



UNIVERSITÀ POLITECNICA DELLE MARCHE
Repository ISTITUZIONALE

GAT-1 mediated GABA uptake in rat oligodendrocytes

This is the peer reviewed version of the following article:

Original

GAT-1 mediated GABA uptake in rat oligodendrocytes / Fattorini, Giorgia; Melone, Marcello; Sánchez Gómez, María Victoria; Arellano, Rogelio O; Bassi, Silvia; Matute, Carlos; Conti, Fiorenzo. - In: GLIA. - ISSN 0894-1491. - STAMPA. - 65:3(2017), pp. 514-522. [10.1002/glia.23108]

Availability:

This version is available at: 11566/244859 since: 2022-05-19T10:06:40Z

Publisher:

Published

DOI:10.1002/glia.23108

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.

This item was downloaded from IRIS Università Politecnica delle Marche (<https://iris.univpm.it>). When citing, please refer to the published version.

(Article begins on next page)

Glia

Copy of e-mail Notification

Glia Published by John Wiley & Sons, Inc.

Dear Author,

Your article page proofs for GLIA is ready for your final content correction within our rapid production workflow. The PDF file found at the URL given below is generated to provide you with a proof of the content of your manuscript. Once you have submitted your corrections, the production office will proceed with the publication of your article.

John Wiley & Sons has made this article available to you online for faster, more efficient editing. Please follow the instructions below and you will be able to access a PDF version of your article as well as relevant accompanying paperwork.

First, make sure you have a copy of Adobe Acrobat Reader software to read these files. This is free software and is available for user downloading at <http://www.adobe.com/products/acrobat/readstep.html>.

Open your web browser, and enter the following web address:

<http://115.111.50.156/jw/retrieval.aspx?pwd=bd12f8325385>

You will be prompted to log in, and asked for a password. Your login name will be your email address, and your password will be bd12f8325385

Example:

Login: your e-mail address

Password: bd12f8325385

The site contains one file, containing:

Author Instructions Checklist

Instructions on the Annotation of PDF Files

Color Reproduction Charge Form

Reprint Order Information

A copy of your page proofs for your article

In order to speed the proofing process, we strongly encourage authors to correct proofs by annotating PDF files. Any corrections should be returned to jrnprod.GLIA@cenveo.com 1 to 2 business days after receipt of this email in order to achieve our goal of publishing your article online 15 days from the day final data was received.

Please see the Instructions on the Annotation of PDF files included with your page proofs. Please take care to answer all queries on the last page of the PDF proof; proofread any tables and equations carefully; and check that

Glia

Copy of e-mail Notification

any Greek characters (especially "mu") have converted correctly. Please check your figure legends carefully.

- answer all queries on the last page of the PDF proof
- proofread any tables and equations carefully
- check your figure(s) and legends for accuracy

Within 1 to 2 business days, please return page proofs with corrections and any relevant forms to:

Production Editor, GLIA

Email: jrnprod.GLIA@cenveo.com

Technical problems? If you experience technical problems downloading your file or any other problem with the website listed above, please contact Balaji/Sam (e-mail: Wiley.CS@cenveo.com, phone: +91 (44) 4205-8810. Be sure to include your article number.

Questions regarding your article? Please don't hesitate to contact me with any questions about the article itself, or if you have trouble interpreting any of the questions listed at the end of your file. **REMEMBER TO INCLUDE YOUR ARTICLE NO. (23108) WITH ALL CORRESPONDENCE.** This will help us address your query most efficiently.

As this e-proofing system was designed to make the publishing process easier for everyone, we welcome any and all feedback. Thanks for participating in our e-proofing system!

This e-proof is to be used only for the purpose of returning corrections to the publisher.

Sincerely,

Production Editor, GLIA

Email: jrnprod.GLIA@cenveo.com



111 RIVER STREET, HOBOKEN, NJ 07030

*****IMMEDIATE RESPONSE REQUIRED*****

Your article will be published online via Wiley's EarlyView® service (wileyonlinelibrary.com) shortly after receipt of corrections. EarlyView® is Wiley's online publication of individual articles in full text HTML and/or pdf format before release of the compiled print issue of the journal. Articles posted online in EarlyView® are peer-reviewed, copyedited, author corrected, and fully citable via the article DOI (for further information, visit www.doi.org). EarlyView® means you benefit from the best of two worlds--fast online availability as well as traditional, issue-based archiving.

Please follow these instructions to avoid delay of publication.

READ PROOFS CAREFULLY

- This will be your only chance to review these proofs. **Please note that once your corrected article is posted online, it is considered legally published, and cannot be removed from the Web site for further corrections.**
- Please note that the volume and page numbers shown on the proofs are for position only.

ANSWER ALL QUERIES ON PROOFS (If there are queries they will be found on the last page of the PDF file.)

- In order to speed the proofing process, we strongly encourage authors to correct proofs by annotating PDF files. Please see the instructions on the Annotation of PDF files. If unable to annotate the PDF file, please print out and mark changes directly on the page proofs.

CHECK FIGURES AND TABLES CAREFULLY

- Check size, numbering, and orientation of figures.
- All images in the PDF are downsampled (reduced to lower resolution and file size) to facilitate Internet delivery. These images will appear at higher resolution and sharpness in the final, published article
- Review figure legends to ensure that they are complete.
- Check all tables. Review layout, title, and footnotes.

RETURN **PROOFS**

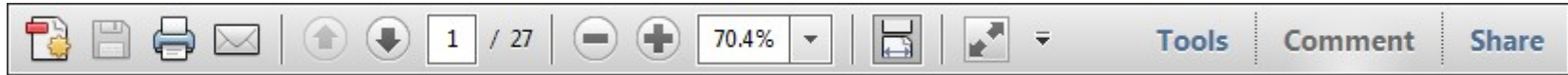
Other forms, as needed

Return corrections immediately via email to jrnprod.GLIA@cenveo.com.

