal was obtained and a one-way analysis of variance performed to detect significant differences among the three populations of terminals. Specific differences between individual populations of terminals were determined by a posteriori Scheffe test.

Results

In area 17 of the cat's visual cortex, 301 boutons containing a population of pleomorphic vesicles (so called F boutons, Beaulieu and Colonnier, 1988) were evaluated. When the presynaptic and postsynaptic membranes could be seen clearly in one of the serial sections (in 86 cases), all boutons containing the small pleomorphic vesicles formed a symmetrical differentiation of the synaptic membranes. Thus the correspondence between the presence of pleomorphic vesicles and the symmetrical differentiation of the synaptic membrane is very high in the visual cortex of the cat. Of the 301 F boutons, 97.0% (292 F boutons) were GABA positive (Table 1). Only 1% (3) of the F boutons were clearly not labelled and were identified as GABA negative. The labelling of 2% (6) of the total of F boutons was equivocal. In these cases, the density of gold particles over the boutons was lower than over adjacent F boutons. If one considers only the distribution of identified elements, 99% (292 of the 295 identified boutons) were GABA positive and only 1% were GABA negative (Table 1). In the different laminae, the distribution of the proportion of F boutons is very similar to that obtained for the total cortical depth.

GABA positive terminals appeared heterogeneous with regard to the shape of the synaptic vesicles. Therefore the mean area and shape factor of the GABA positive boutons containing large ovoid vesicles, the GABA positive terminals containing small pleomorphic vesicles, and the GABA negative boutons containing round vesicles were measured. The mean area of the synaptic vesicles was significantly different among

