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Review

# GABA transporters in the mammalian cerebral cortex: localization, development and pathological implications

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**Abstract**

The extracellular levels of γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the mammalian cerebral cortex, are regulated by specific high-affinity, $Na^+/Cl^-$ dependent transporters. Four distinct genes encoding GABA transporters (GATs), named GAT-1, GAT-2, GAT-3, and BGT-1 have been identified using molecular cloning. Of these, GAT-1 and -3 are expressed in the cerebral cortex. Studies of the cortical distribution, cellular localization, ontogeny and relationships of GATs with GABA-releasing elements using a variety of light and electron microscopic immunocytochemical techniques have shown that: (i) a fraction of GATs is strategically placed to mediate GABA uptake at fast inhibitory synapses, terminating GABA's action and shaping inhibitory postsynaptic responses; (ii) another fraction may participate in functions such as the regulation of GABA's diffusion to neighboring synapses and of GABA levels in cerebrospinal fluid; (iii) GATs may play a role in the complex processes regulating cortical maturation; and (iv) GATs may contribute to the dysregulation of neuronal excitability that accompanies at least two major human diseases: epilepsy and ischemia.


*Theme:* D
*Topic:* GABA

*Keywords:* Amino acid neurotransmitters; Neurotransmitter uptake; Neurons; Glia; Inhibitory synapses

**Contents**



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## 1. Introduction

### 1.1. GABA in the cerebral cortex

The amino acid γ-aminobutyric (GABA) is the main inhibitory neurotransmitter in the cerebral cortex, where it plays a fundamental role in controlling neuronal excitability and information processing [117–119,136,190,194–196,213], neuronal plasticity [7,106,200], and network synchronization [17,27,206].

Most neocortical GABA derives from aspiny nonpyramidal neurons whose axon terminals form symmetric synaptic contacts with both pyramidal and nonpyramidal cells [12,46,69,88,89,93,94,106,115,173,195]. GABAergic neurons are found in all cortical layers, they show a clear preference for layers IV and II–III, and account for 20–30% of all cortical neurons [11,89]. Some GABA also appears to be released by extrinsic axons [4,36,67,68,70,78,81,129,207].

### 1.2. GATs shape GABA's action in the cerebral cortex

Several factors are involved in GABA's postsynaptic effects: presynaptic factors (release probability, number of release sites), factors acting at the cleft (diffusion and transporters), and postsynaptic factors (receptor subtypes, location, number, and interactions with anchoring proteins) [30]. Among those acting at the cleft, high-affinity plasma membrane GABA transporters (GATs) appear to modulate phasic [59,64,134,205] and tonic [104,184,218] GABA-mediated inhibition and GABA spillover [10,58,60,87,101,102,120,121,146,153,177,180,209].

Four cDNAs encoding highly homologous GATs[1] (GAT-1, GAT-2, GAT-3, and BGT-1[2]) have been isolated in rodent and human nervous system [20–22,34,84,122,130,148,171]; they exhibit different ionic dependencies and inhibitor sensitivities, and are differentially distributed within the central nervous system [19,44,83,110,178,182]. The first aim of this paper is to review the distribution and localization of GATs in the adult and developing cerebral cortex to gain insights into their functional roles.

GABA-mediated inhibition exerts a powerful control over cortical neuronal activity, and GABA transport contributes to modulate GABA's action. As altered GATs activity and/or expression are likely to affect markedly cortical function, the second aim of this paper is to provide a brief overview of their possible involvement in the pathophysiology of selected human diseases.

## 2. Localization of GATs in the adult cerebral cortex

### 2.1. GAT-1

GAT-1 is the most copiously expressed GAT in the cerebral cortex. Its general distribution was first described in mapping studies [63,100,168,202,224], and subsequently detailed using in situ hybridization and light and electron microscopic techniques.

In situ hybridization studies in rat neocortex showed that the vast majority of cells expressing GAT-1 are neurons and that some are astrocytes [142]. Neurons expressing GAT-1 mRNA are widely distributed and are more numerous in layers IV and II than in the other layers. All neurons expressing the 67 kDa isoform of the enzyme glutamic acid decarboxylase ($GAD_{67}$), a GABAergic marker, are positive (+) for GAT-1, whereas not all cells expressing GAT-1 mRNA are $GAD_{67}$+; of the latter, some are pyramidal neurons (which are glutamatergic [37,38,49]), suggesting that nonGABAergic neurons also express GAT-1 [142] (see also Ref. [202]).

Immunocytochemical studies showed that GAT-1 immunoreactivity (ir) is associated exclusively with punctate structures resembling axon terminals and fibers in the neocortex of rats, monkeys, and humans [39,47,142] (Figs. 1A and 2A). In the first somatic sensory cortex (SI) of adult rats, GAT-1+ puncta are relatively sparse in layer I, numerous in layers II–III (especially in the lower portion of the latter) and densest in layer IV (Fig. 1A). In infragranular layers, staining intensity is much lower than in layer IV and supragranular layers. Numerous GAT-1+ fibers, usually running obliquely or radially and showing irregularly spaced varicose swellings, are observed in infragranular layers, particularly layer VI [39,142]. In monkey cortex, GAT-1+ puncta exhibit a different distribution: in SI they are more numerous in layers II–IV (as in rat), whereas in homotypical cortices (areas 21 and 46) and in the cingulo-frontal transition cortex (area 32) they are distributed homogeneously [39]. In human cortex (areas 21, 32, and

[1] We use the nomenclature of Guastella et al. [84] and Borden [19] and refer to cDNA clones from rat (r) brain. An analogous, though not identical, nomenclature introduced by Liu et al. [130] identifies cDNA clones from mouse (m) brain encoding four GATs and names them GAT1–GAT4. Whereas mGAT1 is the homolog of rGAT-1, mGAT2, mGAT3, and mGAT4 appear to be the homologs of dog BGT-1, rGAT-2 and rGAT-3, respectively. In addition, rGAT-3 is identical to a rat clone designated as GAT-B by Clark et al. [34].

[2] Whether BGT-1 (for Betaine/GABA Transporter [221]) or its mouse or human homologs contribute to GABA transport in the brain remains to be determined (see Ref. [19] for a detailed description of the discovery, homologies, and pharmacological properties of BGT-1).

