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Different Types of ^{3}H-GABA Accumulating Neurons in the Visual Cortex of the Rat. Characterization by Combined Autoradiography and Golgi Impregnation*

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Summary. Labelled neurons were identified by autoradiography following tangential intracortical injection of [^{3}H]-γ-aminobutyrate (GABA). The addition of cis-1,3-aminocyclohexane carboxylic acid to the GABA solution prevented perikaryal labelling. Labelled neurons were found in each injected layer and in addition they were always present directly above the injection track. The labelling of neurons in layer II. and upper III. following injections in layers V. and VI. can be explained by retrograde axonal transport and indicates that some GABA-ergic neurons project vertically.

Ninety neurons of different types were Golgi impregnated and examined for selective [^{3}H]-GABA uptake. Sixteen of these were labelled. On the basis of dendritic characteristics they were classified as aspiny multipolar neurons with small, medium or large dendritic fields, sparsely spiny multipolar neurons and one neuron was a bipolar cell. Thus Golgi impregnation of their processes reveals that cortical GABA-ergic neurons are a heterogeneous population.

A [^{3}H]-GABA accumulating, aspiny neuron with profoundly branching, "bushy" dendrites and locally arborizing axon in layer VI. was studied in the electron microscope. Its fine structural characteristics were similar to those of other identified non-pyramidal neurons. The existence of several types of cortical GABA-ergic neurons differing in their synaptic connections is discussed.

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Introduction

The increasing evidence that GABA is an inhibitory transmitter in the cerebral cortex (Krnjevic and Schwartz 1976; Curtis et al. 1971; Iversen et al. 1971; Rose and Blakemore 1974; Sillito 1975; Tsumoto et al. 1979) has stimulated experiments designed to study the structural characteristics of GABA-ergic neurons. Two methods have been applied: (i) autoradiographic demonstration of [^{3}H]-GABA following its uptake (Chronwall and Wolff 1980; Hendry and Jones 1981; Hökfelt and Ljungdahl 1972; Somogyi et al. 1981a, b; 1983b; Wolff and Chronwall 1982); (ii) immunocytochemical demonstration of glutamic acid decarboxylase (GAD) (Hendrickson et al. 1981; Ribak 1978), a specific marker enzyme, synthesizing GABA. Both methods reveal GABA-ergic neuronal perikarya and terminals but not the connecting axons or the dendritic arborization of the neurons. Thus it is difficult to relate the chemically characterized GABA-ergic neurons to the rich variety of cortical neuron types revealed by single neuron staining methods such as the Golgi impregnation. Recently we have introduced two procedures to overcome the above limitation. We combined Golgi impregnation with the autoradiographic demonstration of [^{3}H]-GABA, following its selective uptake (Bolam et al. 1983; Somogyi et al. 1981b, 1983b), and in another approach with the immunocytochemical demonstration of GAD (Freund and Somogyi 1983; Somogyi et al. 1983b). The result is that autoradiography or

immunocytochemistry identifies the putative transmitter of the neurons, while the Golgi deposit reveals their processes allowing the identification of neuron types and the tracing of their synaptic connections.

In the present study we applied the combination of autoradiography with Golgi impregnation for the characterization of GABA-ergic neurons and show that they form a heterogeneous population with regard to their dendritic arborization. We also provide light and electron microscopic details of a type of aspiny GABA-ergic neuron in layer VI. Some of these results have been presented in preliminary form (Somogyi et al. 1981b).

Materials and Methods

Three male Wistar albino rats and three male CFY strain albino rats were used. The animals were anaesthetized with chloral hydrate (350 mg/kg) and the visual cortex was injected with [^{3}H]-GABA (60 Ci/mmol, Radiochemical Centre Ltd., Amersham). The radiolabelled amino acid was dried under a stream of N_2 gas and redissolved at a final concentration of 0.33 mM in Krebs bicarbonate containing 134 mM NaCl; 5 mM KCl; 1.25 mM KH_2PO_4; 2 mM $CaCl_2$; 1 mM $MgSO_4$; 22 mM $NaHCO_3$ and 0.18% glucose. Injections were delivered via glass micropipettes (tip diameter 30–50 μm) penetrating at an oblique angle to the surface of the cortex, and perpendicular to the sagittal sinus. The pipettes were advanced 4 mm from the pia, from medial to lateral direction and then gradually withdrawn while [^{3}H]-GABA was ejected using a pressure system. A total of 8–10 μCi was delivered at one injection track, distributed at 4 or 8 sites 1 mm or 0.5 mm apart respectively, over a total time of 5–8 min. One animal usually received two injections into one hemisphere 2–2.5 mm apart rostrocaudally. In two rats one hemisphere received injection of [^{3}H]-GABA along one track whereas along a second injection track, 1.5 mm rostral, a mixture of the same GABA solution and cis-1,3-aminocyclohexane carboxylic acid (ACHC, 100 mM) was delivered. Ten to forty-five minutes after the injections the rats were perfused with a fixative containing 3% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Thereafter the brain was dissected and 1 mm thick slices of the occipital cortex, cut in the sagittal plane, were processed for Golgi impregnation and gold toning (Fairén et al. 1981) as described earlier (Somogyi et al. 1981b) including the illumination of Golgi sections using a Zeiss (Jena) microscope, equipped with heat filter, to enhance gold toning. The 80–90 μm thick sections were dehydrated and embedded in DURCUPAN ACM on slides.

The 80–90 μm thick Golgi impregnated sections were studied and after gold toning the various neuron types around the [^{3}H]-GABA injection sites were mapped. Neurons were included only from area 17 as delineated by Schober and Winkelmann (1975). Test sections were cut from each injection track at various mediolateral levels and the area containing radiolabelled perikarya was established by autoradiographs.

Selected Golgi impregnated neurons within the area of [^{3}H]-GABA uptake in other sections were drawn, photographed and after reembedding 1 μm thick sections were cut from the perikarya and placed on slides for autoradiography. Slides were dipped in Ilford K5 or K2 emulsions and exposed for 3–30 days at 4° C before developing in Kodak D19B and fixing. The sections were stained with a mixture of toluidine blue and Azur II.

