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Synaptic organization of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex

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Summary

Neurons in the monkey somatic sensory and motor cortex were labelled immunocytochemically for the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), and examined with the electron microscope. The somata and dendrites of many large GAD-positive neurons of layers III–VI receive numerous asymmetric synapses from unlabelled terminals and symmetric synapses from GAD-positive terminals. Comparisons with light and electron microscopic studies of Golgi-impregnated neurons suggest that the large labelled neurons are basket cells. Small GAD-positive neurons generally receive few synapses on their somata and dendrites, and probably conform to several morphological types. GAD-positive axons form symmetric synapses on many neuronal elements including the somata, dendrites and initial segments of pyramidal cells, and the somata and dendrites of non-pyramidal cells. Synapses between GAD-positive terminals and GAD-positive cell bodies and dendrites are common in all layers. Many GAD-positive terminals in layers III-VI arise from myelinated axons. Some of the axons form pericellular terminal nests on pyramidal cell somata and are interpreted as originating from basket cells while other GAD-positive myelinated axons synapse with the somata and dendrites of non-pyramidal cells. These findings suggest either that the sites of basket cell terminations are more heterogeneous than previously believed or that there are other classes of GAD-positive neurons with myelinated axons. Unmyelinated GAD-positive axons synapse with the initial segments of pyramidal cell axons or form *en passant* synapses with dendritic spines and small dendritic shafts and are interpreted as arising from the population of small GAD-positive neurons which appears to include several morphological types.

Introduction

Previous studies of the cerebral cortex (Ulmar, 1976; Emson & Lindvall, 1979) have indicated that GABA is synthesized by neurons whose axons do not leave the cortex.

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Candidates for GABAergic intrinsic neurons have been studied by the selective accumulation of tritiated GABA (Hökfelt & Ljungdahl, 1972; Chronwall & Wolff, 1978, 1980; Hamos *et al.*, 1981; Hendry & Jones, 1981) and by the immunocytochemical localization of the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD) (Ribak, 1978; Hendrickson *et al.*, 1981; Houser *et al.*, 1983). In the preceding paper (Houser *et al.*, 1983), it was shown that GAD-positive neurons of the monkey somatic sensory and motor cortex are all non-pyramidal cells and that some can be identified with certain classes of morphologically distinct interneurons. These associations were based on light microscopic observations of the sizes, laminar distributions, proximal dendritic characteristics, and probable axon terminal sites of the immunocytochemically stained neurons. Because previous studies have demonstrated that known classes of cortical interneurons also have characteristic patterns of axonal and synaptic morphology (Somogyi, 1977; Fairén & Valverde, 1980; Peters & Proskauer, 1980; Somogyi & Cowey, 1981), we have examined the ultrastructure of GAD-positive neurons in order to extend the comparisons made at the light microscopic level. The present descriptive report is also intended as a basis for future quantitative studies of GAD-positive neurons and their terminations, and for studies of the relationships between these neurons and defined afferent systems.

Materials and methods

The material for this study was obtained from two adult cynomolgus monkeys (*Macaca fascicularis*). The animals were anaesthetized and preteated with colchicine as described in the preceding paper (Houser *et al.*, 1983). Approximately 75 μl of a 10 μg μl^{-1} solution of colchicine solution were injected slowly over a 3–4 h period into the lateral ventricle, and during this time, multiple 1 μl injections totalling 15–20 μl of the same concentration of colchicine were made into the precentral and postcentral gyri. Two days later the animals were anaesthetized and perfused with a solution of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.12 M Millonig's (1961) phosphate buffer at pH 7.2. The heads were removed and placed overnight in an ice-cold fixative solution similar to the above solution except that it contained no glutaraldehyde. The brains were removed the next day and stored in the buffered paraformaldehyde fixative.

The precentral and postcentral gyri were cut into thin slices that were then sectioned on a Vibratome in the parasagittal plane at a thickness of 100 μm. The sections were rinsed in Millonig's phosphate buffer and processed for GAD immunocytochemistry, utilizing the same GAD antiserum (Wu *et al.*, 1973; Saito *et al.*, 1974) as described in the previous paper. Sections were incubated for 2–18 h in rabbit anti-GAD serum (1:50 or 1:72), and for 2 h each in goat anti-rabbit IgG serum (1:50) and rabbit peroxidase–antiperoxidase Fab complex (1:16). All antisera contained 0.1% Triton X-100 to increase the penetration of the reagents. Between each incubation the sections were washed in phosphate-buffered saline for 2.5 h. Finally the sections were treated with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide for 30 min. Narrow wedges including all cortical layers were cut from these sections and post-fixed in osmium tetroxide, stained *en bloc* with uranyl acetate, dehydrated through an ascending series of ethanols followed by 100% propylene oxide and embedded in an Epon–Araldite mixture.

Thin sections that included all layers or only selected layers of cortex were examined. Most sections were cut at a thickness of 60–70 nm and were collected on 200 mesh copper grids. Several series of 30–60 sections were also cut and collected on Formvar-coated, single slot grids. Thin

sections were cut without previous 'thick' sectioning of the block so that the most superficial sections could be examined in the electron microscope. This procedure was necessary since the penetration of the various antisera employed in the immunocytochemical method is limited. Some thin sections were examined without further heavy metal staining, although most were stained additionally with lead citrate. The sections were examined with either a Zeiss EM-9S or a Hitachi HU-11B electron microscope.

Results

Electron microscopic observations have been made of GAD-positive neurons in all layers of cortical areas 4,3,1 and 2. Since there are no discernible, qualitative differences in the ultrastructure of GAD-positive neurons in these four areas, they will be considered together in the following description.

GAD-POSITIVE SOMATA AND DENDRITES

Previous light microscopic examination of GAD-positive somata has revealed the presence of two size groups: small cell bodies with major diameters less than 15 μm and large cell bodies with major diameters 15 μm or more (Houser *et al.*, 1983). In agreement with the light microscopic findings, small and large GAD-positive somata in these two size ranges are detectable by electron microscopy. The small somata (8–14 μm in diameter) are present in all layers, including layer I, although the greatest numbers are in layers II and IV. The large somata (15–25 μm) are present in the deep half of layer III, in layer V and occasionally in layer VI. In the somatic sensory cortex, large GAD-positive somata are also present in layer IV. The GAD-positive cells are clearly non-pyramidal. They do not exhibit large ascending dendrites that could be interpreted as apical dendrites. Instead dendrites originate from many different parts of the labelled somata. The cytoplasm of GAD-positive somata contains the usual complement of organelles, although the larger labelled somata possess a very high density of mitochondria (Figs. 1a,2). No nuclear inclusions or cytoplasmic organelles were found to be unique to the GAD-positive neurons.

Characteristically, and in agreement with descriptions of non-pyramidal cells in the monkey sensory-motor cortex (Sloper, 1973), the somata of GAD-positive neurons receive both symmetric (Fig. 1b) and asymmetric (Fig. 1c) synaptic contacts. Most large, and some small, GAD-positive somata receive relatively large numbers of synapses (Fig. 1a). However, the majority of small labelled somata in all layers and some large somata in the deeper half of layer III receive only a few synapses in any one section. In the vast majority of cases, synapses with symmetric membrane contacts on the GAD-positive somata are formed by GAD-positive terminals; synapses with asymmetric thickenings are formed by unlabelled terminals.

GAD-positive dendrites of variable size are present in all layers, and they commonly contain numerous mitochondria (Figs. 2,4). Many large GAD-positive dendrites, with diameters of 4 μm or more, originate from large labelled somata (Fig. 2) that are present in layers III to VI. These dendrites receive numerous asymmetric synapses from

unlabelled terminals and symmetric synapses from GAD-positive terminals (Figs. 2,3). Small GAD-positive dendrites, with diameters of 1–3 μm, are present throughout the depth of the cortex and also receive both asymmetric synapses from GAD-negative terminals and symmetric synapses from GAD-positive terminals. Many of the small dendrites are beaded (Fig. 4). Neither the large nor the small GAD-positive dendrites give rise to dendritic spines.

TERMINATIONS OF GAD-POSITIVE AXONS

GAD-positive synaptic terminals are present in all layers, possess large numbers of flattened vesicles and mitochondria, and form only symmetric synapses (e.g. Fig. 5). The terminals vary greatly in size, and form synapses with many different neuronal elements.