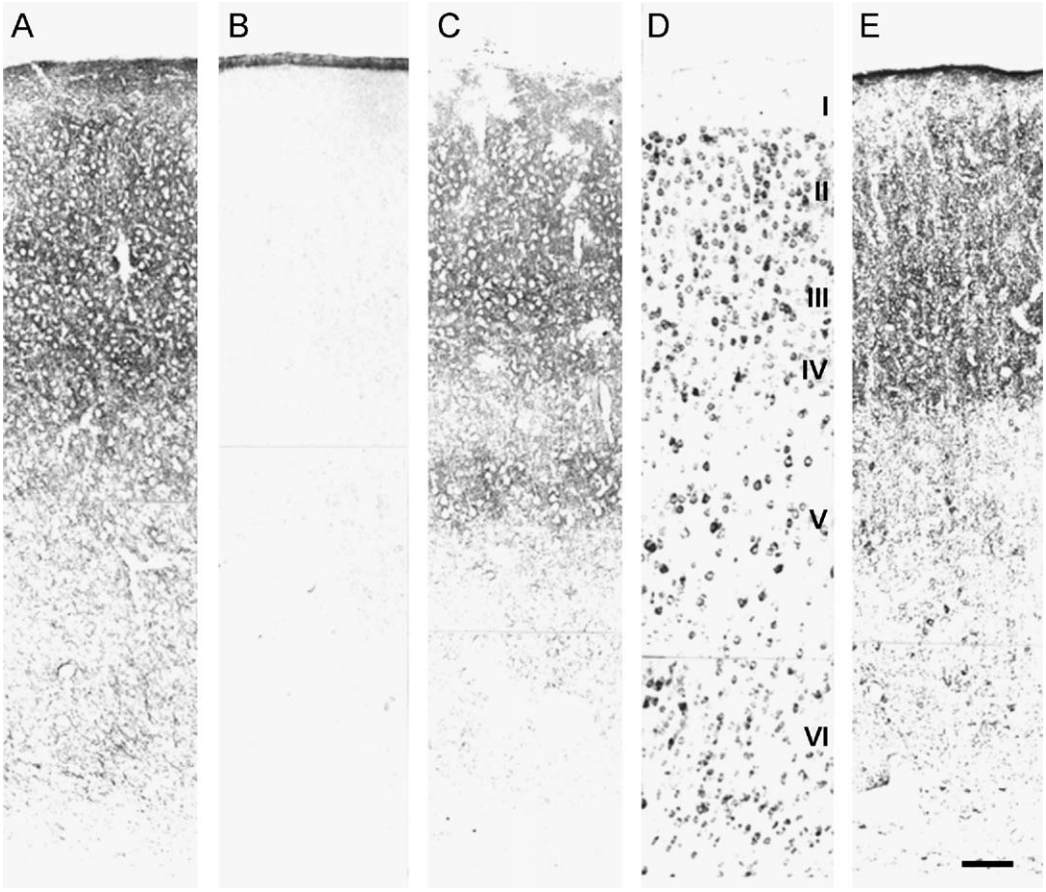


Fig. 1. Distribution of GAT-1 (A), GAT-2 (B), and GAT-3 (C) ir in three adjacent sections of SI cortex in an adult rat [28]. The thionine-stained section (D) is adjacent to those illustrated in A–C; roman numerals indicate cortical layers. The section in E shows the distribution of $GAD_{67}$ ir. (A–C) Sections processed using polyclonal antibodies to GATs obtained from Dr. N.C. Brecha (Dept. of Neurobiol., UCLA, USA) and characterized in previous papers [40,105,142,143]. (E) Section processed using the anti$GAD_{67}$ antibody characterized by Kaufman et al. [112] and purchased from Chemicon (Temecula, CA, USA). Bar: 100 μm for A–E.

46; see Ref. [26]), they display a homogeneous distribution [39]. Comparative analysis of the laminar patterns of GAT-1+ puncta in rat, monkey and humans indicated that the pattern of GAT-1 expression is related to cortical type rather than to species [39]. In all species, GAT-1+ fibers are also present in the white matter underlying the neocortex and are especially numerous in the corpus callosum of rats [39,142].

In all the species studied to date, GAT-1+ puncta have a preferential relationship to the soma and proximal dendrites of cortical neurons [39,47,142]. In layers II–III and V–VI, numerous GAT-1+ puncta are observed around the somata and the proximal portions of the apical dendrites of pyramidal cells (Fig. 2A). In most cases, they distinctly outline unlabeled pyramidal cells, although they are also found around nonpyramidal neurons. The patterns of distribution of $GAD_{67}$+ and GAT-1+ puncta are remarkably similar (Fig. 1; see Refs. [39,142]), as is their relationship to neuronal perikarya and proximal dendrites. Analysis of sections double-labeled with GAT-1 and GABA or GFAP antibodies revealed that the vast majority of GAT-1+ puncta are GABA-positive (Fig. 3) and GFAP-negative (Fig. 2 in Ref. [30]).

The ultrastructural pattern of GAT-1 labeling is similar in all layers of rat, monkey and human cortex [39,142,168]. GAT-1 ir is in axonal and glial profiles (Fig. 2B), whereas somata and dendrites are unlabeled. Axonal labeling is always intense and scattered throughout the axoplasm of thinly myelinated axons and in numerous presynaptic axon terminals forming symmetric synaptic contacts with unlabeled cell bodies or dendrites of all sizes (Fig. 2B). Studies of knock-in mice carrying GAT-1-green fluorescent protein fusions yielded an estimated density of 800–1300 GAT-1 molecules/presynaptic bouton [32]. GAT-1 ir is also found in distal astrocytic processes scattered in the neuropil, sometimes close to GAT-1+ terminals [39,142] (Fig. 2B); this finding is in line with electrophysiological evidence showing the presence of GAT-1-mediated currents in neocortical astrocytes [114].

The cell types giving rise to GAT-1+ puncta have not been determined to date, with a notable exception. Heavily stained GAT-1+ swellings, mostly in layers II–III and VI, are typically aligned in vertically oriented rows below the somata of unlabeled neurons (see Fig. 6C and D in Refs. [39,47]),

