J. Anat. (1994) 185, pp. 315–323, with 5 figures Printed in Great Britain

Distribution of mRNA for the GABA transporter GAT-1 in the rat brain: evidence that GABA uptake is not limited to presynaptic neurons

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(*Accepted 1 March 1994*)

ABSTRACT

Cells containing mRNA for the γ-aminobutyric acid (GABA) transporter GAT-1 were identified in rat brain by in situ hybridisation. They were found in most of the known locations of GABAergic neurons, as defined by the distribution of mRNA for glutamic acid decarboxylase, the synthetic enzyme for GABA. Within the cerebellum there was substantial labelling of basket and stellate cells in the molecular layer, and of Golgi cells but no others in the granule cell layer. Many Purkinje cells were unlabelled while others, particularly in the hemispheres, were moderately labelled. Many of the Purkinje cells negative for GAT-1 mRNA had adjacent intensely labelled small cells whose size and position corresponded to Bergmann glia. Numerical comparison of cells labelling for GAT-1 mRNA and the mRNAs for the two known isoforms of glutamic acid decarboxylase were made on serial sections of cerebral cortex. Cells positive for GAT-1 mRNA were more numerous, indicating that expression of the transporter is not just limited to GABAergic cells and we suggest that it may also be expressed postsynaptically by some non-GABAergic neurons.

Key words: Neurotransmitter uptake; glutamic acid decarboxylase; Purkinje cells; Bergmann glia.

INTRODUCTION

It has long been recognised that the mechanism for termination of a wide range of neurotransmitter signals is the removal of those transmitters from the synaptic cleft by cellular uptake using specific plasma membrane transporters (Iverson & Kelly, 1975). Recently the molecular nature of those transporters has been identified using molecular cloning techniques. The first transporter sequenced was one for γ-aminobutyric acid (GABA), designated GAT-1 (Guastella et al. 1990), which was found to be the prototype for a family of structurally related transporters which couple neurotransmitter influx with a movement of sodium and chloride ions down their electrochemical gradient (Snyder, 1991). Subsequently a second family of neurotransmitter transporters was identified which includes that for glutamate (Amara, 1992).

In the original experiments on neurotransmitter uptake, autoradiography was used to identify the ultimate location of exogenously added radiolabelled transmitter. Label appeared concentrated over the secretory vesicles of synaptic terminals, which was interpreted to mean that the observed uptake was restricted to the presynaptic neuron (Iverson & Kelly, 1975). That conclusion was attractive because it allowed for the economy of neurotransmitter reuse, and it has become a textbook dogma that continues to be repeated (Snyder, 1991; Uhl, 1992).

Recently as a result of detecting GAT-1 mRNA in spinal motoneurons (Snow et al. 1992), which are not thought to be GABAergic, we proposed that GABA uptake might also sometimes be postsynaptic. In such cases the additional location could increase the speed of neurotransmitter removal from the synaptic cleft (Bennett & Lowrie, 1992).

In order to identify other regions where GABA uptake might not be purely presynaptic we have carried out a survey of GAT-1 mRNA distribution through the rat brain, using in situ hybridisation. Localisation of mRNA has the advantage in brain

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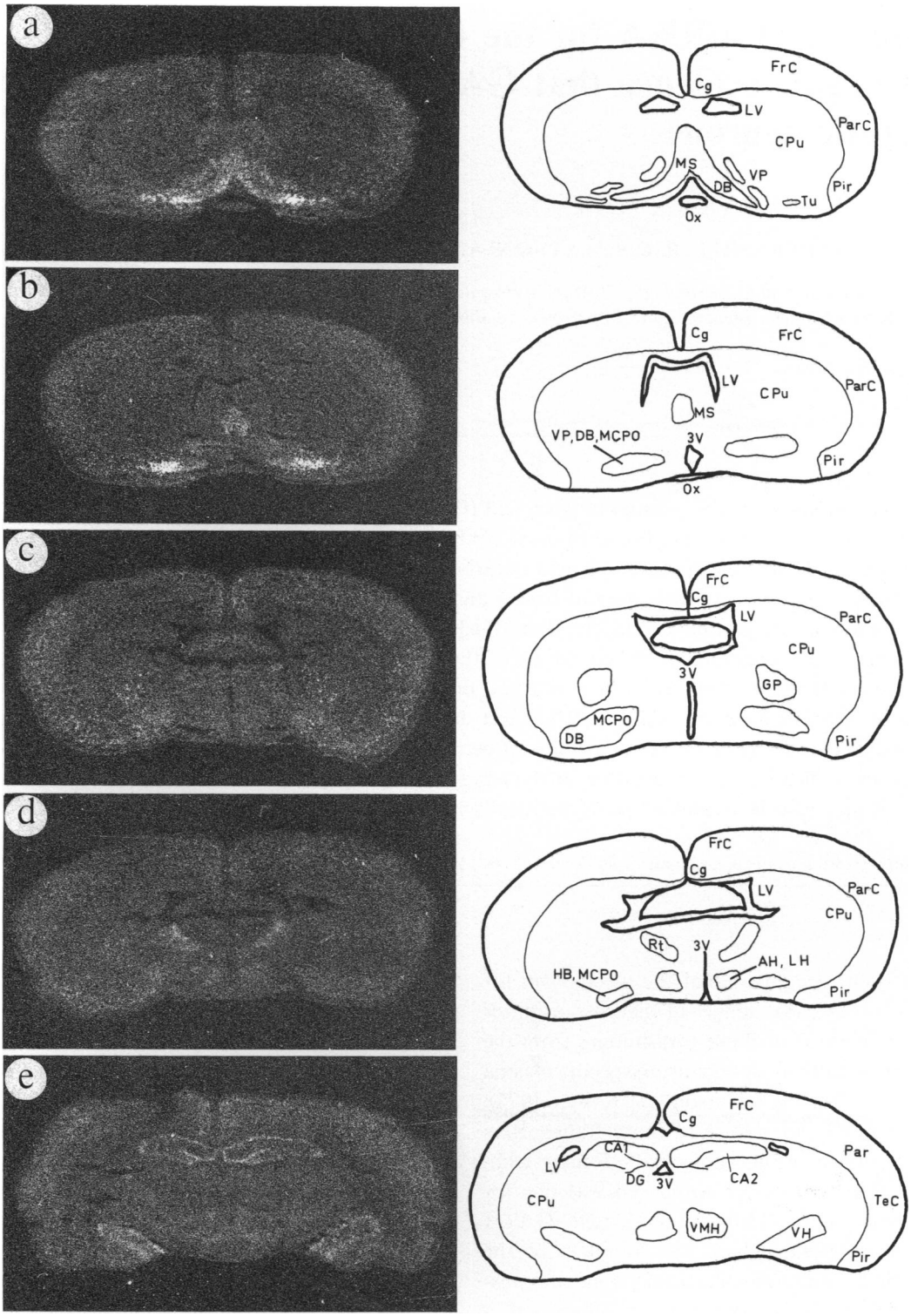