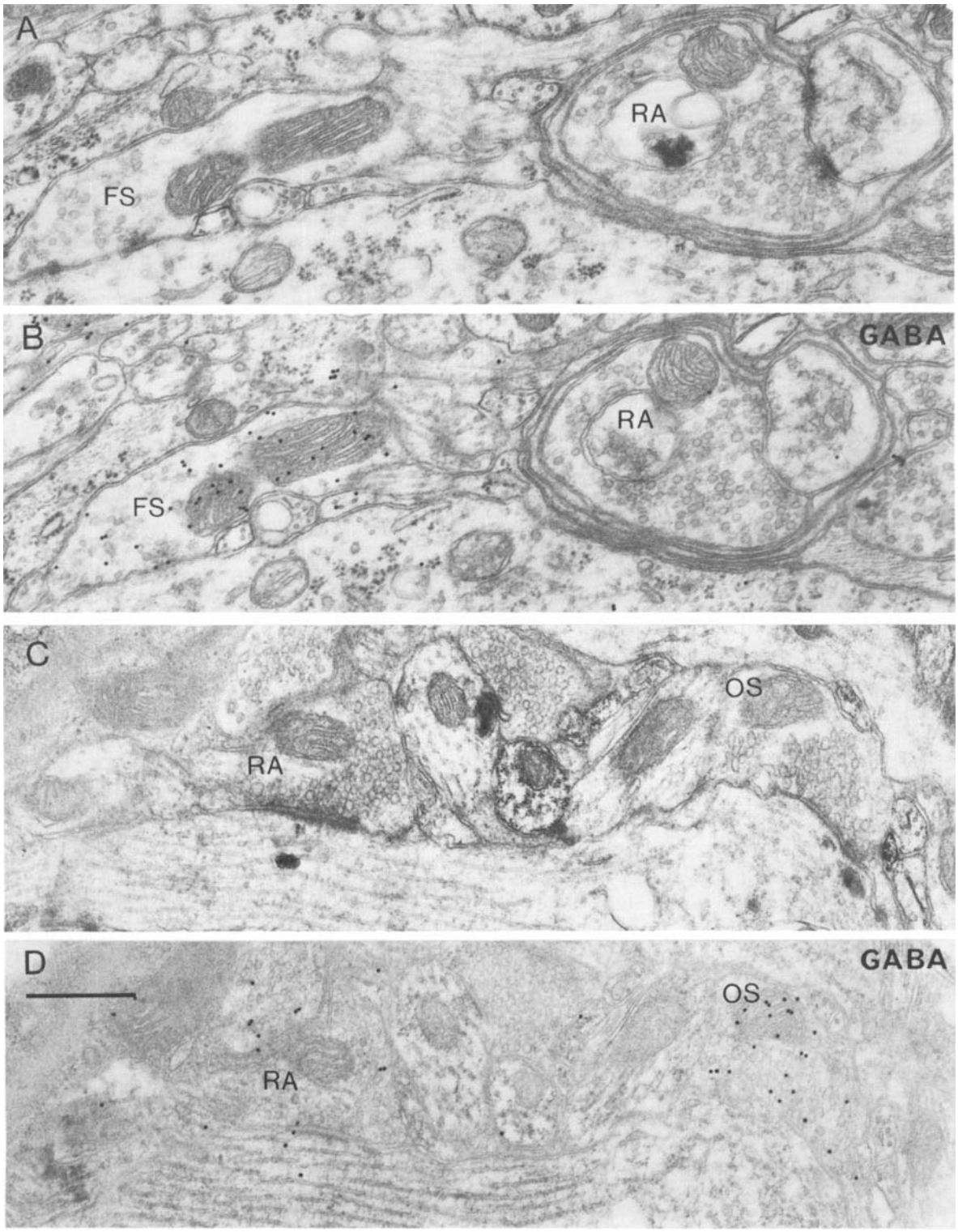

FIG. 1. Immunogold reacted (Figs B and D) and serial nonreacted ultrathin sections (Figs A and C) from cat visual cortex showing GABA negative boutons containing round vesicles (RA), a GABA positive terminal containing small pleomorphic vesicles (FS) and a GABA positive bouton containing large ovoid vesicles (OS). The GABA-positive boutons make type 2 (symmetrical) synapses with a neuronal soma (A and B), and with a dendritic shaft (C and D). All photographs are printed at the same magnification. Scale as in D: 0.5 μm.

TABLE 1. Numbers, proportions and targets of nerve terminals immunoreactive for GABA and containing vesicles of different shapes

Layers	I-II-III	IV	V-VI	Total	%
F-boutons	114	103	84	301	
GABA positive	111	101	80	292	97.0
GABA negative	–	1	2	3	1.0
Uncertain	3	1	2	6	2.0
GABA positive boutons	127	115	100	342	
Pleomorphic vesicles	111	101	80	292	85.4
Ovoid vesicles	5	2	8	15	4.4
Undetermined shape	11	12	12	35	10.2
Synaptic targets	57 (8)	27 (2)	38 (3)	122 (13)	
Spines	16 (0)	7 (0)	7 (0)	30 (0)	24.6
Dendrites	27 (4)	15 (2)	24 (3)	66 (9)	54.0
Somata	8 (4)	3 (0)	5 (0)	16 (4)	13.1
Initial segment	3 (0)	–	–	3 (0)	2.5
Unidentified	3	2	2	7	5.8

F-boutons contained small pleomorphic (flattened) vesicles. Data are from supragranular (I-II-III), granular (IV), and infragranular (V-VI) layers of the visual cortex (area 17) of three cats. Numbers in brackets indicate GABA positive postsynaptic elements.

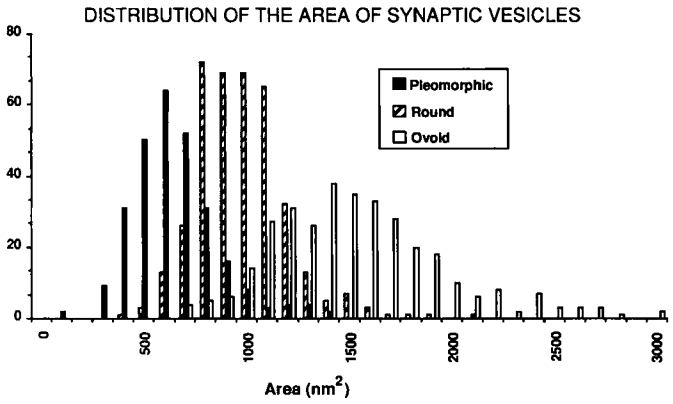

FIG. 2. Distribution of the area of synaptic vesicles in GABA positive terminals containing small pleomorphic vesicles (filled bars; n = 281), GABA negative boutons containing round vesicles (stipled bars; n = 384) and GABA positive boutons containing large ovoid vesicles (empty bars; n = 313).