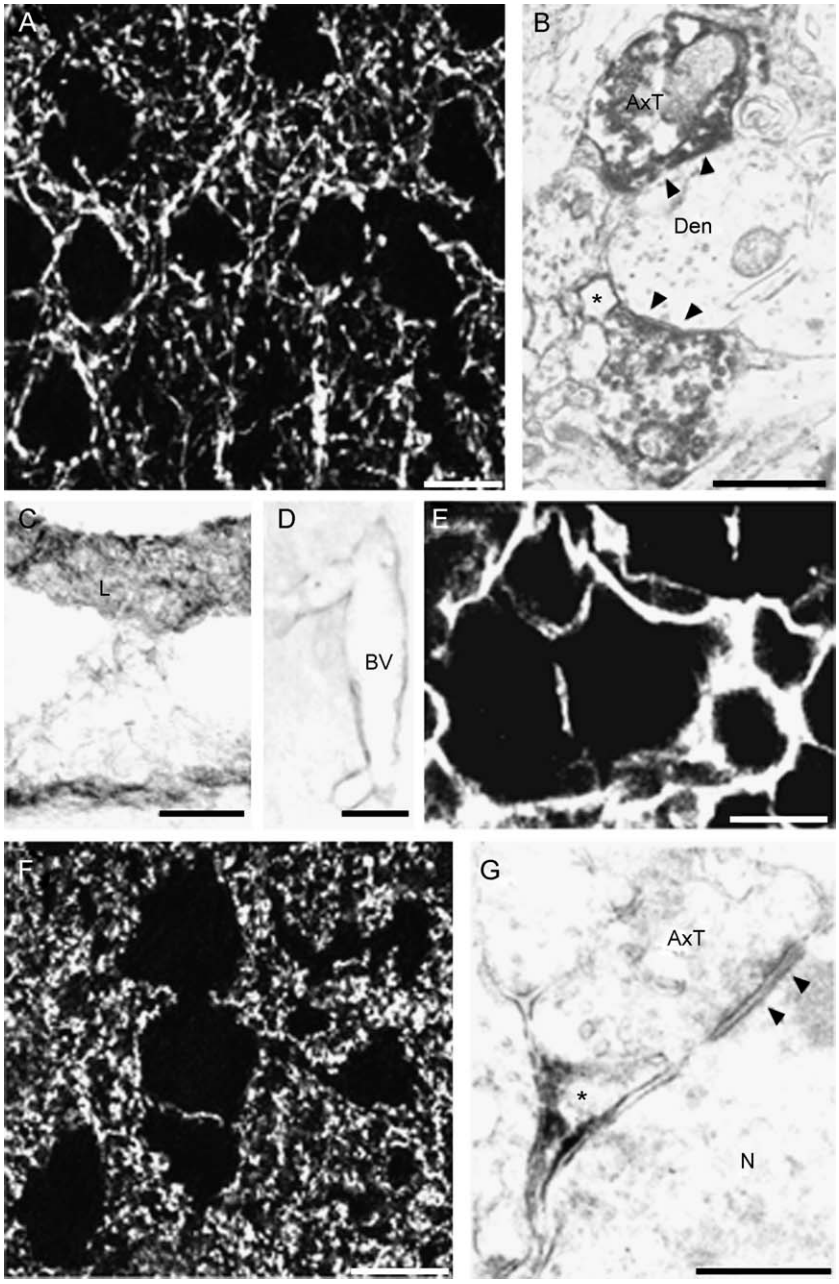


Fig. 2. Localization of GAT-1 (A, B), GAT-2 (C, D, E), and GAT-3 (F, G) ir in cerebral cortex and neighboring structures. (A) GAT-1+ puncta outline unlabeled pyramidal neurons in layer III of rat SI (F. Conti, A. Minelli, and M. Melone, unpublished material). (B) GAT-1 is localized to axon terminals forming symmetric synapses (arrowheads) and to a distal astrocytic process (asterisk) (human cerebral cortex; from [39]). (C–E) GAT-2 ir is prominent in leptomeninges and arachnoid trabeculae (C), around blood vessels (D), and in the epithelial cells of the choroid plexus (E) of rat brain (C and D from: F. Conti, A. Minelli, and M. Melone, unpublished material); E, from Ref. [40]. (F) GAT-3+ puncta outline unlabeled neurons in layer V of rat SI (F. Conti, A. Minelli, and M. Melone, unpublished material). (G) A GAT-3+ distal astrocytic process (asterisk) in the vicinity of an axon terminal forming a symmetric synapse (arrowheads) (from Ref. [143]). AxT, axon terminal; Den, dendrite; BV blood vessel; L, leptomeninges; N, nucleus. Scale bars: 10 μm for A and F, 0.5 μm for B; 20 μm for C and D, 15 μm for E; and 0.25 μm for G.

thus displaying a pattern similar to that of GABAergic chandelier axon terminals [48,161]: these GAT-1+ puncta coexpress parvalbumin [47], a $Ca^{2+}$-binding protein expressed by chandelier cells [50], thus indicating that chandelier cells give rise to some GAT-1+ puncta. In addition, the strong perisomatic labeling of pyramidal cells suggests that basket cells also contribute GAT-1+ puncta [196].

### 2.2. GAT-2

Although in vitro investigations showed high levels of GAT-2 mRNA in some astrocytes [23], early in situ hybridization and immunocytochemical studies failed to detect GAT-2 in brain parenchyma, and reported that it is exclusively expressed in leptomeningeal and ependymal cells

[63,100]. This view has partly been challenged by the demonstration that, beside its strong localization to the leptomeninges—where it is detected in the arachnoid and along the thin arachnoid trabeculae in the subarachnoid space—and to ependymal and choroid plexus (Fig. 2C,E), GAT-2 ir is also present in the cortical parenchyma [40]. In SI cortex, faint GAT-2 ir is found in puncta, cell bodies and processes [40]. GAT-2+ puncta different sizes are scattered in all cortical layers (Fig. 1B) with no apparent laminar segregation; they are localized to glia limitans (Fig. 2C) and outline blood vessels (Fig. 2D) and unlabeled cell bodies. Rare GAT-2+ cells of both neuronal and glial morphology are present in the cortical parenchyma, subcortical white matter and corpus callosum. Scattered GAT-2+ processes are found in the cortical neuropil; of these, some are GFAP+ [40]. However, several GAT-2+ puncta and cell bodies are negative for GFAP [40].

At the ultrastructural level, GAT-2 ir is present in neuronal and non-neuronal cortical cells [40]. Labeled neuronal profiles include perikarya, dendrites and axon terminals, and, occasionally, small myelinated axons. Labeled axon terminals form both asymmetric and symmetric synapses preferentially with distal dendrites and spines. Non-neuronal elements in the cortical parenchyma are astrocytic cell bodies and their proximal and distal processes; the latter, which form perivascular end-feet, are scattered throughout the cortical neuropil, sometimes close to unlabeled terminals with symmetric or asymmetric specializations, but often in areas unrelated to synaptic terminals. GAT-2 ir is also found in astrocytic processes forming glia limitans, in leptomeningeal cells and their processes, in the ependymal cells lining the third ventricle, and in choroid plexus epithelial cells [40].

### 2.3. GAT-3

Contrary to the results of early investigations [34,63,100], GAT-3 ir is not negligible in the cerebral cortex [143] (Fig. 1C). In rat cerebral cortex, GAT-3 ir is localized exclusively to small punctate structures (Fig. 2F) that are difficult to resolve at the light microscope and never appears as labeled fibers or cell bodies [63,100,143]. The highest level of GAT-3 ir is observed in layer IV and in a narrow band corresponding to lower layer V, followed by layers II, III and IV and VI [143] (Fig. 1C). Small patches of tissue exhibiting less intense GAT-3 ir are also found in all cortical layers (Fig. 1C), particularly in layers VI and IV [143]. GAT-3+ puncta form a continuous sheet around the somata of both pyramidal and nonpyramidal neurons in all layers (Fig. 2F). In layers II–III and V, they are also closely associated with the proximal portion of basal and apical dendrites of pyramidal cells. GAT-3+ puncta are more numerous in the neuropil and are generally smaller than the majority of GAT-1+ puncta [143] (compare Figs. 2A and F).

GAT-3 labeling is found exclusively in distal astrocytic processes (Fig. 2G), whereas astrocytic cell bodies and neuronal profiles are always unlabeled [143]. Like those immunoreactive for GAT-1 and GAT-2, GAT-3+ astrocytic processes form perivascular end-feet and are adjacent to axon terminals making either symmetric (Fig. 2G) or asymmetric synaptic contacts with cell bodies or dendrites, or are close to neuronal profiles that do not form synaptic contacts in the plane of section [143].

In surgical samples of human cerebral cortex, GAT-3 ir is found throughout its depth in puncta as well as neurons [139]. GAT-3+ puncta are both dispersed in the neuropil and closely associated with cell bodies, forming a continuous sheet around the somata of both pyramidal and nonpyramidal neurons. Neuronal staining is mostly in perikarya and proximal dendrites of pyramidal neurons; the density of GAT-3+ neurons is variable and unrelated to cortical area. Electron microscopic studies showed GAT-3 ir both in distal astrocytic processes and in neurons and their processes. Since surgical resection induces an acute ischemic situation in the resected sample [111], GAT-3+ neurons may actually not be expressed in humans in physiological conditions. This view is sustained by reports that all GAT-3+ neurons express heat shock protein 70 (HSP70) and that they are numerous in cortical samples from rat cortex resected and processed like human surgical samples [139], and it is reinforced by the detection of GAT-3 ir in rat cortical neurons following transient focal cerebral ischemia [140] (see Section 4.2.). These data could indicate a similar localization of GAT-3 in human and rat cortex, and that GAT-3 is more susceptible than GAT-1 to changes in the energy supply.

