

# UC Irvine

## UC Irvine Previously Published Works

### Title

Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport

### Permalink

<https://escholarship.org/uc/item/8r63t6hc>

### Journal

Brain Research, 140(2)

### ISSN

1385-299X

### Authors

Ribak, Charles E  
Vaughn, James E  
Saito, Kihachi

### Publication Date

1978

### DOI

10.1016/0006-8993(78)90463-8

### Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

## IMMUNOCYTOCHEMICAL LOCALIZATION OF GLUTAMIC ACID DECARBOXYLASE IN NEURONAL SOMATA FOLLOWING COLCHICINE INHIBITION OF AXONAL TRANSPORT\*

CHARLES E. RIBAK, JAMES E. VAUGHN and KIHACHI SAITO

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

(Accepted May 4th, 1977)

---

### SUMMARY

The enzyme that synthesizes the neurotransmitter  $\gamma$ -aminobutyric acid (GABA), glutamic acid decarboxylase (GAD), has been immunocytochemically localized in the somata and dendrites of certain neurons in rat cerebellum and Ammon's horn following colchicine injections into these two brain regions. In the cerebellum, GAD-positive reaction product was observed in the somata and proximal dendrites of Purkinje, Golgi II, basket and stellate neurons. Occasional staining of the proximal portions of axons was also observed in these colchicine-injected preparations. None of the somata or dendrites of these same cell types exhibited reaction product in preparations that were not pretreated with colchicine, although the axon terminals of these neurons were GAD-positive. In Ammon's horn, the somata of a few cells that are classified as probable basket and other short-axon neurons contained detectable concentrations of GAD in preparations that were not pretreated with colchicine. However, following colchicine injections into the Ammon's horn, there was approximately a five-fold increase in the number of GAD-positive somata of basket and other short-axon neurons. There was also a substantial increase in the extent of dendritic staining exhibited by these neurons. Control injections of saline and lumicolchicine produced the same results as those observed in preparations which were not pretreated with colchicine. Thus, the results from the control injections indicate that the increases in somal and dendritic staining are due to a colchicine-mediated inhibition of the somatofugal transport of GAD rather than to a non-specific effect of the drug and/or the injection procedure.

The results of the present study permit the direct identification of the neuronal somata in the cerebellum and Ammon's horn whose synaptic terminals probably use

---

\* A preliminary report of this study was presented at the sixth annual meeting of the Society of Neurosciences in Toronto, Canada, November, 1976.

GABA as their neurotransmitter. On the basis of the present findings, a reasonable explanation for the failure of earlier immunocytochemical studies to detect somal GAD in certain GABAergic neurons is that the axonal transport of GAD appears to occur at a sufficiently rapid rate to limit the somal concentration of GAD to low, undetectable levels.

---

## INTRODUCTION

Glutamic acid decarboxylase (GAD), the synthetic enzyme of the neurotransmitter,  $\gamma$ -aminobutyric acid (GABA), has been localized immunocytochemically within the axon terminals of specific neurons which are either known or strongly suspected of using GABA as their neurotransmitter (e.g. refs. 14, 15, 23 and 28). Furthermore, it has been observed that the somata and dendrites of these neurons do not contain GAD-positive reaction product. This is an unexpected finding because GAD synthesis — like that of other neuronal proteins — mainly occurs within somata<sup>5-7</sup>. It has been suggested previously<sup>28</sup> that this lack of somal staining might be due to either (a) different antigenic properties between somal GAD and GAD within axon terminals, or (b) somal concentrations of GAD which are too low to be detected by the immunocytochemical method employed. However, subsequent to these suggestions<sup>28</sup>, GAD-positive immunocytochemical reaction product was localized within the somata and dendrites of granule and periglomerular neurons of the olfactory bulb<sup>24</sup> using the same antisera and procedures as those reported earlier (e.g. refs. 1, 14, 15, 23 and 28). This finding indicates that somal GAD is in an antigenic form which is very similar to that found within axon terminals, and therefore supports the idea that the lack of GAD-positive reaction product within somata and dendrites of previously examined GABAergic neurons might be due to very low GAD concentrations.

In order to explain the apparent difference in the somal concentrations of GAD, it has been noted<sup>24</sup> that the olfactory bulb neurons differ from those neurons in the cerebellum and spinal cord in that they have numerous presynaptic dendrites while the other GAD-containing neurons exclusively have presynaptic axons. On this basis it has been proposed that variations in somal concentrations of GAD might be achieved by differences in its transport from the somata into the terminal processes of each of the two neuronal classes (i.e., presynaptic dendrite and presynaptic axon neurons)<sup>24</sup>. A rapid transport of newly synthesized GAD from somata to axon terminals in presynaptic axon neurons might prevent the accumulation of detectable concentrations of GAD-positive reaction product within their somata and dendrites. If this suggestion is valid, it should be possible to produce detectable quantities of somal GAD by inhibiting its rapid anterograde transport. Colchicine has been used for this purpose in the present study. The results of this study demonstrate that colchicine inhibition of axoplasmic transport allows for the immunocytochemical detection of GAD in the neuronal somata of known GABAergic presynaptic axon neurons in the cerebellum. In Ammon's horn, it is shown that a few somata contain GAD-positive

reaction product in untreated preparations, but colchicine treatment produces a marked increase in the number of GAD-positive somata and dendrites as well as in the extent of dendritic staining.

#### MATERIALS AND METHODS

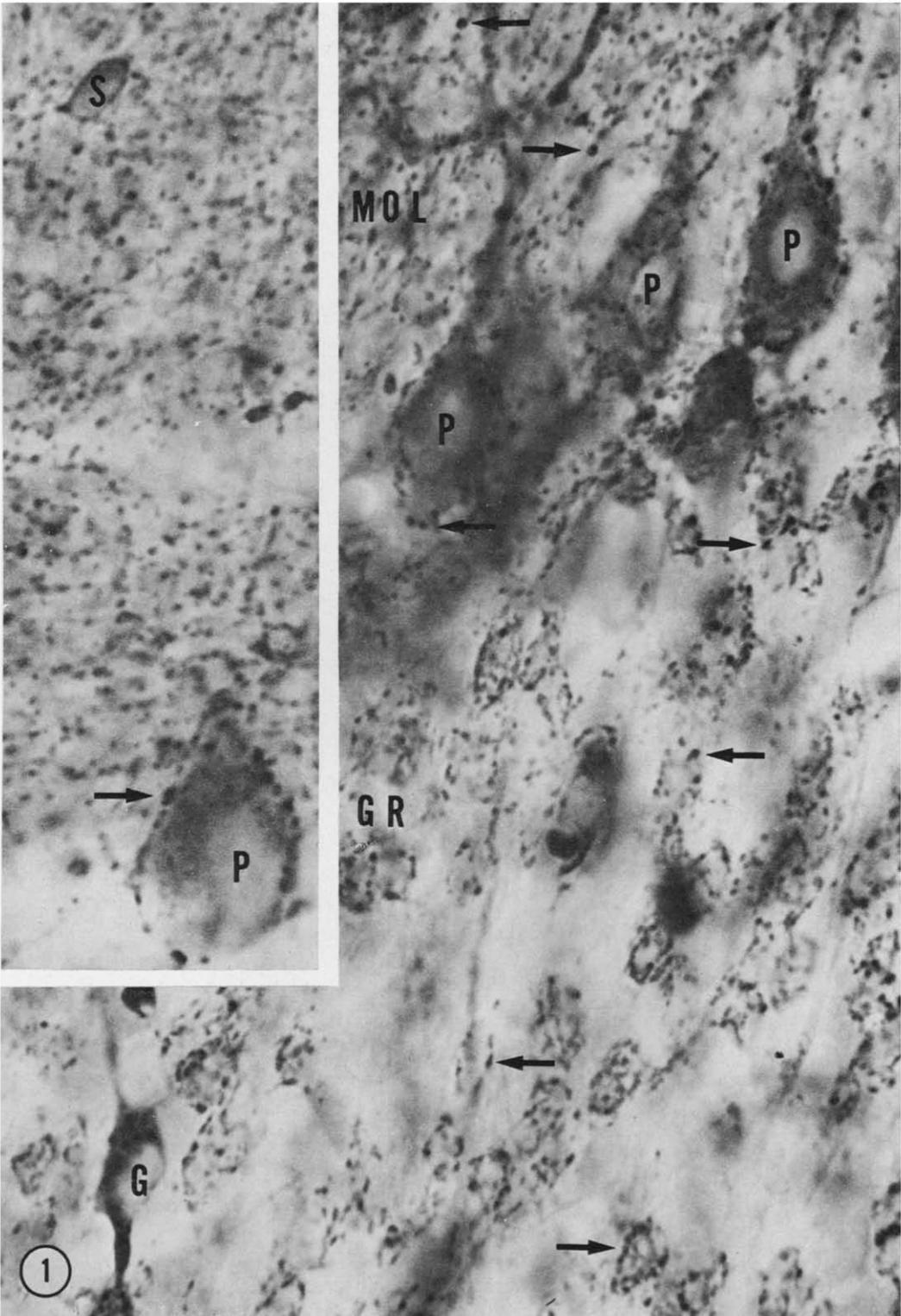
Twelve adult Sprague–Dawley albino rats were used in this study. The cerebellar cortex of three rats was injected with either 2.5 or 10  $\mu\text{l}$  of colchicine (10  $\mu\text{g}/\mu\text{l}$  saline, Sigma Chemical Co.). Six other rats had 2  $\mu\text{l}$  of the same concentration of colchicine injected either directly into the Ammon's horn or into the visual cortex immediately superficial to the Ammon's horn. The remaining three rats received control injections of either 2  $\mu\text{l}$  saline or 2  $\mu\text{l}$  lumicolchicine (10  $\mu\text{g}/\mu\text{l}$  saline) directly into the Ammon's horn. Injections of test and control solutions were administered from a 10- $\mu\text{l}$  Hamilton syringe that was mounted on a micromanipulator, and the cranium of the rats was held stationary during the injections by a stereotaxic head apparatus.