Synapses on pyramidal cells

Pyramidal cells, particularly those with large somata in layers III and V, are major targets of GAD-positive axons. The number of synapses formed by GAD-positive terminals on a pyramidal cell soma in a single section varies from as few as one or two (Fig. 11) to as many as seven. Pyramidal cells receiving the greatest number of synapses from GAD-positive terminals are those of layer V although, in both single and serial thin sections, the number of GAD-positive terminals on neighbouring pyramidal cells in layer V is variable. Generally the somata of the small pyramidal cells in layer II and those of the modified pyramidal cells of layer VI receive relatively few synapses. Frequently, several GAD-positive terminals synapsing with the same pyramidal cell body are found in single or serial thin sections to arise from the same parent axon (Fig. 16), resembling the components of a 'pericellular nest' as described in light microscopic studies of cortical basket cells (e.g. Marin-Padilla, 1969; Jones, 1975). Some axons that give rise to several terminals of this type also have other terminals that contact adjacent dendrites. In other cases GAD-positive terminals, apparently arising from different axons, synapse with the same pyramidal cell.

Most terminals that form synapses with the axon hillocks and the proximal portions of the initial segments of pyramidal cell axons are GAD-positive. Few labelled terminals

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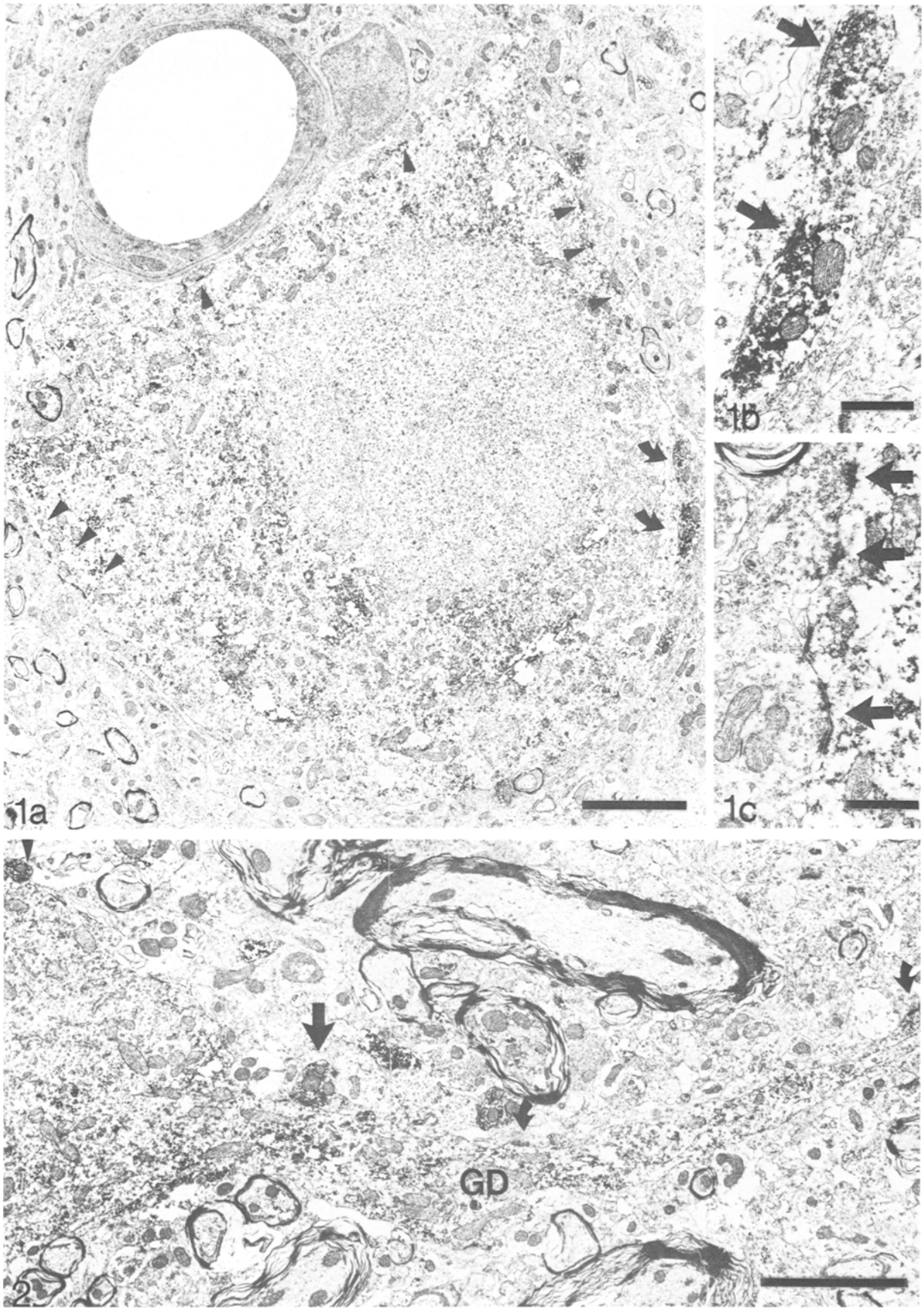
Fig. 1. (a) Large GAD-positive soma in layer V of area 3b receiving numerous synapses from unlabelled terminals (arrowheads and Fig. 1c) and from GAD-positive terminals (arrows and Fig. 1b). Scale bar: 4 μm. (b) Higher magnification of synapses on the large GAD-positive soma in Fig. 1a. The labelled terminals form symmetric synapses (arrows). Scale bar: 1 μm. (c) Asymmetric axosomatic synapses (arrows) formed by unlabelled terminals on the large GAD-positive soma in Fig. 1a. Scale bar: 1 μm.

Fig. 2. Large horizontally oriented GAD-positive dendrite (GD) originating from a large GAD-positive soma in layer V of area 3b. The dendrite and soma receive synapses from GAD-positive terminals (straight arrow, arrowhead). These synapses appeared symmetric at higher magnification. Unlabelled terminals (curved arrows) also contact the dendrite. Scale bar: 8 μm.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2

