Brain Research, 126 (1977) 1–18

Research Reports

GLUTAMATE DECARBOXYLASE LOCALIZATION IN NEURONS OF THE OLFACTORY BULB*

CHARLES E. RIBAK, JAMES E. VAUGHN, KIHACHI SAITO, ROBERT BARBER and EUGENE ROBERTS

Division of Neurosciences, City of Hope National Medical Center, Duarte, Calif. 91010 (U.S.A.)

(Accepted August 13th, 1976)

SUMMARY

Glutamate decarboxylase (GAD), the enzyme that synthesizes the neurotransmitter γ-aminobutyric acid (GABA), has been localized in the rat olfactory bulb by immunocytochemical methods with both light and electron microscopy. The light microscopic results demonstrated GAD-positive puncta concentrated in the external plexiform layer and in the glomeruli of the glomerular layer. In addition, GAD-positive reaction product stained the dendrites and somata of granule and periglomerular cells. The electron microscopic observations confirmed the presence of GAD-positive reaction product within granule and periglomerular somata and dendrites. In electron micrographs of the external plexiform layer, the gemmules which arise from the distal dendrites of granule cells were also observed to be filled with reaction product, and these structures corresponded in size and location to the puncta observed in light microscopic preparations. The gemmules were observed to form reciprocal dendrodendritic synaptic junctions with mitral cell dendrites which lacked reaction product. In the glomeruli, GAD-positive reaction product was observed in the dendritic shafts and gemmules of periglomerular cells which also formed reciprocal dendrodendritic synaptic contacts with mitral/tufted cell dendrites. The localization of GAD in known inhibitory neurons of the olfactory bulb supports the case that these local circuit neurons use GABA as their neurotransmitter.

The present study demonstrates that GAD molecules located within certain neuronal somata and dendrites can be visualized with antisera prepared against GAD that was purified from synaptosomal fractions of mouse brains. This finding suggests

* A part of this study was presented at the 89th annual session of the American Association of Anatomists in Louisville, Ky., April, 1976.

that the lack of GAD staining within somata and dendrites of GABA-ergic neurons noted in previous studies of the cerebellum and spinal cord was probably due to low GAD concentrations, rather than to antigenic differences among GAD molecules located in different portions of the neuron. A striking difference between the granule and periglomerular neurons of the olfactory bulb and the neurons of the cerebellum and spinal cord is that the former have presynaptic dendrites while the latter do not. Since GAD-positive reaction product can be detected in the somata and dendrites of GABA-ergic neurons which have presynaptic dendrites, it is suggested that these neurons may differ from other GABA-ergic neurons with respect to either transport or metabolism of GAD.

INTRODUCTION

Glutamate decarboxylase (GAD), the synthetic enzyme of the neurotransmitter γ-aminobutyric acid (GABA), has been shown to exhibit different activities within the various layers of the olfactory bulb[8]. High levels of GAD activity parallel high concentrations of GABA in the external plexiform layer (EPL), the glomerular layer (GL) and the granule cell layer (GRL). Physiological recordings from mitral cells following stimulation of either the olfactory nerves or the lateral olfactory tract have indicated that the granule and periglomerular cells inhibit the mitral/tufted cell dendrites located in the EPL and GL[7,19,21,29,34]. In addition, the GABA antagonists picrotoxin and bicuculline block the inhibitory effect produced by the granule and periglomerular cells[16,19]. Thus, the data from these studies suggest that both the granule and periglomerular cells of the olfactory bulb are inhibitory interneurons which use GABA as their neurotransmitter.

In the present study, the distribution of GAD in the olfactory bulb has been investigated employing immunocytochemical methods[2,13–15,30–32,36]. The results provide ultrastructural evidence that GAD is present within granule and periglomerular cells, thereby supporting the evidence that these neurons are GABA-ergic.

MATERIALS AND METHODS

Tissue preparation

Adult, Sprague–Dawley rats were anesthetized by intraperitoneal injections of chloral hydrate and were then fixed via intracardiac perfusions[20] with a solution containing 4.0% paraformaldehyde, 0.4% glutaraldehyde (Polysciences) and 0.002% $CaCl_2$ in 0.12 *M* Millonig's[17] phosphate buffer, at pH 7.2 and 37 °C. The olfactory bulbs were dissected from the animals' skulls the following day. Olfactory bulbs from one side of each animal were immersed overnight in a cryoprotectant, 30% sucrose solution. Following rapid freezing, horizontal frozen sections of these specimens were cut at 40 μm on a cryostat[2]. The sections obtained were processed for light microscopic immunocytochemistry. The remaining olfactory bulbs were cut horizontally at 150 μm using a Sorvall TC-2 tissue sectioner. The sections were placed in the phosphate buffer and then processed for electron microscopic immunocytochemistry as described below.

Immunocytochemical procedure

The immunocytochemical procedure employed for specimens used in both light and electron microscopy was similar to that previously described[2,13–15,32,36]. Briefly, sections were incubated in normal rat serum for 30 min and then rinsed in buffer before being incubated for 30 min in either rabbit anti-GAD serum or control rabbit serum. Following a 2.5 h buffer wash, the sections were incubated 30 min in goat antirabbit serum (Antibodies, Inc., Davis, Calif.). Sections were then washed in buffer for 2.5 h, incubated in a peroxidase–antiperoxidase Fab complex[3] for 30 min, and washed again in buffer for 2.5 h before being reacted with 3,3′-diaminobenzidine · 4HCl (Sigma, St. Louis, Mo.) and H_2O_2. The sections for electron microscopy were incubated in the immunocytochemical reagents twice as long as were those for light microscopy.

Morphological preparations

Following the immunocytochemical reactions, the sections for light microscopy were poststained 30 sec in 0.1% aqueous OsO_4 and mounted on glass slides. For electron microscopy, blocks of immunocytochemically treated olfactory bulbs were postfixed 1 h with 2% OsO_4 in 0.12 *M* phosphate buffer[17] at pH 7.2. These specimens were stained en bloc with aqueous uranyl acetate, dehydrated in ethanols followed by propylene oxide and then embedded in Epon–Araldite. The electron microscopic preparations contained all laminae of the olfactory bulbs. Ultrathin sections were cut and randomly mounted on Formvar-coated slot (1 mm × 2 mm) grids for a general study of the ultrastructural localization of GAD in the olfactory bulb. In addition, serial, ultrathin sections were taken for a more detailed analysis of the synaptic relationships of GAD-positive profiles in the glomerular layer. Ribbons of 10 sections each were picked up onto Formvar-coated slot grids, and 100 serial sections were mounted on a total of 10 grids for this glomerular layer analysis. Both random and serial sections were poststained with lead citrate before examination with a Hitachi HU-11B electron microscope.

RESULTS

A densely stained band of reaction product was observed in the external plexiform layer (EPL) in light microscopic preparations of olfactory bulbs that had been incubated in anti-GAD serum. This band merged superficially into an intermittent band of reaction product in the glomerular layer (GL) which filled the interstices of the glomeruli (Fig. 1). The olfactory nerve layer (ONL) was observed to be free of GAD-positive reaction product, but staining was observed in the granule cell layer (GRL). At higher magnifications these loci of reaction product were characterized by numerous GAD-positive puncta which have been shown to correspond to synaptic terminals containing GAD-positive reaction product[2,13,14,32,36]. Some GAD-positive puncta were observed in the GRL, but the highest density was found in the GL and EPL. In some instances the puncta in the EPL appeared to line up adjacent to longitudinally sectioned, mitral cell secondary dendrites (Fig. 4).