Lumicolchicine solutions were prepared from colchicine using the procedure reported by Mizel and Wilson<sup>17</sup>. Briefly, quartz cuvettes containing 0.1 g colchicine dissolved in 1 ml absolute ethanol were irradiated for 4 h using the ultraviolet source of a Reichert fluorescent microscope. The absorption spectra of the resulting lumicolchicine solutions were compared with those of colchicine solutions over the 200–375 nm range. The characteristic colchicine peak at 350 nm (e.g. ref. 27) was absent from the absorption spectra of lumicolchicine solutions. Following the spectrophotometric analysis, the lumicolchicine solutions were evaporated to one-half of the original volumes and then were diluted with saline to a final concentration of 10  $\mu\text{g}/\mu\text{l}$ . All solutions were used for injections on the same day that they were prepared.

The rats were sacrificed by intracardiac perfusions<sup>21</sup> with a solution containing 4.0% paraformaldehyde, 0.2% glutaraldehyde and 0.002%  $\text{CaCl}_2$  in 0.12 M Millonig's<sup>16</sup> phosphate buffer at pH 7.2 and 37 °C. Two of the rats which received colchicine injections directly into the Ammon's horn were sacrificed at 5 and 48 h post-injection, respectively. The remaining rats used in these experiments had a 24-h survival time.

All specimens from the cerebellum and Ammon's horn were removed from the brains on the day following perfusion. For light microscopy, specimens were immersed overnight in a cryoprotectant 30% sucrose solution, rapidly frozen and sectioned on a cryostat at 40  $\mu\text{m}$  (see ref. 1). Sagittal sections were obtained of cerebellar cortex and coronal sections were cut from Ammon's horn. After rinsing in buffer overnight, selected sections which included the injection sites of colchicine or control solutions were processed for GAD immunocytochemistry as described previously<sup>23</sup>. In addition to the routine light microscopic examination of Ammon's horn, neurons containing GAD-positive reaction product within their somata were drawn at  $\times 100$  magnification using a drawing tube. The drawings of both colchicine-treated and control specimens allowed for an analysis of staining differences between the two groups of specimens.

For electron microscopy, unfrozen specimens were sectioned in the same planes as for light microscopy, but they were cut at a thickness of 150  $\mu\text{m}$  using a Sorvall



TC-2 tissue sectioner. Specimens close to the injection sites were placed in phosphate buffer, processed for electron microscopic GAD immunocytochemistry, embedded in Epon-Araldite resin and examined with an electron microscope as described previously<sup>23</sup>.

## RESULTS

### *Localization of GAD in the cerebellum*

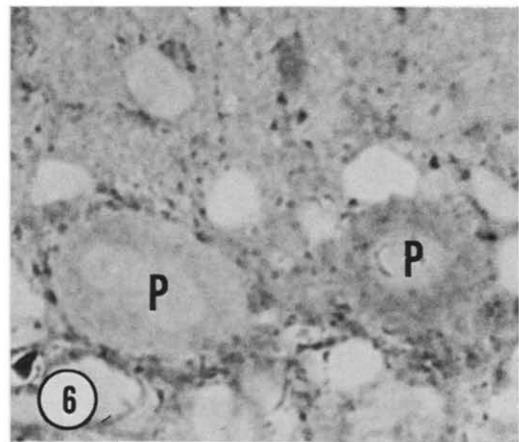
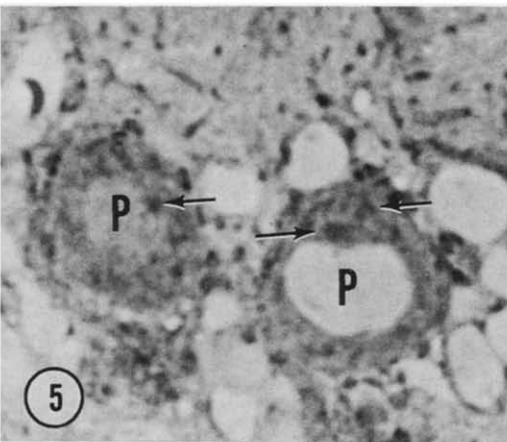
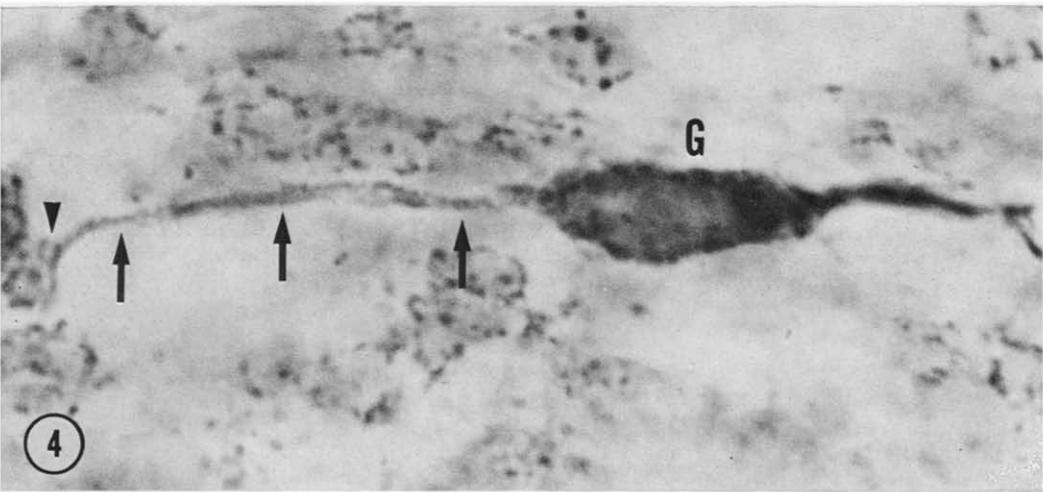
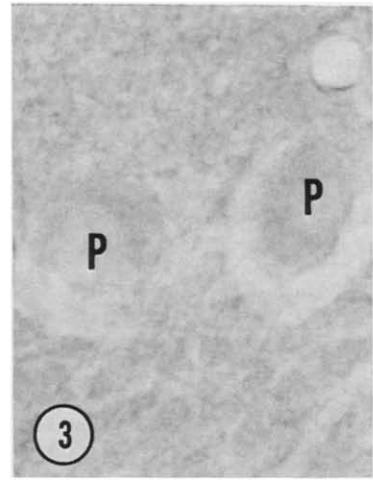
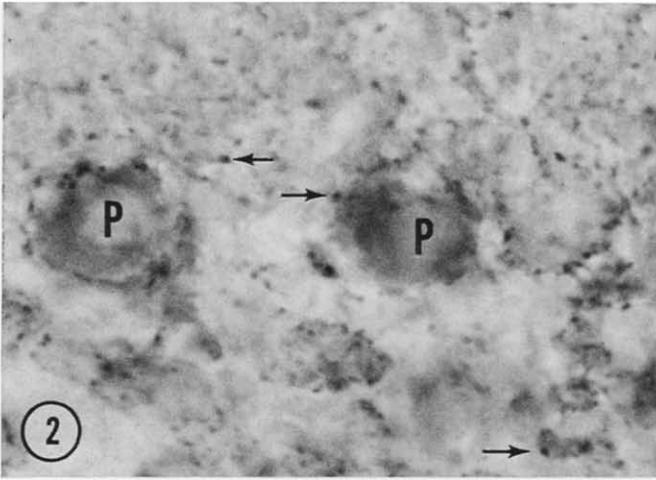
Light microscopy of 40- $\mu\text{m}$  frozen sections of colchicine-injected cerebellar cortex treated with anti-GAD serum showed GAD-positive axon terminals distributed throughout the layers of the cerebellar cortex in the pattern described previously<sup>15,25</sup>. In addition, the proximal dendrites and somata of Purkinje and Golgi II neurons and the somata of basket and stellate cells contained detectable accumulations of GAD-positive reaction product (Figs. 1 and 5). The reaction product appeared in the cytoplasm of the somata and dendrites but was not present within the nuclei. Except for the staining of axon terminals, it was rare to observe GAD-positive staining in axonal-like processes. For example, only a few Golgi II neurons displayed GAD-positive processes emanating from their somata which appeared to be in continuity with some of the stained puncta (axon terminals) that encircle the glomeruli of the granule cell layer (Fig. 4). In these few instances, only the proximal portions of Golgi II axons were stained, and no other neuronal types showed staining of the non-terminal portions of their axons. In all cases, the majority of the GAD-positive somata were located within 100–500  $\mu\text{m}$  of the injection site. In one case, the injection of colchicine formed a cyst in the subdural space between two folia, and somata containing GAD-positive reaction product were observed along the border of the cyst while somata located at a distance from the cyst lacked staining. In all preparations of colchicine-injected cerebella, no GAD-positive granule cell somata were observed regardless of their proximity to the injection site.

