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ORIGINAL ARTICLE

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# Astrocytic processes compensate for the apparent lack of GABA transporters in the axon terminals of cerebellar Purkinje cells

**Abstract** The aim of the present study was to evaluate the expression of two high affinity GABA transporters (GAT-1 and GAT-3) in the rat cerebellum using immunocytochemistry and affinity purified antibodies. GAT-1 immunoreactivity was prominent in punctate structures and axons in all layers of the cerebellar cortex, and was especially prominent around the somata of Purkinje cells. In contrast, the deep cerebellar nuclei showed few if any GAT-1 immunoreactive puncta. Weak GAT-3 immunoreactive processes were present in the cerebellar cortex, whereas GAT-3 immunostaining was prominent around the somata of neurons in the deep cerebellar nuclei. Electron microscopic preparations of the cerebellar cortex demonstrated that GAT-1 immunoreactive axon terminals formed symmetric synapses with somata, axon initial segments and dendrites of Purkinje cells and the dendrites of granule cells. Astrocytic processes in the cerebellar cortex were also immunolabeled for GAT-1. However, Purkinje cell axon terminals that formed symmetric synapses with neurons in the deep cerebellar nuclei lacked GAT-1 immunoreactivity. Instead, weak GAT-1 and strong GAT-3 immunoreactivities were expressed by astrocytic processes that enveloped the Purkinje cell axon terminals. In addition, GAT-3-immunoreactivity appeared in astrocytic processes in the cerebellar cortex. These observations demonstrate that GAT-1 is localized to axon terminals of three of the four neuronal types that were previously established as being GABAergic, i.e. basket, stellate and Golgi cells. GAT-1 and GAT-3 are expressed by astrocytes. The failure to identify a GABA transporter in Purkinje cells is consistent with previous data that indicated that Purkinje cells lacked terminal uptake mechanisms for GABA. The individual glial envelopment of Purkinje cell axon terminals in the deep cerebellar nuclei and the dense immunostaining of GAT-3, and to a lesser extent GAT-1, expressed by astrocytic processes provide a compensatory mechanism for the removal of GABA from the synaptic cleft of synapses formed by Purkinje cell axon terminals.

**Key words** Deep cerebellar nuclei · Cerebellar cortex · Basket cells · Stellate cells · Immunocytochemistry

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## Introduction

The Purkinje cells of the cerebellum are probably the first well-documented GABAergic inhibitory neurons in the mammalian brain (Eccles et al. 1967; Chan-Palay 1977). In addition to Purkinje cells, three other cerebellar cortical neurons – basket, stellate and Golgi cells, were described as having immunoreactivity for the GABA-synthesizing enzyme, glutamate decarboxylase (GAD; McLaughlin et al. 1974; Saito et al. 1974; Ribak et al. 1978; Oertel et al. 1981). Subsequent studies showed that all four cell types express GAD and $\gamma$-aminobutyric acid (GABA; Oertel et al. 1981; Seguela et al. 1984; Ottersen et al. 1987). GABAergic neurons have subsequently been identified by immunocytochemical and in situ hybridization methods (Greif et al. 1991). Thus, the presence of GAD and GABA in Purkinje cells confirmed the physiological and pharmacological findings that indicated these neurons were GABAergic.

In the past 25 years, some doubt has been raised about Purkinje cells being GABAergic because they did not take up $^3$H-GABA when it was placed into the cerebellar cortex, deep cerebellar nuclei or vestibular nuclei (Hökfelt and Ljungdahl 1970; Kelly and Dick 1976; Tol-

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bert and Bantli 1980; Wiklund et al. 1983). In contrast, $^3$H-GABA placed into the cerebellar cortex resulted in the labeling of basket, stellate and Golgi cells (Hökfelt and Ljungdahl 1970, 1972; Schon and Iversen 1972; Chan-Palay 1977; Tolbert and Bantli 1980). These studies indicated that Purkinje cells lack a GABA carrier that can take up GABA from the extracellular space.

Recently, four GABA transporters, GAT-1, GAT-2, GAT-3 and a betaine glycine transporter (BGT-1), have been cloned and found to be expressed in brain (Guastella et al. 1990; Borden et al. 1992; Clark et al. 1992; Lopez-Corcuera et al. 1992; Brecha and Weigmann 1993, 1994; Liu et al. 1993; Durkin et al. 1995). All four of the transporters take up GABA in a $Na^+$ and $Cl^-$ dependent manner, and they share a high degree of predicted amino acid sequence identity (Guastella et al. 1990; Borden et al. 1992) Specific polyclonal antibodies to the GATs have been used in immunocytochemical studies to determine their tissue distribution and cellular localization. GAT-1 is found predominantly in neurons (Clark et al. 1992; Brecha and Weigmann 1994; Ikegaki et al. 1994; Minelli et al. 1995; Johnson et al. 1996; Ribak et al. 1996). GAT-2 is found in ependymal cells, whereas GAT-3 is predominantly expressed in astrocytes (Ikegaki et al. 1994; Durkin et al. 1995; Johnson et al. 1996; Ribak et al. 1996). Specific affinity purified antibodies to GAT-1 and GAT-3 were used in the present study to determine their cellular localization in the cerebellum. The results indicate that the axon terminals of three of the four GABAergic cerebellar neurons contain GAT-1 whereas those of the Purkinje cells lack GABA transporters. In addition, the astrocytic processes that envelop the axon terminals of Purkinje cells are densely labeled for GAT-3 and to a lesser extent GAT-1.

## Methods and materials

### Animals

Adult albino rats (Sprague-Dawley; Harlan, San Diego, Calif.), weighing 180–250 g were used in these studies. They were fed and housed under regular conditions. Care and handling of animals were approved by the Animal Research Committees of the University of California at Irvine and the VAMC-West Los Angeles in accordance with all NIH guidelines.

### Antibodies

Affinity purified, polyclonal antibodies directed to GAT-1 (346J) and GAT-3 (369D) were used in these studies (Minelli et al. 1995, 1996; Johnson et al. 1996; Ribak et al. 1996). The GAT-1 antibody is directed to the last 12 amino acids of the predicted C-terminus of rat GAT-1 (GAT-$1_{588-599}$). The GAT-3 antibody is directed to the last 21 amino acids of the predicted C-terminus of rat GAT-3 (GAT-$3_{607-627}$). These antibodies were affinity purified using either an Epoxy-Sepharose-GAT-$1_{588-599}$ or an Epoxy-Sepharose-GAT-$3_{607-627}$ affinity column (Pharmica Biotech, Piscataway, N.J.).

