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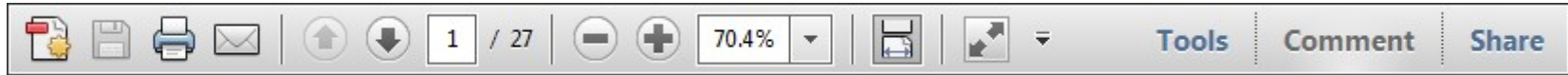
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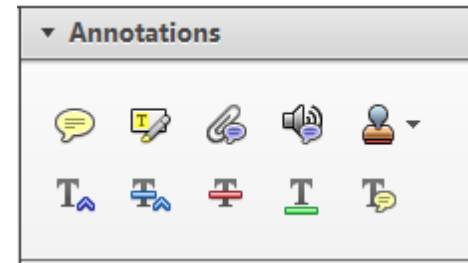
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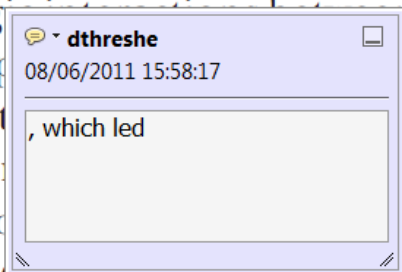


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there is no room for extra profits and the number of firms are zero and the number of firms (net) values are not determined by the number of firms. Blanchard and Kiyotaki (1987), perfect competition in general equilibrium. The classical framework assuming monopoly and perfect competition is that the structure of the sector is determined by an exogenous number of firms.

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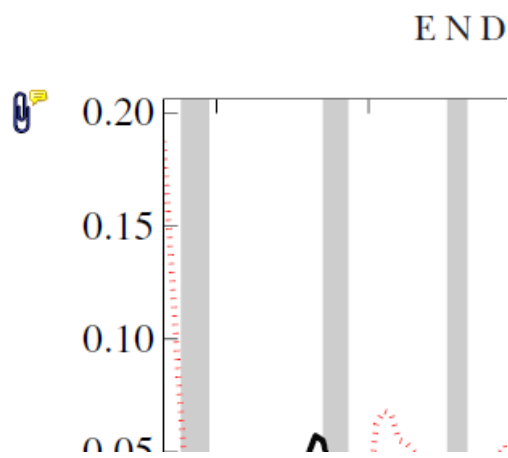
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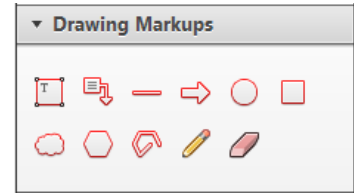
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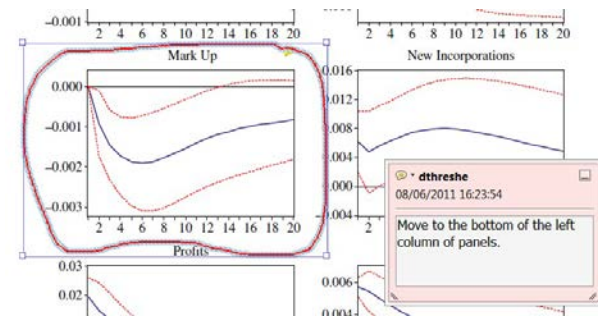
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# GAT-1 Mediated GABA Uptake in Rat Oligodendrocytes

Giorgia Fattorini,<sup>1,2</sup> Marcello Melone,<sup>1,2</sup> María Victoria Sánchez-Gómez,<sup>3</sup>  
Rogelio O. Arellano,<sup>3</sup> Silvia Bassi,<sup>1</sup> Carlos Matute,<sup>3</sup> and Fiorenzo Conti<sup>1,2,4</sup>

Stimulated by the results of a recent paper on the effects of tiagabine, a selective inhibitor of the main GABA transporter GAT-1, on oligodendrogenesis, we verified the possibility that GAT-1 may be expressed in oligodendrocytes using immunocytochemical methods and functional assays. Light microscopic analysis of the subcortical white matter of all animals revealed the presence of numerous GAT-1+ cells of different size (from 3 to 29  $\mu\text{m}$ ) and morphology. An electron microscope analysis revealed that, besides fibrous astrocytes and interstitial neurons, GAT-1 immunoreactivity was present in immature and mature oligodendrocytes. Co-localization studies between GAT-1 and markers specific for oligodendrocytes (NG2 and RIP) showed that about 12% of GAT-1 positive cells in the white matter were immature oligodendrocytes, while about 15% were mature oligodendrocytes. *In vitro* functional assays showed that oligodendrocytes exhibit tiagabine-sensitive  $\text{Na}^+$ -dependent GABA uptake. Although relationships between GABA and oligodendrocytes have been known for many years, this is the first demonstration that GAT-1 is expressed in oligodendrocytes. The present results on the one hand definitely closes the era of "neuronal" and "glial" GABA transporters, on the other they suggest that oligodendrocytes may contribute to pathophysiology of the several diseases in which GAT-1 have been implicated to date.

