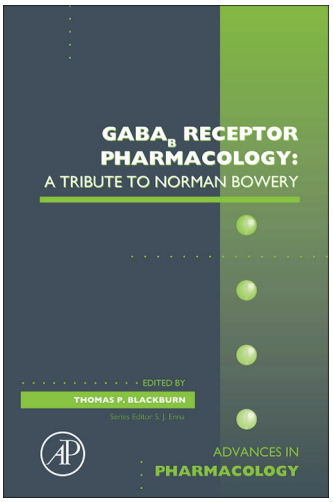


From Guillermo Gonzalez-Burgos, GABA Transporter GAT1:
A Crucial Determinant of GABAB Receptor Activation in Cortical Circuits?.
In: Thomas P. Blackburn and S. J. Enna, editors,
$GABA_B$ Receptor Pharmacology: A Tribute to Norman Bowery (Volume 58).
Academic Press, 2010, p. 175.
ISBN: 978-0-12-378647-0

Guillermo Gonzalez-Burgos

Department of Psychiatry, Translational Neuroscience Program,
University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

GABA Transporter GAT1: A Crucial Determinant of $GABA_B$ Receptor Activation in Cortical Circuits?

Abstract

The GABA transporter 1 (GAT1), the main plasma membrane GABA transporter in brain tissue, mediates translocation of GABA from the extracellular to the intracellular space. Whereas GAT1-mediated uptake could generally terminate the synaptic effects of GABA, recent studies suggest a more complex physiological role. This chapter reviews evidence suggesting that in hippocampal and neocortical circuits, GAT1-mediated GABA

Advances in Pharmacology, Volume 58
1054-3589/10 $35.00

10.1016/S1054-3589(10)58008-6

transport regulates the electrophysiological effects of $GABA_B$ receptor ($GABA_BR$) activation by synaptically-released GABA. Contrasting with synaptic $GABA_A$ receptors, $GABA_BRs$ display high GABA binding affinity, slow G protein-coupled mediated signaling, and a predominantly extrasynaptic localization. Such $GABA_BR$ properties determine production of slow inhibitory postsynaptic potentials (IPSPs) and slow presynaptic effects. Such effects possibly require diffusion of GABA far away from the release sites, and consequently both $GABA_BR$-mediated IPSPs and presynaptic effects are strongly enhanced when GAT1-mediated uptake is blocked. Studies are reviewed here which indicate that $GABA_BR$-mediated IPSPs seem to be produced by dendrite-targeting GABA neurons including specifically, although perhaps not exclusively, the neurogliaform cell class. In contrast, the GABA interneuron subtypes that synapse onto the perisomatic membrane of pyramidal cells mostly signal via synaptic $GABA_ARs$. This chapter reviews data suggesting that neurogliaform cells produce electrophysiological effects onto other neurons in the cortical cell network via $GABA_BR$-mediated volume transmission that is highly regulated by GAT1 activity. Therefore, the role of GAT1 in controlling $GABA_BR$-mediated signaling is markedly different from its regulation of $GABA_AR$-mediated fast synaptic transmission.

I. Introduction

$GABA_B$ receptors ($GABA_BRs$) are prominent in hippocampal and neocortical circuits. Compared with receptors of the $GABA_A$ family ($GABA_ARs$), the distinctive properties of $GABA_BRs$ suggest that these receptors play different roles in regulating the flow of neural activity in cortical circuits. The physiological role of $GABA_BRs$ at the cellular level was identified in early studies [summarized in (Nicoll, 2004)] following the demonstration that GABA acts through receptors different from the bicuculline-sensitive sites (Bowery et al., 1980). Whereas substantial progress has been made in further defining the role of $GABA_BRs$ (Ulrich & Bettler, 2007), the regulation of $GABA_BR$ activation in physiological conditions is still poorly understood. Here, I review findings from experimental studies suggesting that in cortical microcircuits the activity of the GABA transporter 1 is an important determinant of $GABA_BR$ activation. The review focuses on the interplay between GAT1-mediated GABA uptake and $GABA_BRs$ which may play a role in determining the electrophysiological effects of synaptically-released GABA.