### 2.4. Relationship of GATS to GABAergic synapses

Despite some species differences [94], the GABAergic system appears to obey a basic organizational plan in which GABAergic neurons are most numerous in layers II–III and IV, followed by layer VI, while GABAergic axon terminals are densest in layers IV and II–III, moderately dense in layers I and VI, and least dense in layer Va [89,94,106,115,173,194]. The laminar distribution of GAT-1+ and GAT-3+ puncta is therefore strikingly similar to that of GABA-releasing axon terminals (Fig. 1), suggesting that GATs are most expressed in the layers where GABA is released. The vast majority of axon terminals forming symmetric synapses on pyramidal and nonpyramidal neocortical neurons arise from several types of GABAergic nonpyramidal cells (including smooth and sparsely spinous neurons with local plexus axons and basket, chandelier and double-bouquet cells) and all GABAergic axon terminals form symmetric synapses [46,88,94,106,115,173,195]. The findings that the vast majority of GAT-1+ (and rare GAT-2+) puncta are axon terminals forming symmetric synapses [39,40,142], and that few GAT-2+ [40] and some GAT-3+ puncta are astrocytic processes located in the vicinity of

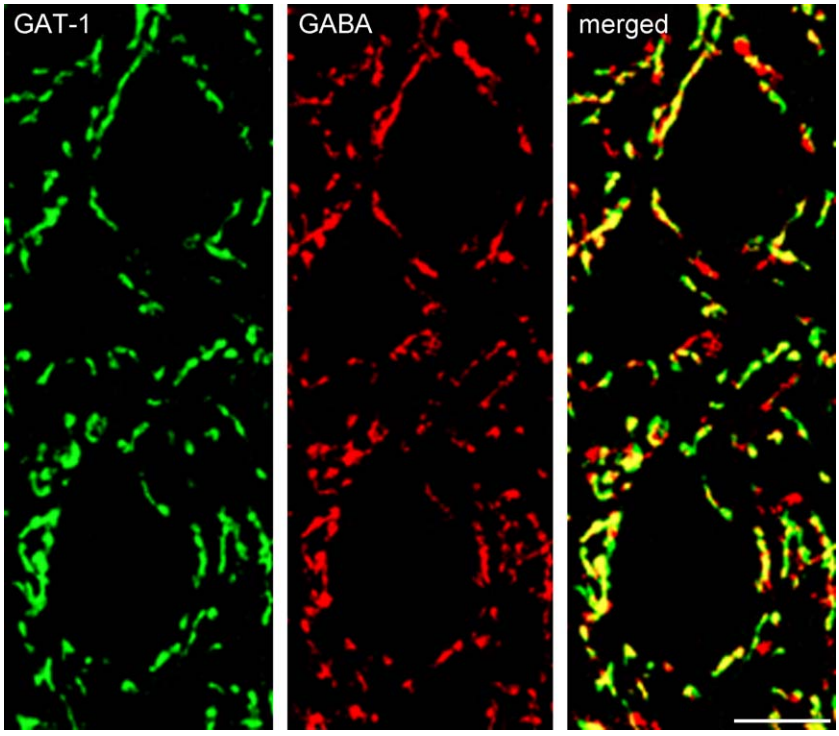


Fig. 3. Most GAT-1+ puncta contain GABA. Single plane confocal images from layer III of rat SI cortex showing that GAT-1 (green)- and GABA (red)-immunoreactive profiles have similar distribution and morphology. Merged image shows a prominent overlap of the two signals (for details on image acquisition and processing, see Refs. [144,145]). Scale bar: 5 μm.

symmetric synapses [143], support the notion of an intimate relationship between GABAergic synapses and GATs. Added to the demonstration that GAT-1+ axon terminals contain GABA (Fig. 3) and that GAT-2+ and GAT-3+ astrocytic processes are located near GABAergic axon terminals [40,143], these data lead to the surmise that GATs located in the vicinity of symmetric synapses are responsible for GABA uptake at inhibitory synapses, thus contributing to terminating GABA's synaptic action and to shaping inhibitory postsynaptic responses in the cerebral cortex [30] (Fig. 4). An electrophysiological study of GABAergic transmission in GAT-1-knock-out mouse hippocampus supports this view and suggests that elevated GABA levels resulting from GAT-1 deficiency may induce post- and presynaptic changes in GABAergic synapses [104].

However, this hypothesis needs to be considered in the light of other data, which do not indicate that the distribution of GATs is coextensive with that of GABAergic synapses; in fact, some GAT-1 is localized to distal astrocytic processes scattered in the neuropil [39,142], GAT-2 is mostly located in neuronal and glial processes that are distant from synapses [40], and a fraction of GAT-3+ puncta is not close to symmetric synapses but at asymmetric, glutamatergic [49] synapses [143]. Nor does all the evidence agree with the view that GATs are located and operate exclusively at GABAergic synapses. Overall, data rather seem to point to a widespread system of GABA uptake in the mammalian cerebral cortex that is more extensive than the GABA-releasing system. This second component of the cortical GABA uptake system is hardly suited to interfere with classic (point-to-point) inhibitory synapses[3] and is probably to be thought of in terms of one of the forms of interneuronal communication that have been ascertained on functional grounds; indeed, classic neurotransmitters such as GABA, glutamate and glycine also produce changes at some distance from their sites of release [2,10,101,102,120,121,177,209]. Although GABA spillover has not been directly demonstrated in mammalian neocortex, but only in the hippocampus [102,153,180,209], cerebellum [58,60,87,146,177] and retina [99], it is conceivable that the function of cortical GATs located at extrasynaptic sites is to regulate the diffusion of the GABA that mediates the cross-talk between neighboring and/or distant synapses

[3] Although Sherrington [187], who introduced the term, viewed the synapse as a physiological concept [185,188] encompassing much of the variety of interactions known at present [186], for the last 50 years this term has been used almost exclusively to indicate the interneuronal junction described by early electronmicroscopists in the mid-1950s [57,158,159]. Since the vast majority of modern localizational and electrophysiological studies use the term synapse in this anatomical acception, we have conformed to this usage. The term is therefore used here to indicate "a presynaptic element containing synaptic vesicles and an apposed postsynaptic element from which it is separated by an intercellular space, the synaptic cleft, 10 to 20 nm wide" [162]. Accordingly, the adjective extrasynaptic is used to indicate an element participating in interneuronal chemical communication located at a distance from a synapse.

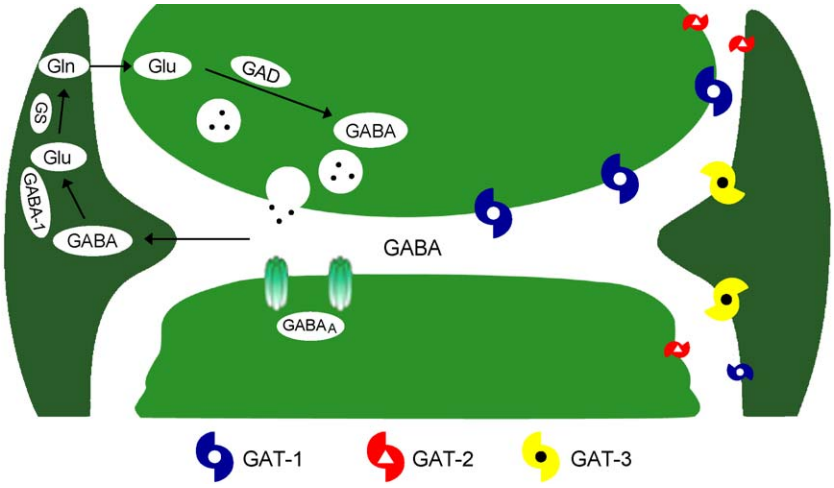


Fig. 4. Highly schematic representation of the localization of GATs at cortical synapses. GAT-1 is shown in blue, GAT-2 in red, and GAT-3 in yellow. Modified from Ref. [30].