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**Key words:** GABA, GABA transporter 1, oligodendrocytes

## Introduction

Tiagabine ((R)-*N*-[4,4-bis-(3-methyl-2-thienyl)but-3-en-1-yl] nipecotic acid) was developed using a structure–activity approach, and shown to bind with high-affinity to the  $\gamma$ -aminobutyric acid (GABA) carrier (Braestrup et al., 1990). Following the cloning and functional characterization of GAT-1 (Guastella et al., 1990), the major GABA transporter in the mammalian central nervous system, it became soon evident that tiagabine specifically interacts with the GAT-1 transporter (Borden, 1996; Borden et al., 1994), and that it is clinically effective as antiepileptic drug (Froestl, 2011; Schousboe and White, 2009; Suzdak and Jansen, 1995). Tiagabine's selectivity toward GAT-1 limits its activity to regions of the CNS in which GAT-1 plays a significant role (neocortex, cerebellum, and hippocampus) (Jasmin et al., 2004). In addition, tiagabine, besides its anticonvulsant effect, has antinociceptive, anxiolytic-like, sedative and

antidepressant-like properties (Jasmin et al., 2004; Salat et al., 2015).

In 2015, a major paper renewed interest on the effects of tiagabine. Zonouzi and colleagues, while investigating cerebellar GABA signaling in a mouse model of diffuse white matter injury (DWMI; a severe neurological syndrome characterized by behavioral, cognitive and motor deficits associated to disruption of subcortical white matter development with hypomyelination), showed that tiagabine administration enhances the cellular progression of NG2 cells and promotes oligodendrogenesis and myelination (Zonouzi et al., 2015).

Tiagabine is a selective inhibitor of GAT-1, a GABA transporter that plays a critical role in the modulation of phasic and tonic GABA<sub>A</sub>R-mediated inhibition—particularly during sustained neuronal activity—(Bragina et al., 2008), and may also contribute to presynaptic homeostasis at GABAergic terminals (Conti et al., 2011). Thus, the effects described by

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Zonouzi et al. (2015) depend on the interaction between tiagabine and its substrate. GAT-1 is reportedly localized exclusively to axon terminals and astrocytic processes (Cherubini and Conti 2001; Conti et al., 2004; see Discussion). Accordingly, the reported effects should be triggered by the action of tiagabine on neurons and/or astrocytes; indeed, Zonouzi et al. (2015) interpret their results as a consequence of the disruption of GABAergic signaling from white matter interneurons to NG2 cells, but did not test alternative possibilities.

However, the possibility exists that the effects of tiagabine reported by Zonouzi depend on its action on GAT-1 expressed by oligodendrocytes, and that this direct action may contribute to explain their observations. We therefore set up to verify the hypothesis that oligodendrocytes express functional GAT-1, focusing on subcortical white matter.

## Materials and Methods

### Animals and Tissue Preparation

Adult male Sprague-Dawley albino rats (190–220 g;  $n = 10$ ; Charles River, Milan, Italy) were used for this study. Their care and handling were approved by the local ethical committee for animal research. Experiments were carried out in accordance with the Council Directive 2010/63EU of the European Parliament and the Council of September 22, 2010 on the protection of animals used for scientific purposes and approved by the local veterinary service. Animals were kept under a dark-light cycle of 12 h and permitted food and water *ad libitum*.

Adult rats were perfused transcardially with a flush of saline followed by freshly depolymerized 4% paraformaldehyde (PFA) in phosphate buffered saline (PB; 0.1 M). Brains were removed, post-fixed in the same fixative for 2 h at 4°C and cut with a vibratome into 60  $\mu\text{m}$ -thick coronal sections (stereotaxic coordinates: from +1.2 to  $-2$  mm from the bregma; Paxinos and Watson, 1982)

**Antibodies.** Source, concentrations, and data on the characterization of primary antibodies used in this study are listed in Table 1.

**Immunoperoxidase.** Free-floating sections were pretreated in 1%  $\text{H}_2\text{O}_2$  for 30 min, preincubated for 1 h in 5% bovine serum albumin (BSA) in PB, and then for 2 h at room temperature plus overnight at 4°C in a solution containing primary antibody (Table 1). The next day, sections were incubated for 20 min in 5% BSA in PB then for 1 h in secondary antibody (Table 1). Sections were subsequently incubated for 40 min in avidin–biotin peroxidase complex (ABC Elite PK6100; Vector; 1:100 in PB), and finally in 0.05% 3,3'-diaminobenzidine tetrahydrochloride in 0.05 M Tris with 0.03%  $\text{H}_2\text{O}_2$ . Finally, sections were washed in PB, mounted on gelatin-coated slides, air dried, coverslipped and finally examined with a Leitz Orthoplan (Wetzlar, Germany) microscope. Method specificity was verified by substituting primary antibodies with PB or BSA.

Sections for pre-embedding electron microscopy studies were post-fixed in 1% osmium tetroxide in PB for 45 min, and contrasted with 1% uranyl acetate in maleate buffer (pH 6.0; 1 h). After dehydration in ethanol and propylene oxide, sections were embedded in

Epon/Spurr resin (Electron Microscopy Sciences, Hatfield, PA), flattened between Aclar sheets (Electron Microscopy Sciences), and polymerized at 60°C for 48 h. Small blocks of subcortical white matter were selected by light microscopic inspection, glued to blank epoxy and sectioned with an ultramicrotome (MTX; Research and Manufacturing Company, Tucson, AZ). The most superficial ultrathin sections ( $\sim 60$  nm) were collected and mounted on 200 mesh copper grids, stained with Sato's lead and examined with a Philips EM 208 electron microscope (Eindhoven, The Netherlands) coupled to a MegaView-II high resolution CCD camera (Soft Imaging System; Munster, Germany). Identification of labeled and unlabeled cells and profiles was based on established morphological criteria (Peters et al., 1991).