II. The Plasma Membrane GABA Transporter 1

The GABA transporter 1 (GAT1) is the predominant plasma membrane GABA transporter in cortical tissue (Guastella et al., 1990), where it is localized in neuronal and glial membranes near synapses (see Section III).

Below I review some of the properties of the GAT1 protein, its role in translocation of GABA across the plasma membrane, and some evidence on the mechanisms regulating GAT1 activity.

A. Properties of the GAT1 Protein and Translocation of GABA by GAT1

The gene encoding the amino acid sequence of GAT1 (*slc6a1*) belongs to the *slc6* family of genes for plasma membrane transporters, together with transporters for dopamine, serotonin, norepinephrine, and glycine, as well as the GABA transporters GAT2, GAT3, and GAT4 (Gether et al., 2006). The GAT1 *slc6a1* gene encodes a 599 amino acid protein with 12 transmembrane domains. To form functional transporter molecules, these proteins are probably assembled as dimers (Gether et al., 2006; Moss et al., 2009).

As the other *slc6* transporters, GAT1 translocates its substrate by co-transport with Na^+ in a process that requires Cl^- (Fig. 1A), although it is not clear whether Cl^- itself is translocated or not (Bicho & Grewer, 2005; Chen et al., 2004). GABA translocation occurs with a relatively slow kinetics, in the order of tens of GABA molecules per second (Bicho & Grewer, 2005; Chen et al., 2004). Such kinetics is significantly slower than the rapid kinetics of synaptic GABA-receptor binding and receptor channel gating (Farrant & Kaila, 2007). Interestingly, basal levels of GAT1 expression in the plasma membrane suggest a density of 300–500 transporters/μm^2 (Chiu et al., 2002; Wang & Quick, 2005). Therefore, depending on their exact membrane localization (see Section III.B. below), GAT1 molecules could provide ample high-affinity (IC50 ~6 nM) binding sites (Borden, 1996) to rapidly buffer synaptically-released GABA independently of its slow translocation rate (Diamond & Jahr, 1997).

GAT1 activity can be inhibited with various pharmacological compounds, some of which are highly selective to block GAT1 as opposed to other GABA transporters (Borden, 1996). Among the GAT1-selective commercially available inhibitors are NO711, also named NNC 711 (1,2,5,6-Tetrahydro-1-[2-[[(diphenylmethylene)amino]oxy] ethyl]-3-pyridinecarboxylic acid hydrochloride), and tiagabine ((*R*)-1-[4,4-bis(3-methylthiophen-2-yl)but-3-enyl] piperidine-3-carboxylic acid). Pharmacological inhibition of GAT1 activity is typically considered to decrease GABA uptake (Borden, 1996)—that is, the translocation of extracellular GABA into the intracellular compartment (Fig. 1A). GAT1 in GABAergic nerve terminals may therefore provide cytosolic GABA to rapidly refill synaptic vesicles without need of GABA synthesis de novo. Glial GAT1-mediated uptake (Fig. 1B) may produce a pool of GABA that is transformed into glutamine and thus is not readily available for neuronal release (Schousboe & Waagepetersen, 2006).