Fig. 1. For legend see facing page.

that it is usually confined to identifiable cell bodies whereas immunocytochemical localisation of the protein (Radian et al. 1990) shows the distribution of synaptic terminals whose cellular origin may be ambiguous. We have found that there are other locations where GAT-1 expression cannot be solely presynaptic, including at least one situation where it appears to be expressed in glial cells, the Bergmann astrocytes.

MATERIALS AND METHODS

Adult female Sprague–Dawley rats of approximately 200 g weight were killed by lethal intraperitoneal

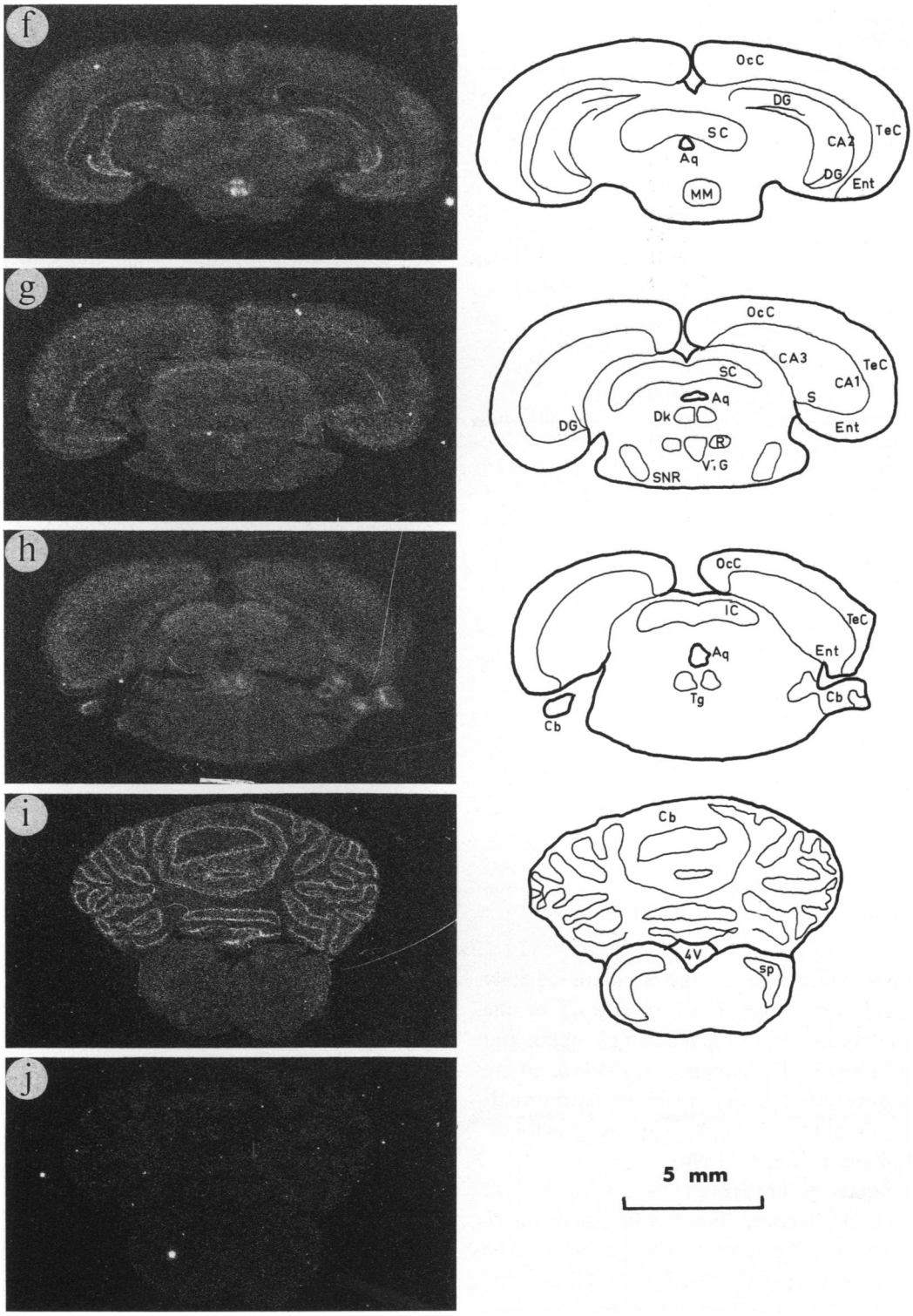

Fig. 1. Distribution of GAT-1 mRNA through the rat brain. (*a*)–(*i*) show a series of coronal sections probed by in situ hybridisation and autoradiographed against film. Adjacent to each panel is a line diagram indicating the major features. (*j*) is a control experiment where hybridisation was carried out, on the adjacent section to that of (*i*), in the presence of a 50-fold excess of unlabelled probe. Abbreviations (after Paxinos & Watson, 1986): 3V, 3rd ventricle; 4V, 4th ventricle; AH, anterior hypothalamic area; Aq, aqueduct; CA1–3, fields of Ammon's horn (hippocampus); Cb, cerebellum; Cg, cingulate cortex; CPu, caudate putamen; DB, diagonal band; DG, dentate gyrus; Dk, nucleus of Darkschewitsch; Ent, entorhinal cortex; FrC, frontal cortex; GP, globus pallidus; HB, nucleus of horizontal limb of diagonal band; LH, lateral hypothalamic area; LV, lateral ventricle; MCPO, magnocellular preoptic nucleus; MM, medial mamillary nucleus; MS, medial septal nucleus; OcC, occipital cortex; Ox, optic chiasma; ParC, parietal cortex; Pir, piriform cortex; R, red nucleus; Rt, reticular nucleus of thalamus; S, subiculum; SC, superior colliculus; SNR, substantia nigra pars reticulata; sp, spinal trigeminal nuclei; TeC, temporal cortex; Tg, tegmental nuclei; Tu, olfactory tubercle; VH, ventral hippocampus; VMH, ventromedial hypothalamic area; VP, ventral pallidum; VTG, ventral tegmentum.

Table 1. *Intensity of labelling of different regions of rat brain with a probe for GAT-1 mRNA**

Brain region	Labelling
Neocortex	0.035
Hippocampus – CA1	0.028
– CA3	0.040
– subiculum	0.031
– dentate gyrus	0.062
Caudate putamen	0.015
Globus pallidus	0.048
Ventral pallidum	0.056
Substantia nigra pars reticulata	0.035
Medial septal nucleus	0.066
Diagonal band	0.074
Bed nucleus	0.050
Magnocellular preoptic nucleus	0.044
Ventral tegmental area	0.045
Anteroventral preoptic nucleus	0.036
Medial preoptic area	0.042
Thalamus – reticular nucleus	0.051
– main nuclei	0.026
Medial mamillary nucleus	0.076
Superior colliculus	0.035
Inferior colliculus	0.018
Nucleus of Darkschewitsch	0.045
Red nucleus	0.038
Superior olivary nucleus	0.025
Tegmental nuclei	0.061
Cerebellum – molecular layer	0.025
– granular layer	0.011
– Purkinje layer	0.050
White matter (corpus callosum)	0.005

* Labelling is expressed in Bq/mm^2, estimated by comparison with a known radioactive standard from the optical density of autoradiographs following hybridisation of sections with [^{35}S]-labelled probe at a specific activity of 0.34 MBq/pmol.