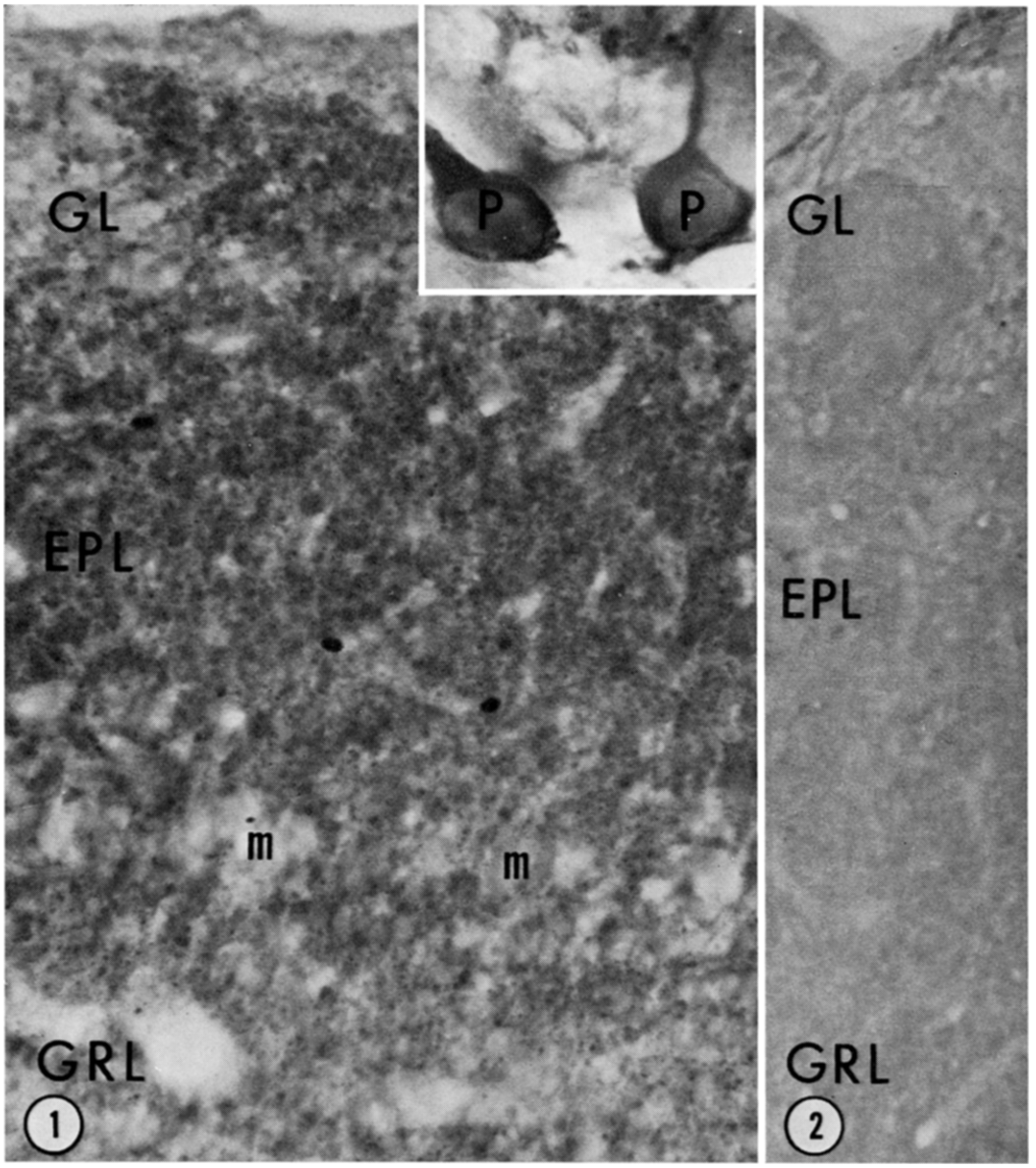
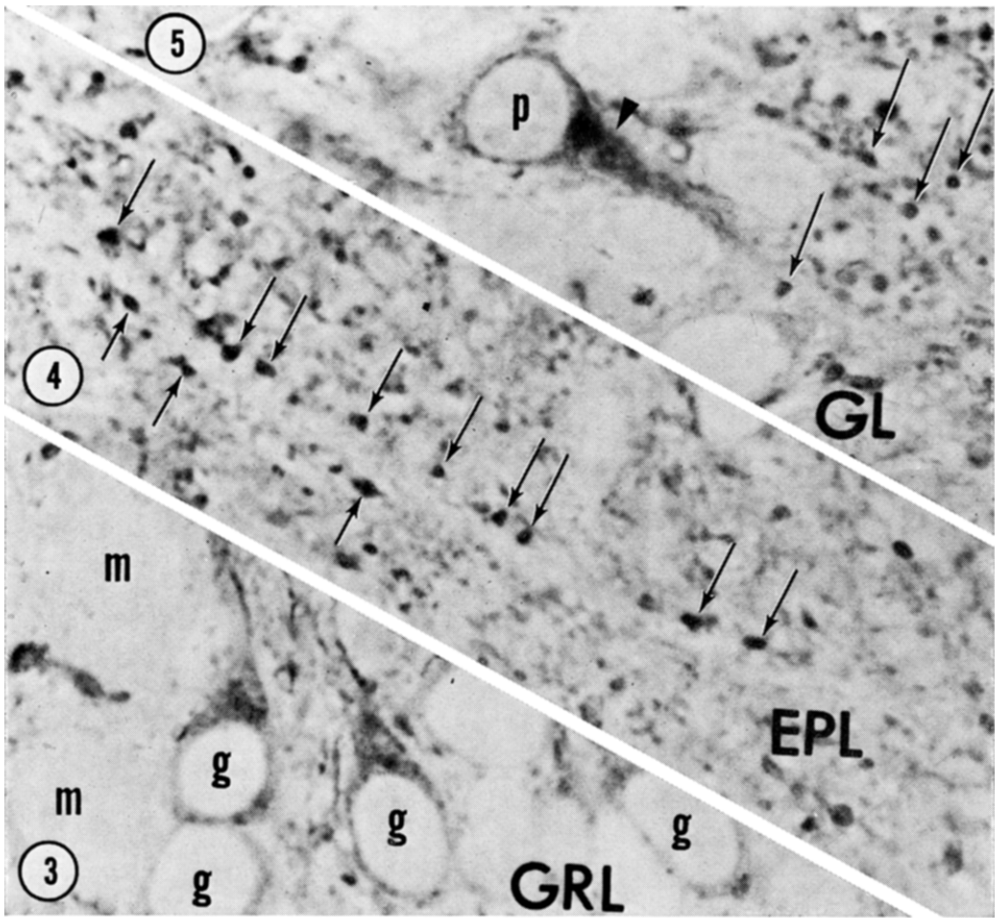


Fig. 1. Frozen section of olfactory bulb incubated in anti-GAD serum showing dense deposits of reaction product in the external plexiform layer (EPL) and within a glomerulus of the glomerular layer (GL). The granule layer (GRL) also shows some staining. At this magnification the reaction product within the somata and dendrites of granule and periglomerular cells is not resolvable. However, the inset shows two, GAD-positive periglomerular cells (P) at a higher magnification ($\times$ 1500). The larger, mitral cell somata (m) appear to be free of reaction product. $\times$ 500.

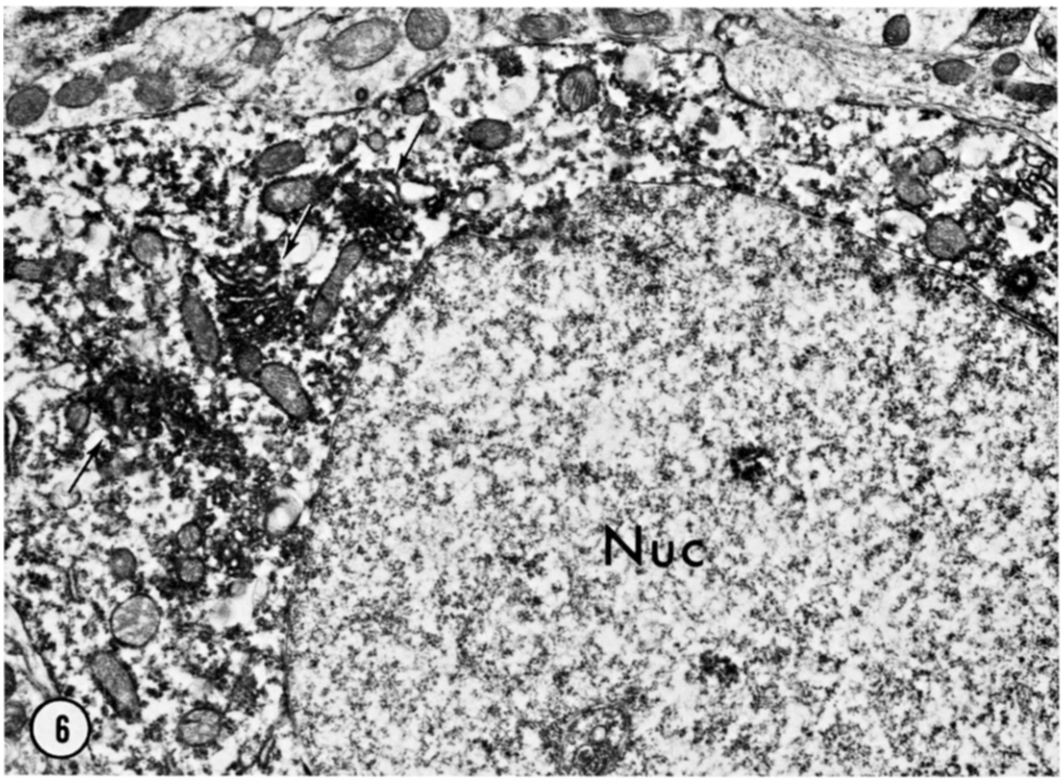
Fig. 2. A frozen section of olfactory bulb incubated in control rabbit serum lacks specific staining. The layers of the olfactory bulb are indicated as they were in Fig. 1. $\times$ 500.