**Immunofluorescence.** Sections were incubated for 1 h in BSA 5% in PB, and then for 2 h at room temperature, and kept overnight at 4°C in a solution containing a mixture of primary antibodies containing GAT-1 and one of the following antibodies: RIP and NG2 (Table 1). The next day, sections were incubated for 20 min in BSA 5% in PB then in a mixture of the appropriate secondary fluorescent antibodies (Table 1). Sections were then mounted, air-dried and coverslipped using Vectashield mounting medium (H-1000; Vector, Burlingame, CA). Images were acquired using a Leica SP2 confocal microscope (Leica Microsystems, Wetzlar, Germany) (pixel size 0.15  $\mu\text{m}$ ). Control experiments with single-labeled sections and sections incubated with either two primary and one secondary antibody or one primary and two secondary antibodies revealed no appreciable fluorochromes bleed-through or antibody cross-reactivity.

**Oligodendrocyte cultures.** Primary cultures of oligodendrocytes derived from the optic nerves of 12-days-old Sprague Dawley rats were obtained as described previously (Barres et al., 1992), with minor modifications (Sánchez-Gómez et al., 2003). Cells were seeded into 24-well plates bearing 12-mm-diameter coverslips coated with poly-D-lysine (10  $\mu\text{g}/\text{ml}$ ) and maintained at 37°C and 5%  $\text{CO}_2$  in a chemically defined medium (Sato medium; Barres et al., 1992). After 2 days *in vitro*, cultures were composed of at least 98% cells positive for O4 antigen. Live cells were immunostained with an oligodendroglial cell-specific marker, mouse anti-O4 (10  $\mu\text{g}/\text{ml}$ , Chemicon International, Ref. MAB345; RRID:AB\_11213138) as described (Arellano et al., 2016). Coverslips were mounted on glass slides with fluorescent mounting medium (Glycergel, Dako, Glostrup, Denmark), and preparations were visualized under a laser scanning confocal microscope (Leica, TCS SP8X).

**[ $^3\text{H}$ ]-GABA uptake assays.** Primary cultures of oligodendrocytes derived from the optic nerves as detailed above. Cells were seeded into 24-well plates bearing 12-mm-diameter coverslips coated with poly-D-lysine (10  $\mu\text{g}/\text{ml}$ ) and maintained at 37°C and 5%  $\text{CO}_2$  in a chemically defined medium (Sato medium; Barres et al., 1992). [ $^3\text{H}$ ]-GABA uptake was assessed at 37°C in 300  $\mu\text{l}$  saline solution containing (in mM): NaCl 140, KCl 5,  $\text{MgCl}_2$  2,  $\text{CaCl}_2$  2, HEPES 10, glucose 4.5 g/L, pH 7.4, or in a sodium-free saline buffer in which sodium was equimolarly replaced with choline. Oligodendrocytes were equilibrated in this buffer for 5 min in the absence or

T1

**TABLE 1: Primary and Secondary Antibodies**

Primary antibodies				
Antibodies	Host <sup>a</sup>	Dilution <sup>b</sup>	Source	Characterization
GAT-1	R	1:400 (EM; IP)	Kindly provided by Dr. N.C. Brecha (Dept Neurobiology, UCLA, Los Angeles, CA) (RRID:AB_2313748)	Guo et al., 2009; Minelli et al., 1995
GAT-1	GP	1:500 (IF)	Synaptic System/274104 (RRID:AB_2620001)	Hu et Quick, 2008; Quick et al., 2004
NG2	R	1:200 (IF)	Chemicon/AB5320 (RRID:AB_91789)	Levine et al., 1993; Stallcup et al., 1981
RIP	M	1:800 (IF)	Chemicon/MAB1580 (RRID:AB_94266)	Friedman et al., 1989; Sakakibara et al., 2008
Secondary antibodies				
Conjugated to	React <sup>a</sup>	Dilution	Source	
Alexa Fluor® 488	GP	1:200	Jackson ImmunoResearch, West Grove, PA/706-546-148	
Alexa Fluor® 594	M	1:200	Jackson ImmunoResearch, West Grove, PA/715-586-150	
Alexa Fluor® 647	M	1:200	Jackson ImmunoResearch, West Grove, PA/715-606-150	
Alexa Fluor® 594	R	1:200	Jackson ImmunoResearch, West Grove, PA/711-586-152	
Biotin-SP	R	1:100	Jackson ImmunoResearch, West Grove, PA/111-066-003	

<sup>a</sup>GP, guinea pig; M, mouse; R, rabbit.

<sup>b</sup>IF, immunofluorescence; EM, electron microscopy; IP, immunoperoxidase.

presence of uptake inhibitor tiagabine (50  $\mu\text{M}$ ; Tocris, Cat No: 4256), and then incubated for 5 min with 100 nM [ $^3\text{H}$ ]-GABA together with unlabeled GABA (10  $\mu\text{M}$ ). Uptake was stopped with two washes in ice-cold phosphate-buffered saline (0.1 M, PBS). Cells were solubilized in 250  $\mu\text{l}$  lysis solution (0.1 N NaOH/0.01% Triton X-100 in 0.1 M PBS) and mixed with 2.5 ml scintillation liquid. Counts per minute were determined in a liquid scintillation counter (Beckman Coulter) and values were expressed as percentage of control (uptake in presence of sodium).