injection of pentobarbitone and the brain immediately removed. Each brain was blocked in two in the coronal plane at the level of the median eminence and frozen in OCT embedding compound (Miles). 10 μm cryostat sections were taken, fixed in paraformaldehyde and defatted in chloroform as previously described (Najlerahim et al. 1990).

30 base oligonucleotide probes (custom synthesised by Oswel DNA Service, Edinburgh) were used, complementary to the appropriate mRNA. The sequences chosen were AGC CTT CTT CTG CAC CTT GAC TAC AAG GGT complementary to bases 85–114 of GAT-1 (Guastella et al. 1990; see also Snow et al. 1992); ATA GAG GTA TTC AGC CAG CTC CAA GCA TTT complementary to bases 1621–1650 of GAD_{67} (Kobayashi et al. 1987; see also Najlerahim et al. 1990); ATA CTC CAT CAT TCT GGC TTT AAT CAC TGG complementary to bases 1665–1694 of GAD_{65} (Erlander et al. 1991). None of these sequences were complementary to other genes in the EMBL sequence database (for up to 6 mismatches). Probes were labelled with [^{35}S]-deoxyadenosine 5′-[α-thio] triphosphate using 3′ terminal deoxynucleotidyl transferase as previously described (Najlerahim et al. 1990).

Hybridisation of sections was carried out for 18 h in a buffer containing 0.78 M sodium ions and 50% formamide as previously described (Najlerahim et al. 1990) at a temperature 15 °C below the calculated melt temperature for each probe. Subsequent washes in 0.15 M sodium chloride, 15 mM sodium citrate (1 × SSC) included 1 h (3 changes) at 15 °C below the recalculated melt temperature. Sections were autoradiographed either by direct apposition to film (Hyperfilm β-max, Amersham) for 14 d as in Figure 1, or by dipping in photographic emulsion (Ilford K5, diluted 1:1 with 2% glycerol) and storing for 6–8 wk at 4 °C before developing and counterstaining with toluidine blue or cresyl violet. In control experiments (Fig. 1*j* and data not shown) specific hybridisation with all probes was abolished by preincubating sections with ribonuclease or by cold displacement using a 50-fold excess of unlabelled probe; in addition no specific hybridisation was observed with a probe of random degenerate sequence (Snow et al. 1992).

Quantitation of silver grains overlying cells was by means of an image analyser with direct video input from a microscope, as previously described (Snow et al. 1992). Regional quantitation was by measuring optical densities on film autoradiographs. The quantitation of cells labelled with particular probes was from dark field photomicrographs of the dipped sections; the identity of micrographs was decoded only after completion of the analysis.

Immunohistochemistry (Fig. 4) was carried out overnight using cryostat sections as above with a rabbit antibody to glial fibrillary acidic protein (GFAP) diluted 1:1000 (Sigma Chemical Company). Detection used biotin-labelled secondary antibody and horseradish peroxidase complexed with avidin (Vector Laboratories) followed by colour development using diamino benzidine and hydrogen peroxide. The slides were lightly counterstained with cresyl violet before mounting.