Specimens of cerebellar cortex from non-injected rats which were incubated in anti-GAD serum showed neither somal nor dendritic staining but displayed the usual pattern of GAD-positive axon terminals<sup>15</sup> (Figs. 2 and 6). Sections from injected (Fig. 3) and non-injected rats which were incubated in control serum showed no specific reaction product within either somata, dendrites or axon terminals.

Electron microscopic preparations of colchicine-injected cerebellar specimens that were incubated in anti-GAD serum contained reaction product in certain pre-

---

Fig. 1. Frozen section (40  $\mu\text{m}$ ) of colchicine-injected cerebellum incubated in anti-GAD serum. The somata and dendrites of Purkinje (P) and Golgi II (G) neurons contain dense, GAD-positive reaction product. Numerous axon terminals (arrows) in the molecular (MOL) and granule (GR) layers are also stained for GAD as are those of basket neurons terminating upon Purkinje somata. The GAD-positive puncta in the granule layer appear to be located mainly within glomeruli.  $\times 1000$ . The inset is a higher magnification photomicrograph from the same cerebellar section as Fig. 1. A Purkinje (P) and a stellate (S) neuron show GAD-positive reaction product within their somata. In addition, the perimeter of the Purkinje soma is studded with GAD-positive axon terminals (e.g. arrow).  $\times 1350$ .



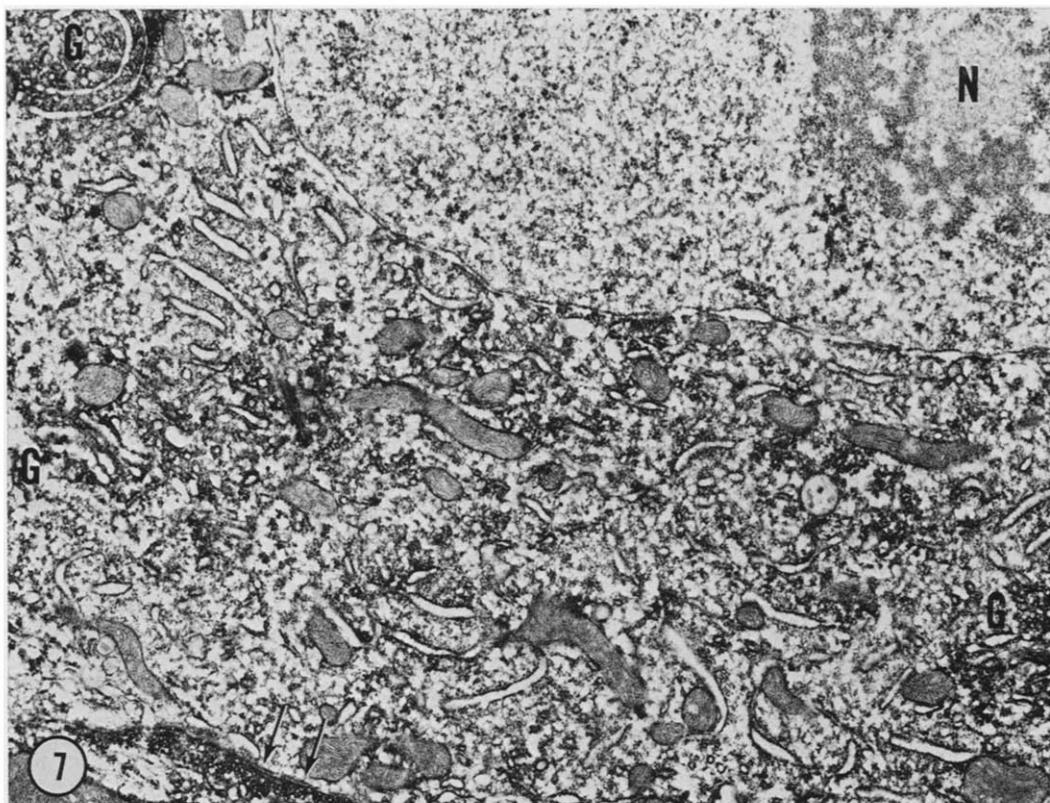


Fig. 7. Electron micrograph of a Purkinje soma from a colchicine-injected cerebellum incubated in anti-GAD serum. The electron-opaque, GAD-positive reaction product appears to be most concentrated around the cisternae of the Golgi apparatus (G). Neither the nucleus nor the nucleolus (N) contain GAD-positive reaction product. A GAD-positive axon terminal of a basket neuron forms a synaptic junction (arrows) with the Purkinje soma.  $\times 17,500$ .