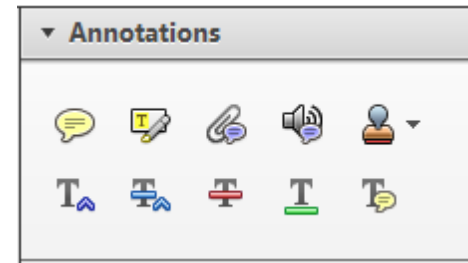
QUESTIONS

Production Editor , GLIA
E-mail: jrnprod.GLIA@cenveo.com
Refer to journal acronym and article production number
(i.e., GLIA 00-0000 for GLIA Record ms 00-0000).

Once you have Acrobat Reader open on your computer, click on the [Comment](#) tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the [Annotations](#) section, pictured opposite. We've picked out some of these tools below:



1. Replace (Ins) Tool – for replacing text.

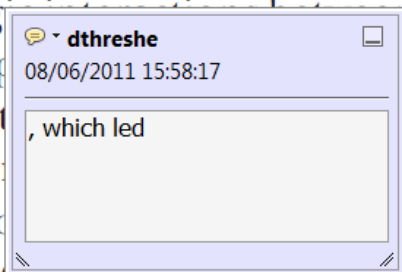


Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the [Replace \(Ins\)](#) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

standard framework for the analysis of microeconomic activity. Nevertheless, it also led to exogenous shocks and a number of strategic decisions. The number of competitors in the industry is that the structure of the main components of the industry level, are exogenous and important. We henceforth use the term 'black box' to refer to the



2. Strikethrough (Del) Tool – for deleting text.



Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the [Strikethrough \(Del\)](#) icon in the Annotations section.

there is no room for extra profits and the number of firms in the industry are zero and the number of firms (net) values are not determined by the industry. Blanchard and Kiyotaki (1987), in their paper on perfect competition in general equilibrium, show that the structure of aggregate demand and supply in the classical framework assuming monopoly is determined by an exogenous number of firms.

3. Add note to text Tool – for highlighting a section to be changed to bold or italic.



Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the [Add note to text](#) icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

dynamic responses of mark-ups are consistent with the VAR evidence.

with the VAR evidence, the VAR model is estimated with the VAR model. The VAR model is estimated with the VAR model. The VAR model is estimated with the VAR model.



4. Add sticky note Tool – for making notes at specific points in the text.



Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the [Add sticky note](#) icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

and supply shocks. Most of the time, the number of firms in the industry is determined by the structure of the industry. The number of firms in the industry is determined by the structure of the industry. The number of firms in the industry is determined by the structure of the industry.



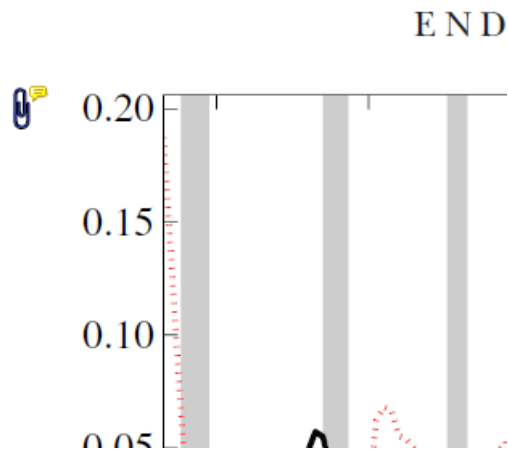
5. Attach File Tool – for inserting large amounts of text or replacement figures.



Inserts an icon linking to the attached file in the appropriate place in the text.

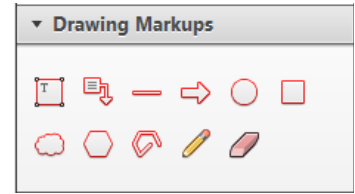
How to use it

- Click on the [Attach File](#) icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



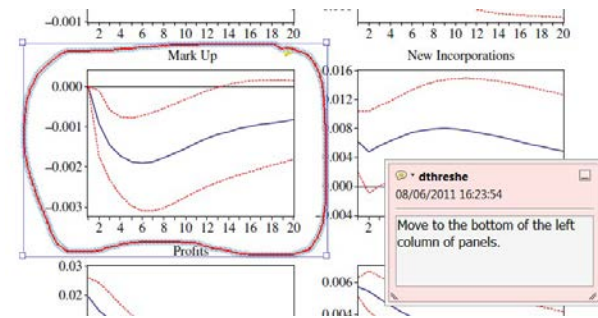
6. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.





Additional reprint purchases

Should you wish to purchase additional copies of your article, please click on the link and follow the instructions provided:

<https://caesar.sheridan.com/reprints/redirect.php?pub=10089&acro=GLIA>

Corresponding authors are invited to inform their co-authors of the reprint options available.

Please note that regardless of the form in which they are acquired, reprints should not be resold, nor further disseminated in electronic form, nor deployed in part or in whole in any marketing, promotional or educational contexts without authorization from Wiley. Permissions requests should be directed to mail to: permissionsus@wiley.com

For information about 'Pay-Per-View and Article Select' click on the following link: wileyonlinelibrary.com/aboutus/ppv-articleselect.html

WILEY

COLOR REPRODUCTION IN YOUR ARTICLE

Color figures were included with the final manuscript files that we received for your article. Because of the high cost of color printing, we can only print figures in color if authors cover the expense. **The charge for printing figures in color is \$550 per figure.**

Please indicate if you would like your figures to be printed in color or black and white. Color images will be reproduced online in *Wiley Online Library* at no charge, whether or not you opt for color printing.

Failure to return this form will result in the publication of your figures in black and white.

JOURNAL GLIA VOLUME _____ ISSUE _____

TITLE OF MANUSCRIPT _____

MS. NO. _____ NO. OF COLOR PAGES _____ AUTHOR(S) _____

<input type="checkbox"/> Please print my figures in black and white	
<input type="checkbox"/> Please print my figures in color	\$ _____
BILL TO:	Purchase
Name _____	Order No. _____
Institution _____	Phone _____
Address _____	
_____	Fax _____
_____	E-mail _____

GAT-1 Mediated GABA Uptake in Rat Oligodendrocytes

Giorgia Fattorini,^{1,2} Marcello Melone,^{1,2} María Victoria Sánchez-Gómez,³
Rogelio O. Arellano,³ Silvia Bassi,¹ Carlos Matute,³ and Fiorenzo Conti^{1,2,4}

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 μm) 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 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.