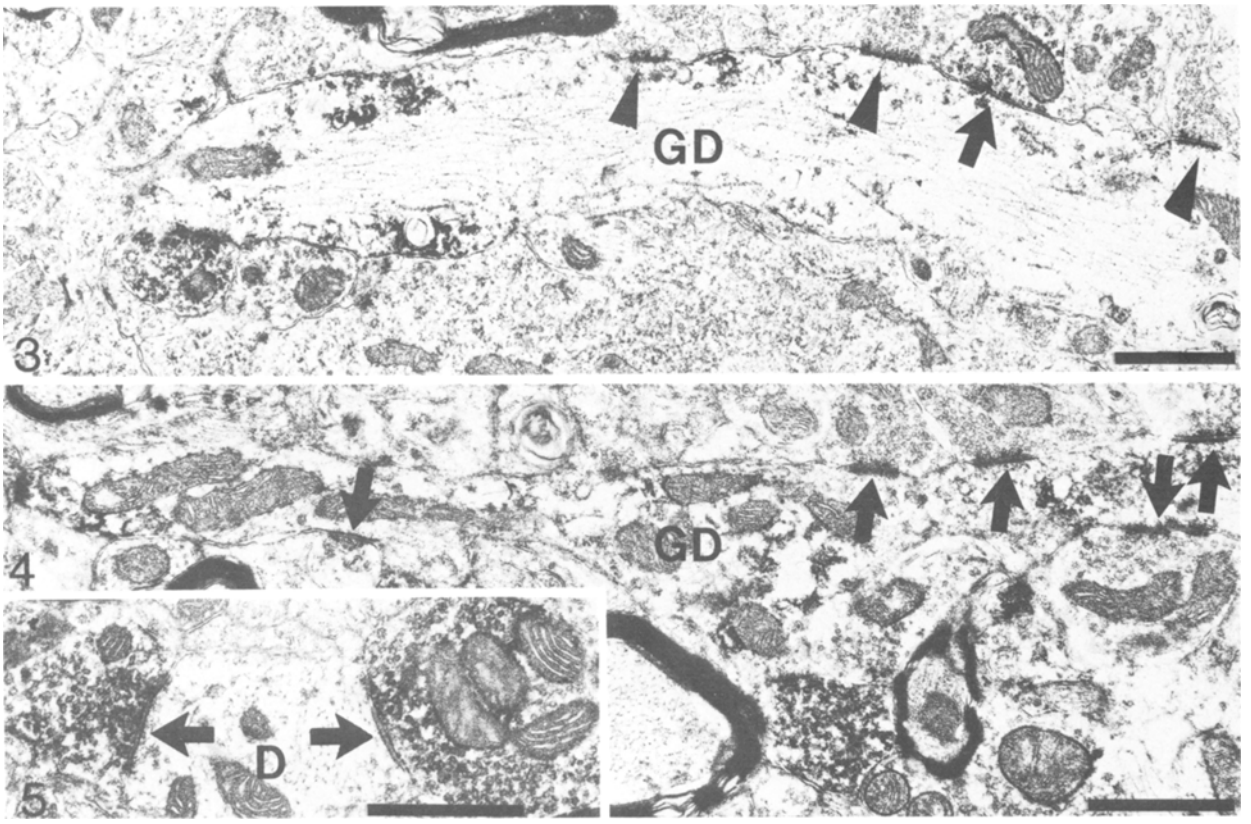


Fig. 3. A large GAD-positive dendrite (GD) in layer IIIB of area 1–2 receives asymmetric synapses (arrowheads) from unlabelled terminals and a symmetric synapse (arrow) from a lightly stained GAD-positive terminal. Other possible synapses, one of which is also GAD-positive, are seen at the left but membrane specializations cannot be seen clearly. Scale bar: 2 μm.

Fig. 4. Thin, varicose GAD-positive dendrite (GD) in layer IV of area 3b. The dendrite receives numerous asymmetric synapses (arrows) from unlabelled terminals. Scale bar: 2 μm.

Fig. 5. Small unlabelled dendrite (D) in layer IV of area 3b receiving symmetric synapses (arrows) from two GAD-positive terminals. The terminal on the left is lightly labelled and thus it is possible to visualize the synaptic vesicles, many of which are flattened. Scale bar: 1 μm.

synapse on axon hillocks, but the number synapsing on many initial segments is rather large (Fig. 7). Adjacent GAD-positive terminals that synapse with an initial segment of a pyramidal cell axon are found sometimes to be connected to the same parent axon. GAD-positive terminals synapsing with the initial segments of pyramidal cell axons also can make synaptic contacts with other processes, usually the shafts of unlabelled dendrites (Fig. 6). Thin, vertically oriented processes that possess the structural characteristics of axon initial segments, but which can not be traced to parent somata,

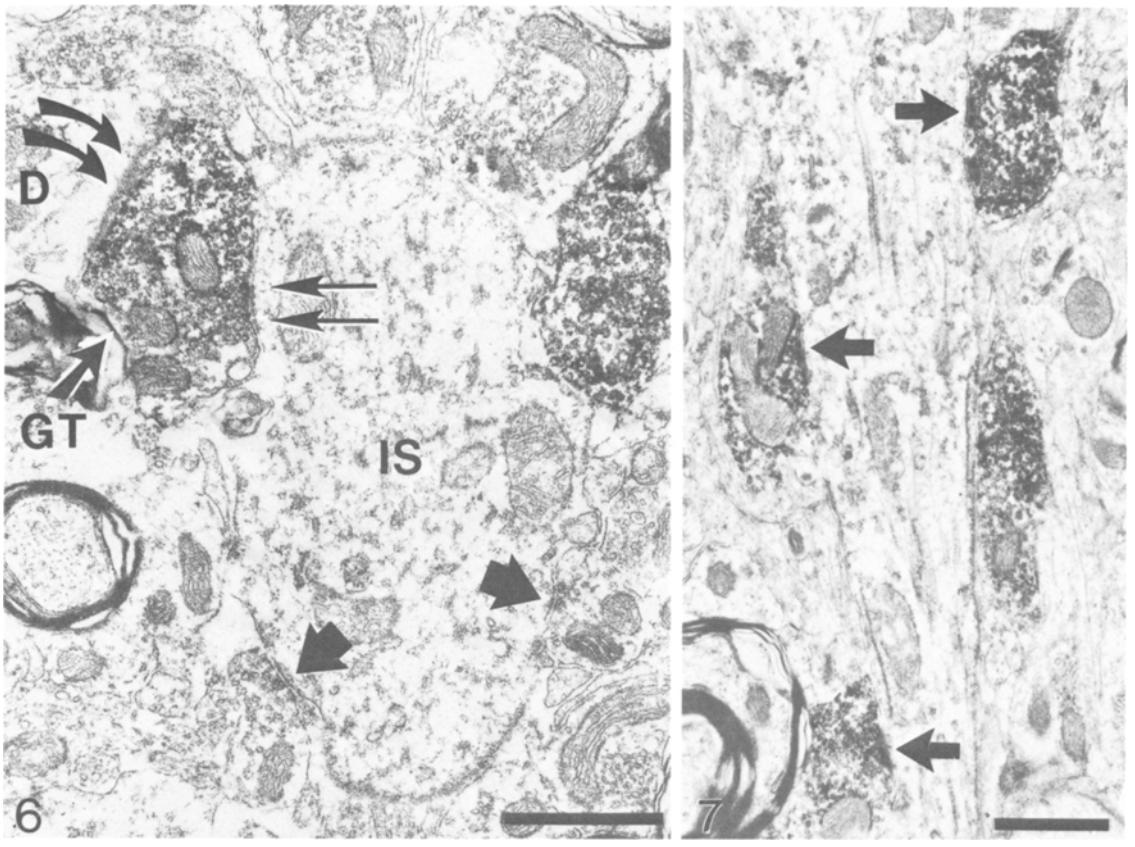


Fig. 6. Profile of an initial segment (IS) of a pyramidal cell axon in layer IIIB of area 3b. The initial segment receives symmetric synapses (double straight arrows) from a GAD-positive terminal (GT) and from two unlabelled terminals (single arrows). The GAD-positive terminal also forms a synapse (double curved arrows) with a dendritic shaft (D). Scale bar: 1 μm.

Fig. 7. Symmetric synapses (arrows) of GAD-positive terminals on the axon initial segment of a layer V pyramidal cell in area 4. Scale bar: 1 μm.

receive symmetric synapses from GAD-positive terminals in layers III and IV. These processes are interpreted as the distal parts of pyramidal cell axon initial segments. In some cases, rows of closely spaced GAD-positive terminals synapse with these isolated processes, but it could not be determined if the labelled terminals all arise from a single axon.

The apical dendrites of pyramidal cells also receive a substantial number of synaptic contacts from GAD-positive terminals (Fig. 10). The proximal portions of these dendrites, when observed in continuity with pyramidal cell bodies, are found to receive symmetric synapses from GAD-positive terminals at approximately the same density as their parent somata (Fig. 11). The GAD-positive terminals usually synapse at rather

widely separated intervals along the apical dendrites, although occasional clusters of labelled terminals are found on the proximal regions of the shafts. More distal portions of the apical dendrites appear to receive relatively fewer synapses from labelled terminals. The branches of apical dendrites and some of the dendritic spines arising from them (Fig. 9) or from the apical shafts themselves, are also contacted by GAD-positive terminals. These synapses are present in all layers. In layer I, GAD-positive terminals synapse on dendrites with features that are characteristic of those formed by the apical tufts of pyramidal cells (Jones & Powell, 1970a; Sloper, 1973) (Fig. 8). Basal dendrites of pyramidal cells also receive symmetric synapses from GAD-positive terminals, and the greatest density of these synapses occurs on the proximal regions of the basal dendrites.

Synapses on isolated dendritic shafts and spines

GAD-positive terminals throughout the depth of the monkey sensory-motor cortex synapse with many profiles of unlabelled dendritic shafts and spines that could not be traced to their parent somata. The largest dendritic shafts and many of the dendritic spines probably arise from pyramidal cells. The dendritic spines and a large population of small processes, either spines or thin dendrites, commonly receive both a symmetric synapse from a GAD-positive terminal and an asymmetric synapse from an unlabelled terminal in a single section (Figs. 9,14). Some terminals forming symmetrical synapses arise from thin, beaded, GAD-positive axons (Fig. 14) that give off multiple *en passant* terminals as they traverse the neuropil.