the three populations of terminals ($p < 0.001$ on a one way ANOVA). The Scheffe test, a posteriori test, also reveals that the mean area of the large ovoid vesicles (1530 ± 227 nm^2) is significantly different from that obtained for the pleomorphic (591 ± 84 nm^2) or round vesicles (922 ± 83 nm^2). These two latter populations were also significantly different ($p < 0.01$) from each other. The shape factor is also significantly different among the three types of terminals ($p < 0.001$). This is, however, due only to a significant difference ($p < 0.01$) between pleomorphic (0.85 ± 0.03) versus round (0.94 ± 0.03) or large ovoid vesicles (0.92 ± 0.04); the latter two not being significantly different ($p < 0.05$).

Figure 2 presents the size distribution of the synaptic vesicles for the three populations of terminals. The distribution appears normal in form for the three types of vesicles with a slight tendency to be skewed to the right, especially for the large ovoid vesicles.

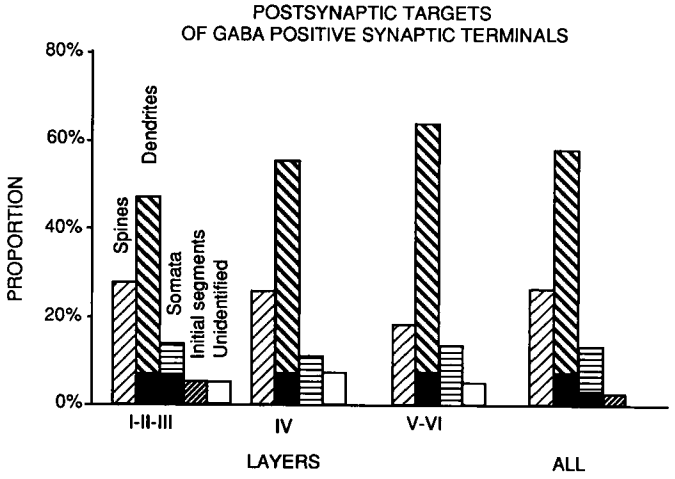

FIG. 3. Distribution of postsynaptic targets to GABA positive synapses in supragranular (I-II-III), granular (IV), and infragranular (V-VI) layers of the cat visual cortex. Black bars represent the proportions of GABA positive postsynaptic elements. The pooled data for the total cortical thickness was calculated as described in the results.

Of the 342 GABA positive profiles containing synaptic vesicles, 292, representing 85.4% of all GABA positive profiles, contained pleomorphic vesicles as shown above. An additional 4.4% (15) of the total number of GABA positive boutons contained a population of vesicles (Fig. 1C,D), larger in size and more ovoid than those in conventional F boutons (see above). In 10.2% of GABA positive terminals, the shape of the vesicles could not be determined unequivocally, because either the number of vesicles in the boutons was insufficient, or there was dirt or folding on the conventionally stained sections. It can be calculated that without this unidentified population, 95% of the GABA positive boutons contained pleomorphic vesicles and 5% contained the large ovoid vesicles. The distribution of GABA positive terminals is relatively similar among the different laminae. Terminals containing a population of pleomorphic vesicles or the large ovoid vesicles were present throughout the cortex and were not restricted to a particular set of layers.

The distribution of GABA positive terminals on different postsynaptic elements is shown in Table 1 and Figure 2. As the majority of GABA positive terminals contain pleomorphic vesicles, the overall distribution of postsynaptic elements to GABA positive boutons essentially reflects the targets of the boutons containing pleomorphic vesicles. In fact, of the 115 identified postsynaptic targets, 113 were made by boutons containing pleomorphic vesicles, only one synapse was made by a bouton containing large ovoid vesicles, and one contact was from a bouton that did not contain enough vesicles for classification. These two latter contacts were on dendrites.