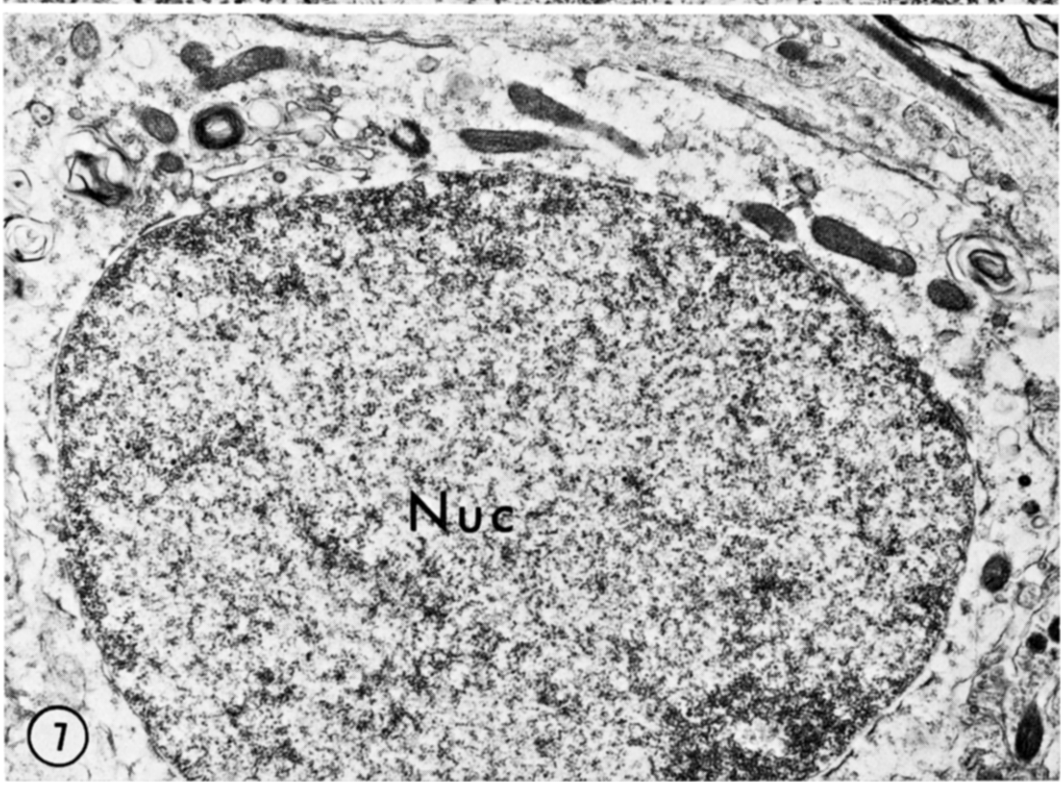


Figs. 3–5. Semithin (1 μm) sections of various layers of the olfactory bulb from a tissue slice incubated in anti-GAD serum.
Fig. 3 shows reaction product within the somata and dendrites of granule cells (g) in the granule cell layer (GRL). The adjacent somata of mitral cells (m) do not exhibit this staining. × 2000.
Fig. 4. illustrates GAD-positive puncta (arrows) lined up adjacent to a longitudinally sectioned, mitral cell secondary dendrite in the external plexiform layer (EPL). × 2000.
Fig. 5 demonstrates somal and dendritic staining in a periglomerular cell (p) in the glomerular layer (GL). The GAD-positive dendrite (arrowhead) is directed toward a glomerulus which contains many GAD-positive puncta (arrows). × 2000.

GAD-positive reaction product was also concentrated within the dendrites and somata of granule and periglomerular neurons in the GRL and GL, respectively (Figs. 1, 3 and 5). In contrast, the somata and dendrites of mitral and tufted cells lacked reaction product. Finally, there was no specific staining within somata, dendrites or punctate structures in sections of olfactory bulb incubated in control serum (Fig. 2).

Observations made with the electron microscope confirmed the presence of GAD-positive reaction product within the somatic and dendritic cytoplasm of granule (e.g., Fig. 6) and periglomerular (e.g., Fig. 13) cells. In addition, the cell types


Nuc
6


Nuc
7

of origin of the GAD-positive puncta in the EPL and the glomeruli were identified in electron microscopic preparations using both random and serial section analysis (see below).

Electron microscopic analysis of the granule cell layer (GRL)

Most of the granule cells contained reaction product in their somata and proximal dendrites in thin sections of the GRL from specimens incubated in anti-GAD serum. The highest concentration of somal reaction product occurred upon the surfaces of the cisternae and small vesicles of the Golgi apparatus (Fig. 6). In addition, substantial GAD-positive reaction product was observed along the surfaces of mitochondria and microtubules. Some reaction product was also associated with the granular endoplasmic reticulum, but this staining was minor in comparison to that of the Golgi apparatus, mitochondria and microtubules.

Somata and dendrites of the short axon cells of the granule cell layer[28] and of the mitral cells[28] did not exhibit reaction product in specimens incubated in anti-GAD serum. Granule cell bodies and dendrites were also free of reaction product in specimens incubated in control rabbit serum (see Figs. 7 and 10).

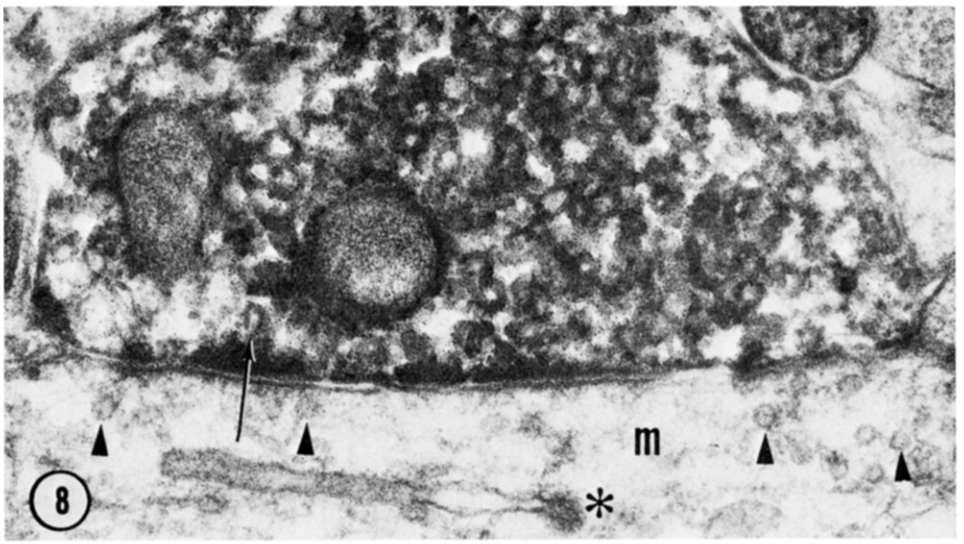
Electron microscopic analysis of the external plexiform layer (EPL)