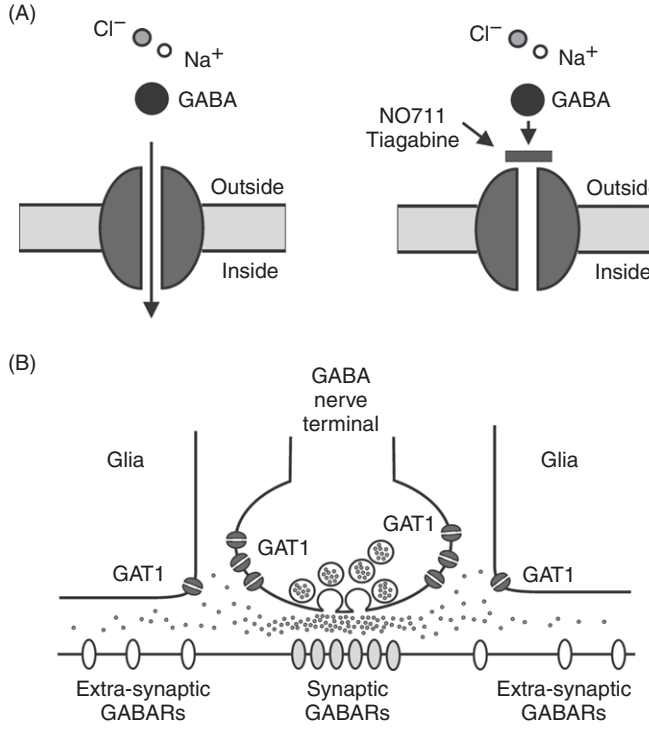


FIGURE 1 (A) Left, Scheme showing that GAT1 translocates GABA through the membrane via a process that requires Na^+ and Cl^-. Na^+ is co-transported by GAT1, contributing to an ionic current typically associated with GABA transport across the membrane. Whether Cl^- is transported as well, is not entirely clear. (A) Right, Several pharmacological compounds inhibit GABA transport in brain tissue, including NO711 and tiagabine, which are selective inhibitors of GAT1. GAT1 inhibitors are thought to mainly inhibit GABA uptake (that is, translocation through the plasma membrane from outside to inside), but can inhibit GABA translocation in the opposite direction as well, in conditions in which GAT1 mediates GABA release (see text). (B) Diagram showing a model, based on electron microscopy studies, for the localization of GAT1 at the ultrastructural level. The model suggests that GAT1 is expressed mainly in neurons, but some expression is observed in the glia as well. In neurons, GAT1 is apparently found mostly in the membrane of presynaptic GABA nerve terminals. The data regarding subcellular localization of GAT1 suggest that the transporter is found in extrasynaptic regions of the presynaptic nerve terminal membrane, relatively distant from the GABA release sites and postsynaptic receptors. This model assumes that neither GAT1-mediated uptake nor the binding of GABA to the transporters (before uptake begins) can effectively control the amount of GABA available to activate synaptic $GABA_A$ receptors after rapid release into the synaptic cleft.

However, glial glutamine can be released and taken up by neurons to be converted into GABA, possibly entering the releasable pool (Cherubini & Conti, 2001). In any event, a clear role of GAT1-mediated GABA uptake is to reduce extracellular GABA levels. An outstanding question is whether

removal of extracellular GABA by GAT1-mediated uptake can influence the activation of GABA receptors by synaptically-released GABA, a topic that will be considered in sections below.

Although GAT1 is thought to mainly mediate GABA uptake, some evidence suggests that GAT1 may translocate GABA in the opposite direction, thus producing non-vesicular GABA release (Attwell et al., 1993). For instance, GAT1 may mediate GABA release when the intracellular Na^+ concentration is increased (Wu et al., 2007). Moreover, the GABA translocation direction can be reversed quickly enough to produce GAT1-mediated GABA release in response to action potentials (Wu et al., 2007). GAT1-mediated release is Ca^{2+}-independent and is insensitive to inhibitors of vesicular GABA release, but is inhibited by the pharmacological GAT1 inhibitor NO711 (Wu et al., 2007).

Under what conditions does GAT1-mediated uptake or release predominate? Whereas answering such question requires additional research, it is interesting to note that in humans, the GAT1 inhibitor tiagabine has antiepileptic effects (Borden, 1996; Laroche, 2007). GAT1 blockade with tiagabine also increases the tonic currents produced by either exogenous or endogenous GABA (Frahm et al., 2001). In addition, electrophysiological experiments in tissue from GAT1 knock-out mice are consistent with an increase in extracellular GABA concentration (Chiu et al., 2005; Jensen et al., 2003). These general observations suggest that in cortical networks GAT1 predominantly operates in the uptake direction (Fig. 1A).