In seven cases (5.8%) it could not be decided whether the postsynaptic structure was a dendritic spine or a small dendrite that did not contain mitochondria in the plane of the section. If the assumption is made that these targets were spines and dendrites in the same proportion as the identified targets, then the proportion of spines can be increased by 1.8% and the proportion of dendritic shafts by 4%. Accordingly, there was about 26.4% of GABA positive synapses on spines, 58% on dendritic trunks, 13.1% on cell bodies, 2.5% on the initial segment of axons in the total cortical thickness (Table 1 and Fig. 3). Of all identified postsynaptic elements, 8% were GABA positive dendrites and 3% were GABA positive somata. Thus the proportion of GABA

positive synapses on GABA positive elements represents 11% of all synaptic contacts made by GABA positive terminals in the striate cortex of the cat.

The proportion of GABA positive synapses on identified spines decreases from 28% in layers I-II-III, to 18% in layers V-VI accompanied by an increase in the proportion of postsynaptic dendrites. Identified dendritic targets comprise 47% in supragranular layers, 56% in layer IV, and 63% in infragranular layers. The proportion on somata does not vary greatly with lamination. The proportion on GABA positive postsynaptic targets is similar throughout the cortex.

Discussion

A high degree of correlation has been found in the present study between boutons immunoreactive for GABA and boutons containing pleomorphic vesicles in the cat striate cortex. Almost all boutons (99%) containing pleomorphic vesicles were GABA positive, and 95% of the GABA positive boutons contained pleomorphic vesicles. The close correlation and the finding that all GABA positive terminals established symmetrical synaptic contacts shows that the GABA-containing bouton population corresponds to previously described boutons making so called flat-symmetrical (FS) synapses (for review see Szentagothai, 1975; Colonnier, 1981). The latter population was characterized without chemical identification on the basis of two ultrastructural features; the boutons contained flattened synaptic vesicles (called pleomorphic in the present study), and they established symmetrical synaptic contacts. The density and laminar distribution of FS synapses has been established in the cat striate cortex (Beaulieu and Colonnier, 1985). It was found that FS synapses constitute 16% of all synapses and provide about 46 million synapses per mm^3 of cortical tissue. Applying this figure to the GABA-containing terminals and adding the population which contains large ovoid vesicles, comprising about 5% of GABA positive boutons, it can be calculated that GABA-containing boutons provide about 48 million synapses per mm^3 of cortical tissue.

The origin of some of the GABA positive boutons with pleomorphic vesicles has been established. In the cat striate cortex several different types of local circuit neurons have been shown to establish symmetrical synapses and to contain pleomorphic vesicles in their terminals (Fairen and Valverde, 1980; Somogyi and Cowey, 1981; De Felipe and Fairen, 1982; Somogyi *et al.*, 1982; Kisvarday *et al.*, 1985; for review see Somogyi, 1989). The origin of the GABA positive terminals containing large ovoid vesicles is not yet known. To our knowledge none of the identified local circuit neurons in the visual cortex have been found to contain such vesicles in their terminals. Although there is no evidence that any thalamic projection to cortex would contain GABA (Fitzpatrick *et al.*, 1984; Montero, 1989), it cannot be excluded that some of the GABA positive boutons have extracortical origin. The demonstration of a GABA immunoreactive projection to the cortex from the basal forebrain (Freund and Gulyas, 1989), and the presence of glutamate decarboxylase in neurons projecting to cortex from the posterior hypothalamus (Vincent *et al.*, 1983) in the rat raises the possibility that some GABAergic terminals are extracortical in origin.