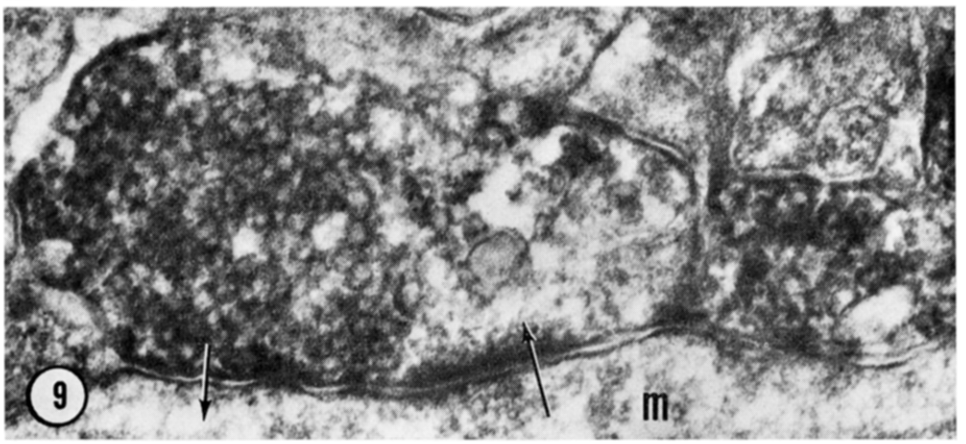
The dendrites of granule cells have been described by many authors[1,4,9,25,26,29,34]. The peripheral dendrites of granule cells ascend into the EPL and bear a number of spine-like structures, or gemmules, upon their surfaces. Electron microscopic analyses have shown that these dendritic gemmules contain flattened synaptic vesicles and form reciprocal synaptic junctions with the shafts of mitral cell dendrites.

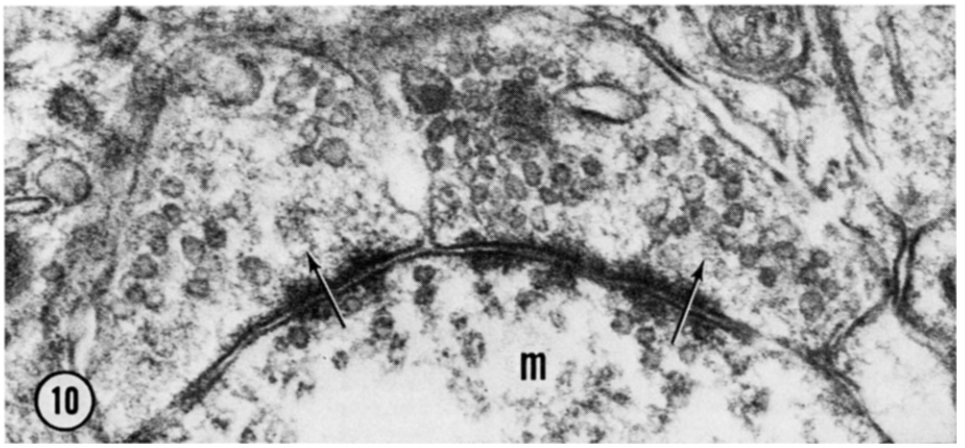
Granule cell gemmules were observed to be filled with GAD-positive reaction product in random, thin sections of the EPL from specimens incubated in anti-GAD serum, (e.g., Figs. 8, 9, 11 and 12). These GAD-positive gemmules were frequently apposed to mitral cell dendrites which contained round synaptic vesicles aggregated at the presynaptic membranes. The GAD-positive gemmules often showed a differential distribution of reaction product. For example, in Fig. 9, a GAD-positive gemmule is shown forming a reciprocal synapse with a mitral cell dendritic shaft. GAD-positive reaction product appears to be concentrated in the part of this gemmule where the synaptic vesicles are clustered (the granule-to-mitral synapse), and appears to be absent from the area of the gemmule where the mitral-to-granule synapse occurs. The mitral-to-granule synapse has no GAD-positive reaction product associated with the round synaptic vesicles aggregated at the presynaptic membrane of the mitral dendrite.

Fig. 6. Electron micrograph of olfactory bulb incubated in anti-GAD serum showing GAD-positive reaction product within the soma of a granule cell. The highest concentration of somal reaction product occurs around the Golgi complex (arrows). The nucleus (Nuc) is free of reaction product. × 16,000.

Fig. 7. Electron micrograph of olfactory bulb incubated in control rabbit serum. The large nucleus (Nuc) of a granule cell occupies most of the field. Reaction product is lacking in the somal cytoplasm. × 16,000.


m
*
8


m
9


m
10

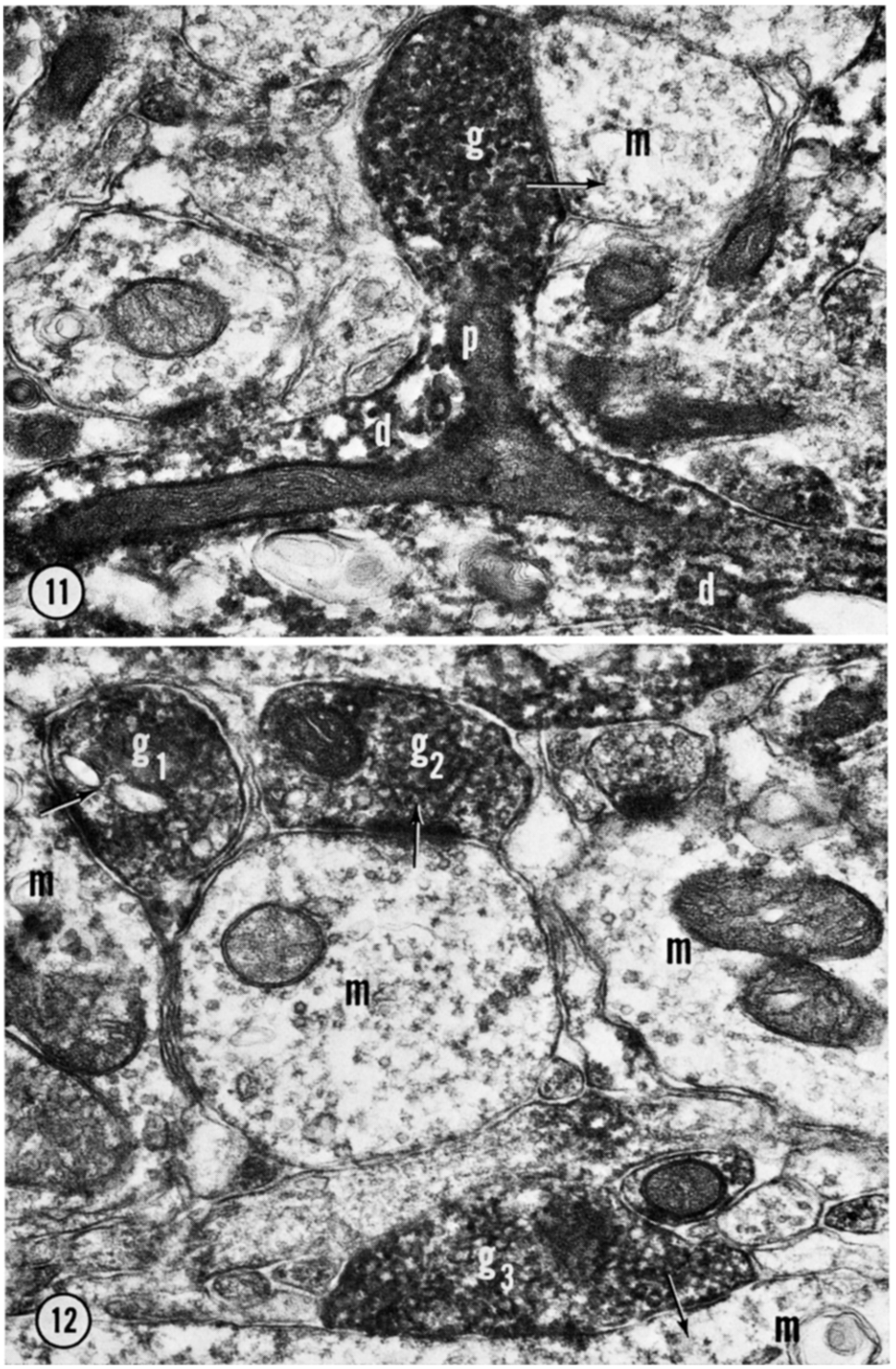
GAD-positive gemmules in the EPL have been observed in continuity with granule cell dendrites which also contain reaction product. In Fig. 11, for example, GAD-positive reaction product is concentrated around the synaptic vesicles in the gemmule and is also distributed along the surfaces of the mitochondria and microtubules in the dendritic shaft. The mitochondrion in Fig. 11 branches into the pedicle of the gemmule, and this funneling of dendritic organelles into gemmules has been described as being typical of granule cell dendrites[20]. The radial orientation of this dendrite to the surface of the olfactory bulb is consistent with the descriptions of the preferred orientation of the peripheral dendrites of granule cells. Thus, the synaptic relationships of the GAD-positive gemmules, their origin from the pedicles of GAD-positive dendrites, and the orientation of the dendrites from which they originate, all indicate that these gemmules belong to granule cells.