Synapses in layer IV

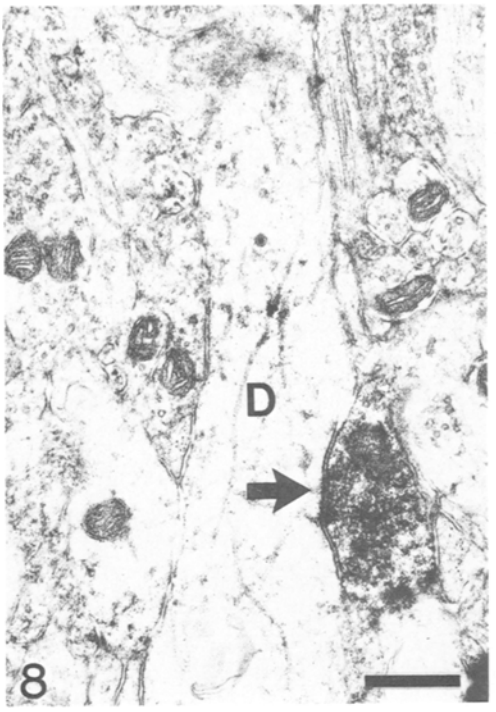
As described in the preceding paper (Houser *et al.*, 1983), layer IV of monkey SI contains a generally high, but variable density of GAD-positive terminals. Qualitatively, patches of increased terminal density are also apparent in the electron microscope. Both within and outside the dense patches, the majority of labelled terminals in layer IV synapse with isolated dendritic shafts of small size and with dendritic spines (Fig. 13).

Fig. 8. Vertically oriented, unlabelled dendrite (D) in layer I of area 3b. The dendrite may be part of the apical dendritic tuft of a pyramidal cell, and it receives a symmetric synapse (arrow) from a GAD-positive terminal. Scale bar: 3 μm.

Fig. 9. A side-branch (D) of an apical dendrite in layer IIIA of area 1–2 gives rise to a spine (S) that receives a symmetric synapse (arrowhead) from a GAD-positive terminal. Unlabelled terminals (arrows) form asymmetric synapses with the spine and with the dendritic shaft. Scale bar: 3 μm.

Fig. 10. Large apical dendrite (AD) of a pyramidal cell in layer V of area 1–2. Five GAD-positive terminals (arrows) could be seen at a higher magnification to form symmetric synapses with the dendrite. Scale bar: 10 μm.

Fig. 11. Pyramidal cell (PC) in layer V of area 3b with GAD-positive terminals near its soma (curved arrows) and its apical dendrite (straight arrows). Synapses appeared symmetric at higher magnification. Scale bar: 10 μm.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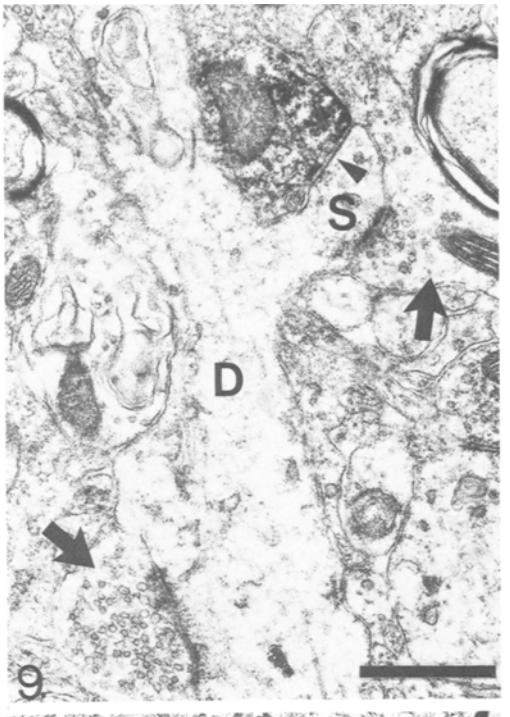
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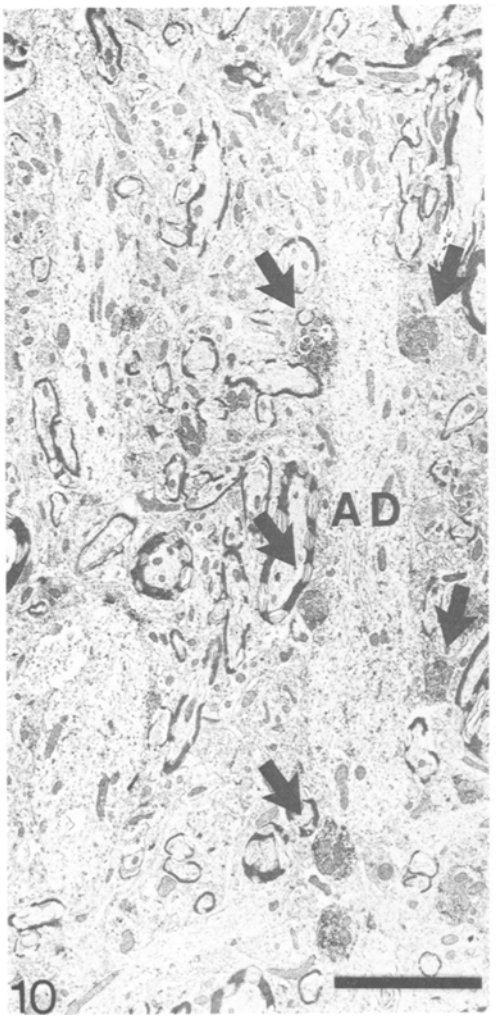
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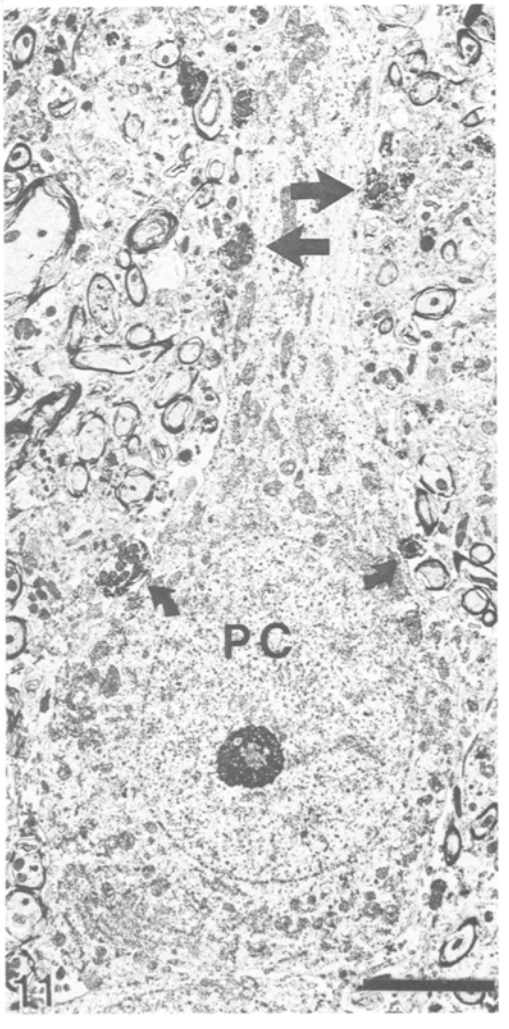
PC
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Other GAD-positive terminals in layer IV synapse on unlabelled neuronal somata (Fig. 12) with morphological features considered to be characteristic of non-pyramidal cells (Jones & Powell, 1970a; LeVay, 1973; Sloper, 1973).

GAD-POSITIVE MYELINATED AXONS

GAD-positive myelinated axons of various diameters (2–5 μm) are found in layers III–VI. Usually the largest axons are oriented horizontally while smaller ones are oriented obliquely or vertically. The myelinated internodes of some axons are found to be GAD-positive, but more commonly only the nodes of Ranvier and the terminals are labelled. In cases where the nodes of Ranvier form synapses, the postsynaptic elements are usually relatively small, unlabelled dendrites, although some large, unlabelled dendrites are also contacted (Fig. 20). Myelinated axons give rise to GAD-positive terminals that form symmetric synapses on somata of both pyramidal and non-pyramidal cells (Figs. 15,17). In most cases, the axosomatic synapses are made by two or more GAD-positive terminals connected by narrow unmyelinated axonal segments that are in turn connected to the myelinated parent axon. The pyramidal cell bodies receiving synapses from GAD-positive myelinated axons are large somata present in layers III and V (Fig. 15). Many non-pyramidal somata receiving such synapses are unlabelled and are found mainly in layer IV (Fig. 17), although large GAD-positive cell bodies in layers III–V also receive synaptic terminals from myelinated axons.