Table 1. Number of silver grains over GABA-accumulating, Golgi-impregnated neurons (A) and over nearby non-accumulating neurons (B) in rat primary visual cortex. Silver grains over neurons Nos. 1 and 12 (see Fig. 5) were too dense for counting

Cell number (see Fig. 3)	Number of silver grains (A)	Number of silver grains over nearest non-accumulating neurons					Mean (B)	Ratio A/B
		1	2	3	4	5		
1	–	–	–	–	–	–	–	–
2	113	21	14	7	9	17	13.6	8.3
3	231	30	52	55	64	30	46.2	5.0
4	88	9	20	25	26	31	22.2	4.0
5	302	53	54	52	49	75	56.6	5.3
6	287	55	77	34	78	87	66.2	4.3
7	117	20	7	18	10	13	13.6	8.6
8	55	11	12	12	13	10	11.6	4.7
9	149	33	10	27	41	37	29.6	5.0
10	235	25	29	29	21	53	31.4	7.5
11	122	15	23	23	20	14	19.0	6.4
12	–	–	–	–	–	–	–	–
13	77	13	7	8	17	15	12.0	6.4
14	184	27	20	18	16	13	18.8	9.8
15	285	50	46	66	36	54	50.4	5.7
16	154	30	23	12	19	32	22.4	6.9

Silver grains were counted using a 100X oil immersion objective, over the perikaryon of the gold toned neuron and over the perikarya of the five nearest neurons (Table 1). Cells were accepted as "labelled" if the number of silver grains over their perikaryon was four times or more higher than the average number of grains over the perikarya of the surrounding "unlabelled" neurons.

The highest number of silver grains over any one of the "unlabelled" neurons was 30% or less than the number of silver grains over the gold toned neuron (exept in one case, cell 4 non-accumulating neuron No. 5 in Table 1, where it was 35.2%). This means that in the case of the 16 cells listed in Table 1, there were no "labelled" neurons among the five nearest cells. The above criterion for accepting a neuron as "labelled" on the basis of grain number over its perikaryon is only an empirical one used in this sample, and other values could prove useful under different conditions.

Following autoradiographic demonstration of [^{3}H]-GABA accumulation in one neuron (No. 12 in Fig. 3), further sections were cut from this cell for electron microscopy. Correlated light and electron microscopy was carried out as described in detail elsewhere (Somogyi et al. 1982).

Results

Pattern of Neuronal Labelling

It has been demonstrated that in autoradiographs of plastic embedded, Golgi impregnated and gold toned sections silver grains are a result of the decay of tritium and not chemical development of the emulsion by metal or reagents in the sections (Bolam et al. 1983; Somogyi et al. 1981b). Accumulations of silver grains were observed over neuronal perikarya