Electron microscopic analysis of the glomerular layer (GL)

The glomerular layer is organized into cellular and neuropil compartments which have been referred to as the periglomerular and the glomerular regions, respectively[22]. Pinching and Powell[22–24] have described the constituents of these regions and their results provide the framework for the present analysis. The periglomerular region contains the somata of external tufted cells, periglomerular (PGL) cells and the superficial short axon cells. The dendrites of the tufted cells and the PGL cells leave the proximity of their somata to enter into the glomerular region where they make synaptic contacts with the olfactory nerve endings and with each other. Short axon cells limit their dendrites to the periglomerular region. The neuropil of the glomeruli contains: (a) the terminal tufts of dendrites from both mitral and tufted cells, (b) the olfactory nerve axon terminals, and (c) the dendrites and gemmules from PGL cells. The dendrites of mitral and tufted cells are grouped together into one category because of their similarities in morphology and connectivity. The PGL dendritic shafts and their gemmules enter into reciprocal synaptic relationships with mitral/tufted dendrites and are also involved in serial synaptic relationships.

The somata and dendrites of many PGL cells in the periglomerular region were stained with GAD-positive reaction product in thin sections from specimens incubated in anti-GAD serum (e.g., Fig. 13). The distribution of the reaction product within the

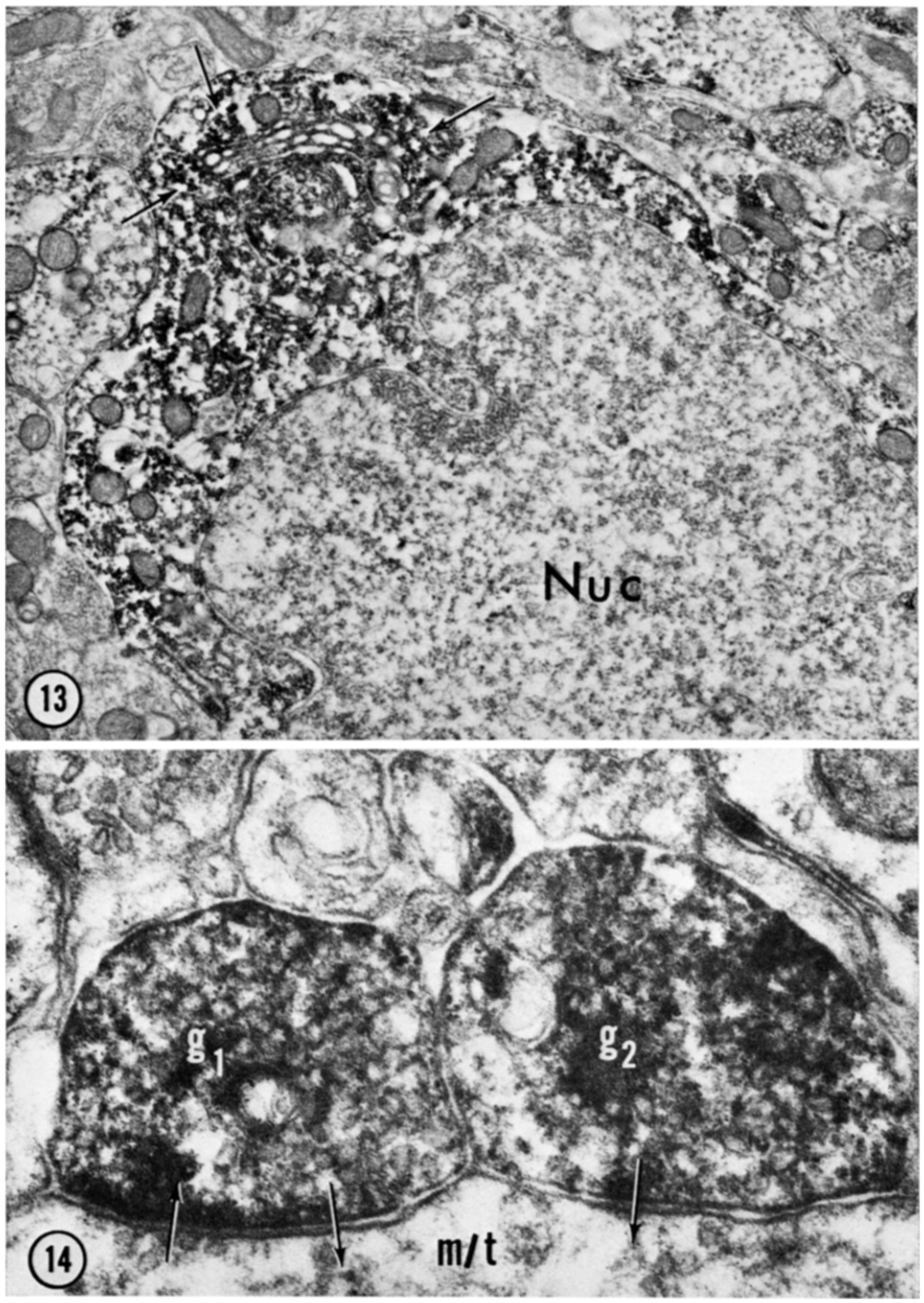
Figs. 8 and 9. Electron micrographs of olfactory bulb sections that were incubated in anti-GAD serum. Both micrographs show a GAD-positive gemmule in the external plexiform layer.
Fig. 8 shows GAD-positive reaction product associated with the surfaces of the synaptic vesicles and mitochondria in a gemmule that is postsynaptic (arrow) to a mitral cell dendrite (m). Round synaptic vesicles (arrowheads) are present in the mitral cell dendrite as well as what appears to be a coated vesicle (asterisk) that is connected with a cistern of smooth endoplasmic reticulum. × 62,000.
Fig. 9 shows a GAD-positive gemmule forming a reciprocal dendrodendritic synapse with a mitral cell dendrite (m). GAD-positive reaction product is concentrated around the synaptic vesicles in the presynaptic part of this gemmule, but it is absent from the portion of the gemmule which is subjacent to the postsynaptic density of the mitral-to-granule synapse. The polarities, or presumed directions of transmission, of these synaptic junctions are indicated by the directions of the arrows. × 62,000.
Fig. 10 is an electron micrograph of olfactory bulb incubated in control rabbit serum. The two gemmules, which are postsynaptic (arrows) to a mitral cell dendrite (m) in the EPL, both lack reaction product. × 62 000.



Figs. 11 and 12. Electron micrographs of olfactory bulb incubated in anti-GAD serum.

Fig. 11. GAD-positive reaction product is concentrated in a granule cell gemmule (g) in the EPL and is also distributed within its dendrite (d) and pedicle (p). The gemmule forms a synapse (arrow) with a mitral cell dendrite (m). × 40,000.