RESULTS

Figure 1 shows the distribution of GAT-1 mRNA detected with a [^{35}S]-labelled synthetic 30-base oligonucleotide probe on a series of representative coronal sections through the rat brain. The distribution is highly specific, with heavy labelling of some individual neurons and no signal over others. The general pattern reflects that previously determined for glu-

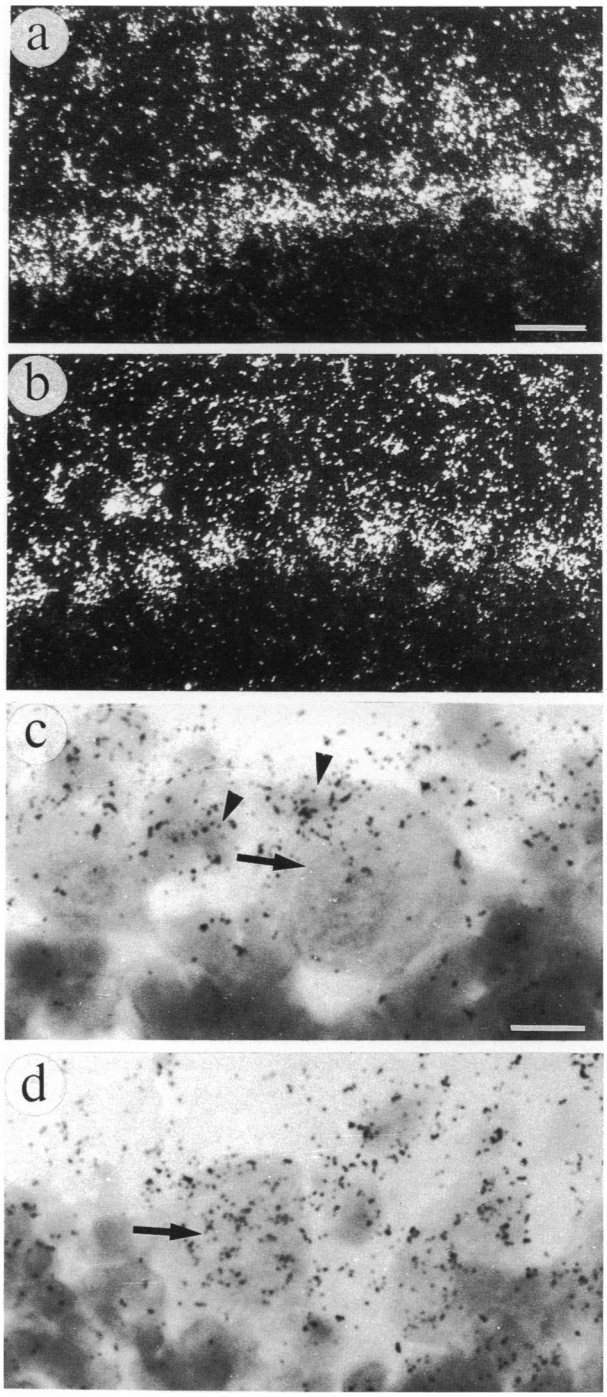

Fig. 2. Location of GAT-1 mRNA in the Purkinje cell layer of the cerebellum. (*a*) Darkfield micrograph of Purkinje cell layer labelled for GAT-1; bar, 50 μm. (*b*) Corresponding area of adjacent section labelled for GAD_{67}. (*c*) and (*d*) bright field micrographs of Purkinje cells (arrowed) illustrating cells unlabelled (*c*) and labelled (*d*) with probe for GAT-1 mRNA; bar, 10 μm. Small labelled cells in the same region are indicated by arrowheads.

tamic acid decarboxylase (GAD) mRNA (Ferraguti et al. 1990; Najlerahim et al. 1990; Erlander et al. 1991). The regional distribution is presented quantitatively in Table 1.

The neocortex displayed moderate GAT-1 mRNA content which appeared fairly consistent between different cortical regions. Closer examination showed autoradiographic signal associated with a proportion of nonpyramidal cells in all laminae.

The laminar architecture of the hippocampal formation was just visible from the autoradiographs. Marked labelling was found in inhibitory neurons of the dentate gyrus and CA3 regions. Nonpyramidal cells of the CA1 and the subiculum appeared to be more moderately labelled.

Within the basal ganglia the caudate putamen was weakly labelled by the probe for GAT-1, whereas the ventral pallidum and globus pallidus had significant labelling. The medial septal nucleus and diagonal band nuclei were strongly labelled, and good hybridisation was also seen in the pars reticulata of the substantia nigra, bed nuclei, magnocellular preoptic nuclei and ventral tegmental area. Closer to the 3rd ventricle, the anteroventral preoptic nuclei and, more caudally, the medial preoptic area were both labelled.

On the whole the diencephalon displayed little labelling for GAT-1. However there was clear labelling in the reticular nuclei of the thalamus and strong labelling of the medial mamillary nuclei associated with the hypothalamus, as well as weaker labelling in other hypothalamic areas.

In the midbrain labelling was prominent in the superficial layers of the superior colliculus while the inferior colliculus was only mildly positive. The nuclei of Darkschewitsch, red nuclei, superior olivary nuclei and tegmental nuclei were all moderately labelled.

In the cerebellum the Purkinje layer appeared to be labelled, together with labelling of the basket and stellate cells of the molecular layer. In the granule layer only Golgi cells were labelled. However closer examination of emulsion dipped sections (Fig. 2*a*) revealed that the labelling of the Purkinje layer for GAT-1 mRNA was not limited to the Purkinje cells themselves in the same way as observed for GAD_{67} mRNA (Fig. 2*b*). Instead Purkinje cell labelling was heterogenous, and there was also labelling of adjacent small cells in this layer. Many Purkinje cells appeared to lack overlying silver grains (Fig. 2*c*), especially in the vermis and intermediate zone, while others showed significant labelling (Fig. 2*d*).