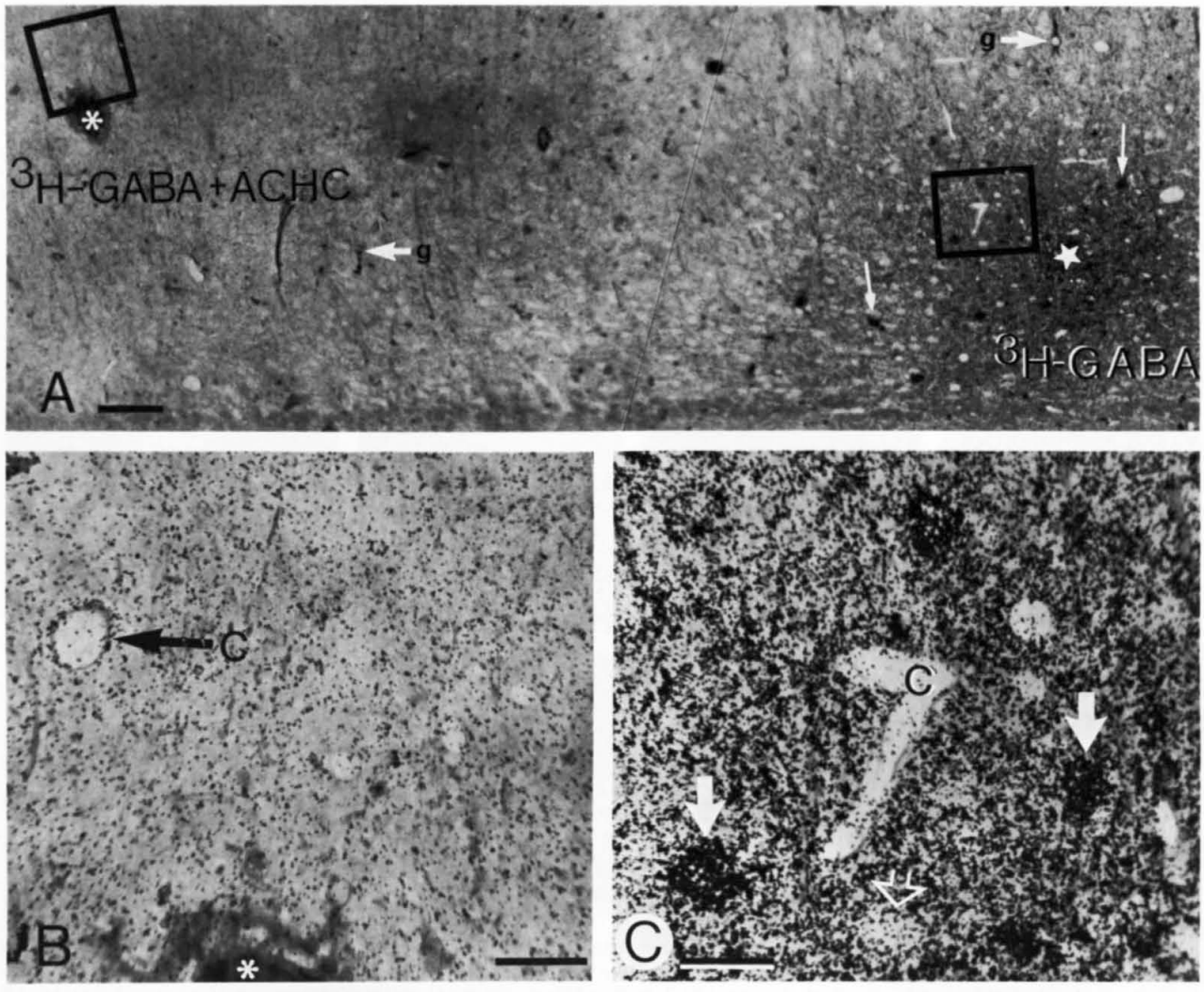

Fig. 1. A Light micrograph of a 1 μm thick sagittal section of the rat visual cortex processed for autoradiography. The section was cut from a Golgi impregnated section, thus outlines of gold toned neurons (thick arrows, g) are present. The cortex was injected with a solution containing [^{3}H]-GABA alone (*star*) or the same concentration of [^{3}H]-GABA but together with ACHC (*asterisk*). The injection capillaries passed at an oblique angle to the pia and nearly perpendicular to the plane of the section. There are numerous labelled neurons, 2 of which are shown by vertical arrows near the site where [^{3}H]-GABA was injected alone. Framed areas are shown in **B** and **C. B** Higher magnification of the injection site on the left in (A) shows only diffuse label in the neuropil and slight accumulation over the wall of a capillary (c). **C** Higher magnification of the injection site on the right in (A). Labelled neurons (*thick arrows*) and unlabelled neurons (*open arrows*) are present in the heavily labelled neuropil. c = capillary. Scales: **A:** 100 μm; **B** and **C:** 20 μm

(Table 1). The neuropil showed only a diffuse distribution of grains with occasional aggregations. The activity around the injection track greatly depends on the survival time; the longer the postinjection survival, the lower the silver grain density observed and the activity over the neuropil decreases more rapidly than that of perikarya. At survival times longer than 20 min the labelling of glial cells and pericapillary cells gradually increases.

As our injection tracks were oblique to the surface of the cortex, the different cortical layers were injected at different medio-lateral coordinates. This provided an opportunity to study the distribution of labelled neuronal perikarya in relation to the injected layer. Neurons in each of layers I–VI. were labelled when the layer was directly injected with [^{3}H]-GABA. The number of labelled neurons gradually decreases from the injection site. Strongly and weakly labelled cells are present both close to the and further away from the injection site. When layers V–VI. were injected the area containing labelled neurons was extended vertically, labelled neurons being found up to layer II even when the injection was in layer VI. Sometimes an upper cell group was

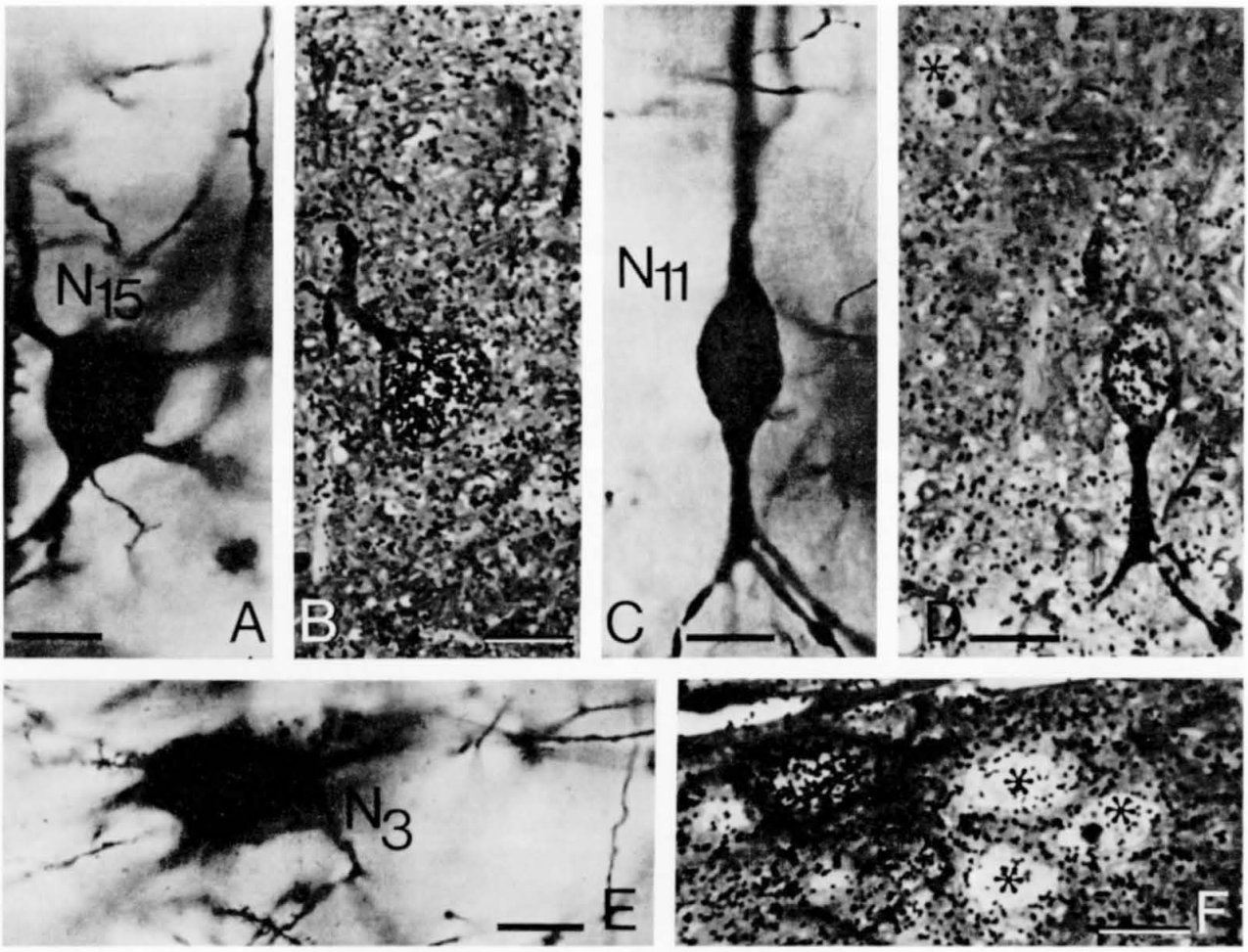

Fig. 2A–F. Golgi impregnated, gold toned neurons with smooth or sparsely spiny dendrites in the rat striate cortex which was injected with [^{3}H]-GABA. **A, C** and **E** are micrographs of different neuron types as seen in the thick Golgi sections. The numbers (Nos. 15, 11, 13) refer to the camera lucida drawings of the same cells shown in Fig. 3. **B, D** and **F** are semithin (1 μm) sections processed for autoradiography. All three neurons show selective accumulation of [^{3}H]-GABA, while neighbouring neurons (*asterisk*) are unlabelled. Scales: 10 μm

observed in layers II–III. and another around the injection track in layers V–VI, separated by a region around layer IV. containing few labelled cells.