[39,142,143]. The slow time course of astrocytic GATs currents recorded in neocortical astrocytes [114] supports this view.

Finally, a third, discrete fraction of GATs is found at sites where it can interact with the cerebrospinal fluid (CSF). This fraction appears to be constituted exclusively of GAT-2 expressed by leptomeninges, ependymal and choroid plexus cells [40,63,100,169]. Although not specific for the cerebral cortex, this fraction may be capable of modifying cortical neuronal activity by sensing local CSF composition [54,150] and regulating GABA transport through the blood–brain barrier [5,109].

Why are there multiple populations of GATs (i.e., of GABA uptake mechanisms) in the cerebral cortex? In heterologous cell systems, GATs exhibit different ionic dependences (e.g., external $Cl^-$) and inhibitor sensitivities [19,82,83]. In addition, they are subject to regulatory mechanisms affecting both their kinetics and density at synapses [127]: these mechanisms depend on substrate concentration [15,167,212], are coupled to factors controlling transmitter release [52,53,167] and hormone levels [90] and may display regional selectivity [90]. Regulatory mechanisms may exert differential, or even opposite effects on different GATs; for instance, pH alterations affect GAT-3 more than that of GAT-1 [82], and protein kinase C activators increase GABA uptake in cells transfected with GAT-1 [42], but reduce GABA transport in primary astrocyte cultures [77]. Furthermore, GATs may also release GABA into the extracellular space in a $Ca^{2+}$-independent, nonvesicular manner at least in vitro [8,128,164,183,189,217] (see also Ref. [103] for data on the olfactory bulb) and, given their functional heterogeneity, different GATs may well exhibit different reversal profiles.

It thus appears that the different responses of GATs to the composition of the extracellular *milieu*, the different regulation of their activity and/or expression, and the possibility of reversing (differentially?) the direction of GABA transport concur to endow the extensive and complex GABA transport system operating in the neocortex with considerable flexibility for the fine regulation of GABA extracellular levels in various physiological and pathological conditions.

## 3. Localization of GATs in the developing cerebral cortex

### 3.1. GAT-1

GAT-1 mRNA and protein first appear at late embryonic stages; their expression, weak in the neonatal cortex, gradually increases in the first two postnatal weeks [66,107,145,219,222]. Before birth, GAT-1 ir is restricted to the marginal zone (with the exception of the entorhinal cortex, where it is diffuse) [107]. At birth, GAT-1 ir is strong in outer layer I and light in the subplate. At P2, the signal increases in layer V and in the lower cortical plate, whereas the upper cortical plate (i.e., the prospective layers II and III) stains faintly. By P5, ir has extended to the entire cortex, with the highest level in layer I and a band of intense labeling occupying layer IV and the differentiating layer III (Fig. 5A). From P10 onward, GAT-1 ir gradually decreases in layer I and increases in all the other layers, most notably in supragranular ones. The mature pattern of expression is reached by P30 [222]. At all ages, GAT-1 ir is prevalently associated with dot-like structures that are distributed in the neuropil during the first postnatal week (Fig. 6A,B) and at later stages aggregate around unstained neuronal profiles, often forming pericellular basket-like structures [222]. Most GAT-1+ puncta are axon terminals, some of which form symmetric synaptic contacts with unlabeled dendrites (Fig. 6C) or with somata in the early phases of development (see Ref. [222]; A. Minelli, P. Barbaresi, and F. Conti, unpublished observations). GAT-1 is also expressed in astrocytic somata and processes (Fig. 6C), in perivascular glia and, transiently, in the somata and dendrites of GABAergic neurons [222].

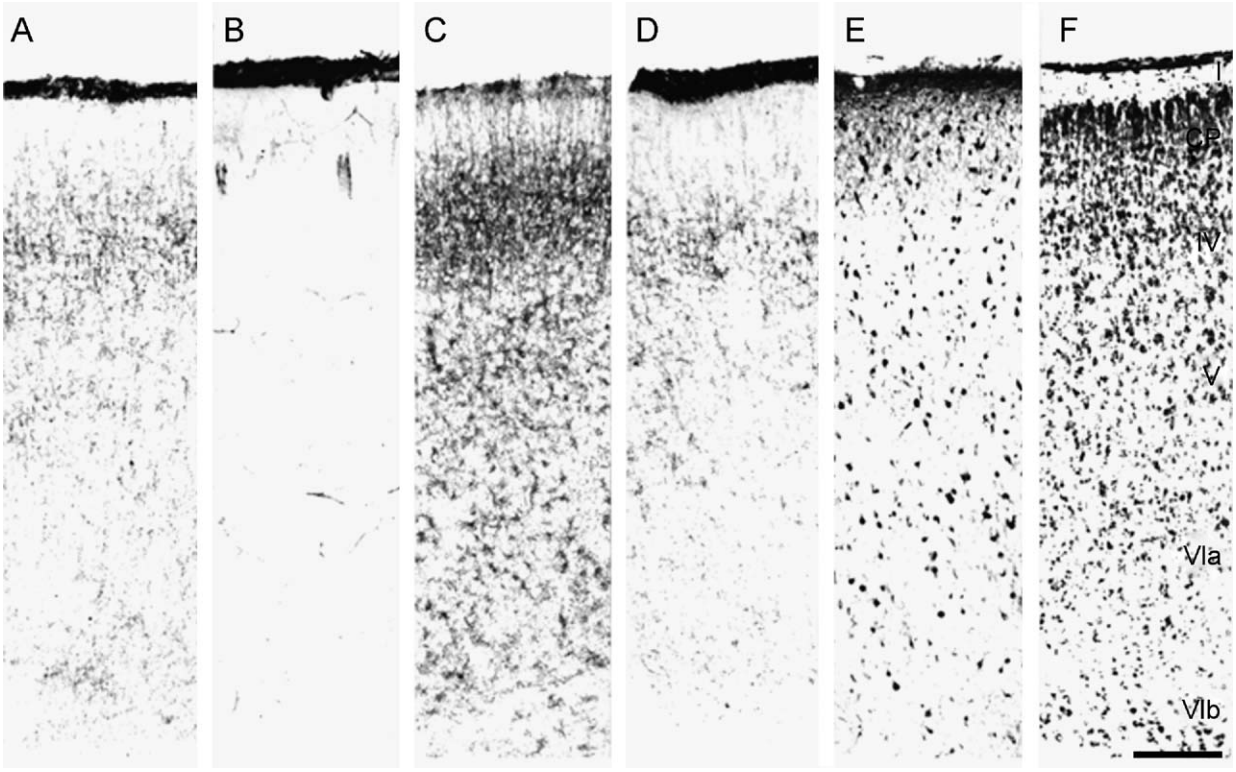


Fig. 5. Distribution of GAT-1 (A), GAT-2 (B), and GAT-3 (C) ir in three adjacent sections of developing SI cortex (P5). The thionine-stained section (F) is adjacent to those illustrated in A–D; roman numerals indicate cortical layers. The section in D illustrates the distribution of VGAT, the one in E shows the distribution of GABA ir (P5). (A–C) Sections processed using the antibodies and procedures referred to in the legend to Fig. 1. (D and E) Sections processed using the antiVGAT (D) and the antiGABA (E) antibodies characterized, respectively, by Chaudry et al. [29] and Matute and Streit [135]. Bar: 100 μm for A–E.