B. Regulation of GAT1-Mediated GABA Transport

GAT1-mediated transport is regulated by controlling its membrane surface expression. Importantly, phosphorylation of several residues in its intracellular loops regulates trafficking of the GAT1 protein (Chen et al., 2004; Quick et al., 2004). A crucial factor regulating phosphorylation-dependent GAT1 trafficking is the extracellular concentration of GABA. A short-term (30 min) raise in extracellular GABA increases GAT1 surface expression by slowing the transporter internalization in a manner dependent on GAT1 phosphorylation on tyrosine (Bernstein & Quick, 1999). Conversely, long-term elevation of extracellular GABA (24 h) reduces GAT1 surface expression by a process involving $GABA_A$ receptor activation and GAT1 phosphorylation on serine (Hu & Quick, 2008). One possibility is that the chronic (hours-long) elevation of extracellular GABA is sensed as a sustained increase in the levels of GABA-mediated inhibition in the circuit. An increased demand for inhibition may then be transduced, by some homeostatic mechanism, into decreased GAT1 levels that potentiate tonic inhibition by increasing the levels of so-called ambient GABA (Ortinski et al., 2006). Regulation of GAT1 levels in the plasma membrane may be an important pathophysiological or compensatory mechanism in several

psychiatric and neurological disorders including schizophrenia (Lewis et al., 2005), epilepsy (Fueta et al., 2003; Lee et al., 2006), cerebral ischemia (Frahm et al., 2004), and alcohol abuse (Hu et al., 2004). Not surprisingly, genetically-engineered GAT1 deficiency in mice produces several behavioral alterations (Cai et al., 2006; Chiu et al., 2005; Gong et al., 2009; Liu et al., 2007).

III. Cellular and Subcellular Localization of $GABA_BRs$ and GAT1

$GABA_BRs$ and GAT1 are relatively ubiquitous and co-localize in multiple cortical and subcortical brain regions. However, a critical issue for understanding the potential functional interactions between $GABA_BRs$ and GAT1 in cortical circuits is their localization at the ultrastructural level. Especially important is the localization of $GABA_BRs$ and GAT1 relative to the GABA release sites. Whereas some ultrastructural studies, reviewed below, examined the localization of $GABA_BRs$ or GAT1, no studies thus far examined whether these two proteins are co-localized, or found within short distance, at the ultrastructural level.

A. Localization of $GABA_BRs$

Functional $GABA_BRs$ most likely are heteromers containing GABAB1 and GABAB2 subunits (Mohler & Fritschy, 1999; Perez-Garci et al., 2006). Immunogold labeling electron microscopy studies show that GABAB1 and GABAB2 subunit proteins co-localize in the plasma membrane of neurons at both pre- and post-synaptic sites (Perez-Garci et al., 2006).

$GABA_BR$ subunits are found in the plasma membrane of nerve terminals at GABAergic/symmetric and glutamatergic/asymmetric synapses (Gonchar et al., 2001; Kulik et al., 2003; Vigot et al., 2006), suggesting that $GABA_BRs$ modulate GABA and glutamate release. Importantly, the plasma membrane of presynaptic nerve terminals in the central nervous system generally lacks excitatory or inhibitory synaptic contacts, raising questions about the source of GABA for presynaptic $GABA_BR$ activation. In GABAergic terminals, some $GABA_BRs$ are found close to the GABA release sites (Gonchar et al., 2001), suggesting those $GABA_BRs$ act as autoreceptors. The $GABA_BRs$ localized in the plasma membrane of glutamatergic nerve terminals are by definition extrasynaptic, and must be activated by GABA diffusing from nearby GABAergic synapses.

In addition to nerve terminals, some $GABA_BRs$ are found in the postsynaptic membrane of GABAergic/symmetric synapses (Gonchar et al., 2001), where they may be directly activated by synaptically-released GABA. However, $GABA_BRs$ are undetectable in the postsynaptic membrane