It is surprising that the vast majority of terminals making symmetrical contacts were found to contain GABA. Previous immunocytochemical localization of neurotransmitter system specific markers has shown that in the cerebral cortex of several species some nerve terminals which make symmetrical synaptic contacts synthesize acetylcholine (Wainer *et al.*, 1984; Houser *et al.*, 1985; de Lima and Singer, 1986), noradrenaline (Papadopoulos *et al.*, 1989), serotonin (Tork *et al.*, 1988), dopamine (Seguela *et al.*, 1988), or contain neuroactive peptides (Hendry *et al.*, 1984; Freund *et al.*, 1986; Peters *et al.*, 1987; Jones *et al.*, 1988). Many neuroactive peptides have been shown to be present in GABA positive neurons (Hendry *et al.*, 1984; Somogyi *et al.*, 1984) therefore it can be assumed that most of the peptide containing terminals were part of our GABA positive sample.

The cholinergic marker enzyme choline-acetyltransferase has been localized in the rat in intrinsic cortical neurons that also contained GABA (Kosaka *et al.*, 1988). Such cells may be absent in the cat (Stichel *et al.*, 1987), where the majority of cholinergic terminals are assumed to originate from neurons of the basal forebrain which have not been thought to contain GABA. However, recent evidence indicates that some of the terminals of the basal forebrain cortical projection forming symmetrical synapses do contain GABA (Freund and Gulyas, 1989) in the rat, and a GABAergic projection has also been suggested to exist in the cat (Fisher *et al.*, 1988). Interestingly Freund and Gulyas (1989) found that in the rat the GABA positive terminals from the basal forebrain preferentially contacted GABA positive dendrites (63% of targets). Terminals originating in the basal forebrain and containing GABA have not been demonstrated in the cat, but in this species the authors found that a proportion of the presumed cholinergic terminals, as identified by their choline acetyltransferase content, also contained immunoreactive GABA (Beaulieu and Somogyi, 1989). Thus some of the GABA positive terminals sampled in the present study may come from neurons that also synthesize acetylcholine.

There is no evidence as yet that any of the monoamine containing terminals would also store GABA in the cortex. Therefore, even assuming that some of the cholinergic terminals were GABA positive, one would expect a proportion of nerve terminals with symmetrical synaptic contacts to be immunonegative for GABA. Since only 1% of the boutons containing pleomorphic vesicles was clearly GABA negative, one possibility is that all terminals lacking GABA contribute only a very small proportion of the symmetrical synapses in the striate cortex of the cat.

In the cerebral cortex GABA has long been shown to act as an inhibitory transmitter (for review see Krnjevic, 1984). Recent *in vitro* experiments, while supporting this view for the effects mediated by the $GABA_A$ receptor, have also demonstrated subtle effects on the firing patterns of cells mediated by $GABA_B$ receptors (Connors *et al.*, 1988). In general the firing rate of cells is reduced by GABA, therefore it is reasonable to propose an inhibitory role for the GABA-containing terminals which form symmetrical synapses and, as shown here, can be equated with the previously described population of boutons containing flat (or pleomorphic) vesicles. Terminals containing pleomorphic or flattened vesicles have for a long time been assumed to exert inhibitory influence (Gray, 1959; Uchizono, 1965; Szentagothai, 1969). Previous qualitative immunocytochemical studies (Ribak, 1978; Fruend *et al.*, 1983; for review see Houser *et al.*, 1984) and our quantitative results largely support this assumption. However, it should be emphasized that while, as a population, the boutons forming symmetrical synapses correspond mainly to the GABA-containing boutons in cortex, the neurochemical nature of any individual bouton cannot be determined with certainty without direct immunocytochemical examination.

The fine structural character of GABAergic nerve terminals is not merely a morphological issue. As our results show, the previously described FS synapses are almost all GABA positive. Therefore the authors use the present and previously published data (Beaulieu and Colonnier, 1985) on the distribution of their targets to calculate and

predict quantitatively the sites of GABAergic influence in the visual cortex. In the present study the authors found more contacts on somata and slightly less spines as targets of GABA-containing terminals than did Beaulieu and Colonnier (1985). Summarizing the two studies it emerges that the major targets of GABA positive synapses are dendritic shafts, which comprise more than half of the postsynaptic elements. About every fourth GABA positive synapse is devoted to dendritic spines. Furthermore neuronal somata are about half as likely to be targets of GABA positive synapses as are spines. Axon initial segments, although the exclusive targets of the GABAergic chandelier cells (Somogyi, 1977), comprise only a small proportion of the total population of postsynaptic elements.