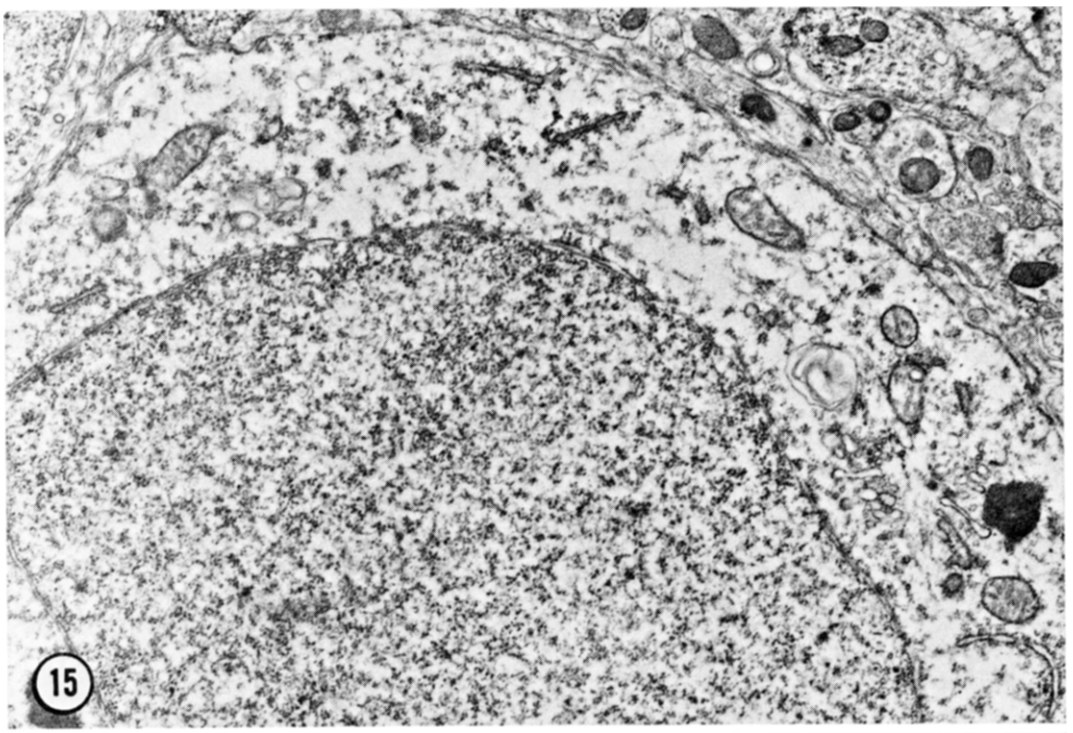
Fig. 12 shows GAD-positive gemmules (g_1–g_3) forming synapses (arrows) with mitral cell dendrites (m) in the EPL. Two of these gemmules (g_1 and g_2) are postsynaptic to mitral cell dendrites, while the other GAD-positive gemmule (g_3) seems to be presynaptic (polarity indicated by direction of arrows). × 40,000.

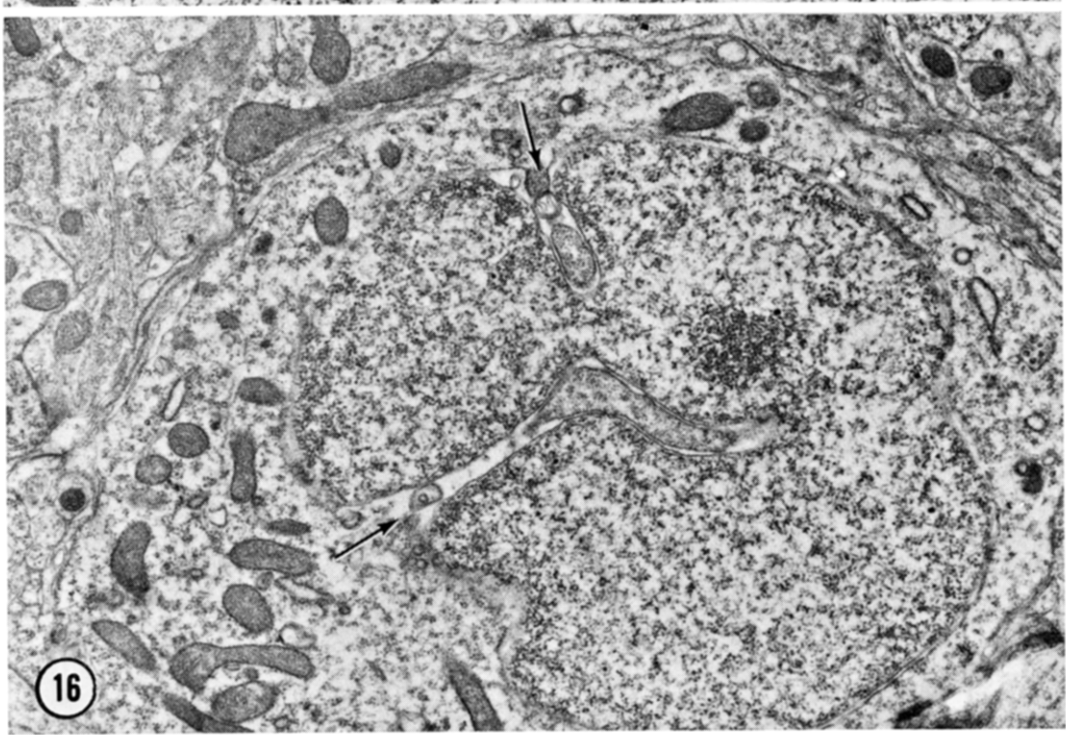


Figs. 13 and 14. Electron micrographs of the glomerular layer of olfactory bulb incubated in anti-GAD serum.

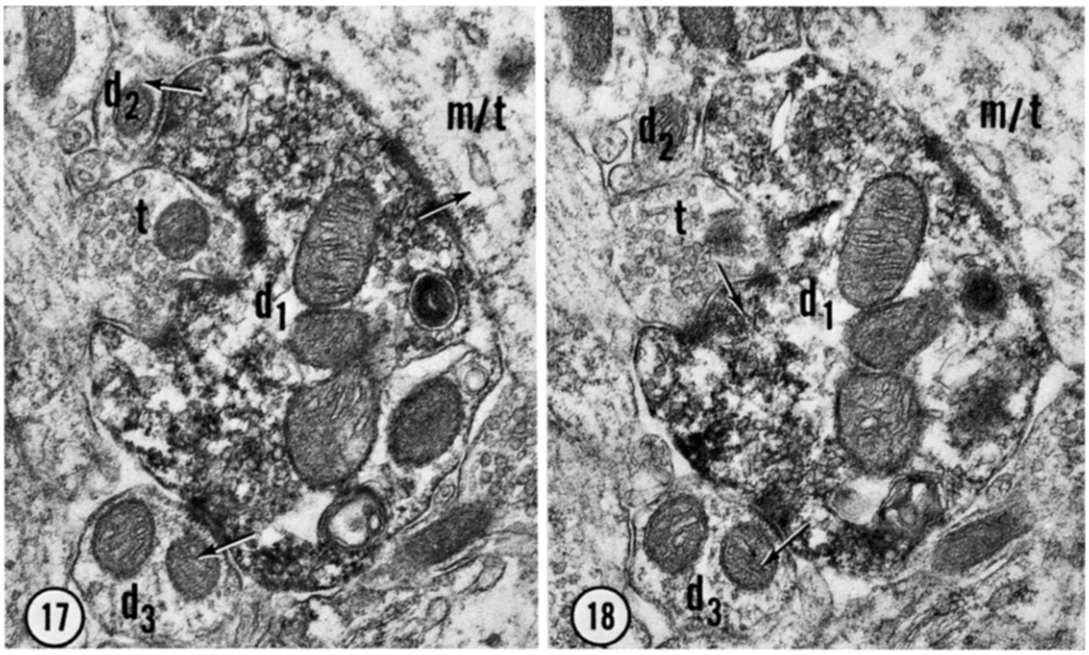
Fig. 13 shows GAD-positive reaction product throughout the soma of a periglomerular cell, but not within its nucleus (Nuc). The somal reaction product is most concentrated around the Golgi complex (arrows). × 16,000.

Fig. 14 shows two GAD-positive gemmules (g_1 and g_2) of PGL cells in a glomerulus. One of the gemmules (g_1) appears to form a reciprocal synapse with a mitral/tufted dendritic shaft (m/t). The other gemmule (g_2) appears to be presynaptic (arrow) to the same dendritic shaft. The polarities of the synaptic junctions are indicated with arrows. × 79,000.





Figs. 15 and 16. Electron micrographs of the glomerular layer of the olfactory bulb incubated in anti-GAD serum.
Fig. 15 shows the soma of an external tufted cell which lacks reaction product. × 16,000.
Fig. 16. shows the soma of a short axon cell with its characteristic indented nuclear envelope (arrows). This neuronal type also lacks reaction product in its soma. × 16,000.



Figs. 17 and 18. Electron micrographs from serial sections of a glomerulus in olfactory bulb incubated in anti-GAD serum. These micrographs show a serial synaptic arrangement involving a periglomerular cell dendrite.