Many of the GAD-positive terminals that arise from myelinated axons synapse on dendritic shafts. Among these are large vertically oriented dendrites ascending through layers III–V that are probably the apical dendrites of layer V or layer VI pyramidal cells. Other large, unlabelled dendrites and many small, unlabelled dendrites in layers III–VI

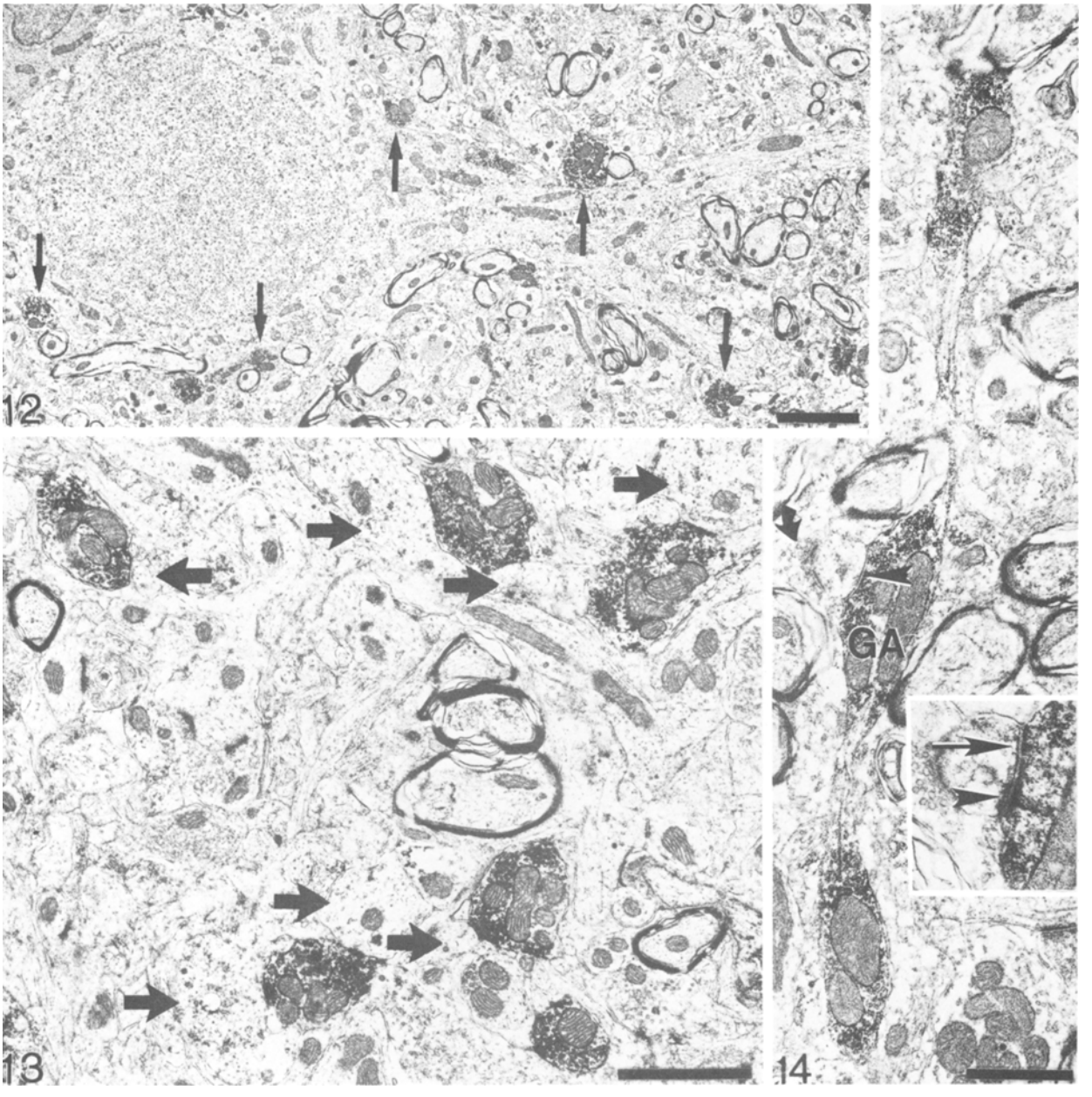
Fig. 12. Small, unlabelled non-pyramidal cell of layer IV in area 1–2. The GAD-positive terminals on its cell body and dendrites (arrows) could be seen at a higher magnification to form symmetric synapses. Scale bar: 3 μm.

Fig. 13. Symmetric synapses between GAD-positive terminals and several unlabelled dendritic shafts and spines (arrows) in layer IV of area 3b. Scale bar: 4 μm.

Fig. 14. Beaded, GAD-positive axon (GA) in layer IV of area 1–2 forming a symmetric synapse (arrowhead) with an unlabelled dendritic spine. The unlabelled spine also receives an asymmetric synapse (curved arrow) from an unlabelled terminal. Inset: serial section of the dendritic spine in Fig. 14 showing a higher magnification view of the symmetric synapse formed by the GAD-positive terminal (arrow) adjacent to a punctum adherens (arrowhead). Scale bar: 2 μm in Fig. 14; 3.5 μm in inset.

Fig. 15. Two GAD-positive terminals arising from a single myelinated axon (MA) synapse (arrows) with the soma of a pyramidal cell in layer V of area 1–2. Scale bar: 2 μm.