Neurons that contain GABA comprise about 20% of all neurons in the striate cortex of the cat (Gabbott and Somogyi, 1986). Unfortunately the average number of synapses on GABA-containing and GABA negative neurons is not known, therefore it cannot be established whether our method quantitatively reveals all the postsynaptic elements that originate from GABA positive cells. The axo-somatic contacts achieved by GABA positive terminals onto GABA positive cells make up about 3% of the total synapses, and give the expected value of approximately 25% for the proportion of GABA positive somatic targets. However, only 15% of dendritic shafts and none of the dendritic spines were GABA positive, resulting in an overall proportion of 11% GABA positive targets postsynaptic to GABA positive terminals. Thus, either the authors' method does not reveal all the GABAergic dendrites or on average GABAergic neurons receive fewer GABAergic synapses on their dendrites than nonGABAergic cortical cells. At present it is not possible to decide between these two alternatives.

The results on the target element distribution of all GABA positive terminals can be compared to the distribution of the postsynaptic elements of individual GABAergic cells. This makes it possible to assess their target selectivity. Several types of GABAergic neuron have been described in the striate cortex of the cat and some information is available on their target distribution from random samples (for review see Somogyi, 1989). None of the cell types reported so far showed the target distribution that would be expected if they randomly picked postsynaptic sites innervated by GABAergic terminals. The degree of target selectivity runs from extreme as in the case of the chandelier cell, terminating exclusively on axon initial segments, to the basket cells terminating on all four categories of postsynaptic sites with different probability, and showing more than average preference for somata (Somogyi *et al.*, 1983; Kisvarday *et al.*, 1985, 1987). In the middle of the range are cells such as the bitufted and neurogliaform cells terminating mainly on dendritic shafts and spines ignoring somata and axon initial segments (Somogyi, 1989). Layer 1 has a type of GABAergic cell terminating largely in this layer (Martin *et al.*, 1989), but its target selectivity cannot be evaluated in the absence of data on the average GABAergic target distribution in layer I.

The possibility that inhibitory GABAergic synapses selectively influence certain excitatory inputs to cortical neurons has been the subject of much speculation. The quantitative results from the present study demonstrate that the vast majority of GABAergic synapses are located on the dendrites and spines of neurons and not on the somata, which would be the ideal site if the role of GABAergic innervation was to prevent the neuron from reaching firing threshold. The more peripherally placed GABAergic synapses may only influence events in their immediate surroundings. In particular the GABAergic synapses situated on dendritic spines, which also receive an excitatory synapse from another terminal, provide an attractive structural design for selective influences (Koch and Poggio, 1983, 1985; Shepherd and Brayton, 1987). However, these are the synapses where physiological testing of any hypothesis is the most difficult due to the uncertainty of how much of the potential or conductance change would be recorded from an intracellular electrode in the somata (for detailed discussion see Martin, 1988). The present study demonstrates that the GABAergic input to spines is not only substantial in absolute numbers, but that it is twice the number of synapses devoted to somata. Thus, although it can be calculated that only about 7.5% of all spines in visual cortex are innervated by both putative excitatory and inhibitory synapses (Beaulieu and Colonnier, 1985), selective distribution of these spines on the postsynaptic cell could have important effects on the gating of excitatory input. For example, GABAergic influence on spines could locally prevent the development of depolarization necessary for the activation of NMDA receptors (Thomson, 1986; for review see Cotman and Iversen, 1987), or it could lead to long-term depression (Stanton and Sejnowski, 1989). Appropriately timed GABAergic input to spines could also influence in a relatively confined space the biochemical processes evoked by excitatory input to the same spine (Riveros and Orrego, 1986; Gamble and Koch, 1987).

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Abbreviations

F bouton	nerve terminals with pleomorphic vesicles
FS	synaptic boutons containing flat vesicles and making symmetrical synapses
GABA	gamma aminobutyric acid
GAD	glutamate decarboxylase
NMDA	N-methyl-d-aspartate
PB	phosphate buffer

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