The specificity of the immunostaining in the cerebellum was demonstrated by the lack of immunostaining in sections when using: (a) preimmune serum in place of the primary antibodies, (b) the GAT-1 antibody which was preadsorbed overnight with $10^{-5}$ M GAT-$1_{588-599}$, (c) the GAT-3 antibody which was preadsorbed

overnight with $10^{-5}$ M GAT-$3_{607-627}$. In addition, the pattern of GAT-1 immunostaining was unchanged when the primary antibody was preadsorbed overnight with $10^{-5}$ M rat GAT-$2_{594-602}$, rat GAT-$3_{607-627}$ or rat glycine transporter-$1_{625-633}$ (GLYT-$1_{625-633}$) (Guastella et al. 1990, 1992; Borden et al. 1992). Finally, the pattern of GAT-3 immunostaining was unchanged when the primary antibody was preadsorbed with $10^{-5}$ M rat GAT-$1_{588-599}$, rat GAT-$2_{594-602}$ or rat GLYT-$1_{625-633}$.

### Immunocytochemistry; light microscopy

Rats were anesthetized with Nembutal (100 mg/kg) and perfused through the heart with 0.12 M PBS (pH 7.4) followed by 4% paraformaldehyde (PFA) in 0.12 M phosphate buffer at pH 7.4. The brain was removed and postfixed in 4% PFA in 0.12 M phosphate buffer at 4°C overnight. Sections were cut at 50 μm in the parasagittal plane with a vibratome and stored in 0.12 M phosphate buffer for immunocytochemical processing.

Tissue sections containing the cerebellum were washed in 0.12 M phosphate buffer, incubated in 10% normal goat serum in 0.12 M PBS for 1 h and washed again. The sections were incubated in antibodies directed to GAT-$1_{588-599}$ (1:500–1:2000) or GAT-$3_{607-627}$ (1:1000) in 0.12 M PBS with 0.5% Triton X-100 and 10% normal goat serum overnight at 4°C. The sections were washed, incubated in biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, Calif.) at a dilution of 1:200 in 1% normal goat serum and 0.12 M PBS for 1 day at 4°C. The sections were washed, incubated in an avidin-biotin peroxidase mixture (Vectastain ABC Kit, Vector Laboratories) for 1 day at 4°C. The sections were then washed, incubated in 0.5% 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.01% $H_2O_2$ for 15–20 min. The sections were washed, mounted onto gelatin-coated slides, dehydrated and coverslipped.

### Immunocytochemistry; electron microscopy

Rats were anesthetized with Nembutal (100 mg/kg) and perfused with 0.1 M PBS (pH 7.4), followed by 200 ml of 4% PFA and 0.1% glutaraldehyde in 0.12 M phosphate buffer at pH 7.4. The brains were removed and placed in 4% PFA solution overnight at 4°C. The next day, brains were removed from PFA and sectioned in the parasagittal plane with a vibratome at 50 μm.

Sections containing the cerebellum were rinsed with 0.12 M PBS for 15 min before they were immersed in 10% normal goat serum in 0.12 M PBS. The sections were then placed in GAT-1 or GAT-3 antibodies and incubated overnight at 4°C. The primary antibody was diluted 1:1000 with 10% normal goat serum and 0.5% Triton X-100. The sections were washed three times in 0.1 M PBS for 15 min in each rinse. All subsequent washings of sections were carried out following this same procedure. Sections were incubated in biotinylated goat anti-rabbit IgG at a dilution of 1:200 and 1% normal goat serum in 0.1 M PBS for 1 h at room temperature. After rinsing the sections in PBS, they were incubated in ABC complex solution for 1 h. The ABC complex was prepared by mixing 1% Avidin DH and 1% biotinylated enzyme with 0.1% Triton X-100 in PBS at pH 7.5. The sections were thoroughly washed before being incubated in 0.5% DAB solution diluted with 0.01% $H_2O_2$ in 0.1 M PBS for 20 min. Finally, the sections were removed and rinsed.

These sections were osmicated with cold (4°C) 1% $OsO_4$ for 30 min, dehydrated in graded ethanol, and embedded in Medcast. Semithin 2-μm sections were cut from these blocks for light microscopic analysis, i.e., photographed and examined to identify the cerebellar cortex and deep cerebellar nuclei. Also, the layers in the cerebellar cortex were mapped for orientation of the thin sections in the transmission electron microscope. Ultrathin sections were cut on a Sorvall MT-5000 microtome, collected on Formvar coated slot grids, stained with lead citrate and uranyl acetate and examined with a Philips CM-10 electron microscope.

## Results

### Light microscopic localization of GAT-1 and GAT-3

#### Cerebellar cortex

The distribution of GAT-1 immunolabeling was similar to that described previously for GAD (Saito et al. 1974). GAT-1 positive puncta surrounded the somata of Purkinje cells (Fig. 1). Purkinje cells also had several puncta associated with the basal portion of their soma, the so-called pinceau region (Palay and Chan-Palay 1974). In addition, GAT-1 immunolabeled puncta and fibers were present in the molecular layer. The granule cell layer displayed GAT-1 puncta interspersed between the granule cells.

The light microscopic distribution of GAT-3 in the cerebellar cortex was much different. Small GAT-3 puncta were found randomly scattered in the three layers (Fig. 1). There were no clusters of GAT-3 puncta along the surfaces of Purkinje cells.

#### Deep cerebellar nuclei

GAT-1 positive immunolabeling was sparse in the deep cerebellar nuclei (Fig. 1). Only a few small puncta were found. They were not concentrated along the surfaces of the neurons in this region.

In contrast, GAT-3 immunolabeled puncta were concentrated on the surfaces of neurons in the deep cerebellar nuclei (Fig. 1). The immunolabeling for GAT-3 continued onto the surfaces of dendrites and outlined most of them. The white matter tracts approaching the deep cerebellar nuclei did not display immunolabeling.

### Electron microscopic localization of GAT-1 and GAT-3

#### Cerebellar cortex

GAT-1 immunoreactivity was localized in axon terminals in all three layers of the cerebellar cortex (Figs. 2, 3). Also, small astrocytic processes displayed immunoreaction product for GAT-1. The labeled axon terminals in the Purkinje cell layer formed symmetric synapses with the somata of Purkinje cells (Fig. 2A,B). The reaction product was distributed heterogeneously in these axon terminals with dense accumulations associated with the plasma membrane and lighter labeling decorating synaptic vesicles and mitochondria. The preterminal portions of these axon terminals also displayed immunoreaction product.