GLIA 2016;00:000–000

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 γ -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_A 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

View this article online at wileyonlinelibrary.com. DOI: 10.1002/glia.23108

Published online Month 00, 2016 in Wiley Online Library (wileyonlinelibrary.com). Received Dec 29, 2015, Accepted for publication Dec 5, 2016.

Address correspondence to Fiorenzo Conti; Department of Experimental and Clinical Medicine, Section of Neuroscience and Cell Biology, Università Politecnica delle Marche, Via Tronto 10/A, 60026 Ancona, Italy. E-mail: f.conti@univpm.it

From the ¹Department of Experimental and Clinical Medicine, Section of Neuroscience and Cell Biology, Università Politecnica delle Marche, Ancona 60026, Italy; ²Center for Neurobiology of Aging, INRCA IRCCS, Ancona 60121, Italy; ³Achucarro Basque Center for Neuroscience, CIBERNED, and Departamento de Neurociencias, Universidad del País Vasco, Leioa, 48940, Spain; ⁴Fondazione di Medicina Molecolare, Università Politecnica delle Marche, Ancona 60026, Italy

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 μm -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% H_2O_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% H_2O_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 (~ 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 μm). 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% 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).

[^3H]-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% CO_2 in a chemically defined medium (Sato medium; Barres et al., 1992). [^3H]-GABA uptake was assessed at 37°C in 300 μl saline solution containing (in mM): NaCl 140, KCl 5, MgCl_2 2, 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 ^a	Dilution ^b	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 ^a	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	

^aGP, guinea pig; M, mouse; R, rabbit.

^bIF, immunofluorescence; EM, electron microscopy; IP, immunoperoxidase.

presence of uptake inhibitor tiagabine (50 μM ; Tocris, Cat No: 4256), and then incubated for 5 min with 100 nM [^3H]-GABA together with unlabeled GABA (10 μM). Uptake was stopped with two washes in ice-cold phosphate-buffered saline (0.1 M, PBS). Cells were solubilized in 250 μl 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 μm^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 μm) 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 μm) and morphology (Fig. 1). Some GAT-1+ cells were small (2–7 μm), roundish in shape and exhibited small processes (Fig. 1A); others were medium-sized (7–13 μm), 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 μm), 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 ± 9.1 cells/ 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 μm ; 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

F2

AQ3

F3

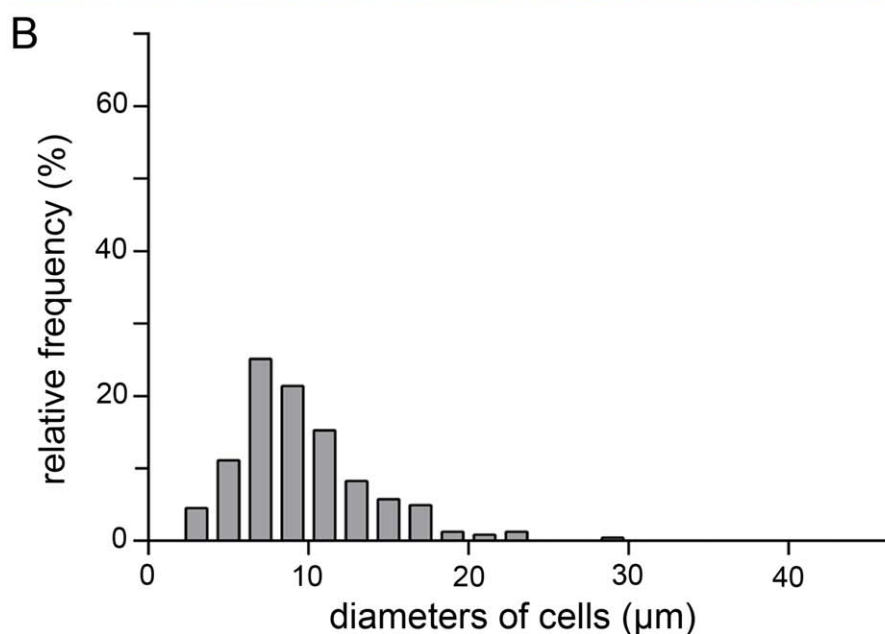
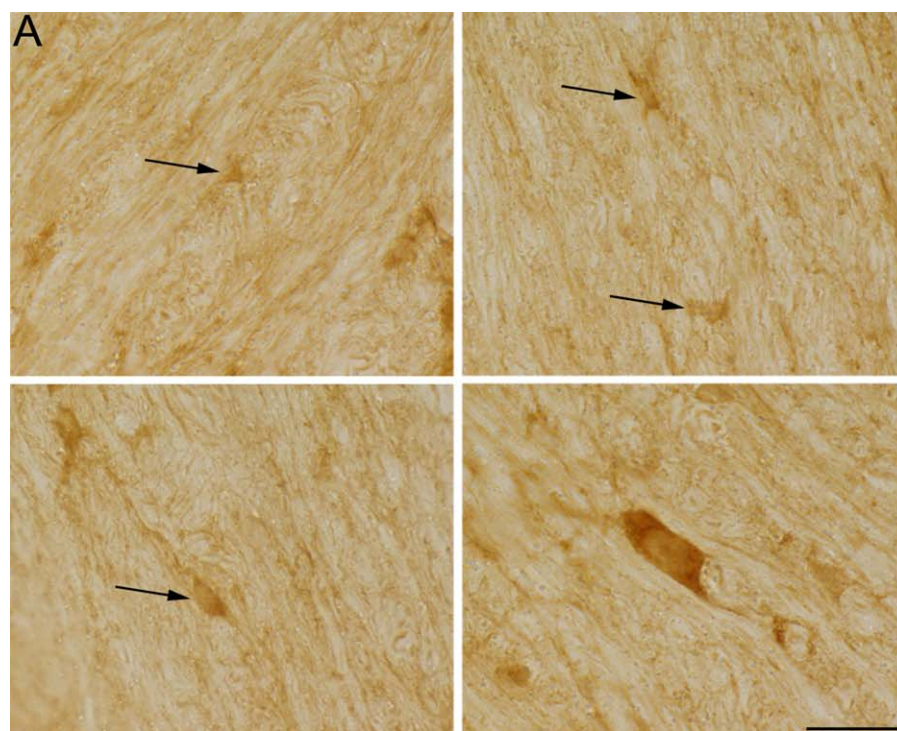