### Data Collection and Analysis

**Light microscopy.** For morphological analysis, a Nikon eclipse E600 (objective 40X) equipped with Nikon DS Camera Control Unit DS-L3 was used. The major diameter of GAT-1+ cells in which a large nucleus, a clear cytoplasm and well defined borders were evident was measured using Image J software (v.1.45s; National Institutes of Health).

**Pre-embedding electron microscopy.** GAT-1 immunopositive profiles were studied from ultrathin sections at the surface of embedded blocks. Data on GAT-1 profiles derived from the analysis of 8–9 ultrathin sections of subcortical white matter for animal (500  $\times$  500  $\mu\text{m}^2$  each; two animals). Data on GAT-1 immunodetection in cortical gray matter derived from layers II/III of parietal cortex (four ultrathin sections for animal; two animals). Microscopic fields used to evaluate GAT-1 immunoreactivity were selected and captured at original magnifications of 12,000 or 20,000 $\times$ .

**Confocal microscopy.** Images from all experimental series were acquired from subcortical white matter (four sections per animal; 10 rats) using a 40 $\times$  lens (numerical aperture 0.75; pinhole 1.0 and image size 512  $\times$  512 pixels, yielding a frame of 286.29  $\mu\text{m}$ ) from a nuclear plane of cells. Signal acquisition was optimized; photomultiplier gain was set so that the brightest pixels were just slightly below saturation, and the offset such that the darkest pixels were just above zero. To avoid bleed-through between green and red (or blue) fluorescence, images were acquired sequentially. To improve the signal/noise ratio, 10 frames/image were averaged. Without reducing the image resolution, each channel was examined separately to identify and count manually immunopositive cells; the two channels were then merged and the number of co-localizing cells was counted.

For all studies, statistical analyses were performed using GraphPrism v.4.0 (GraphPad Software, San Diego, CA).

### Results

Light microscopic analysis of the subcortical white matter of all animals revealed the presence of numerous GAT-1+ cells of different size (from 3 to 29  $\mu\text{m}$ ) and morphology (Fig. 1). Some GAT-1+ cells were small (2–7  $\mu\text{m}$ ), roundish in shape and exhibited small processes (Fig. 1A); others were medium-sized (7–13  $\mu\text{m}$ ), egg-shaped or roundish with regular profiles and a well distinguishable cytoplasm confined in the periphery; these cells also showed small cellular processes (Fig. 1A).

Other medium-sized cells had pyramidal shape and long intensely stained processes, regular profiles, a well distinguishable and a large and eccentric nucleus (Fig. 1A). Finally, we observed cells of considerable size (13–28  $\mu\text{m}$ ), elongated, and with a large central nucleus (Fig. 1A). The frequency distribution of the diameter of GAT-1+ cells in subcortical white matter is shown in Fig. 1B. The density of GAT-1+ cells in subcortical white matter was  $69.1 \pm 9.1$  cells/ $\text{mm}^2$ .