Labeled axon terminals for GAT-1 were also present in the region of the axon initial segment of Purkinje cells in the granule cell layer (Fig. 2C). This region is called the "pinceau" (Palay and Chan-Palay 1974). Several immunolabeled axons were found to run parallel with the axon initial segment. Only a few of these labeled axons

formed symmetric synapses with the axon initial segment in any one thin section. Small labeled axon terminals were found in the deeper parts of the granule cell layer where they formed symmetric synapses with dendrites of granule cells that were also postsynaptic to mossy fibers (not shown).

The GAT-1 positive axon terminals in the molecular layer formed symmetric synapses with dendritic shafts (Fig. 3A,B). Some of the dendrites were of large diameter and arose from Purkinje cells. Smaller dendrites were also postsynaptic to labeled axon terminals and these latter dendrites may arise from basket, stellate or Golgi cells. In the inner molecular layer, GAT-1 positive axon terminals also formed axosomatic symmetric synapses with small somata that had morphological features of basket cells (Fig. 3C).

GAT-3 immunolabeling in the cerebellar cortex was found in astrocytic processes in all of the layers (Fig. 4). The labeling for GAT-3 positive profiles was sparse and so was that for GAT-1 in the deep cerebellar nuclei. GAT-3 immunolabeled profiles occurred in the neuropil near, but not adjacent, to axon terminals forming symmetric synapses.

#### Deep cerebellar nuclei

The deep cerebellar nuclei were analyzed to determine whether the axon terminals of Purkinje cells express GAT-1. GAT-1 immunoreactivity was not observed in axon terminals that formed symmetric synapses with the dendrites and somata of neurons in the deep cerebellar nuclei (Fig. 5). Previous studies showed that most axon terminals from Purkinje cells form symmetric synapses in the deep cerebellar nuclei (Walberg et al. 1976). The only immunolabeling for GAT-1 in the deep cerebellar nuclei was in processes of astrocytes (Fig. 5). Some of these were found in close apposition to the unlabeled axon terminals that formed symmetric axosomatic and axodendritic synapses.

GAT-3 immunolabeling was very prominent in the deep cerebellar nuclei. Very dense immunolabeling occurred in astrocytic processes that surrounded the Purkinje cell axon terminals that form symmetric axosomat-