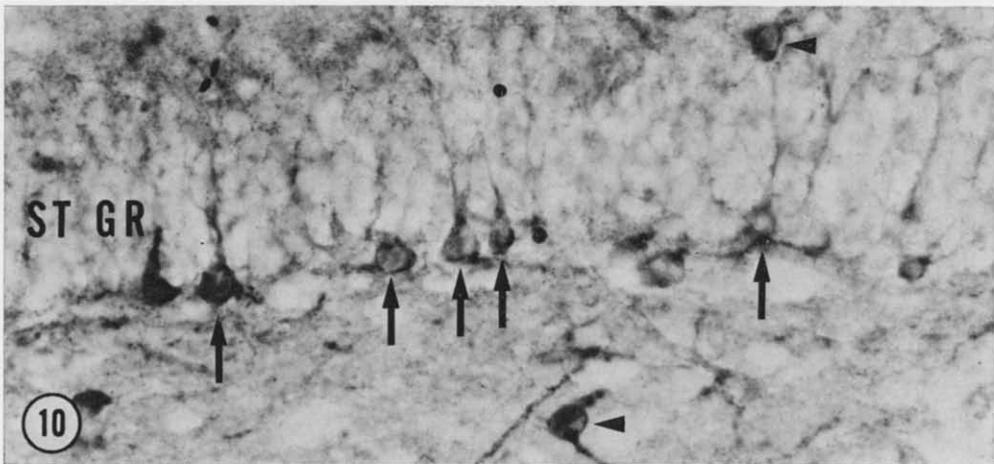
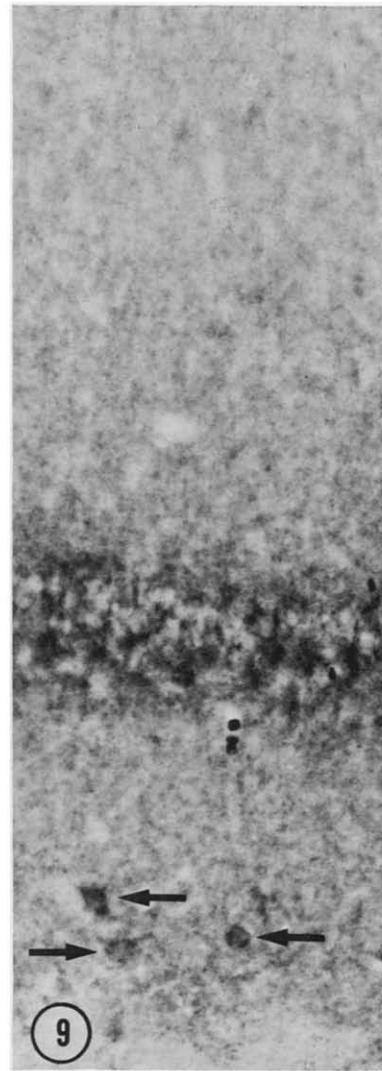
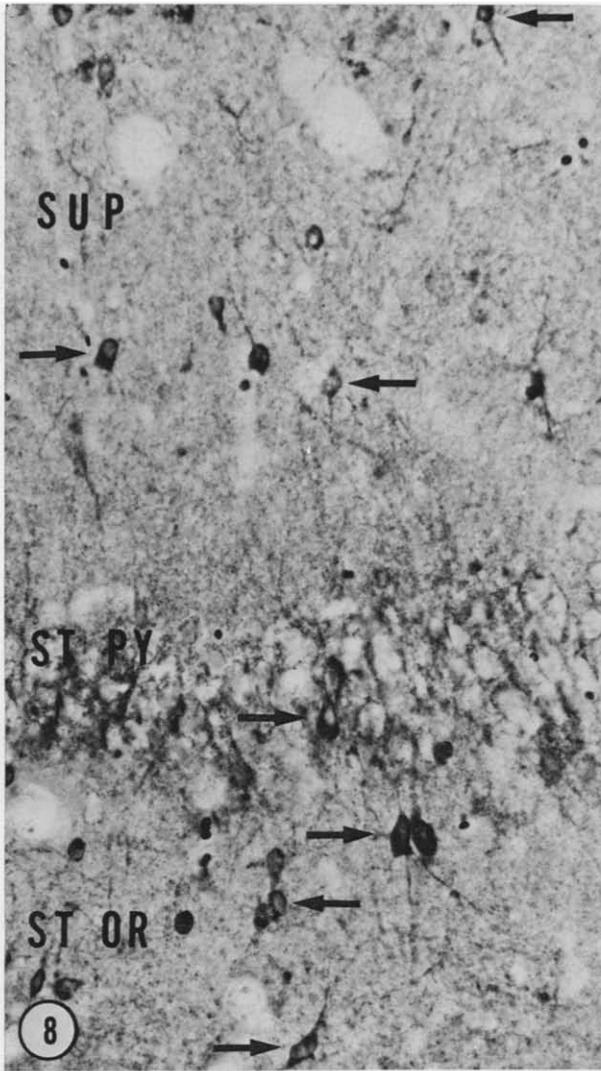
Fig. 2. Frozen section of a non-injected cerebellum incubated in anti-GAD serum. Axon terminals within the molecular, Purkinje and granule layers contain GAD-positive reaction product (arrows), but there is no somal or dendritic staining for GAD. The density of Purkinje somata (P) is due to underlying GAD-positive axon terminals of basket neurons since this somal density is markedly reduced in semithin sections (Fig. 6), and is not present in ultrathin sections which exclude underlying GAD-positive terminals (see refs. 15 and 28).  $\times 900$ .

Fig. 3. Colchicine injected cerebellum incubated in control serum. There is no GAD-positive reaction product present within axon terminals or Purkinje somata (P).  $\times 900$ .

Fig. 4. Colchicine-injected cerebellum incubated in anti-GAD serum. A Golgi II neuron G (G) within the granule layer contains dense, GAD-positive reaction product within its soma and proximal processes. This neuron has a thin axon-like process (arrows) which appears to terminate in a glomerulus (arrowhead).  $\times 1300$ .

Fig. 5. Semithin ( $1 \mu\text{m}$ ) section of colchicine-injected cerebellum incubated in anti-GAD serum. The GAD-positive reaction product appears as indistinct clumps (arrows) in the somata of Purkinje neurons (P). These clumps may correspond to the sites of the Golgi complex since, in electron micrographs, the Golgi complex is associated with concentrated GAD-positive reaction product (see Fig. 7).  $\times 1100$ .

Fig. 6. Semithin ( $1 \mu\text{m}$ ) section of non-injected cerebellum incubated in anti-GAD serum. The Purkinje somata (P) lack GAD-positive reaction product.  $\times 1100$ .



synaptic terminals as described previously<sup>15</sup>. These preparations also contained GAD-positive reaction product within the somata and dendrites of Purkinje (Fig. 7) stellate, basket and Golgi II neurons<sup>19</sup>. The distribution of the reaction product within somata and dendrites of these neurons was similar to that observed in the somata and dendrites of the granule and periglomerular cells of the olfactory bulb<sup>24</sup>. The highest concentration of reaction product occurred adjacent to the cisternae and vesicles of the Golgi apparatus, and reaction product was also present on the surfaces of microtubules and mitochondria (Fig. 7). Electron microscopic preparations treated with control serum revealed no reaction product within any neurons of the cerebellar cortex.

#### *Localization of GAD in Ammon's horn*

Light microscopic preparations of colchicine-injected Ammon's horns which were treated with anti-GAD serum (Figs. 8, 10, 11 and 12) exhibited GAD-positive reaction product within punctate structures that had the same distribution as described previously<sup>1</sup>. Some of the GAD-positive puncta appeared as pericellular baskets around pyramidal and granule cell somata and these puncta corresponded to the known distribution of basket cell axon terminals. In addition, GAD-positive reaction product was observed within the somata and dendrites of about 5 times as many neurons as that observed in non-injected preparations (see below). The extent of dendritic staining of these GAD-positive neurons varied (e.g. Figs. 8, 10, 11 and 12), but it was evident that the dendritic trees were not stained completely. The staining of non-terminal axons from GAD-positive somata rarely occurred.

Drawings which displayed the somal location and the orientation of the proximal dendrites of GAD-positive neurons in Ammon's horn were used as an aid for the classification of these neurons (e.g. Fig. 15). From the distribution of GAD-positive neurons in these drawings, it was evident that they corresponded to the basket and short-axon neuron types described by Ramón y Cajal<sup>22</sup> and Lorente de Nó<sup>13</sup>. Poly-

---

Fig. 8. Colchicine-injected Ammon's horn incubated in anti-GAD serum. Numerous neuronal somata (arrows) and dendrites contain opaque, GAD-positive reaction product in stratum oriens (ST OR), stratum pyramidale (ST PY) and the suprapyramidal layers (SUP). These GAD-positive neurons have been identified as basket and other short-axon neurons (see text). GAD-positive axon terminals appear most prominent surrounding the unstained, hippocampal pyramidal neurons in the stratum pyramidale.  $\times 250$ .

Fig. 9. Lumicolchicine-injected Ammon's horn incubated in anti-GAD serum. Three neuronal somata (arrows) in stratum oriens contain GAD-positive reaction product but do not display dendritic staining. Specimens from control injections, as well as from non-injected specimens of Ammon's horn, did not show nearly as many GAD-positive neuronal somata as that observed in colchicine-injected specimens (also see Figs. 15–18).  $\times 250$ .