This heterogeneity was confirmed by quantifying grain number (Fig. 3). The distribution of grain number among Purkinje cells from all regions of the cerebellum (Fig. 3*a*) showed a substantial number of cells with about the background number of grains while other cells had several times this number. The impression that the labelled cells are not randomly distributed was confirmed: there was a different distribution for the midline lobules compared with the

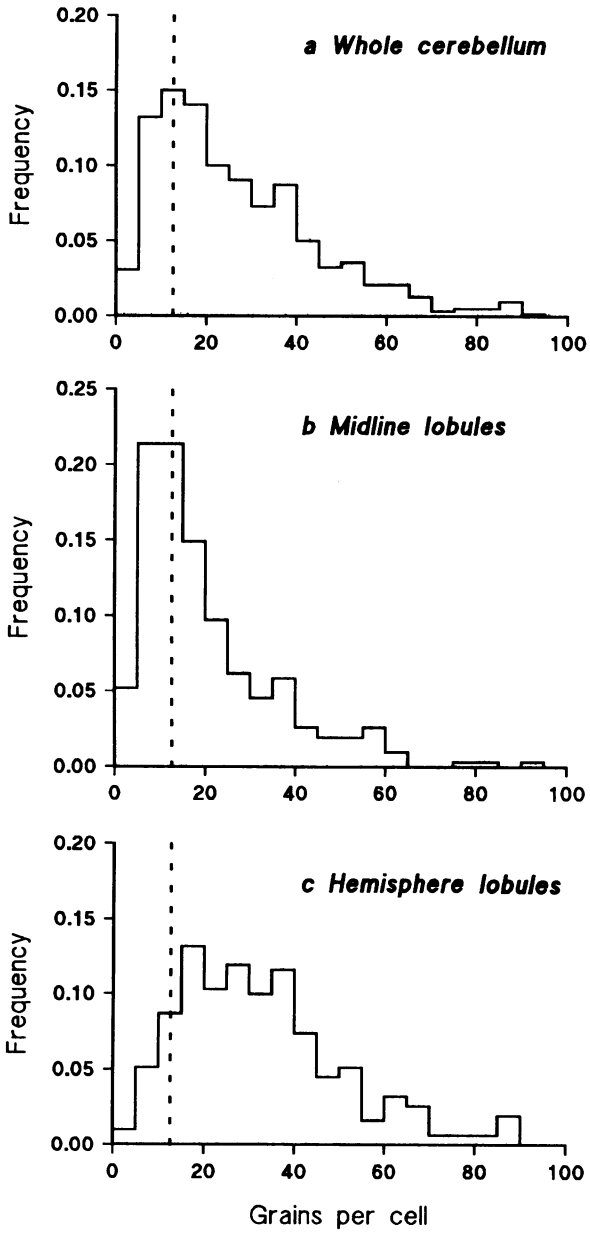

Fig. 3. Frequency distribution of number of silver grains overlying Purkinje cells. An image analyser thresholding routine was used to count silver grains overlying 620 Purkinje cells from all regions of the cerebellum in sections from a single rat brain. (*a*) Complete data set; (*b*) data for 309 cells in midline lobules; (*c*) data for 311 cells in hemisphere lobules. Vertical dashed line indicates background level of labelling (determined as mean number of silver grains overlying Purkinje cells in a control experiment using a random degenerate probe).

hemispheric lobules ($P < 0.001$, 2-tailed Kolmogorov-Smirnov test). In all cerebellar regions a probe for GAD_{67} mRNA labelled all Purkinje cells on adjacent sections.

For many of the apparently unlabelled Purkinje cells, there were seen to be substantial clusters of silver grains on small cells immediately adjacent and at the level of the Purkinje cell necks (Fig. 2*c*). When sections from the same brains were probed with an

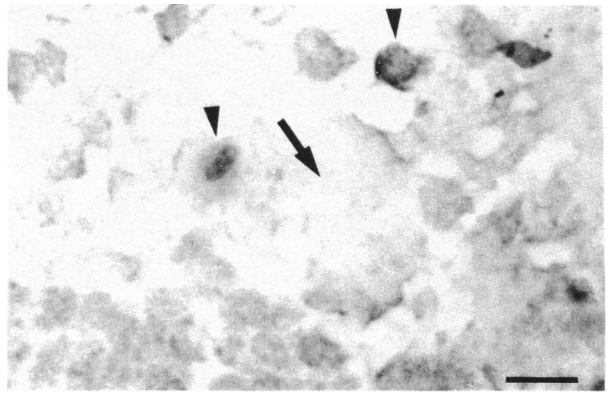

Fig. 4. Location of GFAP in the Purkinje cell layer of the cerebellum by immunohistochemistry. This field shows a Purkinje cell (arrowed) which is partly outlined by immunoreactivity due to astrocytic processes. The small cells in the same region (indicated with arrowheads) are identified as Bergmann glia by their GFAP immunoreactivity. Bar, 10 μm.

antibody to GFAP the cells in this position were positively labelled (Fig. 4) and we conclude that these small cells containing mRNA for GAT-1 were Bergmann astrocytes.

Although the general localisation of cells positive for GAT-1 mRNA corresponded to the regions known to contain GABAergic neurons as defined by the two isoforms of glutamic acid decarboxylase GAD_{67} and GAD_{65} we wished to examine whether the abundance of GAT-1 positive cells was the same as that for the GAD isoforms. This was done by determining the abundance of cells containing the different mRNA species on darkfield micrographs of neighbouring emulsion dipped sections of cerebral cortex, such as those illustrated in Figure 5. The density of labelled cells (i.e. the number of silver grain clusters in a measured area) was counted 'blind' on 27 such sets of micrographs obtained from four cortical regions and three animals before the identity of individual micrographs was decoded and the data tabulated.

The results (Table 2) showed that the probe for GAT-1 labelled more cells in the cortex overall than the probes for either GAD isoform alone or for both together ($P < 0.01$, 2-tailed Student's t test). The same trend was seen when the data was analysed separately for four different cortical regions (Table 2) though only for parietal cortex was the difference statistically significant ($P < 0.01$). The implication is that some of the cells expressing GAT-1 mRNA are non-GABAergic.