**Fig. 1** Photomicrographs of semi-thin 2 μm sections of the cerebellar cortex (**A, B**) and deep cerebellar nuclei (**C, D**). **A** Shows the immunopositive GAT-1 structures in the cerebellar cortex. Labeled axons (*arrowheads*) appear in the molecular layer (*M*) where they contact a Purkinje cell dendrite (*curved arrow*). The somata of Purkinje cells (*P*) are outlined by GAT-1 labeled puncta (*arrows*), that are probably the axons of basket cells. The Purkinje cell on the right also shows immunolabeled axons in the pinceau region (*large arrow*). Only a few puncta are found in the granule cell layer (*G*). **B, C** Show sparse immunolabeling for GAT-3 in the cerebellar cortex, and GAT-1 in the deep cerebellar nuclei, respectively. **C** Shows two somata (*d*) of deep cerebellar neurons. **D** Shows strong immunolabeling (*arrows*) for GAT-3 around the somata of two deep cerebellar neurons (*d*). Nomarski optics. **A** ×1,200, **B** ×1,200, **C** ×1,200, **D** ×1,200
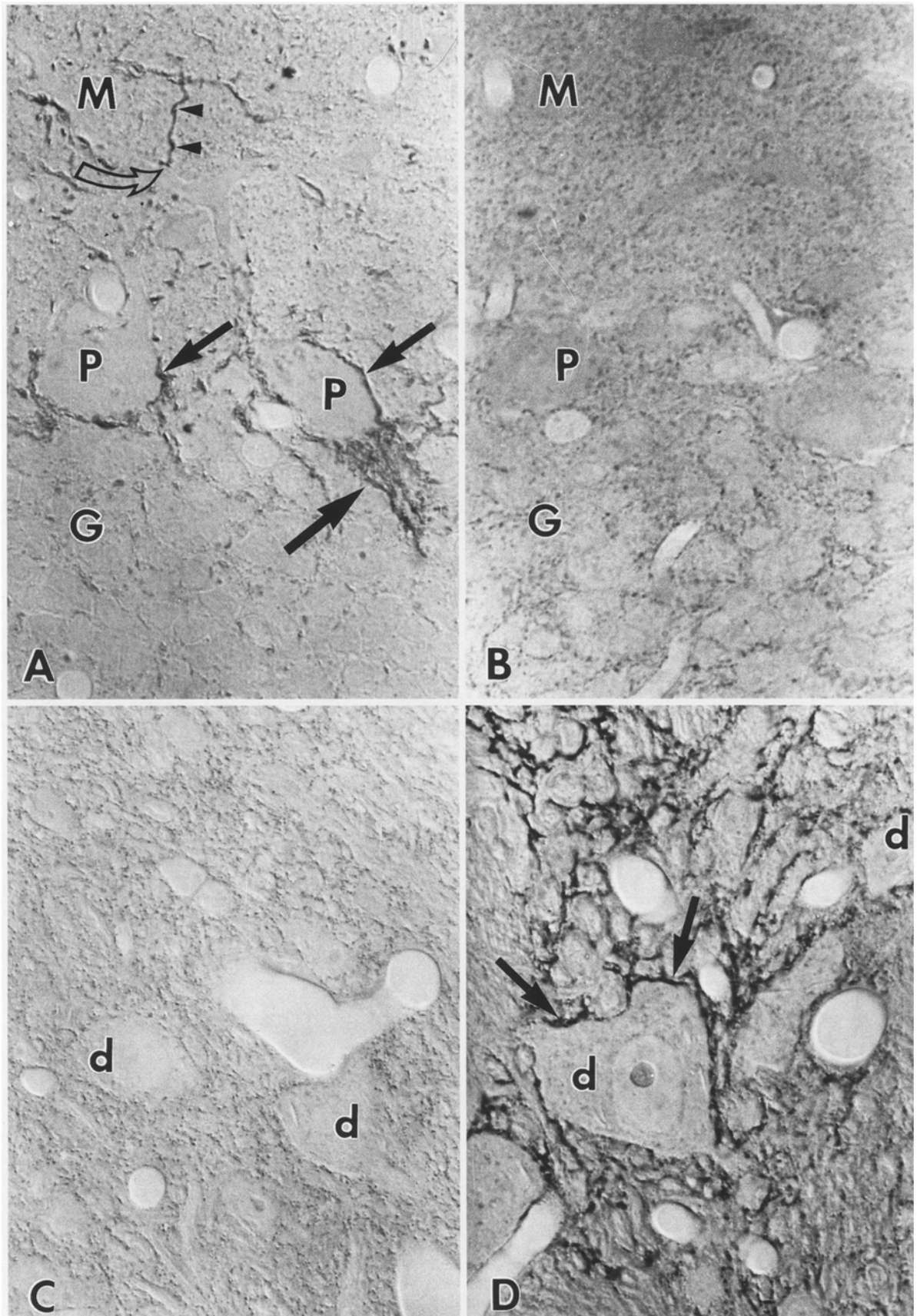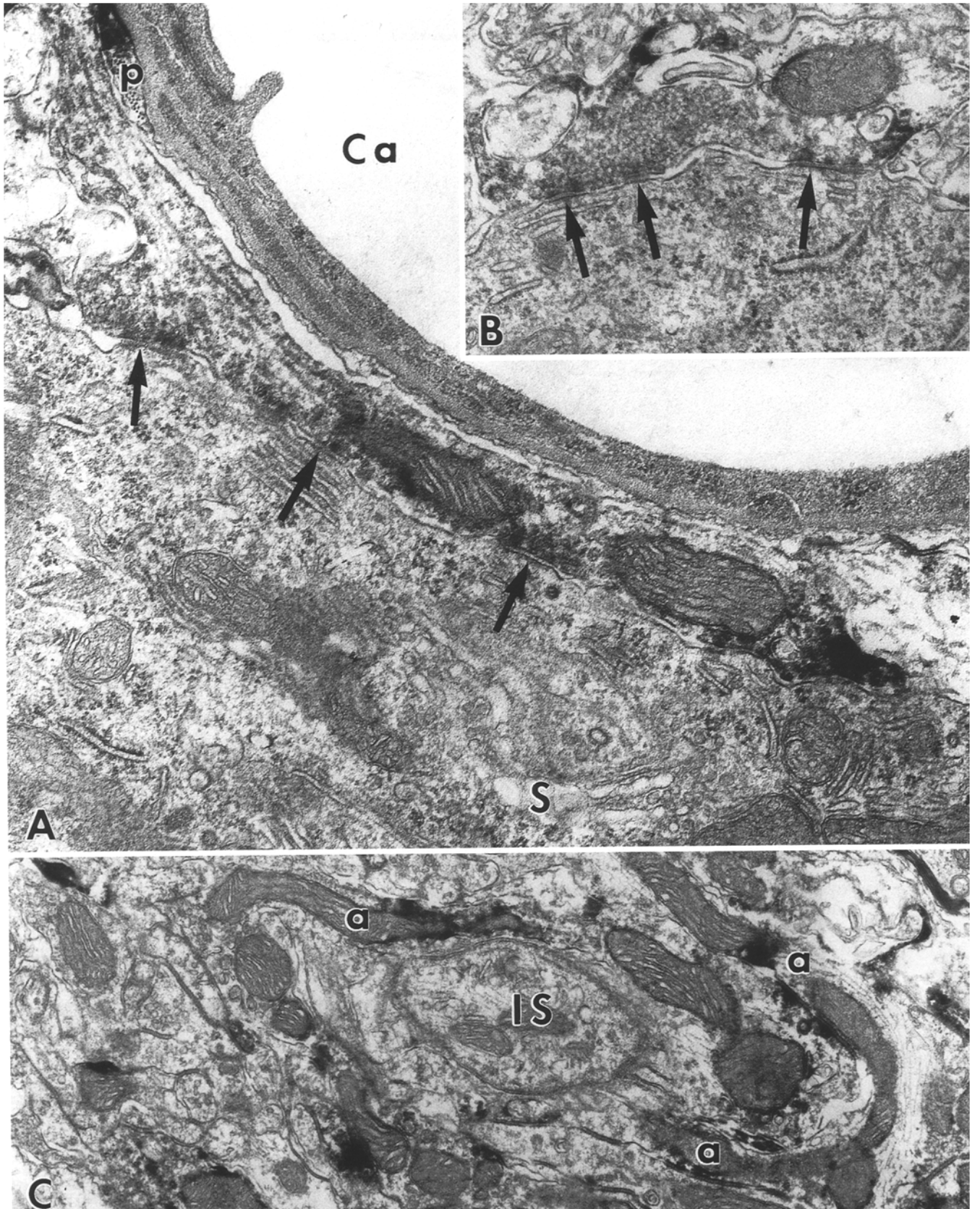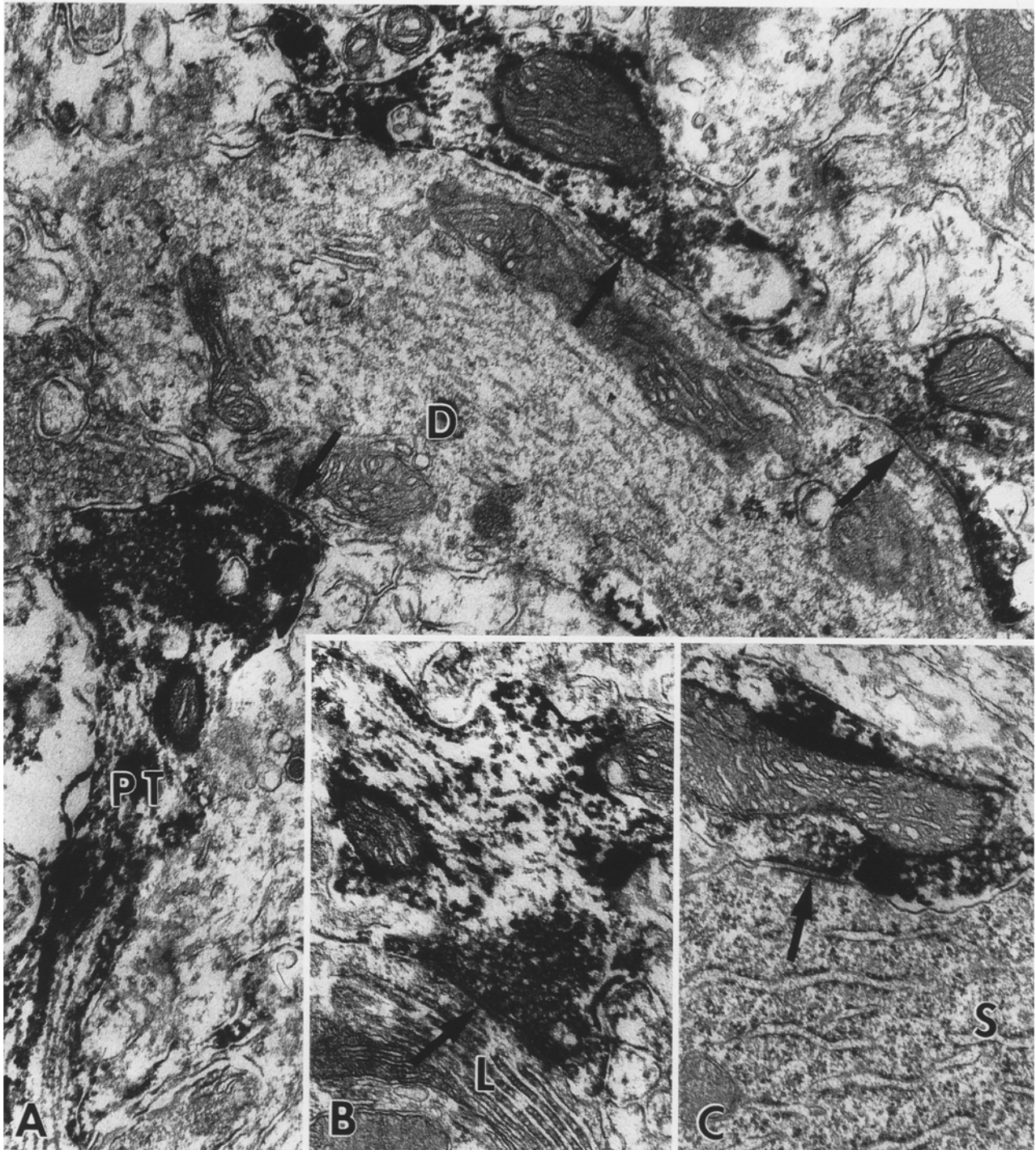
**Fig. 1A–D**