Fig. 10. Suprapyramidal blade of the dentate gyrus from a colchicine-injected Ammon's horn incubated in anti-GAD serum. Immediately deep to the stratum granulosum (ST GR) in the polymorph layer, the somata and apical dendrites of presumptive pyramidal basket neurons (arrows) contain GAD-positive reaction product. Other GAD-positive somata (arrowheads) are located deeper in the polymorph layer and in the molecular layer. The granule cells in the stratum granulosum lack somal staining, but they are outlined by surrounding GAD-positive axon terminals.  $\times 325$ .

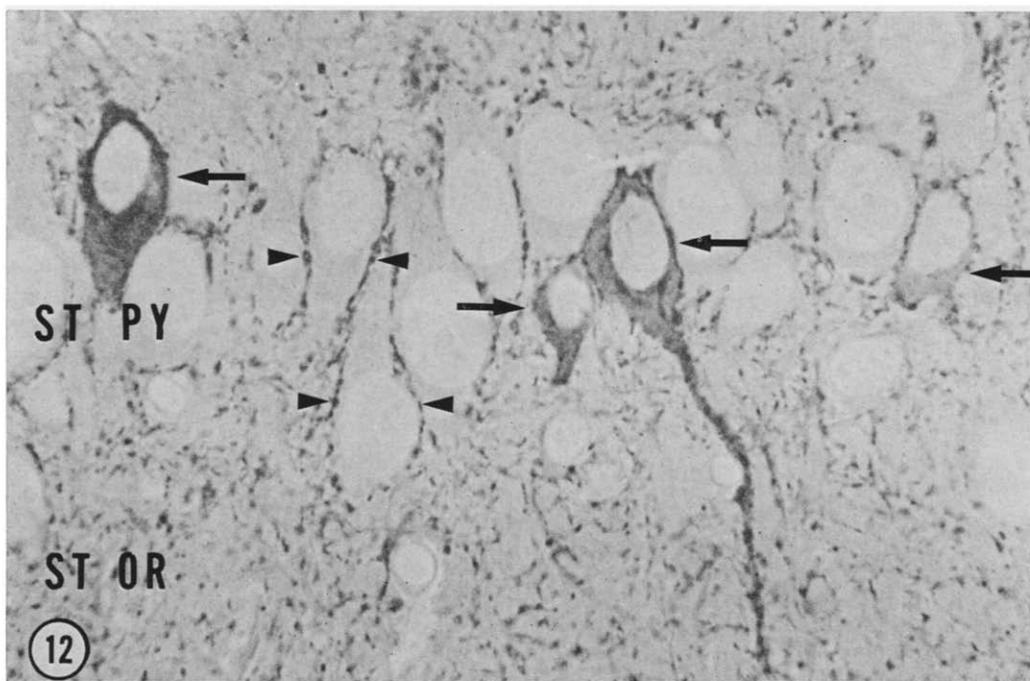
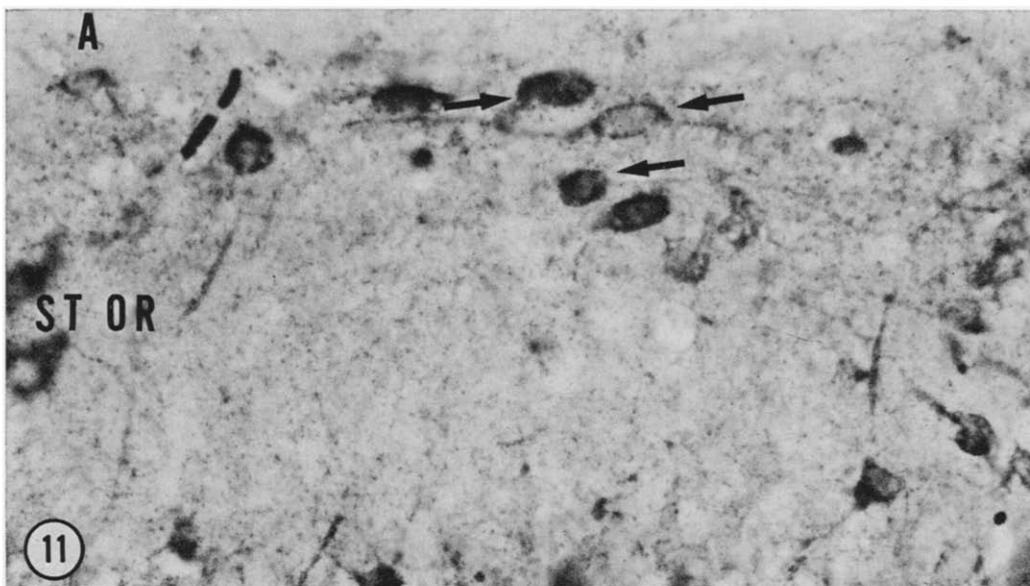


Fig. 11. Frozen ( $40\ \mu\text{m}$ ) section of stratum oriens (ST OR) from a colchicine-injected Ammon's horn incubated in anti-GAD serum. Polygonal and horizontal neurons contain GAD-positive reaction product within their somata and dendrites. Many of the GAD-positive horizontal neurons (arrows) are located along the border between stratum oriens (ST OR) and the alveus (A).  $\times 375$ .

Fig. 12. Semithin ( $1\ \mu\text{m}$ ) section from a colchicine-injected Ammon's horn incubated in anti-GAD serum. The GAD-positive somata of presumptive pyramidal basket neurons (arrows) are interspersed in the pyramidal layer (ST PY) with the unstained somata of hippocampal pyramids. One of the GAD-positive somata has a stained dendrite extending into stratum oriens (ST OR). Numerous GAD-containing puncta (arrowheads) that probably correspond to basket axon terminals (see Fig. 13) surround the pyramidal neurons. Many dense, GAD-positive puncta are also present in strata radiatum and oriens.  $\times 900$ .