Inhibition of [^{3}H]-GABA Uptake by ACHC

Although we used fine capillaries for injection it is unavoidable that some neuronal processes were cut by the injection track and this could have resulted in labelling not through specific uptake but by direct injection of neurons. To check for this possibility we injected [^{3}H]-GABA together with an excess of a specific competitive uptake inhibitor, ACHC. The inhibitor either completely prevented or greatly attenuated the labelling of neuronal perikarya (Fig. 1A, B). Interestingly the total radioactivity retained at the injection site was also substantially reduced (Fig. 1A), possibly because [^{3}H]-GABA, not taken up by cells, was carried away from the injection site either by the circulation or by the cerebrospinal fluid. The accumulation of silver grains around the wall of capillaries was not prevented by ACHC.

Fig. 3. Camera lucida drawings of Golgi impregnated neurons which were found to accumulate [^{3}H]-GABA in different layers (I–VI) of the striate cortex of rat. The neurons all have smooth or sparsely spiny dendrites, but the differences in their shape, size and dendritic arborization indicate that they comprise a heterogenous population. The axon of only one neuron (No. 1) is indicated. Neurons Nos. 3, 11, 12, 15 are also shown in Figs. 2 and 5. Neurons Nos. 4, 7 and 13 were included from Somogyi et al. (1981b) in order to provide a complete picture. Scale: 50 μm