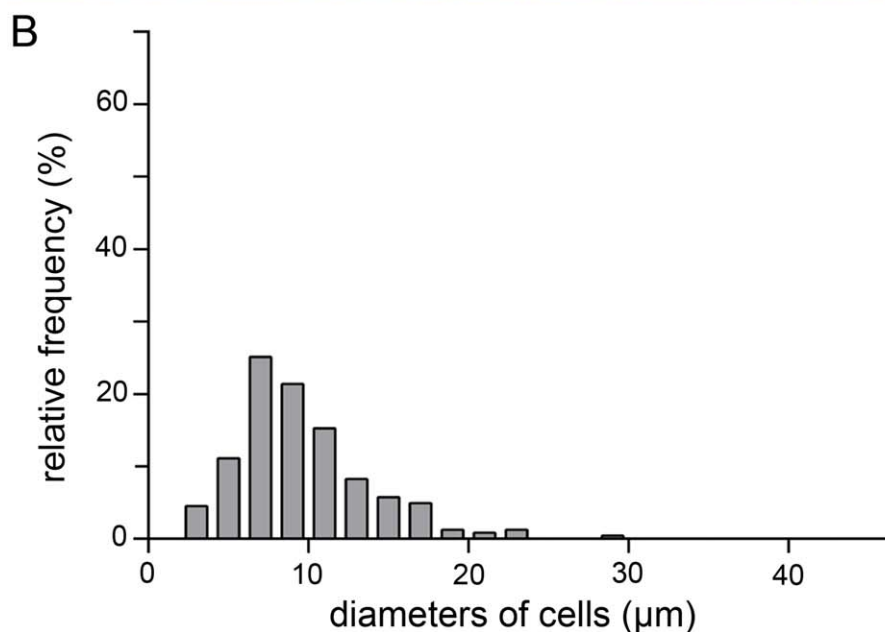
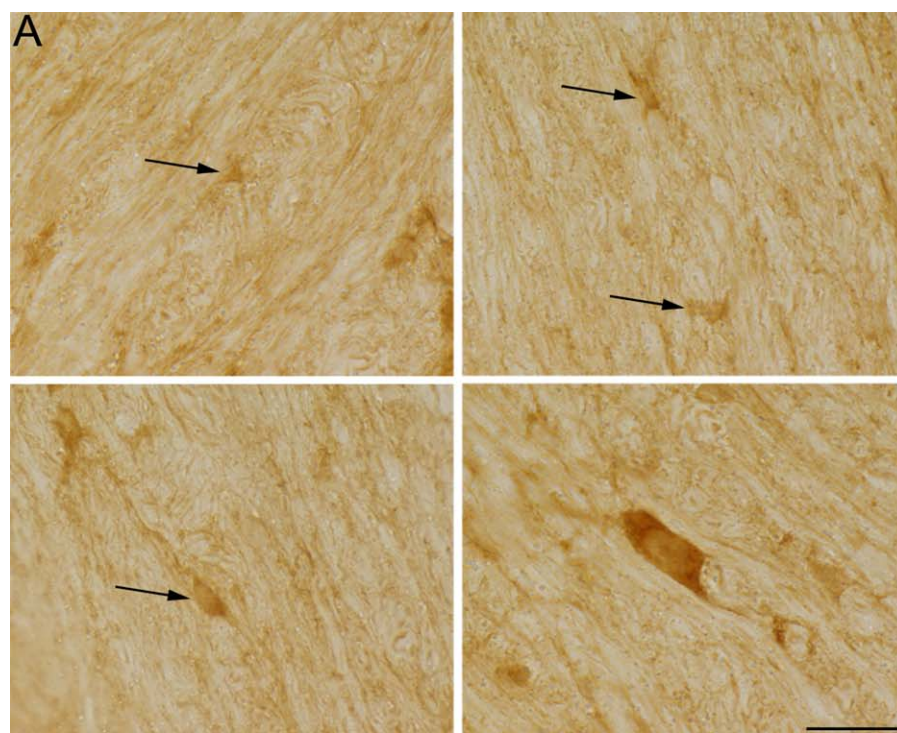
The differences in size and morphology of GAT-1+ cells in subcortical white matter suggested that they may belong to different cell types; accordingly, to verify whether some of these cells are oligodendrocytes, we studied this material at the electron microscope using a pre-embedding method. In line with our previous studies in neocortex (Conti et al., 1998; Melone et al., 2015; Minelli et al., 1995), we observed GAT-1 immunoreactivity in fibrous astrocytes (Fig. 2A), and interstitial neurons (Fig. 2B) (Kostovic and Rakic, 1980; Meyer et al., 1991; Peters et al., 1991). In addition, we found that a subset of GAT-1+ cells showed features typical of immature oligodendrocytes (eccentric nucleus with heavy clumps of condensed chromatin, dark or medium cytoplasmic density a relatively regular shape and a high density of mitochondria; Kaplan and Hinds, 1980) (Fig. 2C), and of mature oligodendrocytes (eccentric nucleus with heavy clumps of condensed chromatin, dark or medium cytoplasmic density, an irregular shape and relatively long profiles; Kaplan and Hinds, 1980; Peters et al., 1991; Vaughan and Peters, 1974) (Fig. 2D). GAT-1 ir was not confined to the cytoplasm of oligodendrocytes, but was also detectable in both proximal and small (diameter <1.5  $\mu\text{m}$ ; Lunn et al., 1997) oligodendrocytic processes. In particular, GAT-1 was observed at the initial segment of proximal processes radiating from the somata (Fig. 2E), was relatively frequent in sectioned peripheral processes (Fig. 2F,G), and occasionally at the cytoplasmic tongues (Peters et al., 1991) of myelin-forming distal oligodendroglial process (Fig. 2H). Finally, in order to verify whether oligodendrocytic expression of GAT-1 was limited to white matter, we extended our electron microscopy analysis to cortical gray matter. Inspection of layers II/III confirmed the presence of GAT-1 ir in the cytoplasm (Fig. 2I,J) and initial segment (Fig. 2K) of oligodendrocytic processes.

Next, to gather information on the percentage of GAT-1+ oligodendrocytes in subcortical white matter, we performed co-localization studies between GAT-1 and NG2 and RIP (NG2 for immature oligodendrocytes, Levine and Nishiyama, 1996; RIP for mature oligodendrocytes, All et al., 2015; Chen et al., 2009; Friedman et al., 1989; Flygt et al., 2013; Kim et al., 2015; Sevc et al., 2014). Confocal microscopic analysis showed that in subcortical white matter co-localization between GAT-1 and these markers (Fig. 3) was as

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F3



**FIGURE 1: Morphology of GAT1+ cells in the subcortical white matter. (A)** Light microscopy revealed the presence of numerous GAT-1+ cells with different size and morphology (arrows point to small and medium-sized cells). **(B)** Frequency distribution of the diameter of GAT-1+ cells in the subcortical white matter. Scale bar: 20 μm.