Fig. 16. Five symmetric synaptic contacts (arrows) are formed by the GAD-positive terminals of a single axon with the soma of a layer V pyramidal cell in area 1–2. The connecting axonal segments between the GAD-positive terminals are indicated by arrowheads. Scale bar: 1 μm.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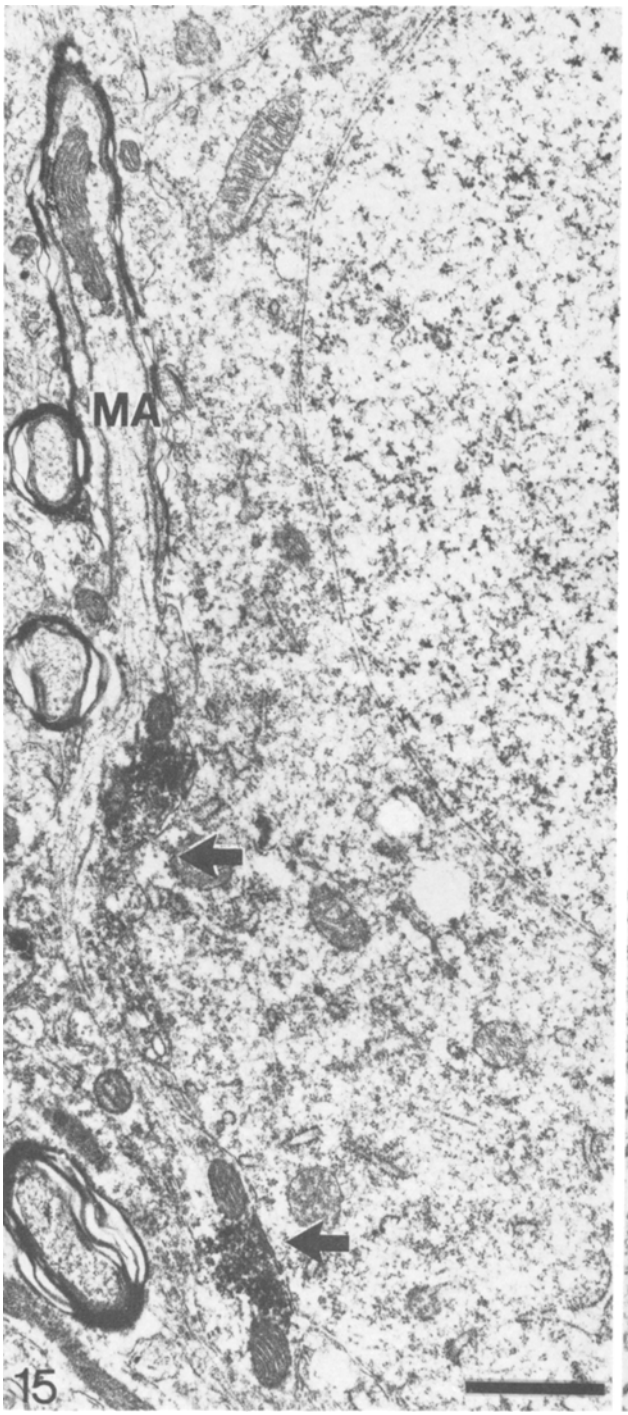
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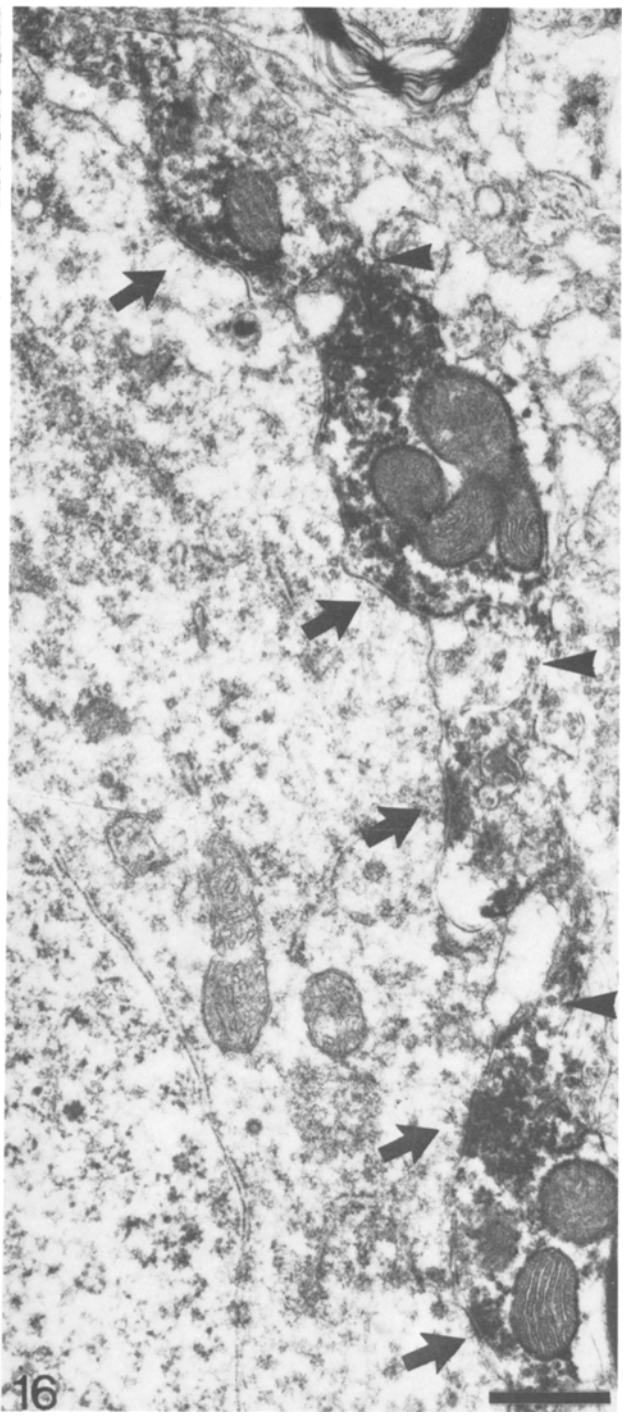
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also receive synapses from GAD-positive myelinated axons (Fig. 18). Some of the large dendrites are cut in transverse section and may be basal dendrites of pyramidal cells. The origin of most of the smaller dendrites is unknown, but some may be the thin, distal branches of pyramidal cell dendrites. However, in one case where a relatively thin dendrite receiving a synapse from a GAD-positive myelinated axon was traced to its

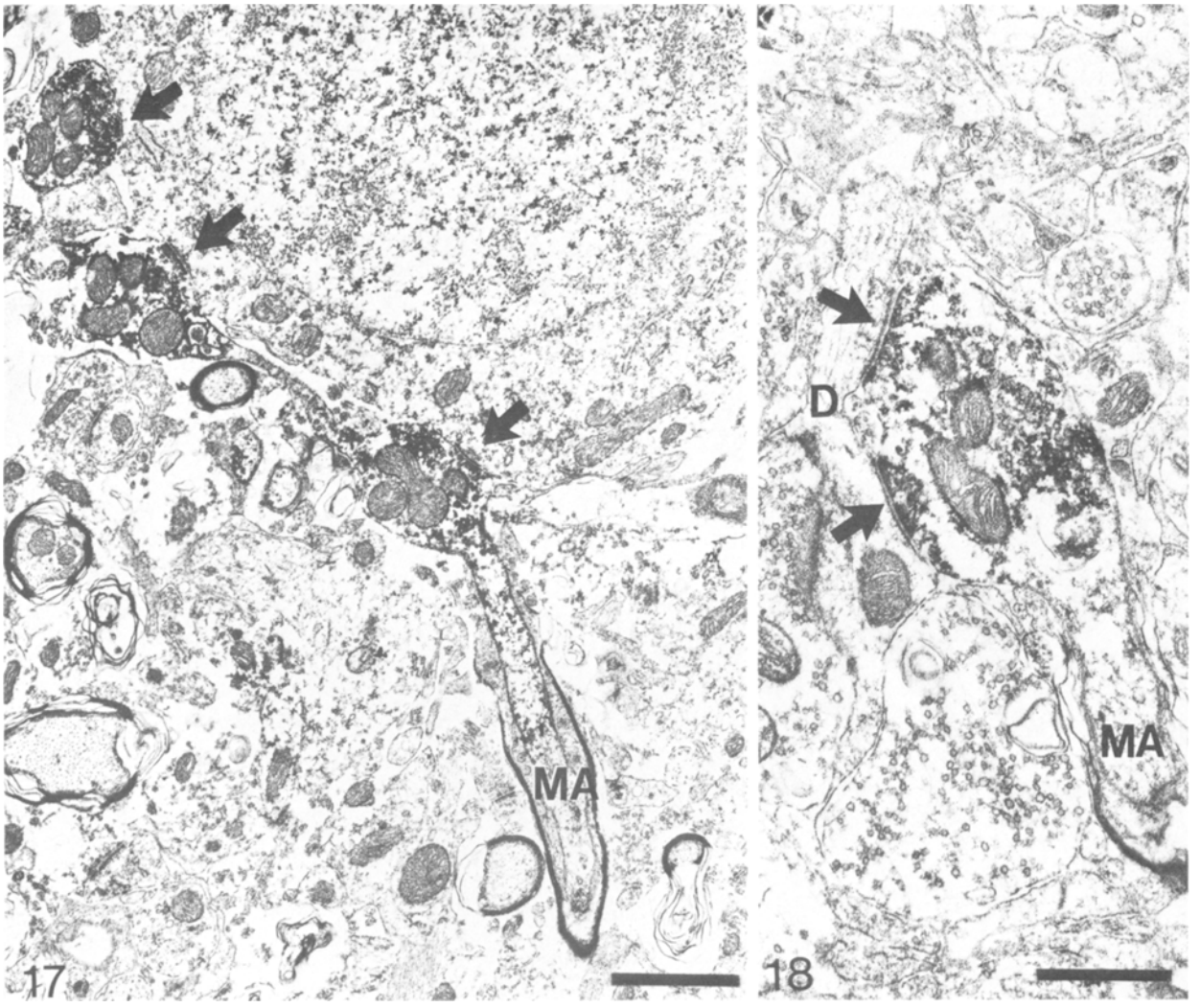

Fig. 17. The soma of an unlabelled non-pyramidal cell in layer IV of area 3b receives symmetric synapses (arrows) from GAD-positive terminals, two of which arise from the same myelinated axon (MA). Scale bar: 3 μm.

Fig. 18. Myelinated axon (MA) in layer IIIB of area 3b gives rise to a GAD-positive terminal that forms symmetric synapses (arrows) with a small unlabelled dendrite (D). Scale bar: 1 μm.

soma, it was found to be a descending dendrite of a small unlabelled layer IV non-pyramidal cell. The terminals of GAD-positive myelinated axons also contact GAD-positive dendrites (Fig. 19). The dendrites, found in layers III–VI, are sufficiently large to have originated from the class of large GAD-positive somata.

A single GAD-positive myelinated axon may synapse with different neuronal processes. For example, the one synapsing with the large GAD-positive dendrite in Fig.