Fig. 2A–C

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◀ **Fig. 2A–C** GAT-1 immunolabeling of axon terminals in the Purkinje cell layer. **A** Shows an elongated GAT-1-immunopositive basket axon terminal that forms three symmetric synapses (*arrows*) with a soma (*S*) of a Purkinje cell located next to a capillary (*Ca*). A small endfoot process (*p*) of an astrocyte also displays immunostaining for GAT-1. **B** Another example of a GAT-1 immunoreactive axon terminal forming axosomatic synapses with a Purkinje cell. Note the three symmetric synapses (*arrows*). **C** Shows several GAT-1 labeled axon terminals (*a*) in the pinceau region of the axon initial segment (*IS*) of a Purkinje cell. **A** ×43,000, **B** ×42,000, **C** ×28,000
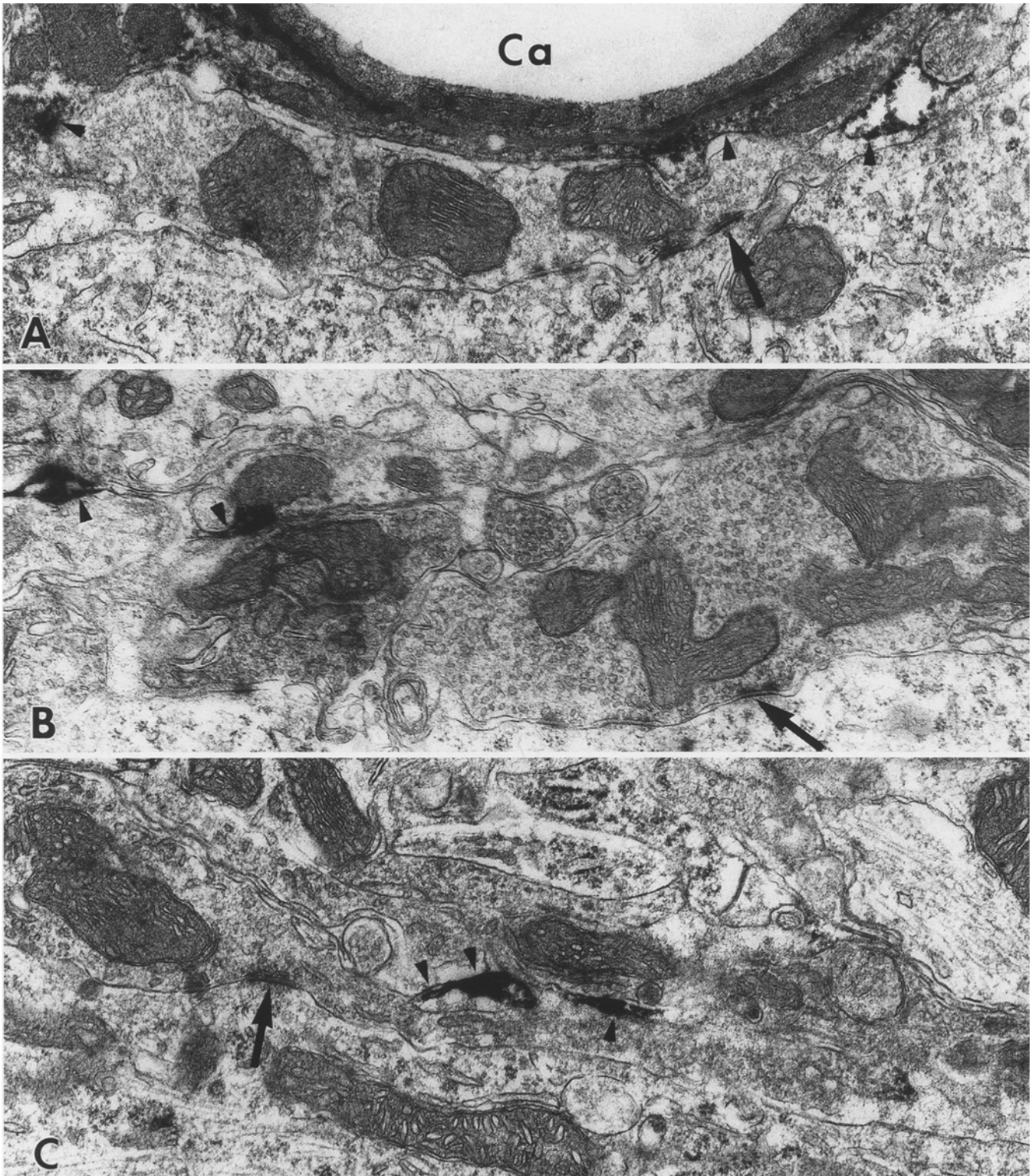
**Fig. 3A–C** GAT-1 immunolabeling of axon terminals in the molecular layer of the cerebellar cortex. **A** Shows three GAT-1 immunoreactive axon terminals that form symmetric synapses (*arrows*) with a medium-sized dendrite (*D*). Note that one of the axon terminals has immunolabeling that continues into its preterminal segment (*PT*). **B** Shows another GAT-1 positive axon terminal that forms a symmetric synapse (*arrow*) with a large dendrite from a Purkinje cell. Note the lamellar body (*L*) in the cytoplasm of the dendrite. **C** Shows a GAT-1 immunolabeled axon terminal that forms a symmetric synapse (*arrow*) with a soma (*S*) of a basket cell located in the inner molecular layer. **A,B** ×43,000, **C** ×42,000
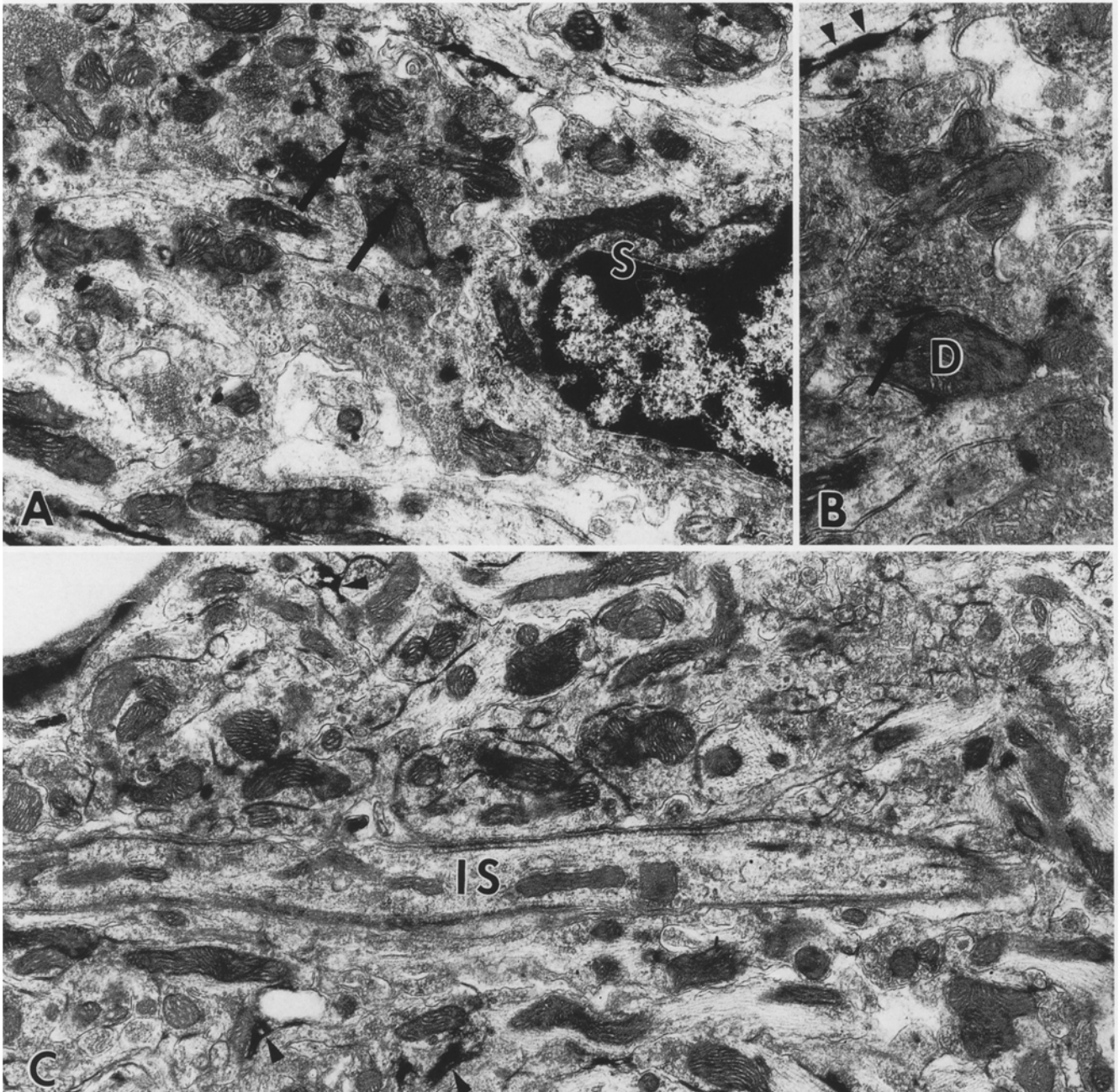
**Fig. 4A–C** GAT-3 immunolabeling in the granule cell layer of the cerebellar cortex. **A** Shows portions of a glomerulus where immunonegative axon terminals of Golgi cells form symmetric synapses (*arrows*) with dendrites of granule cells. A portion of a soma (*S*) of a granule cell is also shown. **B** Shows an enlargement of the synapse with one of these dendrites (*D*), the one on the right. Note that GAT-3 immunoreaction product is found in small processes (*arrowheads*) of astrocytes. **C** Shows an axon initial segment (*IS*) of a Purkinje cell with several adjacent immunonegative axon terminals. GAT-3 immunolabeling is present in astrocytic processes (*arrowheads*). **A** ×17,000, **B** ×30,000, **C** ×16,000