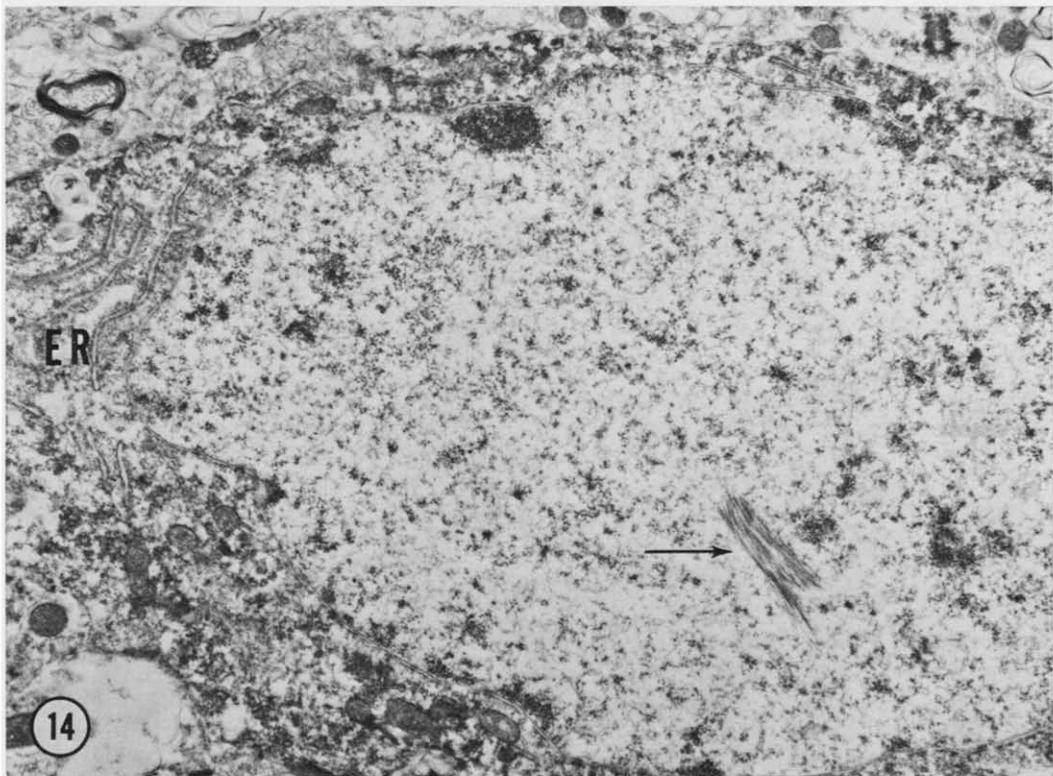
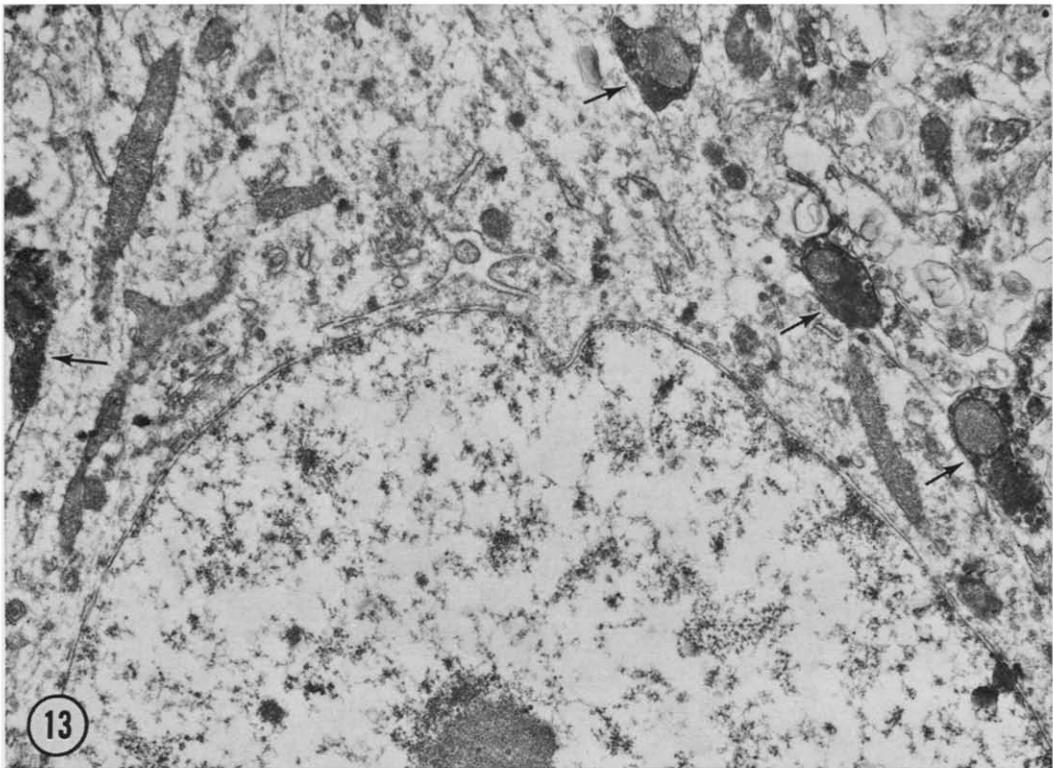
gonal cells in stratum oriens of the hippocampus exhibited dendritic staining extending from their somata for 100–200  $\mu\text{m}$  (Fig. 8). In addition, GAD-positive reaction product filled the somata and bipolar dendrites of many horizontal neurons located near the boundary between stratum oriens and the alveus (Fig. 11). Stratum pyramidale contained a few GAD-positive somata (Figs. 8 and 12) which may correspond to the pyramidal basket cells<sup>13</sup>. The majority of the GAD-positive somata in stratum radiatum and stratum lacunosum corresponded to those of horizontal and other short-axon neurons (Figs. 8 and 15). The hippocampal pyramidal neurons of stratum pyramidale did not contain GAD-positive reaction product.

Many of the GAD-positive neurons in the dentate gyrus were located within the polymorph layer (Fig. 10). These neurons were positioned immediately subjacent to the stratum granulosum, although a few somata appeared to be lodged among the granule neurons (Figs. 10 and 15). The size of these neurons and the apical direction of their proximal dendrite suggested that they were pyramidal basket cells<sup>13,22</sup>. Deeper in the polymorph layer, other short-axon neurons, especially the horizontal neurons<sup>13,22</sup>, were observed to contain GAD-positive reaction product within their somata and dendrites. GAD-positive somata of probable short-axon neurons were not as numerous in the molecular layer as they were in the polymorph layer. The number of these few GAD-positive somata in the molecular layer of the dentate gyrus was greater in the suprapyramidal blade than in the infrapyramidal blade. The granule neurons of the dentate gyrus did not contain GAD-positive reaction product.

Non-injected specimens of Ammon's horn (Fig. 16), as well as specimens from control injections of either lumicolchicine (Figs. 9 and 18) or saline solutions (Fig. 17), did not show nearly as many stained somata, nor nearly the extent of dendritic staining, as that observed in colchicine-injected specimens. As stated above, the number of GAD-positive neurons in colchicine-injected specimens was about 5 times the number observed in these control preparations. However, the distribution and apparent number of axon terminals was not different between the two preparations. Sections from specimens injected with colchicine and incubated in control serum, showed no reaction product within the Ammon's horn.

The various postinjection survival times used in this study did not appear to produce differential effects. For example, the rat, which was sacrificed 48 h after the injection of colchicine into Ammon's horn, revealed similar results to the rats which had a 24-h survival time. In sections incubated in anti-GAD serum, there was no difference in dendritic staining between animals from these two survival times. Also, specimens with 48- and 5-h survival times showed similar numbers and distribution patterns of GAD-positive somata and axon terminals as compared with the specimens with a 24-h survival time.

Electron microscopic preparations of specimens from colchicine-injected Ammon's horn that were incubated in anti-GAD serum, contained reaction product in axon terminals, somata and large dendritic profiles. Some of the GAD-positive axon terminals formed symmetrical synaptic junctions with pyramidal (Fig. 13) and granule cell somata and their dendritic shafts. The GAD-positive terminals forming axosomatic synapses corresponded in location to the basket cell endings as described previous-



ly<sup>2</sup>. These axon terminals had small diameters (0.1–0.3  $\mu\text{m}$ ) and were frequently seen to arise from small diameter (0.1  $\mu\text{m}$ ) preterminal axons which also contained GAD-positive reaction product.

The location of GAD-positive somata in electron microscopic preparations of Ammon's horn coincided with their distribution in light microscopic preparations of 40- $\mu\text{m}$  frozen and 1- $\mu\text{m}$  plastic sections. The distribution of reaction product within GAD-positive somata of basket and other short-axon neurons was similar to the observations in the neurons of the cerebellar cortex (Fig. 14, and compare to Fig. 7). Electron microscopic examination confirmed the light microscopic observations that reaction product was absent from pyramidal and granule cell somata and that reaction product was absent from neurons of Ammon's horn in sections incubated with control serum.

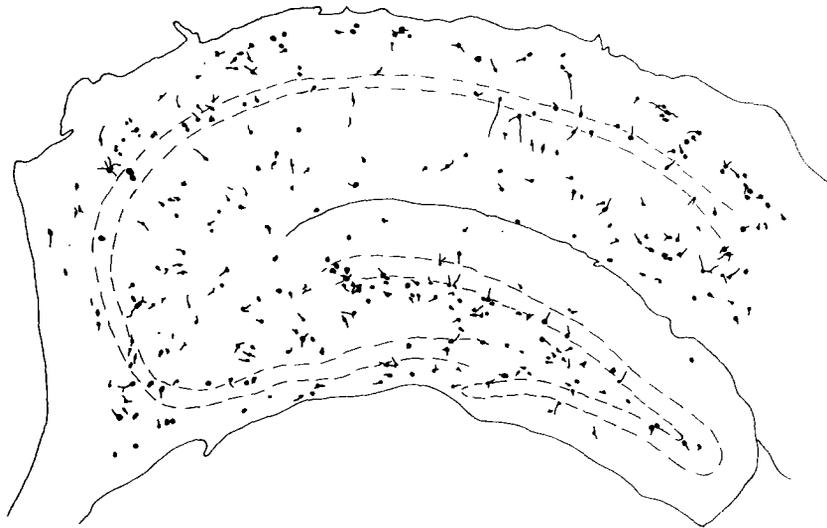
#### DISCUSSION

The results of this study indicate that the use of colchicine produces detectable concentrations of GAD in the somata and dendrites of neurons which previously had detectable concentrations limited to their axon terminals. For example, in the cerebellum, the 4 neuronal types previously described to have GAD within axon terminals<sup>15</sup> (i.e. Purkinje, Golgi II, stellate and basket neurons) were shown to contain GAD within their cell bodies following colchicine injections into the cerebellum. Following colchicine pretreatment, neurons in Ammon's horn which contained GAD in their somata were identified as basket and other short-axon neurons<sup>13,22</sup>. It is suggested that the few neurons which contained GAD within their somata in control and non-injected preparations may have presynaptic dendrites, since a previous study<sup>24</sup> of non-injected specimens has indicated that somal GAD is detected only within neurons whose dendrites form dendrodendritic synapses. However, the proof that some of the GAD-positive neurons in Ammon's horn have presynaptic dendrites must be obtained from detailed electron microscopic studies employing serial section analysis.