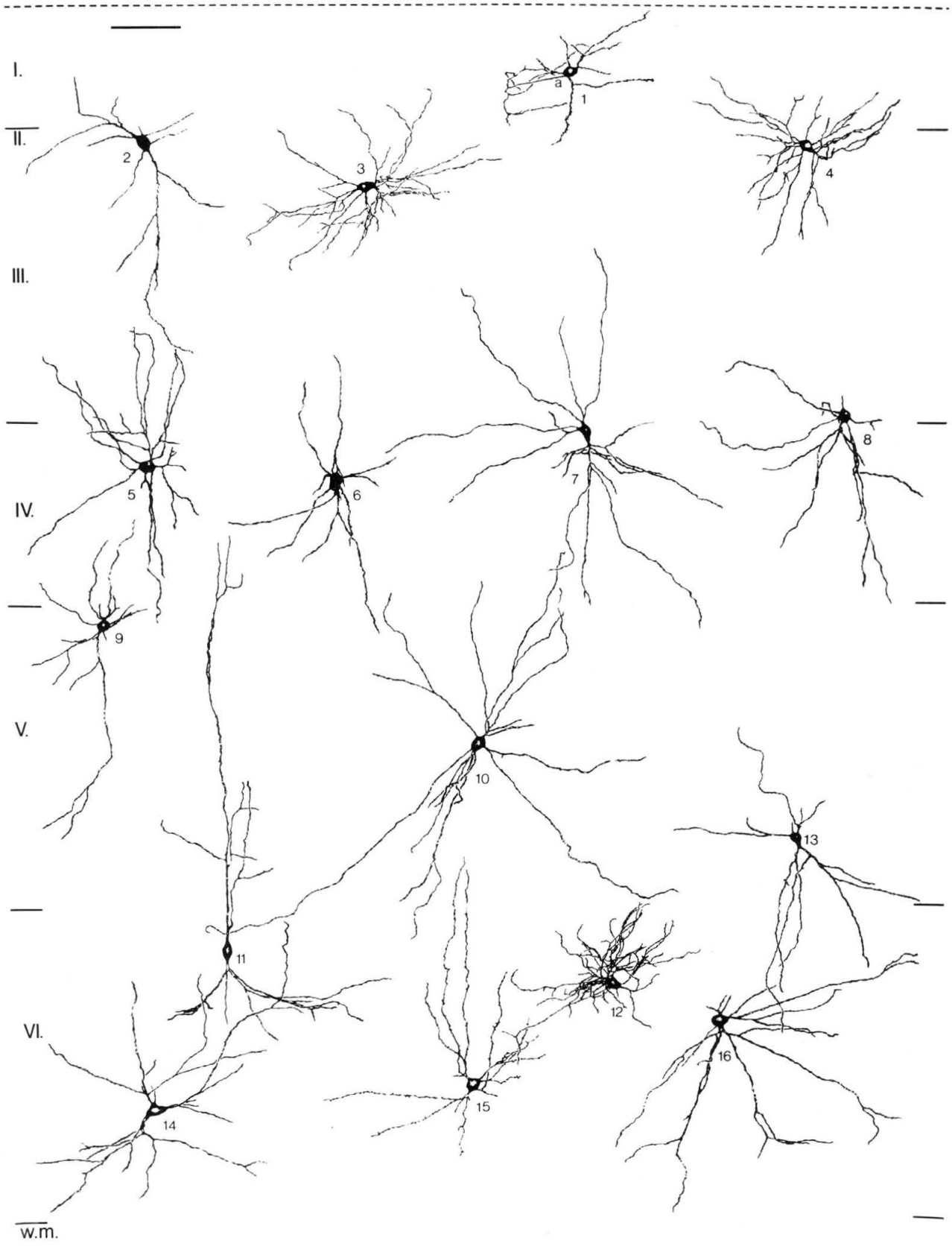

Fig. 3

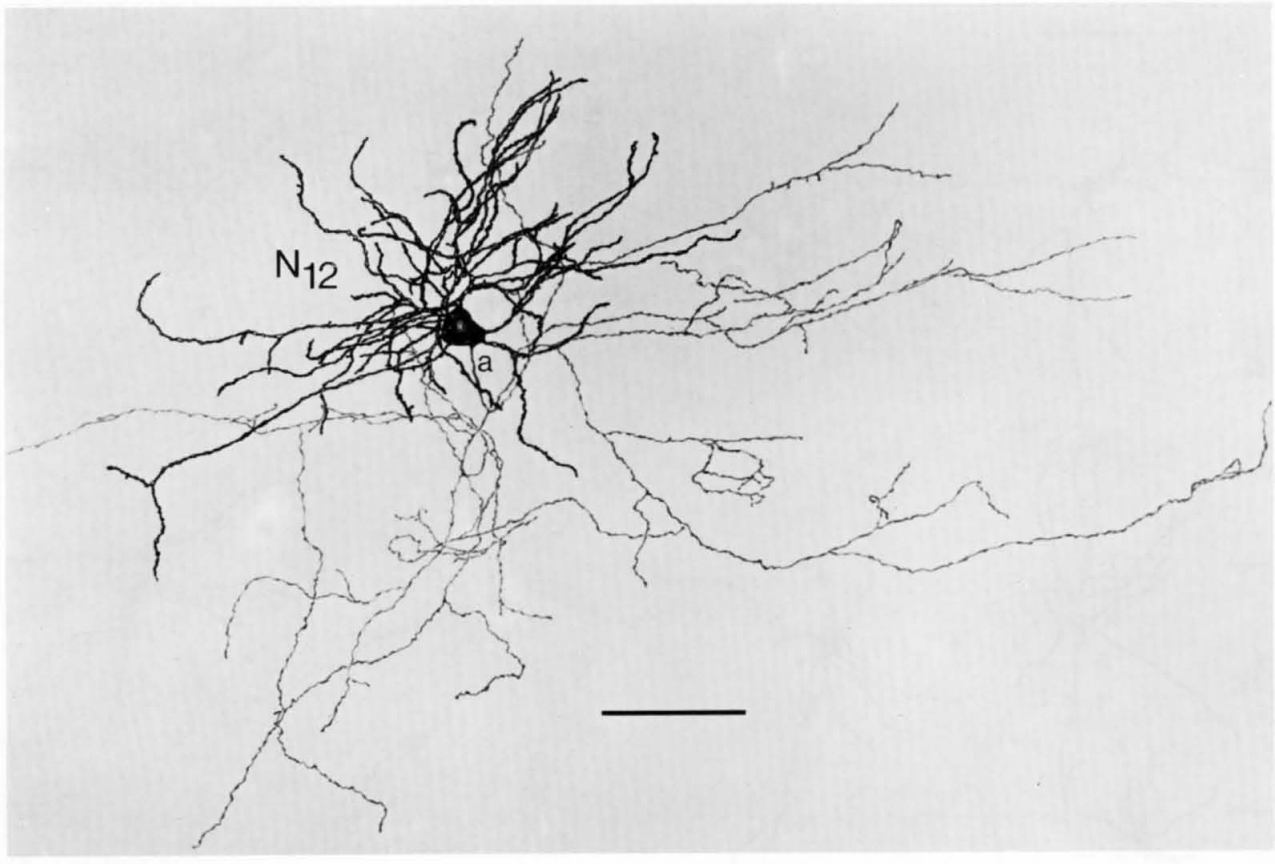

Fig. 4. Camera lucida drawing of a [^{3}H]-GABA accumulating neuron (No. 12 in Figs. 3, 5) with both dendritic (*black*) and axonal (*gray*) arborization in layer VI. Scale: 50 μm

The Types of Golgi Impregnated [^{3}H]-GABA Accumulating Neurons

Previous [^{3}H]-GABA uptake studies and the immunocytochemical localization of glutamate decarboxylase (GAD) suggested that in the visual cortex some non-pyramidal neurons are GABA-ergic. Therefore we tested various types of neurons within the area of [^{3}H]-GABA uptake. In total we tested 41 aspiny or sparsely spiny non-pyramidal neurons from all layers, and 12 spinous multipolar neurons in layer IV. In addition 37 gold toned pyramidal neurons were also tested since the sections of their soma were within the same semi-thin section as those of non-pyramidal neurons under investigation.

Sixteen gold toned neurons were radiolabelled and they all belong to the aspinous and sparsely spinous non-pyramidal population (Fig. 3, Table 1). However, they are not homogenous with regard to size, shape and the distribution of dendrites. In the following description we broadly follow the classification

neurons (Figs. 2 and 3). Within this category three neurons (Nos. 3, 4 and 12) had small dendritic fields, two of them were in layer II and one of them in layer VI. The neuron in layer VI seems different from all the other labelled neurons and will be described later in detail. The other two neurons in layer II had many dendrites which branched frequently and were covering an area about 200–250 μm in diameter. One neuron in layer V (No. 10) had a large dendritic field with long, smooth, rarely-bifurcating dendrites distributed in an area of 400–500 μm in diameter.

Nine of the multipolar neurons (Nos. 2, 5, 6, 7, 8, 9, 13, 14, 16) had medium size dendritic fields 300–400 μm in diameter. These neurons were found in all layers except layer I. The dendritic fields were often elongated in the vertical direction and the predominant orientation of the dendrites was close to vertical. Some of these neurons (Nos. 2, 7, 13) can be considered to be bitufted cells as most of the