FIGURE 1: Morphology of GAT1+ cells in the subcortical white matter. (A) Light microscopy revealed the presence of numerous GAT-1+ cells with different size and morphology (arrows point to small and medium-sized cells). **(B)** Frequency distribution of the diameter of GAT-1+ cells in the subcortical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

COLOR IN ONLINE AND PRINT

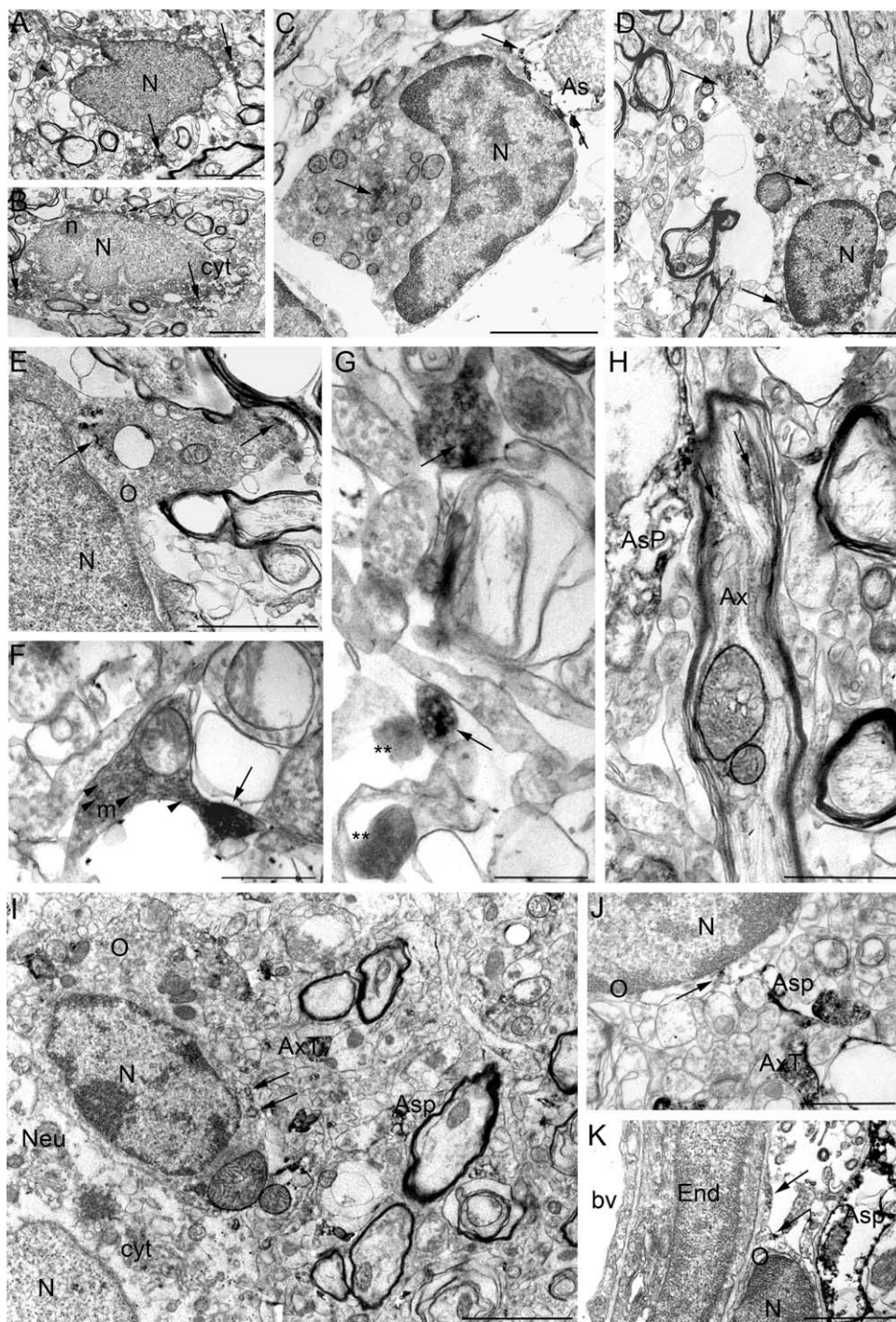


FIGURE 2: Ultrastructural localization of GAT-1 in subcortical white matter. (A,B) Example of a fibrous astrocytes (A) and a neuron (B) with GAT-1 immunoreaction products dispersed in their cytoplasm (arrows). (C,D) GAT-1 immunoreactivity (ir; arrows) of an immature (C) and a mature (D) oligodendrocyte. (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 immunonegative 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 μ m; F, G, 250 nm; H and J, K, 1 μ m.