In addition we observed that the number of cells expressing GAD as detected using probes for both isoforms together was significantly greater than those detected using either probe alone ($P < 0.01$). This

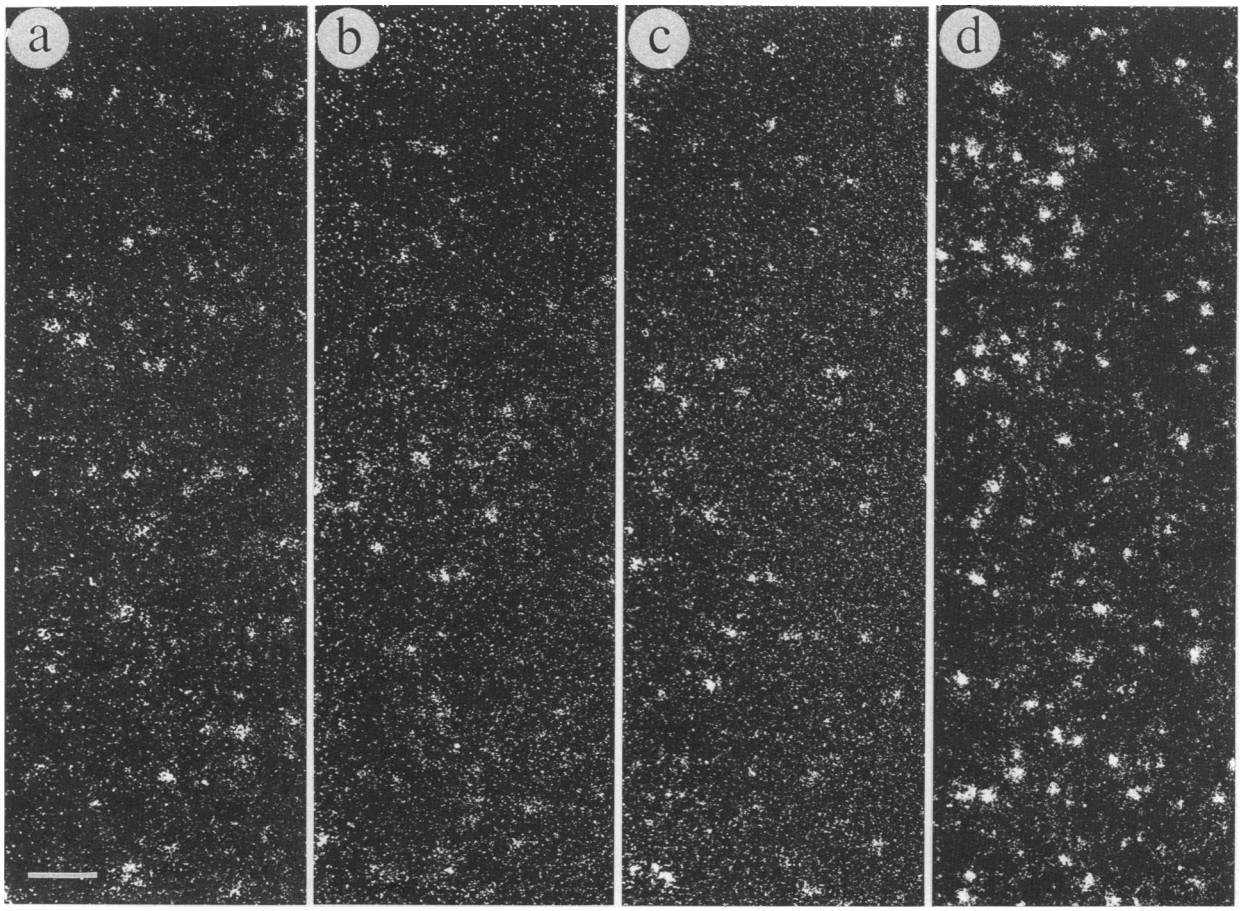

Fig. 5. Cells labelled with probes for GAD_{67}, GAD_{65} and GAT-1 mRNAs in neighbouring sections of temporal cortex. Darkfield micrographs covering laminae 2–6 through the depth of the cortex (pial surface upwards) labelled with (*a*) probe for GAD_{67} mRNA; (*b*) probe for GAD_{65} mRNA; (*c*) mixture of probes for GAD_{67} and GAD_{65} mRNAs; (*d*) probe for GAT-1 mRNA. Bar, 100 μm.

Table 2. *Density of cells labelled for GAD and GAT-1 mRNAs in the cerebral cortex**

Cortical region (no. of measurements)	mRNA probed			
	GAD_{67}	GAD_{65}	$GAD_{67}+GAD_{65}$	GAT-1
Frontal (8)	52±11	66±11	88±18	99±18
Parietal (6)	59±6	65±11	88±9	110±10
Temporal (7)	60±21	72±13	88±32	117±34
Occipital (6)	79±11	78±15	106±28	126±15
All (27)	62±17	70±14	92±24	112±24

* Cells were counted on sets of neighbouring 10 μm sections, and data are expressed as number of labelled cells per mm^2 (mean ± S.D.).

suggests that GABAergic cells differ in the GAD isoform they express, with some cells expressing substantially a single isoform while others express a mixture of both GAD_{67} and GAD_{65}, thus having differing susceptibilities to pyridoxal phosphate concentration (Erlander et al. 1991).

The data from Table 2 on density of labelled cells, together with the data from Table 1 for level of labelling, allow us to calculate that in cortical neurons labelled for GAT-1 mRNA (such as those shown in Fig. 5*d*) there are of the order of 500 probe molecules hybridised per labelled cell.

DISCUSSION

The assumption that GABA transport is presynaptic has become well established (Snyder, 1991) and we find the majority of mRNA for the transporter GAT-1 associated with neurons in known GABAergic brain regions. However, detailed analysis suggests that expression of this GABA transporter isoform is not entirely presynaptic, nor even strictly neuronal.

The earlier evidence for postsynaptic GAT-1 came from spinal motor neurons (Snow et al. 1992). The observation of a hybridisation signal over morphologically identifiable non-GABAergic neurons was fortuitous, although in the course of the present study similar labelling of a subpopulation of large neurons – presumably motor neurons – was seen in the region of the hypoglossal nucleus (not shown). The original observation raised the possibility of non-GABAergic cells expressing GAT-1 postsynaptically in other

regions of the central nervous system such as the cerebral cortex (Snow et al. 1992; Bennett & Lowrie, 1992) and this has been explored in the present paper.