of many other GABA/symmetric synapses (Gonchar et al., 2001). $GABA_BR$ subunits can also be found in the plasma membrane of dendritic shafts and spines in the vicinity of glutamate/asymmetric synapses (Gonchar et al., 2001; Kulik et al., 2003; Kulik et al., 2006; Lopez-Bendito et al., 2002; Vigot et al., 2006). Although the dendritic membrane of pyramidal neurons (including the spines) may receive some GABA synapses (Andrasfalvy & Mody, 2006; Megias et al., 2001; Papp et al., 2001), GABAB1 subunits in dendritic spines and shafts are commonly found in regions of membrane lacking an inhibitory presynaptic partner (Kulik et al., 2003). This suggests that many of the dendritic $GABA_BRs$, which presumably regulate the excitatory effects of glutamate inputs, are extrasynaptic. The extrasynaptic $GABA_BR$ subunits found in dendrites typically co-cluster with Kir3-type K^+ channel proteins (Kulik et al., 2006). Importantly (see Section IV), $GABA_BR$ activation is commonly coupled, via G protein activation, to Kir K^+ channel gating, producing a hyperpolarizing K^+ current (Luscher et al., 1997).

B. Localization of GAT1

Electron microscopy studies of cortical tissue demonstrated that the GAT1 protein is expressed in the plasma membrane of neurons and glial cells. More specifically, GAT1 is frequently found in presynaptic nerve terminals that are part of an inhibitory/symmetric synaptic contact, as well as in glial processes near inhibitory synapses (Fig. 1B) (Conti et al., 1998; Conti et al., 2004; Mahendrasingam et al., 2003; Minelli et al., 1995; Minelli et al., 1996; Ong et al., 1998; Vitellaro-Zuccarello et al., 2003; Yan et al., 1997).

In contrast to the ultrastructural studies of $GABA_BR$ localization reviewed in Section III.A. (which employed immunogold particle methods), most electron microscopy studies of GAT1 localization employed immunocytochemistry based on diaminobenzidine peroxidation for detection of GAT1-positive structures. Using this method, the oxidation reaction product is relatively well confined inside the immunoreactive structure, but does not reveal the specific membrane compartments where the protein of interest is localized. Therefore, unlike the case of the $GABA_BR$ studies, the immunoperoxidase studies of GAT1 localization do not accurately describe if GAT1 proteins are preferentially localized in synaptic or extrasynaptic compartments of the neuronal plasma membrane. The few studies employing immunogold techniques to localize GAT1 in central nervous system tissue found label in the plasma membrane of glial process close to GABA/symmetric synapses as well as in the plasma membrane of presynaptic terminals of GABA/symmetric synapses, where the GAT1 labels are found extrasynaptically, relatively far from the GABA release sites (Conti et al., 1998; Mahendrasingam et al., 2003).

In summary, GAT1 proteins appear to be localized in GABAergic terminals as well as in glial processes near GABA synapses (Conti et al., 2004). The limited data available from immunogold labeling studies show that GAT1 proteins do not localize in very close proximity to GABA release sites or postsynaptic GABA receptors, suggesting that GAT1 activity mainly regulates the GABA concentration in the extracellular space outside the synaptic cleft (Fig. 1B).

IV. $GABA_BR$ Activation by GABA Released from Endogenous Sources

In hippocampal and neocortical circuits GABA is synthesized and released by GABAergic interneurons. While a minority (20–30% of all neurons), interneurons are nevertheless a highly heterogeneous cell type, probably constituted by multiple subtypes (Markram et al., 2004). A crucial question to understand $GABA_BR$ function in cortical circuits is therefore whether $GABA_BRs$ are typically activated by GABA released by all or only by specific subtypes of GABA neurons. Given their highly complex features, classification of GABA neurons is complicated and it is indeed still unclear how many different interneuron subtypes actually exist (Ascoli et al., 2008; Zaitsev et al., 2009). Before considering the importance of GAT1 activity for $GABA_BR$ activation, in this Section I review the different types of electrophysiological responses produced by $GABA_BRs$, when possible identifying the interneuronal source of GABA producing the response.

A. $GABA_BR$-Mediated Inhibitory Postsynaptic Potentials

Inhibitory postsynaptic potentials (IPSPs) can be elicited using extracellular electrical stimulation. Using this approach, pioneer electrophysiological studies demonstrated that synaptic GABA release can elicit IPSPs by activation of $GABA_BRs$ coupled to the gating of a K^+ current (Connors et al., 1988; Dutar & Nicoll, 1988). These $GABA_BR$-mediated IPSPs ($GABA_BR$-IPSPs) thus differ in their ionic basis from IPSPs produced by $GABA_AR$-gated Cl^- currents. $GABA_BR$-IPSPs are significantly slower in rise and decay times than $GABA_AR$-IPSPs (Fig. 2A) (Connors et al., 1988; Deisz, 1999; Dutar & Nicoll, 1988; McCormick, 1989). Such slower kinetics is expected, since $GABA_BRs$ belong to the G protein-coupled receptor family, gating K^+ channels through the various molecular steps involved in signaling via G protein stimulation.