GAT-1 mRNA levels increase earlier than those of the protein: at P1, mRNA is 28% of adult levels and between P10 and P30 it exceeds them by 25–40% [219], whereas protein expression is 10.2% of adult levels at birth, it increases at P2 (24.7%), P5 (41%), and P10 (84.2%), it reaches adult levels at P15 (101%), and peaks above them at P30 (119%) [145]. The transient overexpression of GAT-1 in neuronal and astrocytic cell bodies [222] and the peak above adult levels of GAT-1 mRNA and protein at intermediate and late phases of cortical maturation [85,145,219,222] agree with previous findings that in the developing cortex GABA uptake transiently exceeds adult levels [16,33,43,92,170,172,216] and $V_{max}$ of transport exhibits a parallel trend [43,216].

GAT-1 expression has been studied also in developing human and monkey cortex [65,85]. In human temporal cortex, GAT-1+ puncta appear in the marginal zone at gestational week (GW) 33, they are found in all layers with a density similar to that of the adult by GW 38–39, and attain the mature distribution pattern 1–5 months after birth, when their density exceeds adult levels [85]. Human and rodent cortex thus share a similar spatial pattern of GAT-1 expression during cortical development; in man, however, this expression is anticipated, probably reflecting a corresponding advance of synaptogenesis, which begins early prenatally and peaks a few months after birth [97,98]. In primates, most neocortical synaptogenesis takes place in the embryonic period [24,25,80,225], possibly accounting for the lack of significant changes in the density of GAT-1+ puncta reported in monkey prefrontal cortex during postnatal development [65].

### 3.2. GAT-2

In the rodent brain, GAT-2 mRNA and protein are mainly expressed in the pia-arachnoid membrane and along the arachnoid trabeculae throughout embryonic and postnatal development [66,108,144] (Figs. 5B and 6D). At all ages, parenchymal labeling is weak and uniformly distributed through the cortical wall [144] (Fig. 5B). GAT-2 is robustly expressed by astrocytic processes surrounding blood vessels [108,144], particularly in the early phases of development (Figs. 5B and 6E), when the entire caliber of blood vessels may be completely filled with GAT-2 ir [144]. GAT-2 is also expressed in puncta of various sizes distributed in the neuropil, some of which coexpress synaptophysin [144] (Fig. 6F). At later stages, labeling around blood vessels shrinks to a thin rim [66,144]. From P10 to P15, GAT-2 is also expressed in a small number of astrocytic and neuronal

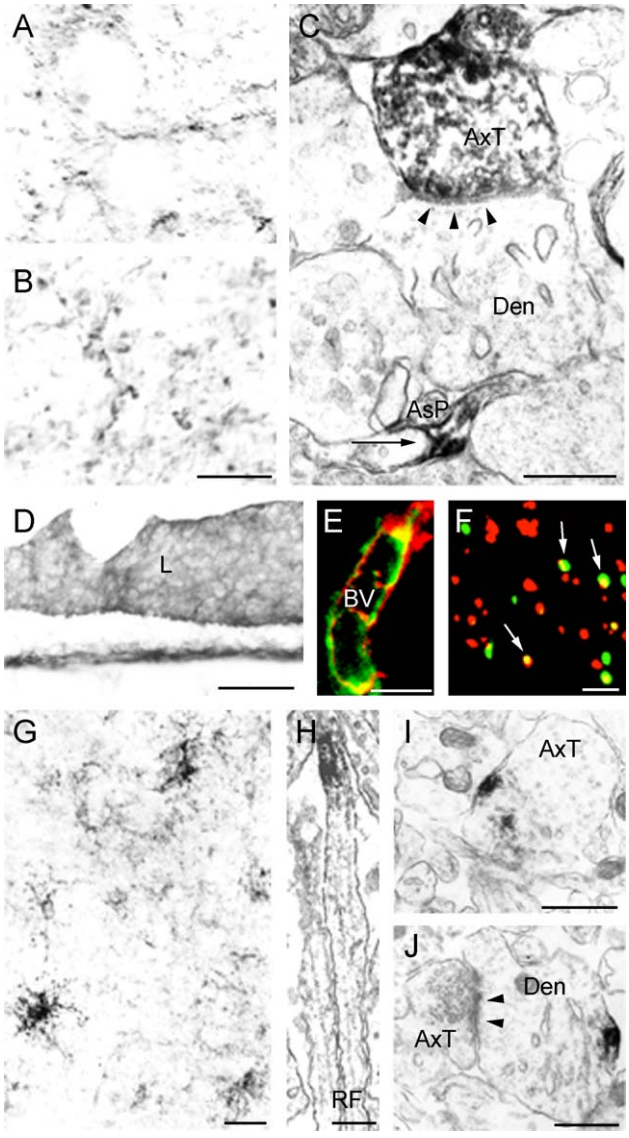


Fig. 6. Localization of GAT-1 (A–C), GAT-2 (D–F), and GAT-3 (G–J) ir in developing SI cortex (P2–P10). (A and B) GAT-1+ puncta and fibers in layers V–VI (P5) (F. Conti, A. Minelli, and M. Melone, unpublished material). (C) GAT-1 is localized to axon terminals forming symmetric synapses (arrowheads) and to distal astrocytic processes (arrow) (P10; P. Barbaresi, A. Minelli, and F. Conti, unpublished material). (D and E) At P5, GAT-2 ir is prominent in leptomeninges (D), and around blood vessels (E, here shown in a section processed simultaneously for the visualization of GAT-2 (green) and GFAP (red); from Ref. [144]). (F) Some GAT-2+ puncta (green) colocalize synaptophysin (red) (white arrows; from Ref. [144]). (G) GAT-3+ astrocytes in layer V of developing SI (P2; F. Conti, A. Minelli, and M. Melone, unpublished material). (H) A GAT-3+ radial glial fiber (P5; from Ref. [144]). (I and J) GAT-3 ir is also in axon terminals (I) and in dendrites (J; the one illustrated here receives an asymmetric synapse [arrowheads] from an unlabeled axon terminal) (P5; from Ref. [144]). AsP, astrocytic process; AxT, axon terminal; BV blood vessel; Den, dendrite; L, leptomeninges; N, nucleus; RF, radial glial fiber. Scale bars: 10 μm for A, B, and G; 30 μm for D and E; 5 μm for F; 0.5 μm for C, H, I, and J.

cell bodies and processes [144]. GAT-2 expression reaches the mature pattern at the end of the second postnatal week [66,144].

### 3.3. GAT-3

GAT-3 expression appears in the marginal and intermediate zones of the cerebral cortex in late embryonic life [66,107], when GAT-3 ir is intense in fasciculate radial structures [107]. GAT-3 mRNA and protein expression increase rapidly in the neonatal brain and until the second postnatal week, when protein expression (which remains relatively stable thereafter [107,144]) diverges from that of mRNA (which decreases) [66].