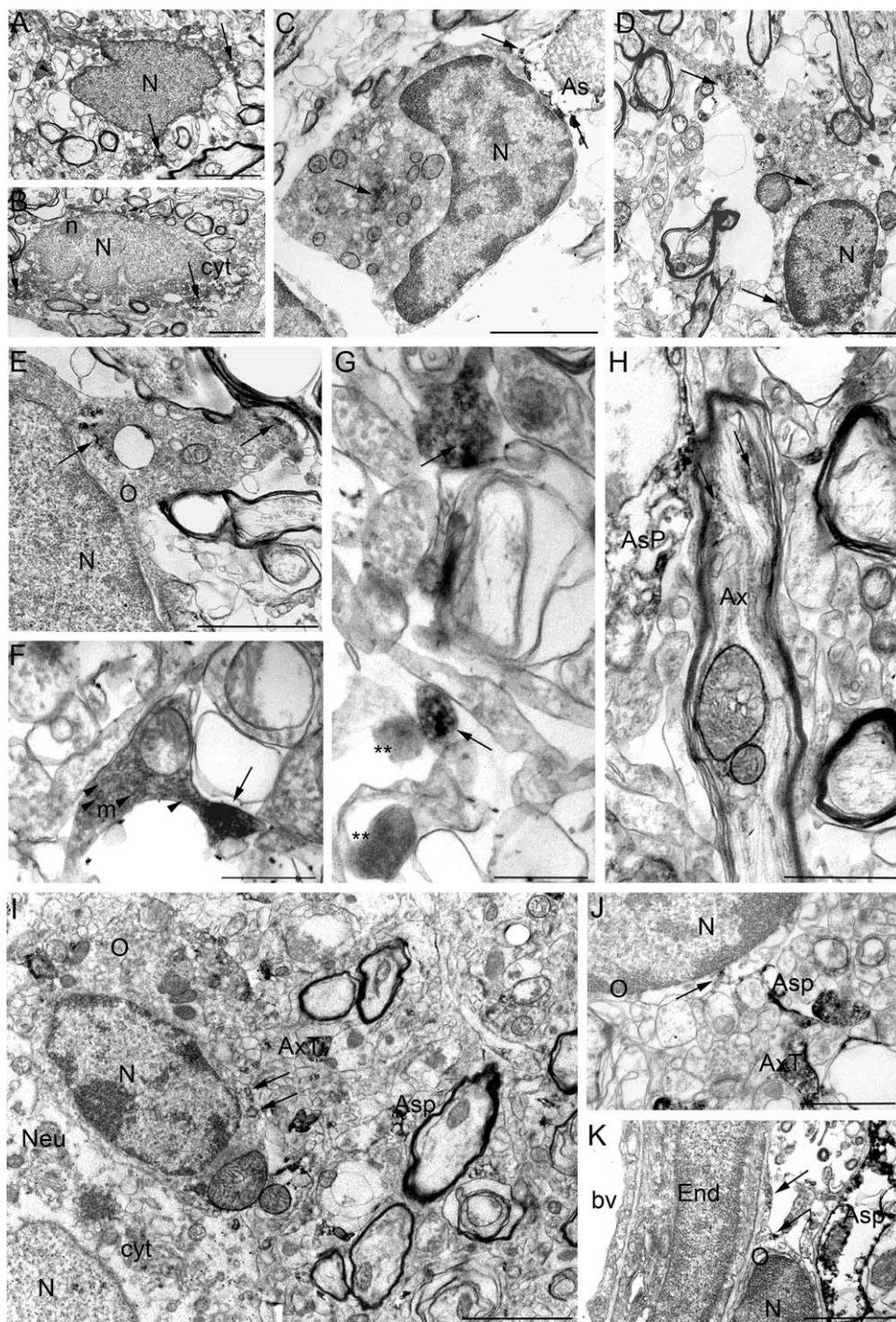
follows: 11.75% ± 2.72% of all GAT-1 positive cells were immature oligodendrocytes (seven animals; 97 fields; 222 cells); and 14.78% ± 2.02% were mature oligodendrocytes (seven animals; 97 fields; 222 cells).

Finally, we assayed in nearly pure oligodendrocyte cultures whether the detected transporter was functional by performing GABA uptake studies with radiolabeled

transmitter. These experiments showed that sodium-dependent GABA uptake was significantly inhibited in the presence of tiagabine (38.6 ± 4.2% of control; *n* = 3; Fig. 4), a potent GABA uptake inhibitor with high affinity and selectivity for the GAT-1 (Laughlin et al., 2002), indicating that GABA uptake in oligodendrocytes is driven by GAT-1 transporter.

F4

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**FIGURE 2:** Ultrastructural localization of GAT-1 in subcortical white matter. (A,B) Example of a fibrous astrocytes (A) and a neuron (B) with GAT-1 immunoreaction products dispersed in their cytoplasm (arrows). (C,D) GAT-1 immunoreactivity (ir; arrows) of an immature (C) and a mature (D) oligodendrocyte. (E-H) Distribution of GAT-1 ir at oligodendrocytic processes. The initial segment of a large proximal oligodendrocytic process radiating from the somata (E) and small peripheral sectioned processes with typical microtubules (m, arrowheads in F) display GAT-1 immunopositive products (arrows). Asterisks in G indicate GAT-1 immuno-negative small oligodendrocytic processes. (H) GAT-1 ir at the cytoplasmic tongues (arrows) of a myelin-forming distal oligodendrocytic process. (I-K) GAT-1 expression in cortical oligodendrocyte. GAT-1 ir (arrows) is detectable in the cytoplasm of cortical oligodendrocytes (I, J). In some cases, the initial segment of proximal oligodendrocytic processes radiating from the somata (K) display GAT-1 immunopositive products (arrows). N, nucleus; n, nucleolus; cyt, cytoplasm; As, Astrocyte; O, Oligodendrocyte; AsP, astrocytic processes; Ax, Axon; Neu, Neuron; Axt, Axon Terminal; bv, blood vessel; End, Endothelial cell. Scale bars: A-E and I, 2  $\mu$ m; F, G, 250 nm; H and J, K, 1  $\mu$ m.