COLOR IN ONLINE AND PRINT

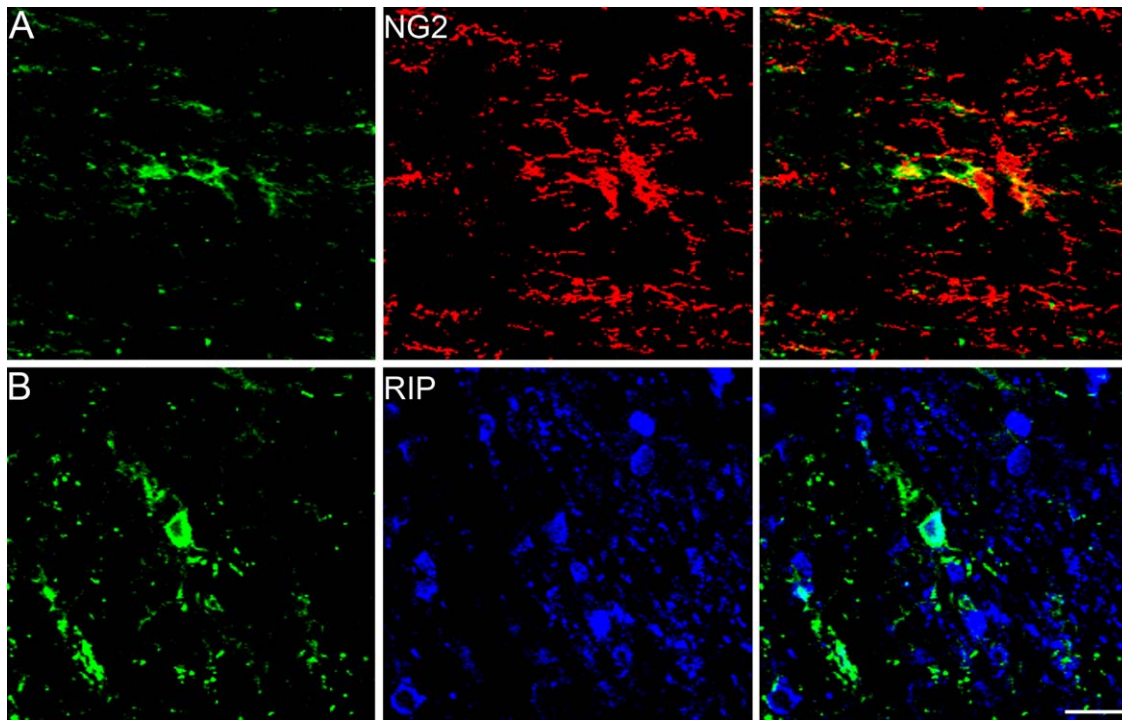


FIGURE 3: Co-localization of GAT-1 ir and oligodendrocyte markers in subcortical white matter. GAT-1+ cells (green; left column) co-expressing NG2 (A) or RIP (B) (blue or red; middle column). Scale bar: 20 μ m.

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 β -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 β -alanine, but not

COLOR IN ONLINE AND PRINT

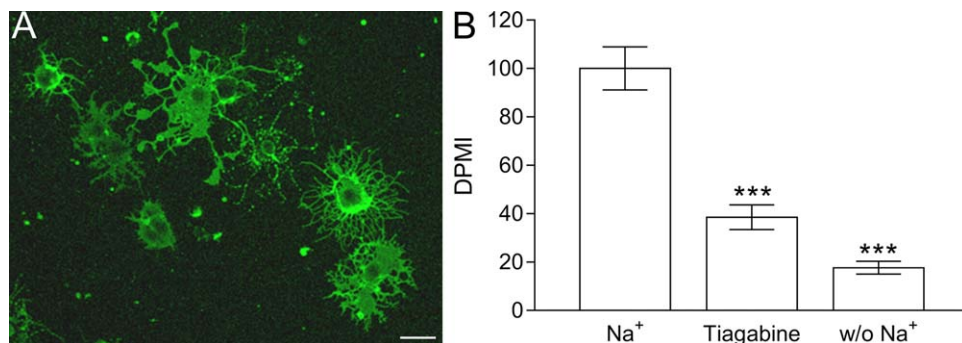


FIGURE 4: GAT-1-mediated GABA uptake in oligodendrocytes. GABA uptake assays were performed on nearly pure oligodendrocytic cultures (A; scale bar: 20 μ m) in control condition (Na⁺), in the presence of tiagabine (a selective inhibitor of GABA transporter GAT-1), or in the absence of Na⁺ (w/o Na⁺; non-specific 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 β -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_AR 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_BR 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.

Acknowledgment

Grant sponsor: Ministero dell'Università e della Ricerca (PRIN 2010/2011; F.C.); Grant sponsor: CIBERNED and MINECO (C.M.); Grant number: SAF2013-45084-R

Authors are grateful to NC Brecha (University of California at Los Angeles, CA) for his generous gift of GAT-1 antibodies.

References

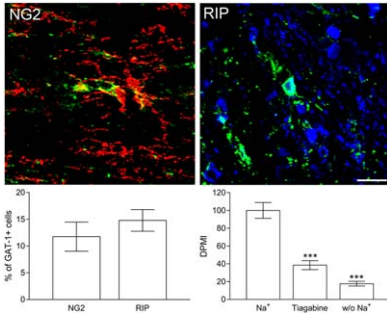
- All AH, Gharibani P, Gupta S, Bazley FA, Pashai N, Chou B-KK, Shah S, Resar LM, Cheng L, Gearhart JD, et al. 2015. Early intervention for spinal cord injury with human induced pluripotent stem cells oligodendrocyte progenitors. *PLoS One* 10:e0116933.
- Arellano RO, Sanchez-Gomez MV, Alberdi E, Canedo-Antelo M, Chara JC, Palomino A, Perez-Samartin A, Matute C. 2016. Axon-to-glia interaction regulates GABA_A receptor expression in oligodendrocytes. *Mol Pharmacol* 89: 63–74.
- Barres BA, Hart IK, Coles HS, Burne JF, Voyvodic JT, Richardson WD, Raff MC. 1992. Cell death and control of cell survival in the oligodendrocyte lineage. *Cell* 70:31–46.
- Beart PM, Johnston GA, Uhr ML. 1972. Competitive inhibition of GABA uptake in rat brain slices by some GABA analogues of restricted conformation. *J Neurochem* 19:1855–1861.
- Berger T, Schnitzer J, Orkand PM, Kettenmann H. 1992. Sodium and calcium currents in glial cells of the mouse corpus callosum slice. *Eur J Neurosci* 4: 1271–1284.
- Bhat R, Axtell R, Mitra A, Miranda M, Lock C, Tsien RW, Steinman L. 2010. Inhibitory role for GABA in autoimmune inflammation. *Proc Natl Acad Sci USA* 107:2580–2585.
- Borden LA. 1996. GABA transporter heterogeneity: pharmacology and cellular localization. *Neurochem Int* 29:335–356.
- Borden LA, Murali Dhar TG, Smith KE, Weinshank RL, Branchek TA, Gluchowski C. 1994. Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. *Eur J Pharmacol* 269:219–224.
- Borden LA, Smith KE, Hartig PR, Branchek TA, Weinshank RL. 1992. Molecular heterogeneity of the gamma-aminobutyric acid (GABA) transport system. Cloning of two novel high affinity GABA transporters from rat brain. *J Biol Chem* 267:21098–21104.