In neonatal cortex, GAT-3 ir is present throughout the cortical wall in fasciculate structures, in puncta scattered in the neuropil, around blood vessels [107,144], and in numerous cells [144] (Figs. 5C and 6G). In many cases, GAT-3+ cells have an astrocytic morphology (Fig. 6G), and express GFAP [144]. Ultrastructural studies showed GAT-3 to be localized to astrocytic somata, perivascular glial processes and radial glial fibers (Fig. 6H). Colocalization studies also showed that at P2–P5 numerous GABAergic neurons and fibers contain GAT-3 ir and some GAT-3+ puncta coexpress synaptophysin. Electron microscopic studies suggest that about 30% of cortical neurons and many axon terminals and dendritic profiles express GAT-3 [144] (Fig. 6I–J). During the second postnatal week, GAT-3 increases in layer IV and supragranular layers; staining increases in the neuropil, it decreases in astrocytic cell bodies and disappears in neurons [144]. From P15 onward, GAT-3 ir displays the mature pattern of expression [143,144].

### 3.4. GATs and the development of GABAergic synapses

In rodents, neocortical inhibitory synaptogenesis is a protracted process that begins in late embryonic life and matures through the first postnatal month [18,51,141] (see Fig. 7). GAT-1 maturation follows the same spatial order as inhibitory synaptogenesis and slightly precedes the full establishment of the morphological features of symmetric synapses [145,222], suggesting a role for GAT-1-mediated GABA transport in the formation and maturation of cortical GABAergic synapses. Since GAT-1 is expressed in axon terminals, it is conceivable that it may modulate GABA concentration in the vicinity of nascent synapses. GABA itself regulates the expression and functional properties of $GABA_A$ receptors [14,75,116,154,155] and chloride transporter KCC2 [14,75,154], whose appearance correlates with the onset of GABA's inhibitory action [96,176,210]. GAT-1-mediated transport may thus contribute to the maturation of point-to-point GABAergic synapses. In this connection, it is worth stressing that in both rodent and human development GAT-1 expression is coordinated with that of other GABAergic presynaptic proteins, i.e., the synthesizing enzyme GAD [85,215] (see Fig. 7) and the vesicular transporter VGAT [145] (see Fig. 7), and parallels that of the α1 subunit of $GABA_A$ receptor [72,125], which participates in mature GABAergic transmission.

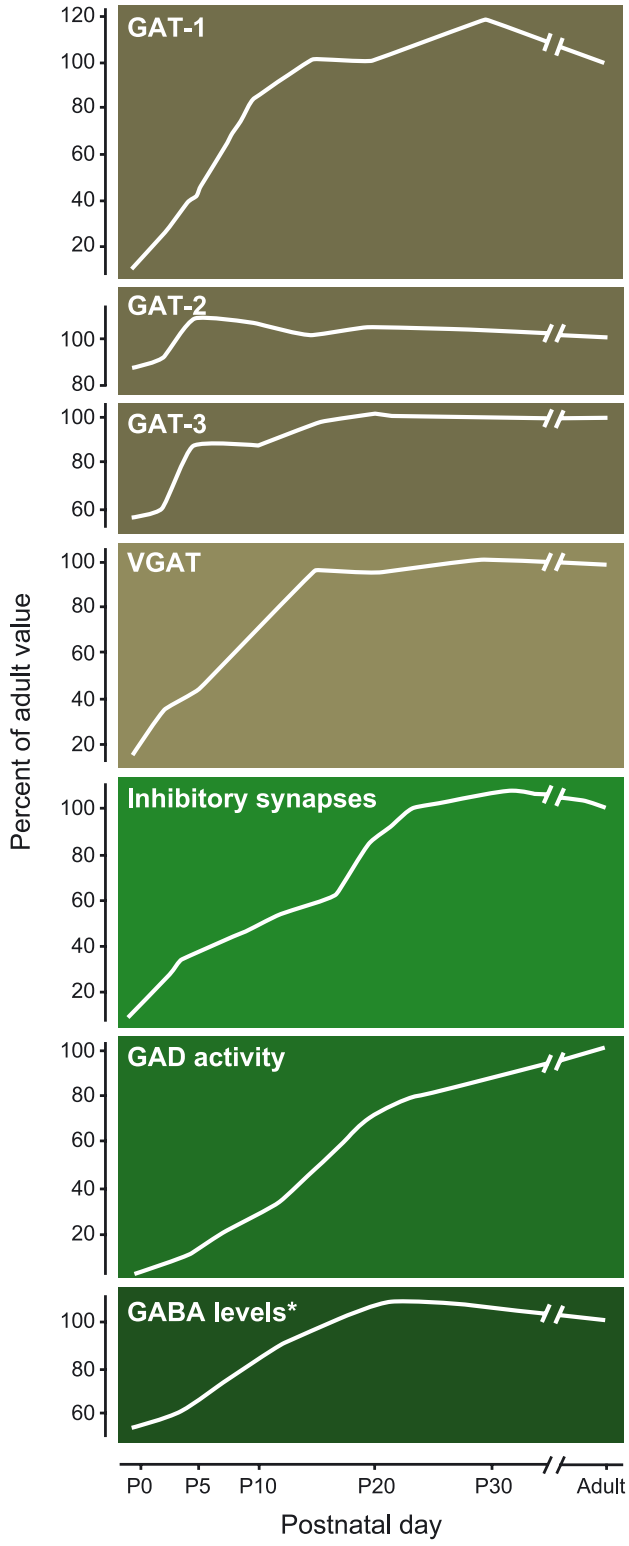


Fig. 7. Highly schematic representation of the postnatal maturation of GATs and other GABAergic markers in rodent cerebral cortex. Data are from: Ref. [145] (GAT-1 and VGAT; immunoblots); A. Minelli, and F. Conti (GAT-2 and GAT-3; gray values; unpublished results); Refs. [51,141] (inhibitory synaptogenesis; density of symmetric synapses); Refs. [43,216] (GAD activity); Ref. [43] (endogenous GABA levels). All data are given as percentage of adult values. *The data on GABA levels are from the whole brain.

Notwithstanding largely immature inhibitory synaptogenesis and vesicular mechanisms for GABA storage and release [18,51,141,145], GABAergic neurons are numerous in neonatal cortex [35,55,208] and endogenous GABA levels are relatively high [43] (see Fig. 7). At this stage, GABA is excitatory [31] and acts in a paracrine mode [56,149,154,155,157] as a trophic factor, influencing several morphogenetic aspects of neuronal maturation [13,123,124,131] via receptor-mediated mechanisms (see Ref. [131,156,197] for data on cerebral cortex). In the neonatal cortex, the only GAT abundantly expressed is GAT-3 [66,144] and GABA uptake is potently inhibited by β-alanine [216], suggesting that extracellular GABA levels at birth are modulated mainly by GAT-3-mediated transport. Since GAT-3 is intensely expressed in the very layers where $GABA_A$ receptors are densest (i.e., outer laminae and cortical plate; see Ref. [72,165,166]), it may be involved in modulating GABA's trophic action.

A currently debated issue regards the sources of non-synaptic GABA release in early brain development. Growth cones arising from GABAergic neurons have been hypothesized to release GABA via reversal transporter activity [9,154,203,204]. Although inhibition of GAT-1-mediated transport does not affect GABA clearance in neonatal hippocampus [56], other transporters, i.e., GAT-3, might be involved, especially in neonatal cortex, where GAT-3 expression far outstrips that of the other GATs. Whereas in the adult neocortex GAT-3 is expressed exclusively in astrocytes [143], at perinatal ages it is also found in a large fraction of cortical GABAergic neurons [144], suggesting that they might release GABA via GAT-3-mediated reversal transport.