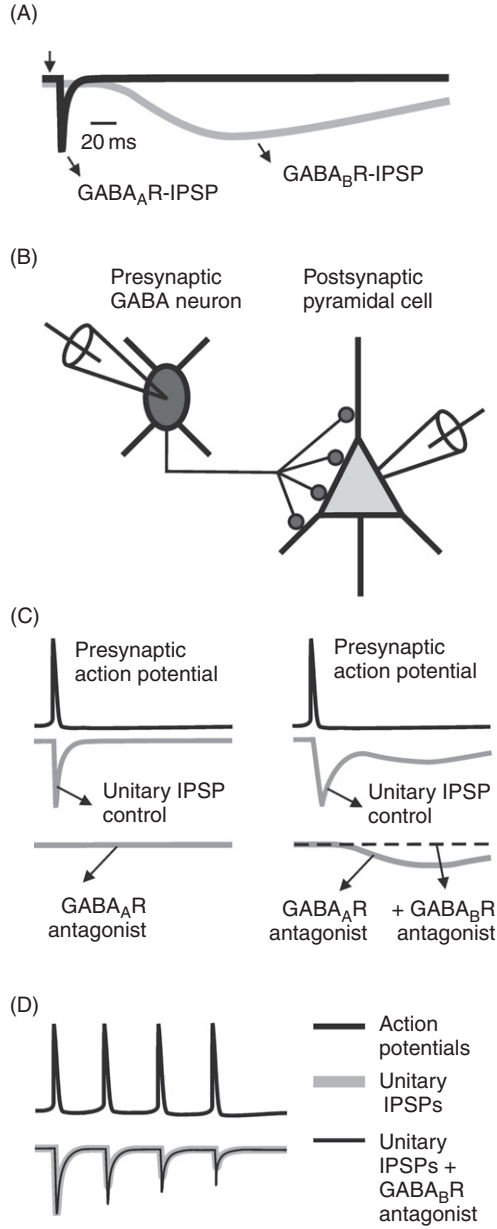
Eliciting $GABA_BR$-IPSPs typically requires stronger extracellular stimulation compared with producing $GABA_AR$-IPSPs (Bertrand & Lacaille, 2001; Dutar & Nicoll, 1988; Scanziani, 2000). Stronger stimulus intensities increase the number of stimulated axons, and therefore the number of active

synapses. Stronger stimuli may be required to elicit $GABA_B$R-IPSPs if at most GABA synapses transmission is purely $GABA_A$R-mediated and stimulating the uncommon $GABA_B$R-containing synapses is a low probability event. A second possibility is that many of the $GABA_B$Rs activated by synaptically-released GABA are extrasynaptic, their activation requiring pooling of extracellular GABA released by multiple synapses. In this case, stronger stimuli may be needed because $GABA_B$R activation requires recruiting synapses above a threshold or minimal number. These two scenarios are not mutually exclusive and actually are both supported by the electron microscopy data reviewed in Section III.A (Gonchar et al., 2001; Kulik et al., 2006; Vigot et al., 2006).