Fig. 17 shows a periglomerular cell dendritic shaft (d_1) containing synaptic vesicles, mitochondria and GAD-positive reaction product. This dendrite (d_1) is presynaptic to a mitral/tufted dendritic shaft (m/t) and to two other dendritic shafts of unknown origin (d_2 and d_3). The arrows indicate the polarity of each synapse. × 35,000.

Fig. 18 is an adjacent section showing the same GAD-positive periglomerular cell dendritic shaft (d_1) as in Fig. 17. In this section, the GAD-positive dendrite (d_1) appears to be postsynaptic to an axon terminal (t) that is probably derived from a centrifugal fiber[27]. Also, d_1 is shown to be presynaptic to the same small dendritic profile (d_3) as in Fig. 17. Since this periglomerular cell dendrite is presynaptic to 3 dendritic profiles and is postsynaptic to both an axon terminal and, as determined in another part of the series, the mitral/tufted dendrite (m/t), this dendrite then forms part of a serial synaptic arrangement. × 35,000.

somata of PGL cells was similar to that found within the somata of the granule cells, i.e., the highest concentration of reaction product occurred around the cisternae and vesicles of the Golgi apparatus. The GAD-positive PGL cells also showed reaction product in their proximal dendrites which entered the glomerular neuropil. The somata and dendrites of the other neuronal types in the periglomerular region (i.e., short axon and external tufted neurons) were observed to be free of reaction product (Figs. 15 and 16).

Profiles filled with GAD-positive reaction product were analyzed using random and serial sections of the glomerular region. These GAD-positive profiles were identified as either gemmules or dendritic shafts of PGL cells on the basis of their synaptic relationships and with the use of serial section reconstructions. For example, in Fig. 14, a GAD-positive profile is shown forming a reciprocal synapse with a mitral/tufted dendritic shaft. This GAD-positive profile was identified as a PGL gemmule because

of its characteristic synaptology[23]. Other GAD-positive gemmules were reconstructed from serial sections and some of them were observed to be in continuity with profiles which exhibited the characteristics of PGL dendritic shafts[23]. Such dendritic shafts also contained GAD-positive reaction product and participated in reciprocal synaptic relationships with mitral/tufted dendrites in the glomerular region. In addition, these GAD-positive dendritic shafts (e.g., Figs. 17 and 18) participated in serial synaptic relationships[23].

DISCUSSION

GABA-ergic neurons of the olfactory bulb

The localization of GAD within granule and periglomerular (PGL) neurons is consistent with the biochemical analysis of GAD activity in the rat olfactory bulb which suggests that both of these cell types use GABA as their neurotransmitter[8]. Our results are also consistent with the results of other studies which suggest that granule and PGL cells inhibit mitral cells[7,19,21,29,34] and that this inhibitory effect is mediated by GABA[16,19]. However, there is also evidence that some PGL cells may use a different neurotransmitter. The results of an immunocytochemical study of another neurotransmitter synthetic enzyme, tyrosine hydroxylase, suggest that some PGL cells in the rat olfactory bulb may use dopamine[10]. This finding could be interpreted as supporting the physiological study which suggests an excitatory role for PGL cells[6]. Since the available immunocytochemical evidence indicates that there are two biochemically distinct types of PGL cells, it is possible that the disagreement concerning the function of PGL cells (cf. refs. 6, 7) may be due to the existence of two, functionally different, types of PGL cells.

Association of GAD-positive reaction product with intracellular organelles

The distribution of GAD-positive reaction product within granule and PGL cells was most dense in its juxtaposition to synaptic vesicles within gemmules and to the Golgi apparatus within neuronal somata. Other sites of reaction product deposition occurred adjacent to microtubules, to the outer membranes of mitochondria and to plasma membranes. These associations of reaction product may be attributable to the binding of GAD to these structures during fixation or to the diffusion of the electron-opaque product formed from the reaction between peroxidase and diaminobenzidine/hydrogen peroxide[36]. However, the possibility exists that some of the observed relationships between GAD-positive reaction product and cellular organelles might be biologically meaningful. For example, GAD-positive reaction product associated with the cisternae and vesicles of the Golgi apparatus might be a reflection of the enzyme's packaging for transport, while the product associated with microtubules might reflect GAD transport from the somata to the synaptic gemmules. The site where a biological association of reaction product with organelles is most supported is the synaptic vesicle (cf. ref. 36). Our observations of a close association of reaction product with synaptic vesicles in the presynaptic portion of gemmules participating in reciprocal dendrodendritic synapses and the absence of both reaction product and

vesicles from the postsynaptic portion of gemmules support the specific vesicle association of GAD proposed on the basis of observations made in the cerebellum[14,15]. These observations all illustrate an extraordinarily high affinity of reaction product for the surfaces of synaptic vesicles which is difficult to view as an artifact. A close relationship between GAD and synaptic vesicles suggests that GABA may be synthesized on or near the membranes of the organelles into which GABA is presumably loaded for synaptic release.

Localization of GAD in somata and dendrites

This study is the first to show an immunocytochemical localization of GAD within somata and dendrites. Previous investigations, using the same antisera to GAD which were used in the present study, have shown that the somata which give rise to certain GAD-positive axon terminals lack GAD-positive reaction product[13,14,32,36]. Possible reasons for this failure to visualize GAD in somata have been discussed recently[36]. One explanation was based on the fact that crude mitochondrial fractions of the brain, predominantly composed of synaptosomes and mitochondria, were used for the biochemical isolation of GAD[37]. On this basis it was suggested that GAD in synaptic terminals might exist in an antigenic form which is somewhat different from that located in non-synaptic neuronal regions. Thus, antisera to synaptosomal GAD might not cross-react with GAD in the rest of the neuron. An alternative explanation[36] suggested that somal GAD is present in low, undetectable concentrations relative to GAD in axon terminals because it might be rapidly transported from its site of synthesis to its sites of utilization. Our observation of GAD-positive reaction product in the cell bodies and dendrites of granule and PGL neurons demonstrates that this GAD is in an antigenic form that is very similar to the GAD in synaptic terminals. Therefore, our results support the latter suggestion that current immunocytochemical methods are not sufficiently sensitive to detect small quantities of freshly synthesized GAD in the cell bodies of certain neurons which transport the enzyme to axon terminals.

A possible reason for the detection of GAD in the somata and dendrites of granule and PGL neurons may relate to the fact that the presynaptic sites of these neurons are primarily dendritic. In striking contrast to this class of neurons, the presynaptic sites of the neurons which do not contain detectable somal and dendritic GAD[13,14,32,36] are exclusively axonal. A preliminary study[31] has demonstrated GAD-positive neuronal somata and dendrites in the lateral geniculate body, the medial geniculate body and the superior colliculus. Since these regions of the central nervous system are also known to contain neurons with presynaptic dendrites[5,11,12,18,35], this finding is consistent with the possibility that somal and dendritic GAD may be detected by current immunocytochemical methods only within neurons which have presynaptic dendrites.

The fact that GAD can be detected in the somata of neurons which transport this enzyme into presynaptic dendrites indicates that such neurons have a higher somal concentration of GAD than that of neurons which do not have presynaptic dendrites. This somal concentration difference suggests that the transport of GAD from the

somata of presynaptic dendrite (PSD) neurons (e.g., granule and PGL cells) is different from that of presynaptic axon (PSA) neurons (e.g., Purkinje, Golgi II, basket and stellate cells). This concentration difference might also be due to variations in size between PSD and PSA neurons. However, this possibility appears unlikely since some cells from the two categories are comparable in size (e.g., granule and stellate cells). Thus, some factors that may contribute to altered transport of GAD are differences in: (a) rates of synthesis of GAD in the cell body; (b) rates of loading GAD onto axonal and dendritic transport mechanisms; (c) velocities of the two transport systems[33] which, in turn, implies two separate transport mechanisms; and (d) rates of GAD utilization at presynaptic sites.

Although the exact mechanisms which might cause alterations in the centrifugal flow of GAD in the two neuronal types (PSD and PSA neurons) remain unresolved, a difference in the transport from the somata into their processes would seem to be a reasonable explanation for the apparent variation in GAD concentrations of certain PSD and PSA cells. This possibility can be tested by blocking axonal flow in PSA cells in order to see if GAD accumulates to detectable levels within their somata. Preliminary experiments using colchicine as a blocking agent of axonal transport indicate that GAD can be detected within the somata and dendrites of PSA neurons[30].

ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr. Jan-Yen Wu for supplying the purified glutamate decarboxylase and Drs. James W. Hinds and Richard E. Wimer for their helpful advice during the course of this study. Also, the authors are grateful for the valuable technical assistance provided by Lynn Anderson, Mariko Nakashima, J. Randall Slemmon and Christine S. Vaughn and for the secretarial assistance of Donna Dreier.

This work was supported by USPHS Grants No. NS-12116 and NS-1615 from the NINDS.

REFERENCES

1 Allison, A. C., The morphology of the olfactory system in the vertebrates, *Biol. Rev.*, 28 (1953) 195–244.
2 Barber, R. and Saito, K., Light microscopic visualization of GAD and GABA-T in immunocytochemical preparations of rodent CNS. In E. Roberts, T. N. Chase and D. Tower (Eds.), *GABA in Nervous System Function*, Raven Press, New York, 1975, pp. 113–132.
3 Barber, R., Vaughn, J. E., Saito, K., McLaughlin, B. J. and Roberts, E., The effects of dorsal rhizotomy on GAD axon terminals in the dorsal horn of the rat spinal cord, In preparation.
4 Cajal, S. Ramón y, *Histologie du Système Nerveux de l'Homme et des Vertébrés, Tôme II*, Maloine, Paris, 1911.
5 Famiglietti, E. V., Jr., Dendro-dendritic synapses in the lateral geniculate nucleus of the cat, *Brain Research*, 20 (1970) 181–191.
6 Freeman, W. J., Relation of glomerular neuronal activity to glomerular transmission attenuation, *Brain Research*, 65 (1974) 91–107.
7 Getchell, T. V. and Shepherd, G. M., Short-axon cells in the olfactory bulb: dendrodendritic synaptic interactions, *J. Physiol. (Lond.)*, 251 (1975) 523–548.
8 Graham, L. T., Distibution of glutamic acid decarboxylase activity and GABA content in the olfactory bulb, *Life Sci.*, 12 (1973) 443–447.

9 Hinds, J. W., Ultrastructure of an anaxonic neuron, the internal granule cell of the olfactory bulb, *Anat. Rec.*, 163 (1969) 199.
10 Hökfelt, T., Halász, N., Lungdahl, Å., Johansson, O., Goldstein, M. and Park, D., Histochemical support for a dopaminergic mechanism in the dendrites of certain periglomerular cells in the rat olfactory bulb, *Neurosci. Lett.*, 1 (1975) 85–90.
11 Lieberman, A. R., Neurons with presynaptic perikarya and presynaptic dendrites in the rat lateral geniculate nucleus, *Brain Research*, 59 (1973) 35–59.
12 Lund, R. D., Synaptic patterns of the superficial layers of the superior colliculus of the rat, *J. comp. Neurol.*, 135 (1969) 179–208.
13 McLaughlin, B. J., Barber, R., Saito, K., Roberts, E. and Wu, J.-Y., Immunocytochemical localization of glutamate decarboxylase in rat spinal cord, *J. comp. Neurol.*, 164 (1975) 305–322.
14 McLaughlin, B. J., Wood, J. G., Saito, K., Barber, R., Vaughn, J. E., Roberts, E. and Wu, J.-Y., The fine structural localization of glutamate decarboxylase in synaptic terminals of rodent cerebellum, *Brain Research*, 76 (1974) 377–391.
15 McLaughlin, B. J., Wood, J. G., Saito, K., Roberts, E. and Wu, J.-Y., The fine structural localization of glutamate decarboxylase in developing axonal processes and presynaptic terminals of rodent cerebellum, *Brain Research*, 85 (1975) 355–371.
16 McLennan, H., The pharmacology of inhibition of mitral cells in the olfactory bulb, *Brain Research*, 29 (1971) 177–184.
17 Millonig, G., Advantages of a phosphate buffer for OsO_4 solutions in fixation, *J. appl. Phys.*, 32 (1961) 1637 (Abstract).
18 Morest, D. K., Dendrodendritic synapses of cells that have axons: The fine structure of the Golgi type II cell in the medial geniculate body of the cat, *Z. Anat. Entwickl.-Gesch.*, 133 (1971) 216–246.
19 Nicoll, R. A., Pharmacological evidence for GABA as the transmitter in granule cell inhibition in the olfactory bulb, *Brain Research*, 35 (1971) 137–149.
20 Peters, A., The fixation of central nervous tissue and the analysis of electron micrographs of the neuropil with special reference to the cerebral cortex. In W. J. H. Nauta and S. O. E. Ebbesson (Eds.), *Contemporary Research Methods in Neuroanatomy*, Springer, New York, 1970, pp. 56–76.
21 Phillips, C. G., Powell, T. P. S. and Shepherd, G. M., Responses of mitral cells to stimulation of the lateral olfactory tract on the rabbit, *J. Physiol. (Lond.)*, 168 (1963) 65–88.
22 Pinching, A. J. and Powell, T. P. S., The neuron types of the glomerular layer of the olfactory bulb, *J. Cell Sci.*, 9 (1971) 305–345.
23 Pinching, A. J. and Powell, T. P. S., The neuropil of the glomeruli of the olfactory bulb, *J. Cell Sci.*, 9 (1971) 347–377.
24 Pinching, A. J. and Powell, T. P. S., The neuropil of the periglomerular region of the olfactory bulb, *J. Cell Sci.*, 9 (1971) 379–409.
25 Price, J. L. and Powell, T. P. S., The morphology of the granule cells of the olfactory bulb, *J. Cell Sci.*, 7 (1970) 91–124.
26 Price, J. L. and Powell, T. P. S., The synaptology of the granule cells of the olfactory bulb, *J. Cell Sci.*, 7 (1970) 125–155.
27 Price, J. L. and Powell, T. P. S., An electron-microscopic study of the termination of the afferent fibres to the olfactory bulb from the cerebral hemispheres, *J. Cell Sci.*, 7 (1970) 157–187.
28 Price, J. L. and Powell, T. P. S., The mitral and short axon cells of the olfactory bulb, *J. Cell Sci.* 7 (1970) 631–652.
29 Rall, W., Shepherd, G. M., Reese, T. S. and Brightman, M. W., Dendrodendritic synaptic pathway for inhibition in the olfactory bulb, *Exp. Neurol.*, 14 (1966) 44–56.
30 Ribak, C. E. and Vaughn, J. E., Immunocytochemical localization of GAD in somata and dendrites of GABA-ergic neurons following colchicine treatment, *Neurosci. Abstr.*, 2 (1976) 796.
31 Ribak, C. E., Vaughn, J. E. and Saito, K., Immunocytochemical localization of glutamate decarboxylase (GAD) in the olfactory bulb, *Anat. Rec.*, 184 (1976) 512–513.
32 Ribak, C. E., Vaughn, J. E., Saito, K., Barber, R. and Roberts, E., Immunocytochemical localization of glutamate decarboxylase in rat substantia nigra, *Brain Research*, in press.
33 Schubert, P. and Kreutzberg, G. W., Parameters of dendritic transport. In G. W. Kreutzberg (Ed.), *Advances in Neurology, Vol. 12*, Raven Press, New York, 1975, pp. 255–268.
34 Shepherd, G. M., Synaptic organization of the mammalian olfactory bulb, *Physiol. Rev.*, 52 (1972) 864–917.
35 Tigges, M. and Tigges, J., Presynaptic dendrite cells and two other classes of neurons in the superficial layers of the superior colliculus of the chimpanzee, *Cell Tiss. Res.*, 162 (1975) 279–297.

36 Wood, J. G., McLaughlin, B. J. and Vaughn, J. E., Immunocytochemical localization of GAD in electron microscopic preparations of rodent CNS. In E. Roberts, T. N. Chase and D. B. Tower (Eds.), *GABA in Nervous System Function*, Raven Press, New York, 1975, pp. 133–148.
37 Wu, J.-Y., Purification, characterization, and kinetic studies of GAD and GABA-T from mouse brain. In E. Roberts, T. N. Chase and D. B. Tower (Eds.), *GABA in Nervous System Function*, Raven Press, New York, 1975, pp. 7–55.