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**Fig. 5A–C** GAT-1 immunolabeling in the deep cerebellar nuclei. **A–C** Show that axon terminals forming symmetric synapses (*arrows*) with somata (in **A,B**) or dendrites (**C**) of deep cerebellar neurons lack immunostaining for GAT-1. In contrast, small astrocytic processes (*arrowheads*) that are found near these terminals display GAT-1 immunoreaction product (*Ca* capillary). **A** ×32,000, **B** ×31,000, **C** ×37,000
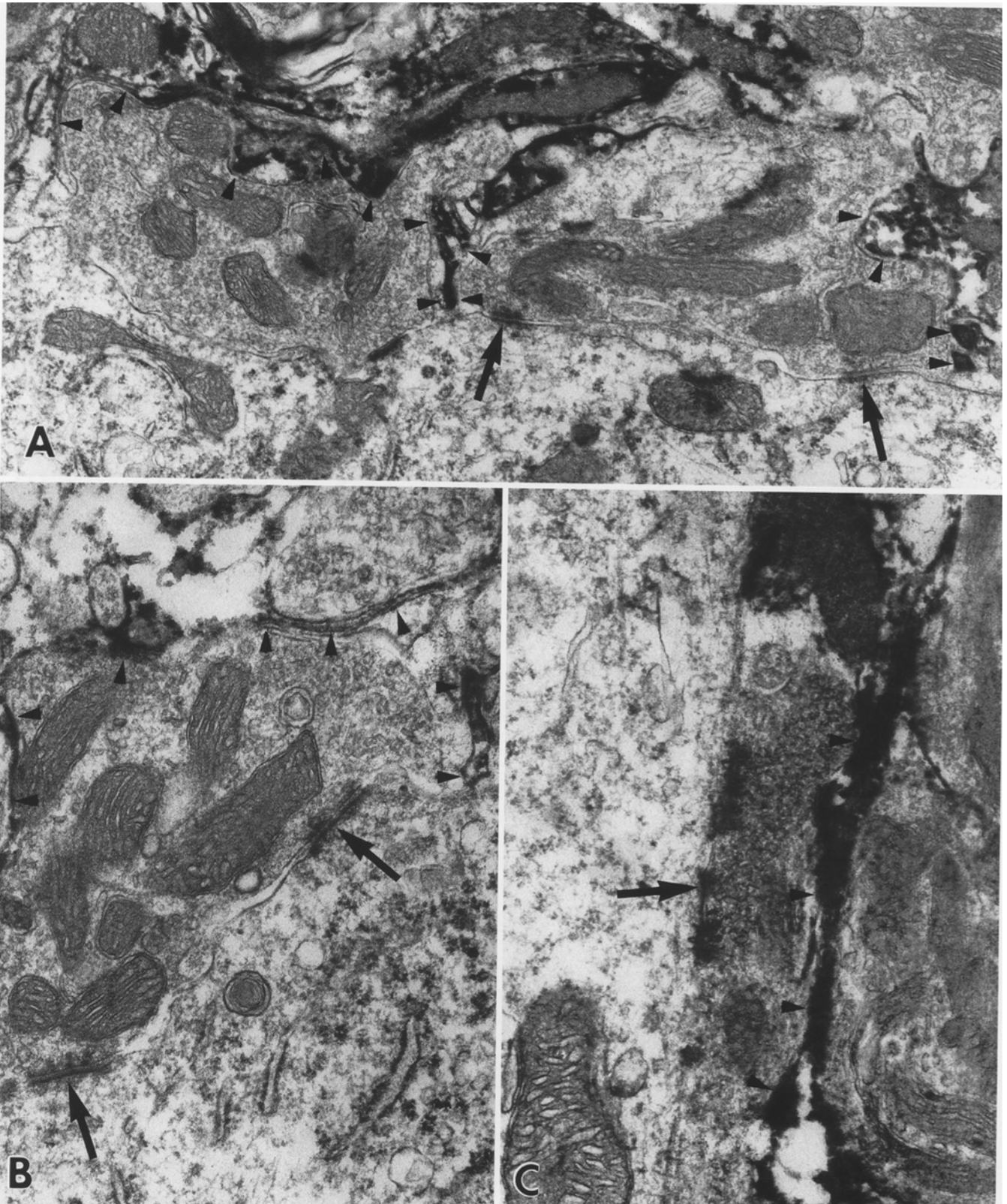
**Fig. 6A–C** GAT-3 immunolabeling of astrocytic processes (*arrowheads*) in the deep cerebellar nuclei. **A–C** Show several immunonegative axon terminals that form symmetric synapses (*arrows*) with somata (**A,B**) and dendrites (**C**) and GAT-3 immunopositive astrocytic processes (*arrowheads*) that envelop them. **A** ×32,000, **B** ×43,000, **C** ×43,000
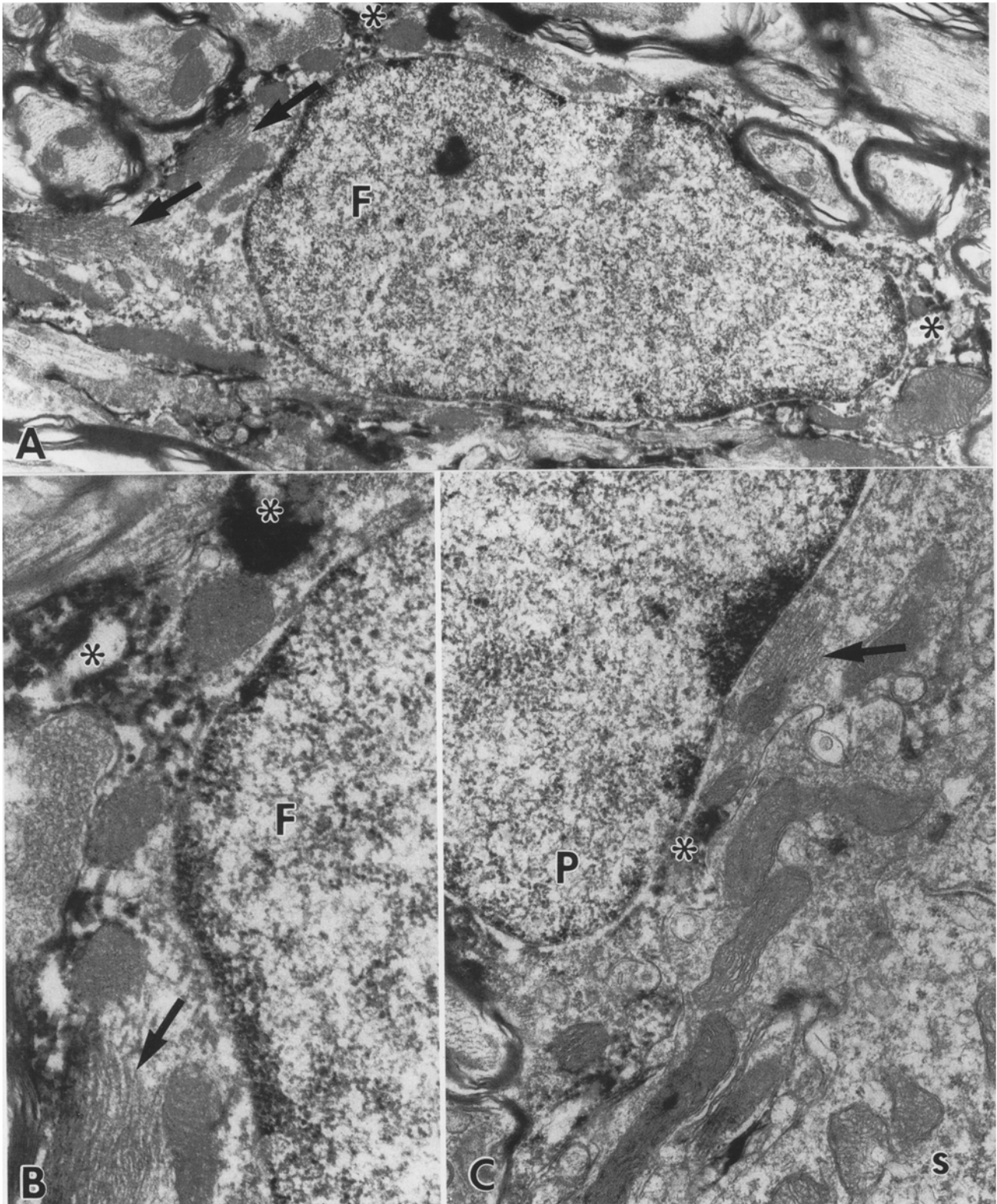
**Fig. 7A–C** GAT-3 immunolabeling of the perikaryal cytoplasm of astrocytes in deep cerebellar nuclei. **A** Show a fibrous astrocyte (*F*) in the white matter that contains GAT-3 immunoreaction product (*asterisks*). Note that the glial filaments (*arrows*) have no immunolabeling associated with them. **B** An enlargement of the top portion of this astrocyte to show the GAT-3 immunolabeling (*as-terisks*) and glial filaments (*arrow*). **C** Shows a portion of a protoplasmic astrocyte (*P*) with GAT-3 immunolabeling (*asterisk*). The glial filaments (*arrow*) show no immunolabeling. An adjacent soma (*s*) of a neuron is immunonegative. **A** ×15,000, **B** ×37,000, **C** ×29,000