Using extracellular electrical stimulation, the identity of the stimulated axons (that is, to what GABAergic neuron subtype such axons belong) cannot be determined. Importantly, interneuron subtypes are broadly divided into those synapsing at or near the pyramidal cell soma (perisomatic-targeting) versus those making synapses in more distal dendrites (dendritic-targeting). Do $GABA_B$Rs mediate responses at specific compartments of the postsynaptic cell membrane? Applying focal extracellular stimulation, it is feasible to elicit in pyramidal cells IPSPs by selective activation of perisomatic-targeting versus dendritic-targeting GABAergic axons (Bertrand & Lacaille, 2001; Gonzalez-Burgos et al., 2009; Gulledge & Stuart, 2003). Using focal stimulation, we found recently that $GABA_B$R-IPSPs are preferentially evoked by stimulation of dendritic-targeting synapses (Gonzalez-Burgos et al., 2009). Importantly, such dendritic $GABA_B$R-IPSPs co-exist with dendritic $GABA_A$R-IPSPs and are produced with the same stimulus strength (Gonzalez-Burgos et al., 2009). These results suggest that $GABA_B$Rs predominantly mediate signaling by interneurons that target pyramidal cell dendrites.

B. Postsynaptic $GABA_B$R Activation During Unitary Synaptic Transmission

Although synaptic stimulation with extracellular electrodes may in cases be targeted to specific membrane compartments, this approach still has multiple limitations. For instance, it cannot determine whether the $GABA_A$R-IPSPs and $GABA_B$R-IPSPs originate at the same or separate synapse populations (Nurse & Lacaille, 1997). A state-of-the-art method to stimulate well-identified GABAergic synaptic connections is simultaneous recording from synaptically-connected pairs of neurons (Debanne et al., 2008). In these experiments, current is injected into a neuron to elicit an action potential while recording membrane potential or current simultaneously in another cell (Fig. 2B). Action potentials produced in a GABAergic interneuron synaptically-connected onto a postsynaptic cell produce responses called “single-axon” or “unitary” IPSCs/IPSPs. Unitary IPSCs/



IPSPs therefore result from activation of a single GABA neuron, although they typically involve multiple synaptic contacts between the presynaptic axon and the postsynaptic cell (Fig. 2B). In paired recordings, the recorded neurons can be identified by their morphology, biochemical and electrical properties. Moreover, using GABA receptor antagonists, whether $GABA_B$Rs or $GABA_A$Rs mediate the unitary IPSCs/IPSPs can be determined (Fig. 2C).

In recordings from synaptically-connected pairs, $GABA_A$R antagonists completely abolish the unitary responses evoked by a variety of interneuron subtypes (Fig. 2C, left), including the fast-spiking basket cells and chandelier neurons in the hippocampus (Ali et al., 1999; Bartos et al., 2002; Buhl et al., 1994; Buhl et al., 1995; Buhl et al., 1995; Doischer et al., 2008; Thomson & Destexhe, 1999; Thomson et al., 2000) and neocortex (Gonzalez-Burgos et al., 2005; Szabadics et al., 2006; Szabadics et al., 2007; Tamas et al., 1997; Tamas et al., 1998; Tamas et al., 2003; Thomson & Destexhe, 1999; Thomson et al., 1996). Non-fast-spiking basket cells similarly produce fast unitary IPSCs/IPSPs consistent with being mediated by $GABA_A$Rs (Galarreta et al., 2008; Glickfeld & Scanziani, 2006; Glickfeld et al., 2008; Hefft & Jonas, 2005). Basket cells (fast-spiking and non-fast spiking) and chandelier neurons innervate pyramidal