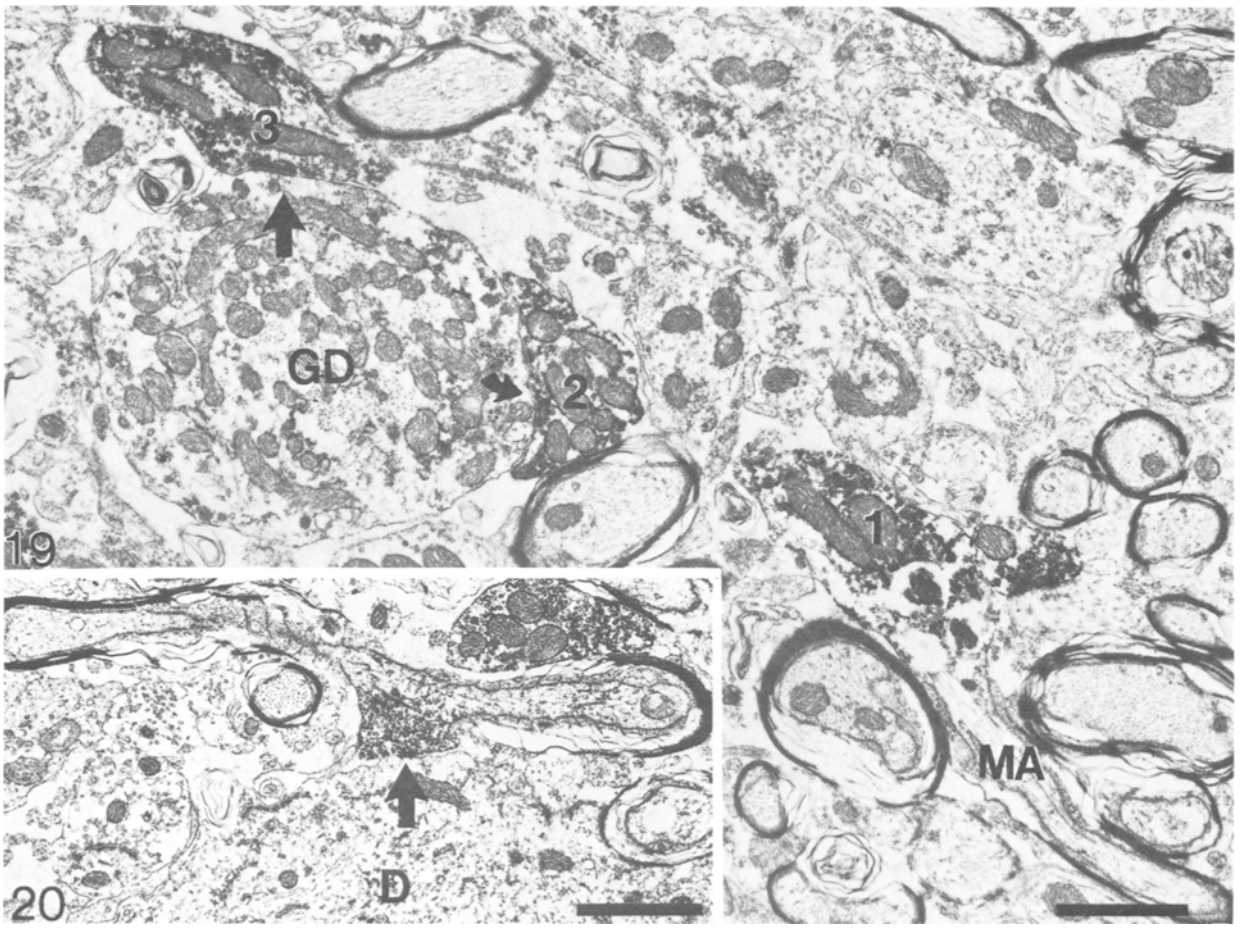

Fig. 19. Two GAD-positive terminals (2 and 3) in layer V of area 3b forming symmetric synapses (straight and curved arrows) with a large GAD-positive dendrite (GD) that was more heavily labelled in serial sections. Also in serial sections, terminal 3 was found to originate from the same myelinated axon (MA) as GAD-positive terminal 1. Terminal 2, however, arose from a different axon. Scale bar: 3 μm.

Fig. 20. A GAD-positive terminal arises from the node of Ranvier of a myelinated axon. The terminal forms a symmetric synapse (arrow) with a large unlabelled dendrite (D) in layer V of area 1–2. Scale bar: 3 μm.

19 also contacts other dendrites. When followed through a series of 40 sections, it is found to give rise to four terminals in addition to the one synapsing with the labelled dendrite. Two of the terminals each synapse on both a medium-sized and a small dendrite, while the other two synapse on small dendrites. All of these other dendrites are unlabelled.

SYMMETRIC SYNAPSES FORMED BY UNLABELLED TERMINALS

It appears that GAD-positive terminals form the vast majority of symmetric synapses in the monkey sensory-motor cortex. In many thin sections all terminals synapsing with the somata of pyramidal cells are found to be GAD-positive. However, in some sections it is possible to identify some unlabelled terminals forming symmetric synapses onto pyramidal cell bodies, initial segments of pyramidal cell axons (Fig. 6) or isolated dendrites. Such unlabelled terminals can be adjacent to other well-labelled terminals. It is possible that these terminals are truly GAD-negative and contain transmitters other than GABA. However, it must be emphasized that, in immunocytochemistry, technical problems such as incomplete penetration of large reagent molecules make interpretation of unlabelled elements extremely difficult.

Discussion

In previous studies (Hendry & Jones, 1981; Houser *et al.*, 1983) it was noted that GABA neurons of the monkey sensory-motor cortex are all non-pyramidal and form a heterogeneous population. The ultrastructural observations of GAD-positive neurons in the present study confirm and extend these light microscopic findings. They show that GABA neurons have axons that terminate on many different neuronal elements and in patterns that are typical of certain morphologically defined intrinsic cortical neurons. They also suggest that the GAD-positive neurons receive synapses from many sources, including other GAD-positive cells.

LARGE GAD-POSITIVE NEURONS – BASKET CELLS

In previous studies, the large intrinsic neurons of the monkey sensory-motor cortex that accumulate tritiated GABA or that contain GAD-positive reaction product were interpreted as being the basket cells defined in Golgi preparations (Hendry & Jones, 1981; Houser *et al.*, 1983). In agreement with the previous studies, large GAD-positive cell bodies were found in the present study in layers IIIB to VI, layers that contain the somata of basket cells in the monkey SI cortex (Jones, 1975). The results of the present study also show that the large GAD-positive cell bodies receive many symmetric and asymmetric synapses. This feature has been described as characteristic of the largest non-pyramidal cell somata in monkey cortex (Sloper, 1973).

The major distinguishing features of basket cells are their axons that give rise to 'pericellular nests' surrounding the somata and primary dendrites of pyramidal cells (Cajal, 1911; Marin-Padilla, 1969, 1971, 1974; Jones, 1975). The failure of basket cell axons

in adult animals to impregnate with the Golgi method beyond the initial segment (Jones, 1975) and the presence of myelinated axons arising from large multipolar non-pyramidal cells in rat visual cortex (Peters & Proskauer, 1980) and in monkey sensory-motor cortex (Sloper & Powell, 1979a) indicate that basket cells possess myelinated axons. It has also been shown that horseradish peroxidase-labelled myelinated axons in cat striate cortex form pericellular basket-like terminations on pyramidal cell bodies and proximal dendrites (Holländer & Vanegas, 1981). The findings of the present study indicate that myelinated axons forming pericellular basket terminations on the somata and dendrites of pyramidal cells are GAD-positive, lending further support to the suggestion (Hendry & Jones, 1981; Houser *et al.*, 1983) that basket cells are a class of GABAergic cortical intrinsic neuron.

Basket cell axons have been interpreted as terminating only on pyramidal cells in the human cerebral cortex (Marin-Padilla, 1969, 1971, 1974). However, the terminations of GAD-positive myelinated axons on the somata and dendrites of non-pyramidal cells may indicate that basket cell terminations are more heterogeneous. An alternative possibility is that the GAD-positive myelinated axons arise from more than one class of intrinsic neuron. Some classes of Golgi-impregnated non-pyramidal neurons in the visual cortex of rats, cats and monkeys have been found to possess myelinated axons, but unlike GAD-positive axon terminals they form asymmetric synapses (Peters & Kimerer, 1981; Somogyi & Cowey, 1981). Thus at present it would appear that basket cells may be the major, possibly the sole, class of GAD-positive neuron within the cerebral cortex to possess myelinated axons.