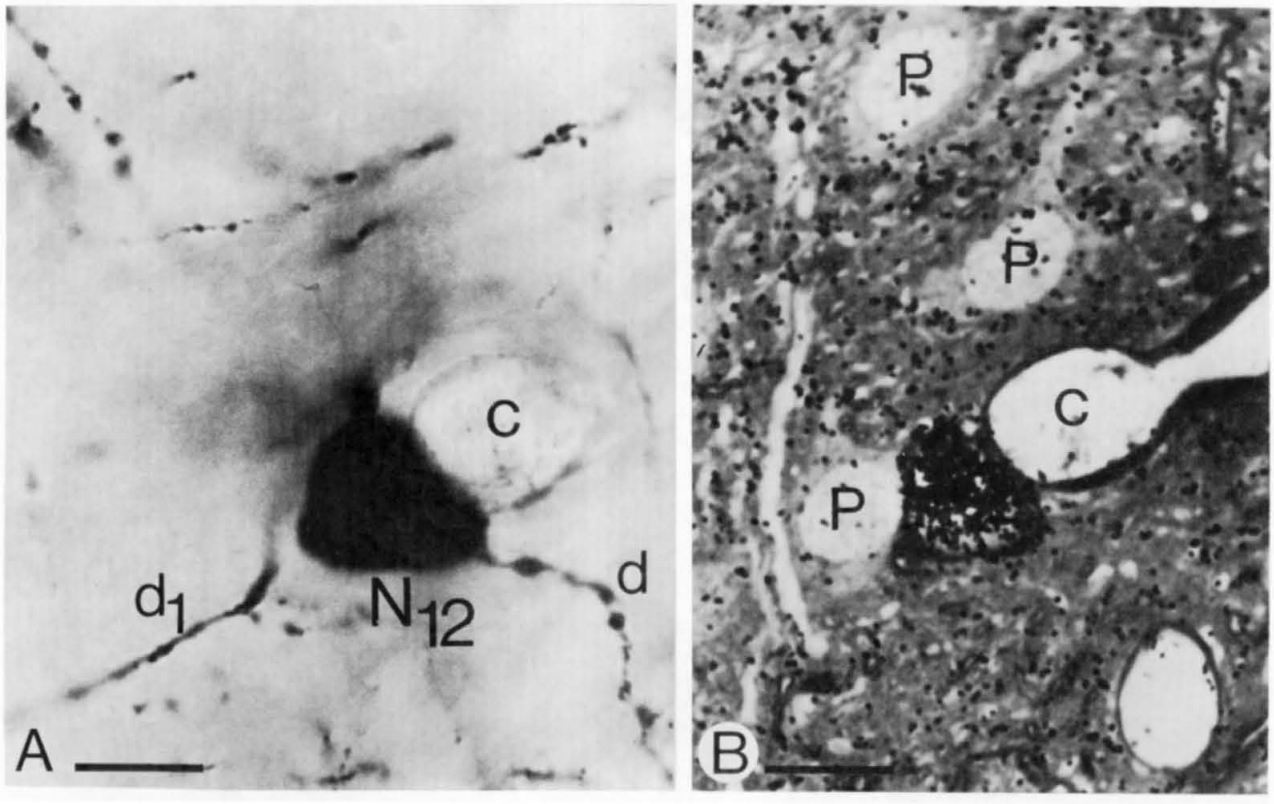

Fig. 5. A Light micrograph of a Golgi impregnated neuron (No. 12 in Figs 3 and 4) in layer VI. of the rat striate cortex. Two dendrites (d, d_1) are indicated and are also shown in Fig. 6 at the electron microscope level. **B** Semithin section of the same neuron processed by autoradiography shows that this neuron selectively accumulates [^{3}H]-GABA while neighbouring pyramidal neurons (P) are not labelled. The neuron is very strongly labelled after only 6 days exposure. A capillary (c) serves as reference in the two sections. Scale: 10 μm

Two neurons (Nos. 1, 15) had several spines and appendages on their dendrites (Figs. 2A, B and 3). Therefore they are considered as representatives of the sparsely spiny multipolar neuron type. Although the aspinous multipolar neurons may have occasional protrusions on their dendrites, the two sparsely spinous neurons had conspicuously more and are different from both aspinous multipolar and spinous multipolar population (Feldman and Peters 1978). The neuron in layer I (No. 1) had a small dendritic field.

One neuron (No. 11) selectively accumulating [^{3}H]-GABA (Fig. 2C, D) in layer VI. was a *bipolar type* as its dendrites originated from two major vertically-oriented shafts. The upper dendrites ascended vertically 450 μm to layer IV. The axon originated from the lower dendritic shaft vertically.

With the exception of four cells in layer VI. (Nos. 12, 14, 15, 16) the dendrites of [^{3}H]-GABA accumulating neurons extended into more than one layer. This is especially true for neurons in and around layer IV. which have dendrites penetrating into layers III. and V.

Four neurons (Nos. 1, 3, 12 and 14) had axons impregnated beyond the initial segment. The axons of neurons in layer I and II arborized locally within the dendritic field. The axon of the neuron in layer VI (No. 12) is described below.

A Small "Bushy" Cell Accumulating [^{3}H]-GABA in Layer VI

The most strongly labelled neuron (No. 12 in Fig. 3 and also Figs. 4 and 5) was different in many ways from the others. Two such "bushy" neurons have been observed so far both of them in layer VI. We have not been able to find description of similar neurons in the visual cortex on rodents. Therefore we studied one of them, which was labelled by [^{3}H]-GABA, in more detail. This neuron (Fig. 4) had short, undulating, slightly varicose dendrites originating from all parts of the perikaryon. The dendritic arborization was very compact occupying a sphere 150–200 μm in diameter. The thicker primary dendrites gave rise to numerous secondary branches