- Braestrup C, Nielsen EB, Sonnewald U, Knutsen LJ, Andersen KE, Jansen JA, Frederiksen K, Andersen PH, Mortensen A, Suzdak PD. 1990. (R)-N-[4,4-bis(3-methyl-2-thienyl)but-3-en-1-yl]nipecotic acid binds with high affinity to the brain gamma-aminobutyric acid uptake carrier. *J Neurochem* 54:639–647.
- Bragina L, Marchionni I, Omrani A, Cozzi A, Pellegrini-Giampietro DE, Cherubini E, Conti F. 2008. GAT-1 regulates both tonic and phasic GABA(A) receptor-mediated inhibition in the cerebral cortex. *J Neurochem* 105:1781–1793.
- Brecha NC. 1992. Expression of GABA_A receptors in the vertebrate retina. *Prog Brain Res* 90:3–28.
- Brecha NC, Weigmann C. 1994. Expression of GAT-1, a high-affinity gamma-aminobutyric acid plasma membrane transporter in the rat retina. *J Comp Neurol* 345:602–611.
- Charles KJ, Deuchars J, Davies CH, Pangalos MN. 2003. GABA_B receptor subunit expression in glia. *Mol Cell Neurosci* 24:214–223.
- Chen Y, Wu H, Wang S, Koito H, Li J, Ye F, Hoang J, Escobar SS, Gow A, Arnett HA, et al. 2009. The oligodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination. *Nature Neurosci* 12:1398–1406.
- Cherubini E, Conti F. 2001. Generating diversity at GABAergic synapses. *Trends Neurosci* 24:155–162.
- Cheung G, Kann O, Kohsaka S, Faerber K, Kettenmann H. 2009. GABAergic activities enhance macrophage inflammatory protein-1alpha release from microglia (brain macrophages) in postnatal mouse brain. *J Physiol* 587:753–768.
- Clark JA, Amara SG. 1994. Stable expression of a neuronal gamma-aminobutyric acid transporter, GAT-3, in mammalian cells demonstrates unique pharmacological properties and ion dependence. *Mol Pharmacol* 46:550–557.
- Clark JA, Deutch AY, Gallipoli PZ, Amara SG. 1992. Functional expression and CNS distribution of a beta-alanine-sensitive neuronal GABA transporter. *Neuron* 9:337–348.
- Conti F, Melone M, De Biasi S, Minelli A, Brecha NC, Ducati A. 1998. Neuronal and glial localization of GAT-1, a high-affinity gamma-aminobutyric acid plasma membrane transporter, in human cerebral cortex: with a note on its distribution in monkey cortex. *J Comp Neurol* 396:51–63.
- Conti F, Vitellaro-Zuccarello L, Barbaresi P, Minelli A, Brecha NC, Melone M. 1999. Neuronal, glial, and epithelial localization of gamma-aminobutyric acid transporter-2, a high-affinity gamma-aminobutyric acid plasma membrane transporter, in the cerebral cortex and neighboring structures. *J Comp Neurol* 409:482–494.
- Conti F, Melone M, Fattorini G, Bragina L, Ciappelloni S. 2011. A Role for GAT-1 in Presynaptic GABA Homeostasis? *Front Cell Neurosci* 5:2-
- Conti F, Minelli A, Melone M. 2004. GABA transporters in the mammalian cerebral cortex: localization, development and pathological implications. *Brain Res Brain Res Rev* 45:196–212.
- Cummins CJ, Glover RA, Sellinger OZ. 1982. Beta-alanine uptake is not a marker for brain astroglia in culture. *Brain Res* 239:299–302.
- Flygt J, Djupsjö A, Lenne F, Marklund N. 2013. Myelin loss and oligodendrocyte pathology in white matter tracts following traumatic brain injury in the rat. *Eur J Neurosci* 38:2153–2165.
- Friedman B, Hockfield S, Black JA, Woodruff KA, Waxman SG. 1989. In situ demonstration of mature oligodendrocytes and their processes: an immunocytochemical study with a new monoclonal antibody, rip. *Glia* 2:380–390.
- Froestl W. 2011. An historical perspective on GABAergic drugs. *Future Med Chem* 3:163–175.
- Gavrilovic J, Raff M, Cohen J. 1984. GABA uptake by purified rat schwann cells in culture. *Brain Res* 303:183–185.
- Guastella J, Nelson N, Nelson H, Czyzyk L, Keynan S, Miedel MC, Davidson N, Lester HA, Kanner BI. 1990. Cloning and expression of a rat brain GABA transporter. *Science* 249:1303–1306.
- Hamilton NB, Clarke LE, Arancibia-Carcamo IL, Kougioumtzidou E, Matthey M, Káradóttir R, Whiteley L, Bergersen LH, Richardson WD, Attwell D. in press. Endogenous GABA controls oligodendrocyte lineage cell number, myelination, and CNS internode length. *Glia*. doi: 10.1002/glia.23093. [Epub ahead of print].
- Iversen LL, Kelly JS. 1975. Uptake and metabolism of gamma-aminobutyric acid by neurones and glial cells. *Biochem Pharmacol* 24:933–938.
- Jasmin L, Wu MV, Ohara PT. 2004. GABA puts a stop to pain. *Curr Drug Targets CNS Neurol Disord* 3:487–505.
- Jones GP, Neal MJ. 1976. Selective inhibition of neuronal GABA uptake by cis-1,3-aminocyclohexane carboxylic acid. *Nature* 264:281–284.
- Kanner BI, Bendahan A. 1990. Two pharmacologically distinct sodium- and chloride-coupled high-affinity gamma-aminobutyric acid transporters are present in plasma membrane vesicles and reconstituted preparations from rat brain. *Proc Natl Acad Sci USA* 87:2550–2554.
- Kaplan MS, Hinds JW. 1980. Gliogenesis of astrocytes and oligodendrocytes in the neocortical grey and white matter of the adult rat: electron microscopic analysis of light radioautographs. *J Comp Neurol* 193:711–727.
- Kettenmann H, Ransom BR. 2013. *Neuroglia*. New York: Oxford University Press.
- Keynan S, Suh YJ, Kanner BI, Rudnick G. 1992. Expression of a cloned gamma-aminobutyric acid transporter in mammalian cells. *Biochemistry* 31:1974–1979.
- Kim JB, Lee H, Araúz-Bravo MJ, Hwang K, Nam D, Park MR, Zaehres H, Park KI, Lee S-JJ. 2015. Oct4-induced oligodendrocyte progenitor cells enhance functional recovery in spinal cord injury model. *EMBO J* 34:2971–2983.
- Kirchhoff F, Kettenmann H. 1992. GABA triggers a [Ca²⁺]_i increase in murine precursor cells of the oligodendrocyte lineage. *Eur J Neurosci* 4:1049–1058.
- Kostovic I, Rakic P. 1980. Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. *J Neurocytol* 9:219–242.
- Kuhn SA, van Landeghem FK, Zacharias R, Farber K, Rappert A, Pavlovic S, Hoffmann A, Nolte C, Kettenmann H. 2004. Microglia express GABA(B) receptors to modulate interleukin release. *Mol Cell Neurosci* 25:312–322.
- Larsson OM, Johnston GA, Schousboe A. 1983. Differences in uptake kinetics of cis-3-aminocyclohexane carboxylic acid into neurons and astrocytes in primary cultures. *Brain Res* 260:279–285.
- Laughlin TM, Tram KV, Wilcox GL, Birnbaum AK. 2002. Comparison of antiepileptic drugs tiagabine, lamotrigine, and gabapentin in mouse models of acute, prolonged, and chronic nociception. *J Pharmacol Exp Ther* 302:1168–1175.
- Levi G, Wilkin GP, Ciotti MT, Johnstone S. 1983. Enrichment of differentiated, stellate astrocytes in cerebellar interneuron cultures as studied by GFAP immunofluorescence and autoradiographic uptake patterns with [3H]D-aspartate and [3H]GABA. *Brain Res* 312:227–241.
- Levine JM, Nishiyama A. 1996. The NG2 chondroitin sulfate proteoglycan: a multifunctional proteoglycan associated with immature cells. *Perspect Dev Neurobiol* 3:245–259.
- Levine JM, Stincone F, Lee YS. 1993. Development and differentiation of glial precursor cells in the rat cerebellum. *Glia* 7:307–721.
- Lewis DA, Gonzalez-Burgos G. 2006. Pathophysiologically based treatment interventions in schizophrenia. *Nat Med* 12:1016–1022.
- Lin SC, Bergles DE. 2004. Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. *Nat Neurosci* 7:24–32.
- Liu J, Huang D, Xu J, Tong J, Wang Z, Huang L, Yang Y, Bai X, Wang P, Suo H, Ma Y, Yu M, Fei J, Huang F. 2015. Tiagabine protects dopaminergic neurons against neurotoxins by inhibiting microglial activation. *Sci Rep* 5:15720
- Liu QR, Lopez-Corcuera B, Mandiyan S, Nelson H, Nelson N. 1993. Molecular characterization of four pharmacologically distinct gamma-aminobutyric acid transporters in mouse brain. *J Biol Chem* 268:2106–2112.
- Luyt K, Slade TP, Dorward JJ, Durant CF, Wu Y, Shigemoto R, Mundell SJ, Varadi A, Molnar E. 2007. Developing oligodendrocytes express functional GABA(B) receptors that stimulate cell proliferation and migration. *J Neurochem* 100:822–840.