The number of cells containing GAT-1 mRNA was compared with those that are GABAergic by the criterion of expressing one of the isoforms of GAD. About 15–20% of cells expressing GAT-1 in the cortex appear to be non-GABAergic. Those cells could be glia or postsynaptic neurons. Both neurons and astrocytes isolated from cerebral cortex have been shown to contain a GABA transport system (Larsson et al. 1986) but their differing pharmacology has led to the conclusion that the transporter molecule is different in the two cell types with GAT-1 being the neuronal isoform (Lopez-Corcuera et al. 1992). We therefore propose a possible postsynaptic location of GAT-1 expression in some cortical neurons.

Postsynaptic GAT-1 expression must presumably be explained by a need at some synapses for a faster removal of GABA from the synaptic cleft that can be accommodated by presynaptic and glial uptake routes alone. Such synapses must be the exception rather than the rule since the vast majority of non-GABAergic neurons must receive GABAergic inputs but only a proportion express GAT-1, presumably those having a special functional requirement for rapid termination of GABAergic signals. Previous evidence consistent with the existence of non-GABAergic neurons in the cerebral cortex which possess a GABA uptake mechanism have come from a comparison of GABA immunoreactivity with that of GAD (Kosaka et al. 1988) and a study suggesting that intracellular GABA can influence the functioning of the GABA receptor (Wood & Davies, 1989).

Our conclusion appears to contradict the well established observation that exogenous radiolabelled GABA applied to brain became localised over secretory vesicles of presumed GABAergic cells (reviewed by Iverson & Kelly, 1975). However, in those original autoradiography experiments the very high concentration of neurotransmitter within secretory vesicles, together with the relatively low degree of labelling observed, meant that if there were any other location with a higher specific radioactivity of transmitter but at a considerably lower concentration it would probably have been missed.

The proposal that a neurotransmitter transporter molecule can be expressed postsynaptically means that it will not be possible to use transporters automatically as cell-specific markers to identify neurons not otherwise unequivocally identifiable, as suggested by Uhl (1992). However some other transporters are exclusively presynaptic: the 5-hydroxytryptamine transporter is expressed only in known serotonergic locations (Blakely et al. 1991, and our unpublished results). If that can be established independently for a transporter where no other cell-specific marker is available, such as some of the amino acid neurotransmitters, then the transporter would become an invaluable tool for mapping neuron types.

The heterogenous labelling of Purkinje cells in the cerebellum for GAT-1 mRNA is interesting as all of these cells are well established as GABAergic. The simplest explanation is simply that they are heterogenous only in terms of the type of GABA transporter expressed, since there have very recently been shown to be at least three other isoforms (Liu et al. 1993) which will not be detected with our probe. Of those GAT-2 is thought to be the glial form (Lopez-Corcuera et al. 1992) and GAT-3 is predominantly expressed outside the CNS though it is detected in neonate brain (Liu et al. 1993). The other isoform, GAT-4, is neuronal and transports GABA with particularly high affinity, but is not abundantly expressed in the cerebellum compared with other regions such as the brainstem (Liu et al. 1993); whether it is expressed in Purkinje cells has not yet been reported.

That Purkinje cells in the vermis region tend to show different neurotransmitter handling properties compared with those in the hemispheres presumably reflects other differences between the cells in these two regions. Purkinje cells in these locations differ with respect to their projections to the deep cerebellar nuclei and their olivocerebellar input (Voogd et al. 1985). A heterogeneity in the response of Purkinje cells to adrenalectomy showed a similar anatomical distribution (Ahima et al. 1992). Although cells positive and negative for GAT-1 mRNA were found in all locations, the distribution does not obviously correspond to the parasagittal bands seen for expression of some other proteins of neurotransmitter metabolism (Chan-Palay et al. 1982) and for the zebrin antigen (Hawkes, 1992).

Many of the Purkinje cells which lacked GAT-1 mRNA had a close association with small cells which did label for GAT-1; these were identified as Bergmann astrocytes by GFAP content. Rattray & Priestley (1993) have recently independently reported GAT-1 mRNA in these glia. The obvious explanation is that Bergmann glia are important in terminating GABAergic transmission in this region since they have been shown to contain GABA transaminase which is the degradative enzyme for GABA (Lolova & Davidoff, 1990). However the GABA uptake system in glia usually appears to be due to the GAT-2

isoform which confers the characteristic pharmacology (Lopez-Corcuera et al. 1992). Radian et al. (1990) have previously shown GABA transporter immunoreactivity by electron microscopy in glial processes, presumably of Bergmann astrocytes, in the Purkinje cell region of the cerebellum; however the polyclonal antibody used in that study was not thought to distinguish GAT-1 and GAT-2.

It is interesting that we find Bergmann glia express GAT-1 which otherwise appears to be restricted to neurons. It could be speculated that this population of astrocytes might in some way be specialised to play a direct role in cell–cell signalling. They have been shown to express some isoforms of both GABA and glutamate receptors (Wisden et al. 1989; Monyer et al. 1991; Ortega et al. 1991; Gallo et al. 1992; Laurie et al. 1992) and to contain the excitatory amino acid homocysteic acid (Cuenod et al. 1990).

ACKNOWLEDGEMENT

This work was supported in part by a grant from the Medical Research Council.

REFERENCES

AHIMA RS, TAGOE CN, HARLAN RE (1992) Type II corticosteroid receptor-like immunoreactivity in the rat cerebellar cortex: differential regulation by corticosterone. *Neuroendocrinology* **55**, 683–694.

AMARA SG (1992) A tale of two families. *Nature* **360**, 420–421.

BENNETT JP, LOWRIE MB (1992) Are neurotransmitter carriers cell-specific markers? *Trends in Neurosciences* **15**, 483.

BLAKELY RD, BERSON HE, FREMEAU RT, CARON MG, PEEK MM, PRINCE HK et al. (1991) Cloning and expression of a functional serotonin transporter from rat brain. *Nature* **354**, 66–70.