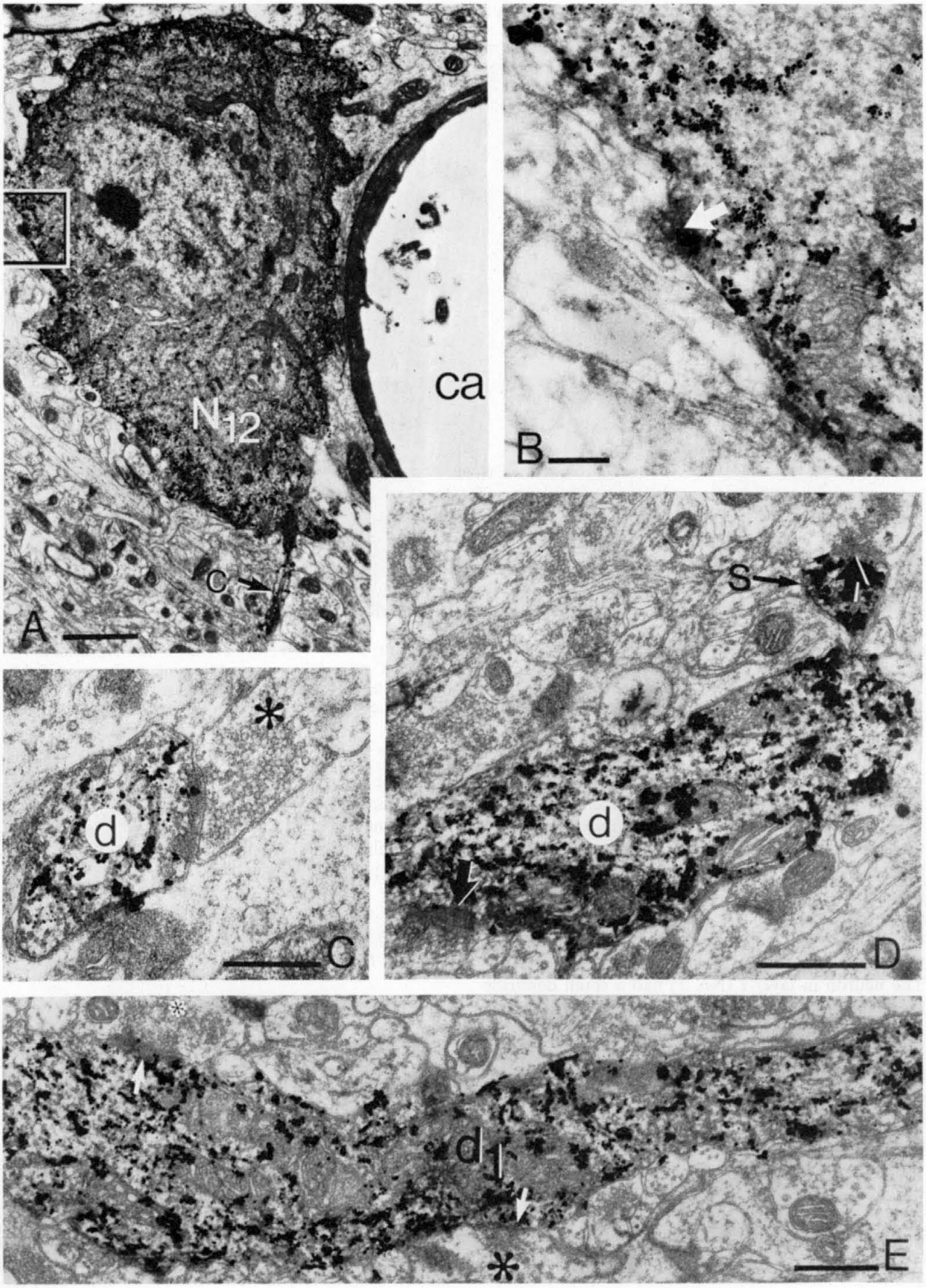

Fig. 6A–E

usually originating in a short distance of the soma giving the neuron a characteristic "bushy" appearance. The dendrites had very few, short spines. The axon initial segment originated from the base of the soma and soon divided into slender, varicose branches which themselves divided several times. The centre of the axon arborization was below the dendritic field and collaterals run as far as 270 μm from the perikaryon occupying an area twice the size of the dendritic field. The [^{3}H]-GABA injection track was within the area of the axonal arborization.

The fine structural features of the "bushy" [^{3}H]-GABA accumulating neuron were studied in order to compare this neuron to previously studied other identified neuron types. The perikaryon had a scalloped appearance with rather dark cytoplasm partly due to the high number of ribosomes (Fig. 6A, B). The neuron was rich in rough endoplasmic reticulum and mitochondria. The nucleus had several invaginations and contained a large amount of heterochromatin. A single cilium was found to originate from the soma (Fig. 6A). The dendrites were rich in microtubules and mitochondria both in their proximal and distal portions. The beaded portions had groups of mitochondria occupying the enlarged segments.

The perikaryon received very few synaptic contacts, only three were seen after serial sectioning of one third of the soma. These synapses were established by small boutons containing pleomorphic vesicles (Fig. 6B). More boutons made synaptic contact with the dendrites, most of them establishing asymmetrical junctions and containing clear round vesicles (Fig. 6C–E). Similar boutons contacted the few spines (Fig. 6D). Occasionally symmetrical synaptic contacts were also observed (Fig. 6E).

Discussion

Types of GABA-ergic Neurons in the Visual Cortex of Rat

The selective accumulation of [^{3}H]-GABA seems to be a good marker for GABA-ergic neurons in the cortex. Neurons which accumulate the labelled amino acid (Chronwall and Wolff 1980; Hendry and Jones 1981; Hökfelt and Ljungdahl 1972; Somogyi et al. 1981a, b; Wolff and Chronwall 1982) are similar to those which have been shown to contain the GABA synthesizing enzyme, glutamate decarboxylase (Hendrickson et al. 1981; Ribak 1978). Similarly, there seems to be agreement between autoradiographic and immunohistochemical studies of GABA-ergic neurons in the cerebellum (Hökfelt and Ljungdahl 1972; Saito et al. 1974; Schon and Iversen 1972), olfactory bulb (Halász et al. 1959; Ribak et al. 1977) and neostriatum (Bolam et al. 1983; Ribak et al. 1979).

The present study demonstrates that the types of GABA-ergic neuron in the rat visual cortex are heterogeneous. This conclusion is based on Golgi impregnation of their dendritic arborization which revealed differences in dendritic density, field size, orientation and spine density.

Despite the differences, all labelled neurons belong to a broad category, the aspinous or sparsely spinous non-pyramidal neurons. Neither pyramidal cells nor the spinous multipolar neurons (Feldman and Peters 1978) which in the rat are probably homologous to spiny stellate cells in the cat (Garey 1976; Lund et al. 1979; Hornung and Garey 1981) and monkey (Lund 1973; Valverde 1971) were labelled. This proves the selectivity of the uptake but it does not indicate that all aspinous cells are GABA-ergic. In the present study only 39% of the examined neurons of this latter category were labelled by [^{3}H]-GABA, even though those which were not labelled were also situated within the area of perikaryal labelling. One possible explanation for the lack of [^{3}H]-GABA accumultion is that these neurons did not have the high affinity system and therefore are not GABA-ergic. However, it is also possible that their axons were outside the effective injection area where the concentration of [^{3}H]-GABA was high enough to result in perikaryal labelling through retrograde axonal transport. Thus the lack of labelling does not necessarily indicate that the neurons are not GABA-ergic. Recent evidence suggests that the [^{3}H]-GABA injection site can be at considerable distance from the perikarya which become labelled by retrograde axonal transport (Hunt and Künzle 1976; Somogyi et al. 1981a, 1983a; Streit et al. 1979). Thus it is possible that all perikaryal labelling is a result of uptake at the terminals, implying that a