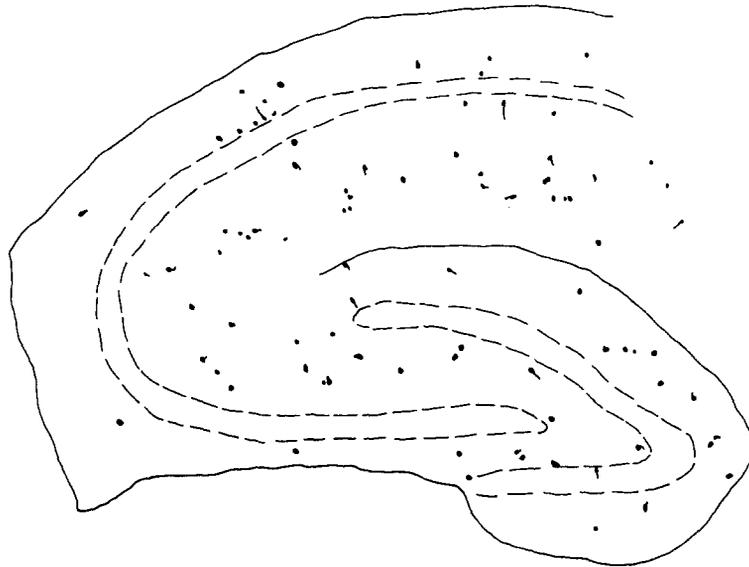
The use of colchicine for the production of detectable somal concentrations of GAD provides an effective neuroanatomical tool for the determination of the distribution of GABAergic neurons in the central nervous system. Such colchicine preparations may be used in conjunction with other experimental neuroanatomical methods (i.e. anterograde degeneration) to provide information concerning the connectivity of GABAergic neurons.

---

Figs. 13 and 14. Electron micrographs of colchicine-injected hippocampus incubated in anti-GAD serum. Fig. 13 shows an unstained soma of a hippocampal pyramidal neuron that has 4 GAD-positive axon terminals in contact with its surface (arrows).  $\times 14,500$ . Fig. 14 shows a GAD-positive neuronal soma located in stratum oriens. The opaque GAD-positive reaction product is sparsely scattered throughout the perikaryal cytoplasm but it is absent from the region of granular endoplasmic reticulum (ER). The nucleus contains an intranuclear rod (arrow).  $\times 12,000$ .

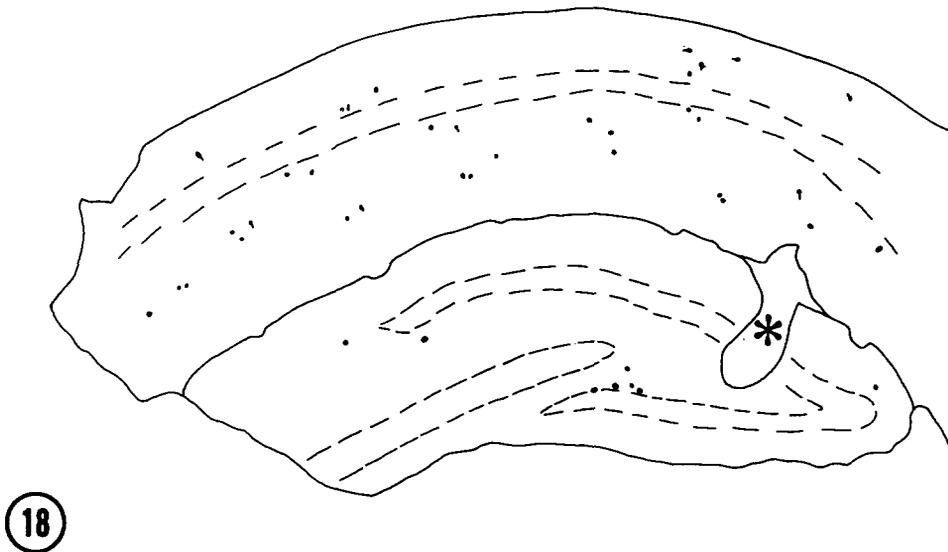
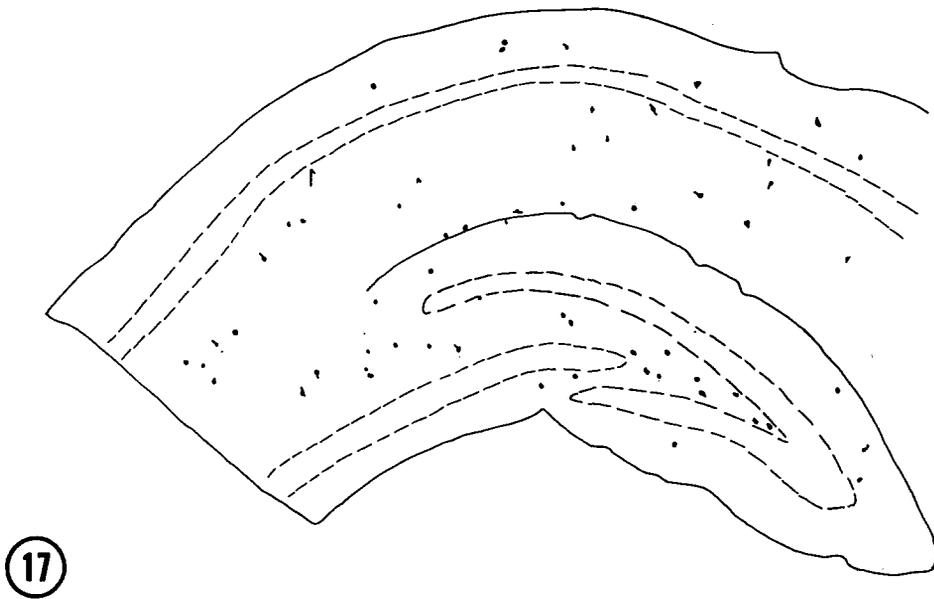


15



16

Figs. 15-18. Drawings of 40- $\mu$ m frozen sections from Ammon's horn showing the number and distribution of GAD-positive somata present in injected and control preparations. Strata granulosum and pyramidale are outlined with dashed lines. Fig. 15 shows numerous GAD-positive somata present in a colchicine-injected preparation of Ammon's horn. The proximal dendrites of most of the GAD-positive somata also display GAD-positive staining. Fig. 16 shows the GAD-positive somata observed in a non-injected preparation of Ammon's horn, and Fig. 17 is a drawing from a saline-injected Ammon's horn. Fig. 18 is from a lumicolchicine-injected Ammon's horn, and the injection site is indicated by an asterisk (\*). The number of GAD-positive neurons in the colchicine-injected preparation (Fig. 15) is about 5 times as great as the number observed in control-injected (Figs. 17 and 18) and non-injected (Fig. 16) specimens. The presence of some GAD-positive somata within preparations which did not receive colchicine suggests that these neurons may have presynaptic dendrites similar to the GAD-positive somata present in non-injected preparations of olfactory bulb (see text).  
 $\times 35$ .



*Rationale for the use of colchicine*

Colchicine has been used by other investigators to increase the concentrations of various substances within neuronal somata<sup>4,9,10</sup>. The effectiveness of this procedure apparently involves the ability of colchicine to interrupt axonal transport<sup>11,12</sup> so that proteins synthesized in the somata are prevented from being transported to axon terminals. Thus, newly synthesized proteins are accumulated in the region where they

are synthesized, the cell body. A successful application of this procedure for the immunocytochemical detection of somal GAD must also depend upon the ability of anti-GAD serum to cross-react with somal GAD. Since a previous study<sup>24</sup> has provided evidence that somal GAD is in an antigenic form, very similar to that of the GAD in synaptic terminals, it was anticipated that somal accumulations of GAD produced by colchicine would be detected by a GAD immunoperoxidase method.