ic and axodendritic synapses (Fig. 6). The density of immunoreaction product in these astrocytic processes with GAT-3 was greater than that for GAT-1 (Figs. 5, 6). The somata of astrocytes also displayed large amounts of GAT-3 immunoreaction product (Fig. 7). The labeling in the somata of astrocytes avoided the bundles of glial filaments in their cytoplasm (Fig. 7B).

## Discussion

One of the major findings of this study is that GAT-1, and not GAT-3, is found in the axon terminals of three GABAergic cell types in the cerebellum, but not in the fourth type, the Purkinje cells. That is, axon terminals of basket cells forming symmetric axosomatic synapses with Purkinje cell bodies expressed GAT-1. Likewise, axon terminals of stellate and Golgi cells were also immunopositive for GAT-1. These findings are consistent with results from a previous study of the cerebellar cortex using polyclonal antibodies to a partially purified GABA transporter (Radian et al. 1990). However, the present study showed that the axon terminals of Purkinje cells in the deep cerebellar nuclei appeared to lack immunostaining for GAT-1. The lack of GAT-1 immunoreactivity in Purkinje cells is consistent with previous data from in situ hybridization studies that showed little or no labeling for GAT-1 mRNA over Purkinje cells (Rattray and Priestley 1993; Durkin et al. 1995). This observation is also consistent with several previous studies that indicated Purkinje cells do not take up $^3$H-GABA into their cell bodies from their axon terminals (Hökfelt and Ljungdahl 1970, 1972; Schon and Iversen 1972; Kelly and Dick 1976; Chan-Palay 1977; Tolbert and Bantli 1980; Wiklund et al. 1983).

It is unclear why Purkinje cells do not express GAT-1 or GAT-3. While GATs were demonstrated to exhibit Na$^+$ and Cl$^-$ dependent, high affinity uptake for GABA in heterologous cell systems (Guastella et al. 1990; Borden et al. 1992; Clark et al. 1992), there is evidence that GABA transporters may also release GABA into the synaptic cleft and extracellular space in a Ca$^{++}$ independent, non-vesicular manner (Pin and Bockaert 1989; Levi and Raiteri 1993). Do certain GABAergic neurons require GAT-1 for neurotransmitter uptake because they are metabolically more active and thus have a greater demand for GABA? Or do more active GABAergic neurons release GABA via GAT-1 during increased activity, such as that found in epileptic foci (During et al. 1995)? The lack of GAT-1 in Purkinje cells may indicate that these neurons are not particularly active. In any event, it is clear that Purkinje cells do not possess the ability for uptake of GABA.

Another major finding is that GAT-1 and GAT-3 are localized to astrocytic processes in the cerebellum. This result is consistent with the finding that Bergmann glial cells express GAT-1 mRNA in the cerebellar cortex and GAT-3 mRNA labeling occurs in glia in the deep cerebellar nuclei (Rattray and Priestley 1993; Durkin et al.

1995). The labeling of astrocytic processes in the cerebellar cortex for GAT-1 was lighter than that for GAT-3 in the deep cerebellar nuclei.

In the deep cerebellar nuclei, the moderate expression of GAT-1 and the prominent expression of GAT-3 by astrocytes suggest that GABA released by Purkinje cell axon terminals is taken up by glial cells. Interestingly, not only were the astrocytic processes enveloping axon terminals more immunoreactive for GAT-3 than GAT-1, but the somata of astrocytes in the deep cerebellar nuclei were observed to be immunolabeled for GAT-3. The dense glial labeling for GAT-3 accentuated the unique envelopment of the Purkinje cell axon terminals by astrocytic processes. This relationship was previously described by Chan-Palay (1977) who concluded that astrocytes play a role in the isolation of synapses, a principle that has been generalized to many brain regions (Peters et al. 1991). The presence of GABA transporter in these glial processes and not in the Purkinje cell axon terminals that use GABA provides a functional meaning for this intimate glial relationship that is not found universally in the brain. For example, GABAergic neurons in the substantia nigra are apposed to each other without intervening glia (Ribak et al. 1976). If these latter axon terminals possess GAT-1, then it would indicate that they do not have to rely on glia for the removal of GABA from the synaptic cleft.

The Purkinje cells represent an interesting GABAergic neuronal type among those found in the cerebellar cortex. They are projection neurons with axon terminals that terminate upon neurons in the deep cerebellar nuclei and Deiters' nucleus. However, unlike the other GABAergic cell types, the Purkinje cell does not take up or retrogradely transport $^3$H-GABA (Tolbert and Bantli 1980; Wiklund et al. 1983). Thus, Purkinje cells seem to lack the uptake mechanisms for GABA. These findings, together with our results and the results of others show Purkinje cells lack GAT-1, GAT-2 and GAT-3 immunoreactivities and mRNAs (Radian et al. 1990; Rattray and Priestley 1993; Ikegaki et al. 1994; Swan et al. 1994; Durkin et al. 1995). This example of a GABAergic neuron that lacks GABA transporter does not appear to be unique in the brain; Rattray and Priestley (1993) showed that neurons in the inferior colliculus and the thalamic reticular nucleus do not express GAT-1 mRNA, even though there were neurons in these regions that expressed high levels of GAD$_{67}$ mRNA. It would be interesting to know whether the GABAergic neurons in these regions share any common functional properties with Purkinje cells.

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