Given the scarce neuronal and astrocytic expression of GAT-2 throughout development [66,108,144], its effect on GABA-mediated responses, if any, appears of minor import. The main function of GAT-2 in developing cortex seems to relate to the regulation of GABA levels in CSF and of GABA transport across the blood–brain barrier. Interestingly, the GAT-2+ astrocytic processes surrounding blood vessels are denser in neonatal than adult cortex [108,144], a finding that lends support to recent data showing a faster rate of GABA uptake in immature compared with adult rat brain due, at least partly, to greater maximal transport [5]. Notably, GAT-3+ puncta also surround neonatal cortex blood vessels [144], possibly contributing to effective GABA transport in the neonatal brain and to regulating the amount of neurotrophic GABA available to proliferating and migrating neurons during pre- and perinatal development [154].

## 4. Expression of GATs in neurological diseases

This section does not address all the neuropsychiatric diseases in which changes in GABA transport have been described or could play a role. Rather, it is a succinct

overview of the data linking the cortical expression of GATs to the few conditions in which such a role appears likely.

### 4.1. Epilepsy

An increase in GAT-1+ interneurons has been reported in rat neocortex 24 h after corticotropin-releasing hormone-induced seizures [152] and a transient increase in GAT-3 mRNA (but not protein) expression has been described in amygdala-kindled rats 1 h after the last seizure [91].[4] These findings suggest that seizure activity is associated with an upregulation of neocortical GATs expression. Recent data showing that transgenic mice overexpressing GAT-1 exhibit increased susceptibility to chemically induced seizures, although they do not display spontaneous seizure activity [95], and that enhanced GAT-1-mediated GABA transport is associated with seizures in genetically epileptic mouse strain [73] support this possibility.

Evidence for a downregulation of GATs function in the epileptic neocortex has also been reported: (i) cortical GABA uptake is reduced in different genetic mouse and rat models of epilepsy [41,201]; (ii) GAT-1 mRNA expression is reduced in the neocortex of genetically epilepsy-prone rats [3]; and (iii) GAT-1 is reduced, particularly in perisomatic axon terminals, in the sensorimotor cortex of a rat pilocarpine model of temporal lobe epilepsy [191]. Finally, GAT-1 is reduced and abnormally distributed in the neocortex of patients with temporal lobe epilepsy and focal dysplasia [199].

Interpreting these studies is difficult since they employ different models (which may involve different pathophysiological mechanisms with different temporal patterns) and, most importantly, given the inherent difficulty of isolating primary modifications of GATs expression from secondary, adaptive changes. Increased GATs expression should lower extracellular GABA levels, thus contributing to the origin and spread of epileptic activity; in line with this view, selective GAT-1 blockers (i.e., tiagabine and NNC-711; for reviews see Refs. [1,126,132,137,138,198]) and glial (possibly GAT-3-mediated) GABA uptake inhibitors (i.e., SNAP-5114, NNC 05-2045 and *N*-methyl-exo-THPO; [44,74,79,163,178,182,211]) have been shown to possess anticonvulsant activity. Alternatively, since GABA transporters can work in reverse [8,103,128,164,183,189,217], an upregulation of GATs following sustained neuronal activity may induce a compensatory increase of GABA release via a nonvesicular, transporter-mediated mechanism [62,76,218]. Similarly, the substantial reduction in perisomatic GAT-1+ terminals reported in epileptic neocortex [191] (see also Ref. [6,179] for the hippocampus) is in line with the notions that loss of the GABAergic cells innervating the soma and initial axon segment of pyramidal neurons, i.e., basket and chandelier cells [71], is crucial to seizure onset [45,174,175,192,193], but that the reduction of GABA transport and GATs expression may represent a compensatory response homeostatically modulating neuronal overexcitation [198]. Moreover, reduced GATs expression in epileptic tissue may exacerbate epileptiform activity by decreasing the scope for GABA heterotransport [62,76,160].

### 4.2. Ischemia

Following reports that GABA uptake inhibitors tiagabine and NCC-711 exert neuroprotective effects [151,220,223], the cortical expression of GAT-1 and GAT-3 was investigated in a rat model of transient focal ischemia [140]. The study showed that, unlike GAT-1 expression, GAT-3 expression is markedly and selectively affected by ischemia: (i) GAT-3+ puncta (i.e., distal astrocytic processes) are reduced in the perilesional cortex; and (ii) numerous cortical neurons, the vast majority of which are pyramidal, become GAT-3+, particularly in the perilesional cortex; these neurons are not apoptotic (GAT-3+ neurons are TUNEL negative) and express HSP70.

These observations agree with the notion that ischemia induces hyperexcitability in rat neocortex and that in the perilesional cortex neuronal hyperexcitabilty is, at least partly, determined by reduced GABAergic activity [61,86,133,147,181,214]. Since the novel expression of GAT-3 is mostly detected in perikarya of cortical pyramidal neurons, which are important targets of GABAergic action, an increase in GAT-3 molecules at postsynaptic sites may conceivably increase GABA uptake, thereby reducing the amount of GABA available for binding to receptors and increasing neuronal excitability.

A known physiological instance of cortical neurons expressing GAT-3 is early development [145] (see Sections 3.3 and 3.4), thus indicating that during ischemia GAT-3 expression undergoes a revertion to its immature pattern. Reactivation of embryonic gene expression patterns may occur in the brain after injury-induced hypoxia (for review, see Ref. [113]). Although neuronal GAT-3 in ischemic brain may serve different functions and have different consequences for the organism than in developing neocortex, it is tempting to speculate that it may be part of a genetic "reprogramming" occurring as a response to injury.

## 5. Comments and conclusions

Given its crucial role in the normal functioning of the cerebral cortex, an enormous research effort has been directed in the past decades at investigating GABAergic transmission. Most studies have focused on pre- and postsynaptic factors (in particular, GABA receptors), generating a vast body of data on their role in information

[4] In line with these findings, compounds not acting preferentially on GAT-1 and selective astroglial GATs inhibitors possess significant anticonvulsant activity in chemically and sound-induced seizures [44,74,79,163,178,182,211].

processing and in the pathophysiology (and in some cases, the therapy) of human diseases. GATs studies are lagging behind, despite their obvious importance in cortical physiology (GATs contribute to shaping inhibition in intracortical circuits as well as to modulating cortical output). The data reviewed here suggest the existence in the cerebral cortex of a complex and heterogeneous system mediating GABA uptake that may have the potential for sustaining the sophisticated adaptive mechanisms allowing the normal functioning of the cortical GABAergic system. Similarly, the complex and asynchronous maturation patterns of GATs revealed in the developing neocortex suggest that GABA transport may contribute both to the cascade of events leading to inhibitory synaptogenesis and to neuronal maturation during early cortical development. Overall, although many details are still missing, the anatomy of the cortical uptake system in both the adult and the developing cerebral cortex appears to be sufficiently understood to allow the study of its dynamic physiological features.

The studies of the expression of GATs in epilepsy and cerebral ischemia (or in their animals models) do not provide definite answers on whether the changes observed have pathophysiological implications. Nevertheless, they show that these changes are compatible with a role for GATs in brain dysfunctions that are either intrinsic features of the pathophysiology of the disease (i. e., epilepsy) or are responsible for the genesis of some of its symptoms (i. e., cerebral ischemia). Besides fostering further research into the involvement of GATs in human diseases, we hope that these preliminary data will promote the development of a larger set of specific agonists and antagonist for use both at the bench and in the therapeutic armamentarium.

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