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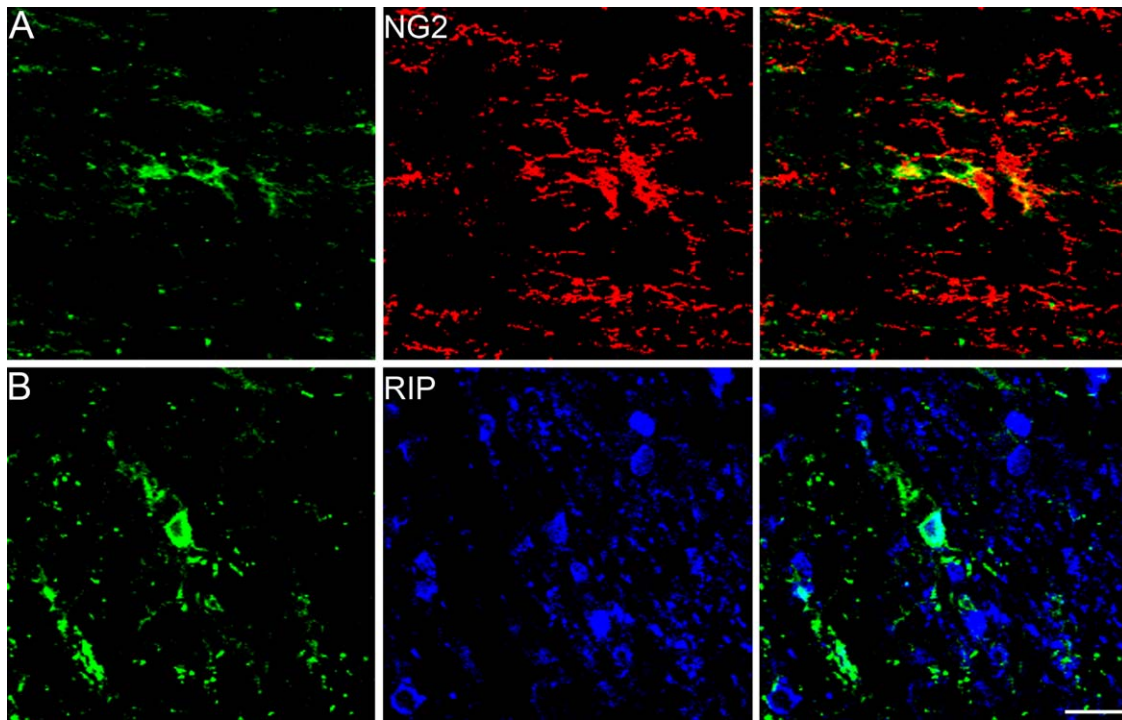


FIGURE 3: Co-localization of GAT-1 ir and oligodendrocyte markers in subcortical white matter. GAT-1+ cells (green; left column) co-expressing NG2 (A) or RIP (B) (blue or red; middle column). Scale bar: 20  $\mu\text{m}$ .

### Discussion

Early biochemical and pharmacological studies on GABA uptake into nerve terminals and/or surrounding glial processes led to the notion that neurons and astrocytes express different GABA transporters (Iversen and Kelly, 1975; Mabjeesh et al., 1992), and that “neuronal” and “glial” GABA transporters were separable on the basis of pharmacological criteria (Cummins et al., 1982; Levi et al., 1983; Reynolds and Herschkowitz, 1986). Application of newly developed cloning techniques revealed that four cDNAs encoding GABA transporters (GATs) are localized in the rodent and human nervous system: GAT-1, GAT-2, GAT-3, and BGT-1 (Borden et al., 1992, 1994; Brecha, 1992; Clark et al., 1992; Guastella

et al., 1990; Liu et al., 1993). It was soon shown that GABA uptake by GAT-1 is strongly inhibited by *cis* 3-aminocyclohexane carboxylic acid (ACHC) and, to a lesser extent, by 2,4-diaminobutyric acid, but not by  $\beta$ -alanine (Guastella et al., 1990; Keynan et al., 1992). These properties were considered typical of “neuronal” transporters (Beart et al., 1972; Iversen and Kelly, 1975; Jones and Neal, 1976; Larsson et al., 1983; Mabjeesh et al., 1992). In contrast, GAT-2 and GAT-3 exhibit pharmacological properties associated with “glial” transporters (Gavrilovic et al., 1984; Iversen and Kelly, 1975; Kanner and Bendahan, 1990; Mabjeesh et al., 1992; Schon and Kelly, 1974), since GABA uptake by GAT-2 and GAT-3 is strongly inhibited by  $\beta$ -alanine, but not

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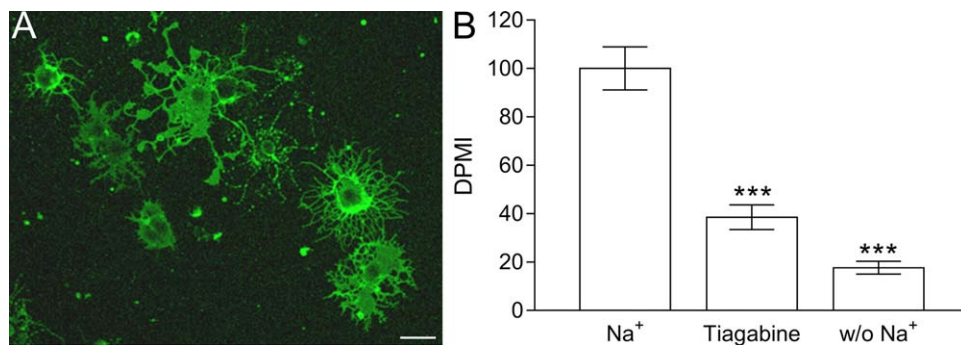