CHAN-PALAY V, PALAY SL, WU JY (1982) Sagittal cerebellar microbands of taurine neurons: immunocytochemical demonstration by using antibodies against the taurine-synthesizing enzyme sulfinic acid decarboxylase. *Proceedings of the National Academy of Science of the USA* **79**, 4221–4225.

CUENOD M, DO KQ, GRANDES M, MORINO P, STREIT P (1990) Localization and release of homocysteic acid, an excitatory sulfur-containing amino acid. *Journal of Histochemistry and Cytochemistry* **38**, 1713–1715.

ERLANDER MG, TILLAKARATNE NJK, FELDBLUM S, PATEL N, TOBIN AJ (1991) Two genes encode distinct glutamate decarboxylases. *Neuron* **7**, 91–100.

FERRAGUTI F, ZOLI M, ARONSSON M, AGNATI LF, GOLDSTEIN M, FILER D et al. (1990) Distribution of glutamic acid carboxylase messenger RNA-containing nerve cell populations of the male rat brain. *Journal of Chemical Neuroanatomy* **3**, 377–396.

GALLO V, UPSON LM, HAYES WP, VYKLICKY L, WINTERS A, BUONANNO A (1992) Molecular cloning and developmental analysis of a new glutamate receptor subunit isoform in cerebellum. *Journal of Neuroscience* **12**, 1010–1023.

GUASTELLA J, NELSON N, NELSON H, CZYZYK L, KEYNAN S, MIEDEL MC et al. (1990) Cloning and expression of a rat brain GABA transporter. *Science* **249**, 1303–1306.

HAWKES R (1992) Antigenic markers of cerebellar modules in the adult mouse. *Biochemical Society Transactions* **20**, 391–395.

IVERSON LL, KELLY JS (1975) Uptake and metabolism of γ-aminobutyric acid by neurones and glial cells. *Biochemical Pharmacology* **24**, 933–938.

KOBAYASHI Y, KAUFMANN DL, TOBIN AJ (1987) Glutamic acid decarboxylase cDNA: nucleotide sequence encoding an enzymically active fusion protein. *Journal of Neuroscience* **7**, 2768–2772.

KOSAKA T, TAUCHI M, DAHL JL (1988) Cholinergic neurons containing GABA-like and/or glutamic acid decarboxylase-like immunoreactivities in various brain regions of the rat. *Experimental Brain Research* **70**, 605–617.

LARSSON OM, GRIFFITHS R, ALLEN IC, SCHOUSBOE A (1986) Mutual inhibition kinetic analysis of γ-aminobutyric acid, taurine and β-alanine high-affinity transport into neurons and astrocytes: evidence for similarity between the taurine and β-alanine carriers in both cell types. *Journal of Neurochemistry* **47**, 426–432.

LAURIE DJ, SEEBURG PH, WISDEN W (1992) The distribution of 13 $GABA_A$ receptor mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *Journal of Neuroscience* **12**, 1063–1076.

LOLOVA I, DAVIDOFF M (1990) Immuno- and histochemical data on changed GABA transmission in aged rat cerebellum. *Journal für Hirnforschung* **31**, 423–428.

LOPEZ-CORCUERA B, LIU QR, MANDIYAN S, NELSON H, NELSON N (1992) Expression of a mouse brain cDNA encoding a novel γ-aminobutyric acid transporter. *Journal of Biological Chemistry* **267**, 17491–17493.

LIU QR, LOPEZ-CORCUERA B, MANDIYAN S, NELSON H, NELSON N (1993) Molecular characterization of four pharmacologically distinct γ-aminobutyric acid transporters in mouse brain. *Journal of Biological Chemistry* **268**, 2106–2112.

MONYER H, SEEBURG PH, WISDEN W (1991) Glutamate-operated channels: developmentally early and mature forms arise by alternative splicing. *Neuron* **6**, 799–810.

NAJLERAHIM A, HARRISON PJ, BARTON AJL, HEFFERNAN J, PEARSON RCA (1990) Distribution of messenger mRNAs encoding the enzymes glutaminase, aspartate aminotransferase and glutamic acid decarboxylase in rat brain. *Molecular Brain Research* **7**, 317–333.

ORTEGA A, ESHHAR N, TEICHBERG VI (1991) Properties of kainate receptor/channels on cultured Bergmann glia. *Neuroscience* **41**, 335–349.

PAXINOS G, WATSON C (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd edn. London: Academic Press.

RADIAN R, OTTERSON OP, STORM-MATHISEN J, CASTEL M, KANNER BI (1990) Immunocytochemical localization of the GABA transporter in rat brain. *Journal of Neuroscience* **10**, 1319–1330.

RATTRAY M, PRIESTLEY JV (1993) Differential expression of GABA transporter-1 messenger RNA in subpopulations of GABA neurones. *Neuroscience Letters* **156**, 163–166.

SNOW H, LOWRIE MB, BENNETT JP (1992) A postsynaptic GABA transporter in rat spinal motor neurones. *Neuroscience Letters* **143**, 119–122.

SNYDER SH (1991) Vehicles of inactivation. *Nature* **354**, 187.

UHL GR (1992) Neurotransmitter transporters (plus): a promising new gene family. *Trends in Neuroscience* **15**, 265–268.

VOOGD J, GERRITS NM, MARANI E (1985) Cerebellum. In *The Rat Nervous System*, vol. 2 (ed. G. Paxinos), pp. 251–291. Sydney: Academic Press.

WISDEN W, MCNAUGHTON LA, DARLISON MG, HUNT SP, BARNARD EA (1989) Differential distribution of $GABA_A$ receptor mRNAs in bovine cerebellum – localization of alpha 2 mRNA in Bergmann glial layer. *Neuroscience Letters* **106**, 7–12.

WOOD JD, DAVIES M (1989) Regulation of the γ-aminobutyric acid A receptor by γ-aminobutyric acid levels within the postsynaptic cell. *Journal of Neurochemistry* **53**, 1648–1651.