Light microscopic observations of Golgi-stained preparations from human cerebral cortex have led some investigators to suggest that extrinsic afferent axons also contribute to the pericellular baskets terminating around pyramidal cell somata (Cajal, 1911; Marin-Padilla, 1972). However, the somata of pyramidal cells receive exclusively symmetric synapses (e.g. Jones & Powell, 1970a; Peters & Kaiserman-Abramof, 1970; LeVay, 1973; Sloper, 1973), and all cortical afferents studied to date terminate in asymmetric synapses (e.g. Jones & Powell, 1970b; Sloper & Powell, 1979b; LeVay & Sherk, 1981). Furthermore, evidence from the present study indicates that the vast majority of the terminals forming axosomatic synapses with pyramidal cells are GAD-positive, and the results from undercutting the cortical grey matter indicate that no extrinsic afferent system is GABAergic (see above). It is likely, then, that the axons interpreted previously as extrinsic afferents are instead the ascending branches of basket cell axons.

GABAergic synaptic transmission has been implicated in the inhibition between neighbouring columns of cells in the cat visual cortex, and this inhibition is thought to be a major factor in orientation selectivity (Sillito, 1975, 1979). Similarly, in the monkey somatic sensory cortex, activation of one column in response to peripheral stimulation is accompanied by inhibition of neighbouring columns (Mountcastle & Powell, 1959). Intercolumnar inhibition of this type has been proposed to be a function of the cortical basket cells (Jones, 1981). These cells and their axons are oriented perpendicular to the

central sulcus (Marin-Padilla, 1974; Jones, 1975) and extend horizontally for more than a millimetre (Marin-Padilla, 1969, 1971, 1974; Jones, 1975) before terminating (Gatter *et al.*, 1978). Evidence from the present study indicates that the GAD-positive myelinated axons presumed to be from basket cells terminate in large numbers on the cell bodies and dendrites of both pyramidal and non-pyramidal neurons. The basket cells in one column appear to be in an optimal position to influence the activity of both the pyramidal (output) and the intrinsic neurons in neighbouring columns. Not only do basket cells appear to receive the terminations of extrinsic afferents (Sloper & Powell, 1979b; Shanks & Powell, 1981), but also their myelinated axons probably have relatively fast conduction velocities, thus ensuring that intercolumnar inhibition would occur rapidly.

SMALL GAD-POSITIVE NEURONS – SEVERAL TYPES

As discussed in the preceding paper (Houser *et al.*, 1983), several classes of small non-pyramidal neurons appear to be GAD-positive. Substantial evidence exists to suggest that the chandelier cells of Szentágothai & Arbib (1974) are GAD-positive (Peters *et al.*, 1982) and that they give rise to axons that terminate in vertical rows on the distal parts of the initial segments of pyramidal cell axons (Somogyi, 1977; Fairén & Valverde, 1980; Peters *et al.*, 1982). In the present study, rows of GAD-positive axon terminals typical of chandelier cell axon terminals also were observed on parts of initial segments apparently at some distance from axon hillocks. On parts of initial segments nearer to axon hillocks, GAD-positive terminals often did not form rows, and some synapsed with both the initial segment and with adjacent dendritic shafts. Therefore it appears likely that the axons of at least two classes of GAD-positive neurons synapse on the axon initial segments of some pyramidal cells; the chandelier cell axon distally and a second, as yet unidentified, type proximally. Analysis of the somal size, laminar distribution and dendritic morphology of some small GAD-positive neurons led to the suggestion that a class of cortical intrinsic neuron called spider web (Cajal, 1911), clewed (Valverde, 1971) or type 5 (Jones, 1975) may also be GAD-positive (Houser *et al.*, 1983). Axons of these cells have been described as highly beaded. Beaded GAD-positive axons were found in the present study to contact by multiple *en passant* synapses the shafts and spines of unlabelled dendrites mainly in layer IV. These axons may arise from spider-web cells, but any firm conclusion must await a direct ultrastructural correlation of GAD-positive axons with morphologically identified spider-web cell axons.

SYNAPSES OF GAD-POSITIVE TERMINALS ON NON-PYRAMIDAL CELLS

In SI, GAD-positive terminals synapse in rather large numbers on non-pyramidal cells of layer IV that are unlabelled in our material. Although there is a possibility that some of these cells are GABAergic neurons that failed to stain due to limited access to colchicine or to insufficient penetration of the various immunocytochemical reagents, it appears likely that at least some of the unlabelled somata in layer IV do not contain GAD and, thus, use a neurotransmitter other than GABA. The presence of a population of

non-GABAergic neurons in layer IV of monkey SI is consistent with evidence showing that approximately half the layer IV neurons in this area do not accumulate tritiated GABA (Hendry & Jones, 1981). Such cells may consist mainly of the population of spiny non-pyramidal cells of layer IV – type 7 cells (Jones, 1975). Spiny non-pyramidal cells have been shown to be a major target of thalamocortical axons in mouse SI and in rat visual cortex (Peters *et al.*, 1979; White & Rock, 1980). In the monkey, these cells send narrow bundles of axons vertically into the supragranular layers (Szentágothai, 1973; Jones, 1975), and terminate in asymmetric synapses on the apical dendrites of pyramidal neurons (LeVay, 1973). Inhibition of these spiny non-pyramidal cells of layer IV by GABAergic interneurons might, therefore, suppress the vertical transmission of thalamic input through the cortex. In the present study, GAD-positive myelinated axons were found to terminate on profiles typical of the spiny cells, suggesting that one class of neuron that may produce such a suppression is the large basket cell. However, the beaded unmyelinated GAD-positive axons, which arise from other cells, are also possible sources of terminations on the spiny non-pyramidal cells.

In agreement with the results of previous studies (Ribak, 1978; Houser *et al.*, 1983), GAD-positive terminals were found to synapse with both large and small GAD-positive neurons. Since physiological studies have demonstrated that GABA has inhibitory influences in the cerebral cortex (Krnjević, 1974), the presence of large numbers of synapses between GAD-positive terminals and the somata and dendrites of GAD-positive neurons would suggest the existence of inhibitory inputs to interneurons that are themselves inhibitory. This would provide a substrate for intracortical disinhibition. Synapses between inhibitory neurons in the cortex would be expected from intracellular recordings which show that all cortical neurons, including presumed inhibitory ones, display disynaptic IPSPs in response to afferent stimulation (Watanabe *et al.*, 1966; Toyama *et al.*, 1974). The type of GAD-positive cells giving rise to axons that terminate on other GAD-positive cells is not fully known, but evidence from the present study indicates that some GAD-positive myelinated axons, suggested to be from basket cells, may terminate on other GAD-positive basket cell dendrites and somata.

MULTIPLE GABAERGIC INPUTS

Data from previous studies (Ribak, 1978; Peters *et al.*, 1982; Houser *et al.*, 1983) and from the present study indicate that individual cortical neurons, particularly pyramidal cells, probably receive axonal terminations from several classes of GAD-positive intrinsic neurons. The terminations on pyramidal cells of two such types of neurons, the basket cells and the chandelier cells, is fairly clear, and other classes of GAD-positive neurons also may synapse with pyramidal cells. As yet there is no information that different GABAergic neurons might exert differential effects on their target neurons. However, it has been suggested that, in the cat and monkey visual cortex, several receptive field properties such as orientation selectivity, direction selectivity and the hypercomplex property (end-stop inhibition) are produced by separate intracortical inhibitory mechanisms (Schiller *et al.*, 1976; Hammond, 1978; Orban *et al.*, 1979; Bishop *et al.*, 1980),

each of which may be GABA mediated (Sillito, 1975, 1977, 1979). Analogous receptive field properties such as selectivity for the orientation of a stimulus and the direction of its movement across the skin have been reported in neurons of the monkey SI (Whitsel *et al.*, 1972; Costanzo & Gardner, 1980). These properties are due to inhibitory mechanisms (Gardner & Costanzo, 1980) possibly of intracortical origin. It may be, therefore, that separate classes of inhibitory GABAergic cortical neurons are activated under different stimulus conditions, and this may lead to differential effects on target neurons. It would be important, therefore, to determine if the different types of receptive field properties are the result of a selective distribution of particular GABAergic axon types or to differences in the number or efficiency of these synapses on a particular recipient cell type.

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