- Mabjeesh NJ, Frese M, Rauen T, Jeserich G, Kanner BI. 1992. Neuronal and glial gamma-aminobutyric acid+ transporters are distinct proteins. *FEBS Lett* 299:99–102.
- Melone M, Barbaresi P, Fattorini G, Conti F. 2005. Neuronal localization of the GABA transporter GAT-3 in human cerebral cortex: a procedural artifact? *J Chem Neuroanat* 30:45–54.
- Melone M, Ciappelloni S, Conti F. 2015. A quantitative analysis of cellular and synaptic localization of GAT-1 and GAT-3 in rat neocortex. *Brain Struct Funct* 220:885–897.
- Meyer G, Gonzalez-Hernandez T, Galindo-Mireles D, Castañeyra-Perdomo A, Ferres-Torres R. 1991. The efferent projections of neurons in the white matter of different cortical areas of the adult rat. *Anat Embryol* 184:99–102.
- Minelli A, Brecha NC, Karschin C, DeBiasi S, Conti F. 1995. GAT-1, a high-affinity GABA plasma membrane transporter, is localized to neurons and astroglia in the cerebral cortex. *J Neurosci* 15:7734–7746.
- Nguyen MV, Felice CA, Du F, Covey MV, Robinson JK, Mandel G, Ballas N. 2013. Oligodendrocyte lineage cells contribute unique features to Rett syndrome neuropathology. *J Neurosci* 33:18764–18774.
- Paxinos G, Watson C. 1982. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press.
- Peters A, Palay SL, Webster Hd. 1991. *The Fine Structure of the Nervous System: Neurons and their Supporting cells*. New York: Oxford University Press. xviii, 494 pp.
- Reynolds R, Herschkowitz N. 1986. Selective uptake of neuroactive amino acids by both oligodendrocytes and astrocytes in primary dissociated culture: a possible role for oligodendrocytes in neurotransmitter metabolism. *Brain Res* 371:253–266.
- Ribak CE, Tong WM, Brecha NC. 1996. GABA plasma membrane transporters, GAT-1 and GAT-3, display different distributions in the rat hippocampus. *J Comp Neurol* 367:595–606.
- Robinson AP, White TM, Mason DW. 1986. Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognizing complement receptor type 3. *Immunology* 57:239–247.
- Sakakibara S, Nakadate K, Tanaka-Nakadate S, Yoshida K, Nogami S, Shirataki H, Ueda S. 2008. Developmental and spatial expression pattern of alpha-taxilin in the rat central nervous system. *J Comp Neurol* 511:65–80.
- Salat K, Podkowa A, Kowalczyk P, Kulig K, Dziubina A, Filipek B, Librowski T. 2015. Anticonvulsant active inhibitor of GABA transporter subtype 1, tiagabine, with activity in mouse models of anxiety, pain and depression. *Pharmacol Rep* 67:465–472.
- Sánchez-Gómez MV, Alberdi E, Ibarretxe G, Torre I, Matute C. 2003. Caspase-dependent and caspase-independent oligodendrocyte death mediated by AMPA and kainate receptors. *J Neurosci* 23:9519–9528.
- Schon F, Kelly JS. 1974. The characterisation of [3H]GABA uptake into the satellite glial cells of rat sensory ganglia. *Brain Res* 66:289–300.
- Schousboe A, White HS. 2009. Glial modulation of excitability via glutamate and GABA transporters. In: Schwartzkroin PA, editor. *Encyclopedia of basic epilepsy research*. Oxford: Academic Press. pp 397–401.
- Scimemi A. 2014. Structure, function, and plasticity of GABA transporters. *Front Cell Neurosci* 8:161.
- Sevc J, Matiašová A, Kútna V, Daxnerová Z. 2014. Evidence that the central canal lining of the spinal cord contributes to oligodendrogenesis during postnatal development and adulthood in intact rats. *J Comp Neurol* 522:3194–3207.
- Stallcup WB. 1981. The NG2 antigen, a putative lineage marker: immunofluorescent localization in primary cultures of rat brain. *Dev Biol* 83:154–165.
- Su J, Yin J, Qin W, Sha S, Xu J, Jiang C. 2015. Role for pro-inflammatory cytokines in regulating expression of GABA transporter type 1 and 3 in specific brain regions of kainic acid-induced status epilepticus. *Neurochem Res* 40:621–627.
- Suzdak PD, Jansen JA. 1995. A review of the preclinical pharmacology of tiagabine: a potent and selective anticonvulsant GABA uptake inhibitor. *Epilepsia* 36:612–626.
- Swanson GT, Umbach JA, Gundersen CB. 1994. Glia of the cholinergic efferent nucleus of Torpedo are the source of the cDNA encoding a GAT-1-like GABA transporter. *J Neurochem* 63:12.
- Tanaka Y, Tozuka Y, Takata T, Shimazu N, Matsumura N, Ohta A, Hisatsune T. 2009. Excitatory GABAergic activation of cortical dividing glial cells. *Cereb Cortex* 19:2181–2195.
- Vaughan DW, Peters A. 1974. Neuroglial cells in the cerebral cortex of rats from young adulthood to old age: an electron microscope study. *J Neurocytol* 3:405–429.
- Vélez-Fort M, Maldonado P, Butt A, Audinat E, Angulo M. 2010. Postnatal switch from synaptic to extrasynaptic transmission between interneurons and NG2 cells. *J Neurosci* 30:6921–6929.
- Verkhatsky A, Butt AM. 2013. *Glial Physiology and Pathophysiology*. Oxford: Wiley.
- Von Blankenfeld G, Trotter J, Kettenmann H. 1991. Expression and Developmental Regulation of a GABA_A Receptor in Cultured Murine Cells of the Oligodendrocyte Lineage. *Eur J Neurosci* 3:310–316.
- Wu Y, Wang W, Diez-Sampedro A, Richerson GB. 2007. Nonvesicular inhibitory neurotransmission via reversal of the GABA transporter GAT-1. *Neuron* 56:851–865.
- Yamauchi A, Uchida S, Kwon HM, Preston AS, Robey RB, Garcia-Perez A, Burg MB, Handler JS. 1992. Cloning of a Na⁽⁺⁾- and Cl⁽⁻⁾-dependent betaine transporter that is regulated by hypertonicity. *J Biol Chem* 267:649–652.
- Zonouzi M, Scaffidi J, Li P, McEllin B, Edwards J, Dupree JL, Harvey L, Sun D, Hübner CA, Cull-Candy SG, Farrant M, Gallo V. 2015. GABAergic regulation of cerebellar NG2 cell development is altered in perinatal white matter injury. *Nat Neurosci* 18:674–682.