Fig. 6A–E. Electron microscopic details of the Golgi stained, gold toned neuron (No. 12), which in Fig. 5 was shown to accumulate [^{3}H]-GABA. **A** The soma emitting a cilium (c) is seen near a capillary (ca). Framed area is shown in **B. B** A small bouton makes an axo-somatic synapse (*arrow*). **C** A distal dendrite (d) receives an asymmetrical synapse from a bouton with round vesicles (*asterisk*). **D** A proximal dendrite (d) emitting a spine (s) receives asymmetrical synaptic contacts both on the dendritic shaft and the spine (*arrows*). **E** The dendrite (d_1) shown in Fig. 5 receives an asymmetrical synaptic contact from a bouton (*small asterisk*) and a symmetrical contact from another bouton (*large asterisk*). Arrows indicate synaptic contacts. Scales: **A:** 1 μm; **B:** 0.2 μm; **C–E:** 0.5 μm

neuron becomes labelled only if a substantial proportion of its boutons are within the injection area.

Inhibition of GABA Uptake by ACHC

The lack of neuronal labelling in the presence of an excess of ACHC a competitive neuronal uptake inhibitor (Bowery et al. 1976) provides evidence that labelling occurs by an active uptake process (Bolam et al. 1983; Somogyi et al. 1983a). The use of an uptake inhibitor also greatly reduces the radioactivity retained at the injection site, possibly because [^{3}H]-GABA which was not taken up by neurons becomes metabolized and transported by the circulation. It seems that glial uptake of [^{3}H]-GABA is inhibited to a lesser extent by ACHC because the walls of capillaries exhibit accumulation of silver grains. This is in agreement with pharmacological studies (Bowery et al. 1976), suggesting that ACHC mainly blocks neuronal uptake.

Intracortical Pattern of Labelled Neuronal Perikarya

Previous studies (Chronwall and Wolff 1980; Hendry and Jones 1981; Hökfelt and Ljungdahl 1972; Wolff and Chronwall 1982) employed vertical injections for the delivery of the isotope which results in a cylindrical distribution of labelled cells around the injection track. Using tangential injections it has been demonstrated that the distribution of labelled perikarya in the monkey visual cortex depends on the laminar position of the injection site (Somogyi et al. 1981a, 1983a). In the monkey the labelled neurons are organized vertically, with the longest intracortical GABA-ergic projection descending to layers V and VI from perikarya in layer II (Somogyi et al. 1981a). A similar pattern was observed in the present study following [^{3}H]-GABA injections in the deep layers, but the separation of the labelled neuron groups, one around the injection site in layer VI and the other in layer II and upper III was not as sharply defined as in the monkey. Nevertheless the presence of labelled neurons up to layer II after deep layer injections shows that the vertical organization of GABA-ergic neurons is a basic feature of the cortex, and is present in the rat visual cortex.

A Small GABA-ergic Neuron in Layer VI

Previous Golgi studies of the rat visual cortex (Feldman and Peters 1978; Hedlich and Winkermann 1982; Parnavelas et al. 1977; Werner et al. 1979) do not describe the type of small neuron with "bushy" dendrites which was labelled very strongly by [^{3}H]-GABA in the present study. This neuron somewhat resembles the neurogliform cells in cat (Szentágothai 1973; Tömböl et al. 1976) and rat (Werner et al. 1979) but it has more dendrites and a wider axonal arborization. The fine structural features of the "bushy" cell are in many ways similar to those of smooth and sparsely spinous stellate cells studied in the visual cortex of rat (Peters and Fairén 1978). However, our GABA-ergic neuron in layer VI had much fewer synaptic contacts on the perikaryon.

Axons of GABA-ergic Neurons

Only four of the GABA labelled neurons had axons impregnated. Several of the others had impregnated axon initial segments but the axon subsequently became myelinated and failed to impregnate. The four impregnated axons, including that of the "bushy" cell, did not form special terminal configurations such as pericellular baskets, or vertical axo-axonic segments (Somogyi 1977). The varicose axon collaterals follow a tortuous course without obviously being associated with a particular postsynaptic element. Although electron microscopic studies have not been carried out on the gold-toned axons, the "bushy" cell and the other two GABA-accumulating multipolar neurons with axons do not seem to be specific with regard to postsynaptic targets. In contrast another interneuron, the axo-axonic or chandelier cell, which makes synapses with the axon initial segments of pyramidal neurons (Fairén and Valverde 1980; Peters et al. 1982; Somogyi 1977, Somogyi et al. 1982) has absolute specificity for the postsynaptic element. Immunocytochemical demonstration of GAD in axo-axonic boutons in the visual cortex of rat indicates that axo-axonic cells are probably GABA-ergic (Peters et al. 1982). Certainly in the hippocampus there is evidence from counts of immunoreactive axo-axonic terminals for the GABA-ergic nature of axo-axonic or chandelier cells (Somogyi et al. 1983c).

Another GABA-ergic interneuron candidate is the cell type which makes multiple synaptic contacts with perikarya and dendrites of pyramidal neurons (Martin et al. 1983). In the rat visual cortex a multipolar stellate cell has been shown to provide such boutons (Peters and Proskauer 1980) and in the rat again most if not all boutons making synaptic contacts onto the perikarya of pyramidal neurons, contain GAD (Ribak 1978). The multipolar stellate cell (Peters and Proskauer 1980) is different from both chandelier cells and axon bearing [^{3}H]-GABA accumulating neurons in the present study.

From the survey of the axons of probable GABA-ergic neurons it can be concluded that they are a heterogeneous population, a conclusion reached also from the visualization of their dendrites in this study, and from the study of their synaptic input (Wolff and Chronwall 1982). Thus it is likely that the role of the various GABA-ergic neuron types in the cortex is determined not only by their transmitter but also by their afferent and efferent synaptic connections.

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