FIGURE 4: GAT-1-mediated GABA uptake in oligodendrocytes. GABA uptake assays were performed on nearly pure oligodendrocytic cultures (A; scale bar: 20  $\mu\text{m}$ ) in control condition (Na<sup>+</sup>), in the presence of tiagabine (a selective inhibitor of GABA transporter GAT-1), or in the absence of Na<sup>+</sup> (w/o Na<sup>+</sup>; non-specific uptake) (B). Data were expressed as % of decays per minute (DPMI) of control, and represent the mean  $\pm$  SEM of at least three independent experiments (\*\*\*)  $P < 0.001$  vs. control).

by ACHC (Borden et al., 1992; Clark and Amara, 1994; Clark et al., 1992). GABA uptake by the fourth GABA transporter, BGT-1, is not inhibited by ACHC or  $\beta$ -alanine (Liu et al., 1993; Yamauchi et al., 1992). Thus, the classical subdivision between neuronal and glial GABA transporters was perpetuated. Only when GAT-1 expression started to be carefully studied at the cellular level, it emerged immediately that this GABA transporter was expressed in retinal Muller cells (Brecha and Weigmann, 1994), in the electromotor nucleus of *Torpedo* (Swanson et al., 1994), and in neocortical and hippocampal astrocytic processes (Minelli et al., 1995; Ribak et al., 1996). These early observations were soon replicated in most brain regions, leading to the conclusion that GAT-1 is expressed by both neurons and astrocytes, that astrocytic labeling was not an occasional finding (Minelli et al., 1995), and that it was evident also in human cerebral cortex (Conti et al., 1998). Only recently, the concept that GAT-1 is not exclusively neuronal appear to have gained consensus. A recent quantitative analysis of GAT-1 in cerebral cortex showed that 54% of GAT-1 positive profiles were neuronal and 42% were astrocytic (Melone et al., 2015). Interestingly, GAT-3, a presumed “glial” GABA transporter, is expressed also in brainstem and cortical neurons (Clark et al., 1992; Melone et al., 2005, 2015), and GAT-2, also a presumed “glial” GABA transporter, is expressed also in epithelial cells and, although at very low level, in neurons too (Conti et al., 1999). The present demonstration that GAT-1 is expressed in oligodendrocytes thus definitely closes the era of “neuronal” and “glial” GABA transporters.

Although this is the first demonstration that GAT-1 is expressed in oligodendrocytes, our findings are not surprising given the known relationships between GABA and oligodendrocytes (Hamilton et al., in press; Verkhratsky and Butt, 2013). Functional GABA<sub>A</sub>R have been demonstrated in oligodendrocyte progenitor cells (OPCs) and in oligodendrocytes (Arellano et al., 2016; Berger et al., 1992; Kirchhoff and Kettenmann, 1992; Lin and Bergles, 2004; Vélez-Fort et al., 2010; Von Blankenfeld et al., 1991), and GABAergic synapses from neurons are known to contact OPCs (Lin and Bergles, 2004; Tanaka et al., 2009). In addition, GABA<sub>B</sub>R are expressed by OPCs, and are down-regulated in myelinating oligodendrocytes (Charles et al., 2003; Luyt et al., 2007). On this basis, it has been proposed that GABA regulates oligodendrocyte lineage proliferation (Verkhratsky and Butt, 2013). GAT-1 may contribute to the effects of GABA on oligodendrocytes by regulating GABA levels, either by taking up GABA or by releasing it, if working in the reverse mode (Scimemi, 2014; Wu et al., 2007).

The present observations may have important pathophysiological implications. First, GAT-1 has been implicated in several devastating human diseases, for example, epilepsy

and schizophrenia (Conti et al., 2004; Lewis and Gonzalez-Burgos, 2006). The present demonstration that GAT-1 is expressed also in oligodendrocytes may indicate that these glial cells contribute to some aspects of their pathophysiology. Second, the paper that stimulated the present study shows that GAT-1 contribution to the pathophysiology of DWM (Zonouzi et al., 2015) may be related to oligodendrocytes. Given the well-known involvement of oligodendrocytes in pathophysiological conditions (Verkhratsky and Butt, 2013), it is conceivable that oligodendrocytic GAT-1 may play a role in other neuropsychiatric diseases: the recent demonstration that a loss of mature oligodendrocytes is detectable in an animal model of Rett syndrome (Nguyen et al., 2013), in which GABAergic synaptic transmission is altered, may represent such an example.

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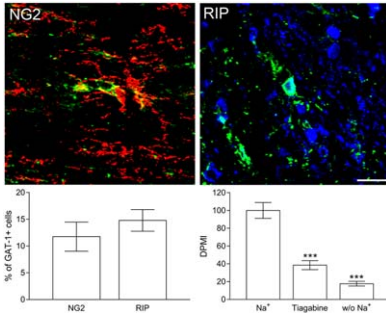
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- About 26% of GAT-1+ cells in subcortical white matter are mature and immature oligodendrocytes;
- Functional studies showed that oligodendrocytes exhibit Na<sup>+</sup>-dependent GABA uptake, and that it was significantly inhibited by tiagabine.

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