SGML and CITI Use Only DO NOT PRINT



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

WILEY
Author Proof

AQ1: Please check whether grant information is OK as typeset.

AQ2: Please note that as per journal style multiple paragraphs are not allowed in abstract section. Hence changed to single paragraph.

AQ3: Please provide complete details for (Lunn et al., 1997, Guo et al., 2009, Hu et Quick, 2008, Quick et al., 2004, and Stallcup et al., 1981) in the reference list or delete the citation from the text.

AQ4: Please provide complete author list for All et al., 2015.

AQ5: There is no mention of (Bhat et al., 2010, Cheung et al., 2009, Kettenmann and Ransom, 2013, Kuhn et al., 2004, Liu et al., 2015, Robinson et al., 1986, Stallcup, 1981, and Su et al., 2015) in the text. Please insert a citation in the text or delete the reference as appropriate.

AQ6: Please update “Hamilton et al., in press.

AQ7: Please check whether Table 1 layout is OK as typeset.

AQ8: Please confirm that given names (red) and surnames/family names (green) have been identified correctly.

Please confirm that the funding sponsor list below was correctly extracted from your article: that it includes all funders and that the text has been matched to the correct FundRef Registry organization names. If a name was not found in the FundRef registry, it may be not the canonical name form or it may be a program name rather than an organization name or it may be an organization not yet included in FundRef Registry. If you know of another name form or a parent organization name for a not found item on this list below, please share that information.

FundRef name	FundRef Organization Name (Country)	FundRef DOI	Grant IDs
Ministero dell'Università e della Ricerca (PRIN 2010/2011; F.C.)	Ministero dell'Istruzione, dell'Università e della Ricerca	10.13039/501100003407	
CIBERNED and MINECO (C.M.)	Ministerio de Economía y Competitividad	10.13039/501100003329	SAF2013-45084-R