*Rationale for injection of saline and lumicolchicine solutions*

Although our results show that colchicine produces detectable concentrations of somal GAD, it is possible that this effect is not due to a colchicine-mediated inhibition of axonal transport. For example, damage from injections could have disrupted axons, and this damage may have caused the effect. Another manner in which colchicine may have caused this effect indirectly is by producing an increase in membrane permeability that would have allowed for better penetration of the large immunocytochemical reagents into the somata. In addition, an increase in permeability could have been due to membrane damage caused by the injection procedure itself.

Control injections of saline and luminolchicine were used in order to test whether the observed effect of colchicine is due to its ability to block axonal flow or if it is based on one of the possibilities mentioned above. The fact that saline injections did not increase the number of GAD-positive somata over the number observed in non-injected specimens indicates that the colchicine effect is not due to damage caused by the injection procedure. Furthermore, injections of lumicolchicine, a structural isomer of colchicine which has the same membrane effect as colchicine<sup>27</sup>, did not increase the number of stained somata above that found in non-injected specimens. This indicates that the colchicine effect is not due to a drug-induced increase in membrane permeability.

In contrast to colchicine, lumicolchicine does not readily bind with tubulin<sup>27</sup>. This relative inability of lumicolchicine to bind with tubulin, as well as its inability to inhibit fast axoplasmic transport<sup>20</sup>, support the contentions that (1) colchicine's ability to produce somal staining for GAD is specifically due to the drug's effect on axonal transport and (2) the effect of colchicine on axonal transport involves its binding with tubulin. Whether or not the tubulin involved in axonal transport is associated with microtubules<sup>18,26</sup> and/or with some axonal membrane system<sup>3,8</sup> remains unclear.

*Differences in the somatofugal transport of GAD in PSA and PSD neurons*

Unlike most of the neurons observed in this study, the GAD-positive neurons in the olfactory bulb<sup>24</sup> have dendrites which are presynaptic to other dendrites, and they can be classified as presynaptic dendrite (PSD) neurons. In contrast, the GAD-positive neurons in the cerebellum only have axon terminals as their presynaptic sites, and they can be classified as presynaptic axon (PSA) neurons. The present study shows that a somal localization of GAD within PSA neurons depends upon an inhibition of fast axonal transport, while the investigation of the olfactory bulb<sup>24</sup> shows that such a localization within PSD neurons does not require any experimental alteration

of somatofugal transport. Therefore these observations support the previous suggestions<sup>24</sup> that a somatofugal transport of GAD may be effected more rapidly in PSA neurons than in PSD neurons, and that this is probably the basis for the observed differences in GAD staining of PSA and PSD neuronal somata.

#### ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. Eugene Roberts' vital role in coordinating the immunochemical studies essential to the present investigation. We also wish to thank Dr. Jan-Yen Wu for supplying the purified glutamic acid decarboxylase. In addition, we acknowledge the helpful criticisms of our manuscript received from Drs. Richard Hammerschlag, Dee Ann Matthews, and Richard Wimer, the expert technical support given by Lynn Anderson, Robert P. Barber, Donald L. Cimarusti, Mariko Nakashima, J. Randall Slemmon and Christine S. Vaughn, and the secretarial assistance of Donna Dreier.

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

#### REFERENCES

- 1 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. B. Tower (Eds.), *GABA in Nervous System Function*, Raven Press, New York, 1976, pp. 113-132.
- 2 Blackstad, T. W. and Flood, P. R., Ultrastructure of hippocampal axo-somatic synapses, *Nature (Lond.)*, 198 (1953) 542-543.
- 3 Byers, M. A., Structural correlates of rapid axonal transport: evidence that microtubules may not be directly involved, *Brain Research*, 75 (1974) 97-113.
- 4 Dahlström, A., Effect of colchicine on transport of amine storage granules in sympathetic nerves of rat, *Europ. J. Pharmacol.*, 5 (1968) 111-113.
- 5 Droz, B., Synthèse et transfert des protéines cellulaires dans les neurones ganglionnaires étude radioautographique quantitative en microscopie électronique, *J. Microscopie*, 6 (1967) 201-228.
- 6 Droz, B. and Leblond, C. P., Migration of proteins along the axons of the sciatic nerve, *Science*, 137 (1962) 1047-1048.
- 7 Droz, B. and Leblond, C. P., Axonal migration of proteins in the central nervous system and peripheral nerves as shown by radioautography, *J. comp. Neurol.*, 121 (1963) 325-346.
- 8 Droz, B., Rambourg, A. and Koenig, H. L., The smooth endoplasmic reticulum: structure and role in the renewal of axonal membrane and synaptic vesicles by fast axonal transport, *Brain Research*, 93 (1975) 1-13.
- 9 Hökfelt, T. and Dahlström, A., Effects of two mitosis inhibitors (colchicine and vinblastine) on the distribution and axonal transport of noradrenaline storage particles, studied by fluorescence and electron microscopy, *Z. Zellforsch.*, 119 (1971) 460-482.
- 10 Hökfelt, T., Kellerth, J.-O., Nilsson, G. and Pernow, B., Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons, *Brain Research*, 100 (1975) 235-252.
- 11 Karlsson, J. O. and Sjöstrand, J., The effect of colchicine on the axonal transport of protein in the optic nerve and tract of the rabbit, *Brain Research*, 13 (1969) 617-619.
- 12 Kreutzberg, G. W., Neuronal dynamics and axonal flow. IV. Blockage of intra-axonal enzyme transport by colchicine, *Proc. nat. Acad. Sci. (Wash.)*, 62 (1969) 722-728.
- 13 Lorente de Nó, R., Studies on the structure of the cerebral cortex. II. Continuation of the study of the Ammonic system, *J. Psychol. Neurol. (Lpz.)*, 46 (1934) 113-177.
- 14 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.

- 15 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.
- 16 Millonig, G., Advantages of a phosphate buffer for OsO<sub>4</sub> solutions in fixation, *J. appl. Phys.*, 32 (1961) 1637.
- 17 Mizel, S. B. and Wilson, L., Nucleoside transport in mammalian cells. Inhibition by colchicine, *Biochemistry*, 11 (1972) 2573-2578.
- 18 Ochs, S., Fast transport of materials in mammalian nerve fibers, *Science*, 176 (1972) 252-260.
- 19 Palay, S. and Chan-Palay, V., *Cerebellar Cortex: Cytology and Organization*, Springer, New York, 1974.
- 20 Paulson, J. C. and McClure, W. O., Microtubules and axoplasmic transport. Inhibition of transport by podophyllotoxin: an interaction with microtubule protein, *J. Cell Biol.*, 67 (1975) 461-467.
- 21 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, pp. 56-76.
- 22 Ramón y Cajal, S., *Estructura del asta de Ammon*, *Anat. Soc. Esp. Histol. Nat. Madrid.*, 22 (1893) 53-114. English translation, *The Structure of Ammon's Horn*, Charles C Thomas, Springfield, Ill., 1968, 78 pp.
- 23 Ribak, C. E., Vaughn, J. E., Saito, K., Barber, R. and Roberts, E., Immunocytochemical localization of glutamate decarboxylase in rat substantia nigra, *Brain Research*, 116 (1976) 287-298.
- 24 Ribak, C. E., Vaughn, J. E., Saito, K., Barber, R. and Roberts, E., Glutamate decarboxylase localization in neurons of the olfactory bulb, *Brain Research*, 126 (1977) 1-18.
- 25 Saito, K., Barber, R., Wu, J.-Y., Matsuda, T., Roberts, E. and Vaughn, J. E., Immunohistochemical localization of glutamate decarboxylase in rat cerebellum, *Proc. nat. Acad. Sci. (Wash.)*, 71 (1974) 269-273.
- 26 Schmitt, F. O., Fibrous proteins — neuronal organelles, *Proc. nat. Acad. Sci. (Wash.)*, 60 (1968) 1092-1101.
- 27 Wilson, L., Bamburg, J. R., Mizel, S. B., Grisham, L. M. and Creswell, K. M., Interaction of drugs with microtubule proteins, *Fed. Proc.*, 33 (1974) 158-166.
- 28 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, 1976, pp. 133-148.