FIGURE 2 (A) Scheme comparing the fast time-course of $GABA_A$R-IPSPs (black trace) with the significantly slower time-course of $GABA_B$R-IPSPs (gray trace). Note that relative to the stimulus (arrow), the onset of the $GABA_B$R-IPSPs is substantially delayed compared to the $GABA_A$R-IPSP. In addition, the rising phase of the $GABA_B$R-IPSP is much slower and its decay time is at least ten-times slower compared with the $GABA_A$R-IPSP. (B) A drawing illustrating the typical experimental arrangement used to obtain electrophysiological recordings from synaptically-connected pairs of neurons. In this example, a presynaptic GABA neuron makes a synaptic connection, composed of four synaptic contacts, onto a postsynaptic pyramidal cell. The glass electrode pipettes can be used to inject stimuli or to record membrane potential or current from either neuron. The IPSPs produced in this kind of study are called single-axon or unitary IPSPs, even though they involve multiple synaptic contacts. (C) Examples of $GABA_A$R- and $GABA_B$R-mediated unitary IPSPs observed in recordings from synaptically-connected pairs. The example on the left shows a case in which the presynaptic GABA neuron produces in the postsynaptic cell a unitary IPSP purely mediated by $GABA_A$Rs. The great majority of GABA neuron subtypes identified thus far produce unitary IPSPs of this kind, including basket cells that target the perisomatic region of the pyramidal cell membrane, as well as some dendrite-targeting GABA neuron subtypes. The example on the right illustrates a unitary IPSP produced by the combined activation of $GABA_A$ and $GABA_B$ receptors. Only interneurons of the neurogliaform cell subtype have so far been clearly identified to produce unitary IPSPs with these characteristics. Note that the time-course of the $GABA_A$R-IPSP produced by neurogliaform cells is substantially slower than the time-course of $GABA_A$R-IPSPs produced by other interneuron subtypes. (D) Illustration of the autoreceptor effects produced on unitary $GABA_A$R-IPSPs by presynaptic $GABA_B$R in some types of GABA synapses. Note that, in control conditions, the strength of subsequent $GABA_A$R-IPSPs produced by stimulus trains shows progressive depression. After application of a $GABA_B$R antagonist, the strength of subsequent $GABA_A$R-IPSPs increases, showing that $GABA_B$Rs contribute to the IPSP depression. The initial unitary IPSP in the train is, however, unaltered by the $GABA_B$R antagonist, showing that autoreceptor effects are produced by GABA released by a recently preceding stimulus.

cells onto the perisomatic membrane compartment (Somogyi et al., 1998). Thus, perisomatic-targeting GABA neurons in general transmit via $GABA_A$Rs without significant contribution of postsynaptic $GABA_B$Rs (Freund & Katona, 2007). In addition, the unitary IPSCs/IPSPs produced by various dendrite-targeting interneuron subtypes in hippocampus and neocortex are similarly mediated by postsynaptic $GABA_A$R activation (Ali & Thomson, 2008; Bertrand & Lacaille, 2001; Kapfer et al., 2007; Murayama et al., 2009; Vida et al., 1998).

Whereas most GABA neuron subtypes produce purely $GABA_A$R-mediated unitary IPSCs/IPSPs (Fig. 2C, left), recent studies identified an interneuron subtype producing unitary $GABA_B$R-IPSPs. In paired recordings, interneurons of the neurogliaform cell class produce in pyramidal neurons slow unitary IPSPs that are never completely abolished by $GABA_A$R antagonists (Fig. 2C, right) (Tamas et al., 2003). Moreover, unitary IPSPs produced by neurogliaform cells after $GABA_A$R blockade rise and decay slowly, have long onset latency and are blocked by $GABA_B$R antagonists (Tamas et al., 2003). Therefore, neurogliaform cells produce unitary IPSPs simultaneously mediated by $GABA_A$Rs and $GABA_B$Rs (Fig. 2C, right). Interestingly, when stimulated repetitively, GABA release by neurogliaform cells exhausts quickly, eventually depressing almost completely the unitary IPSP (Tamas et al., 2003).

Are neurogliaform cells the only interneuron subtype producing $GABA_B$R-IPSPs by synaptic GABA release? The quick and strong depression of GABA release observed during repetitive stimulation of neurogliaform cells differs from the finding that repetitive extracellular stimulation produces $GABA_B$R-IPSPs more efficiently than single stimuli (Gonzalez-Burgos et al., 2009; Isaacson et al., 1993; Scanziani, 2000). These findings suggest that in addition to neurogliaform cells, other interneuron subtypes produce unitary $GABA_B$R-IPSPs. In one study, interneurons different from the neurogliaform cell class produced, exclusively during repetitive stimulation, slow unitary IPSPs insensitive to $GABA_A$R antagonists (Thomson & Destexhe, 1999). Moreover, in some cases, the slow $GABA_B$R-IPSPs were observed in the absence of a $GABA_A$R-mediated IPSP (Thomson & Destexhe, 1999), contrasting with the neurogliaform cell unitary IPSPs mediated by both $GABA_B$R and $GABA_A$Rs (Tamas et al., 2003). Such results confirm that some non-neurogliaform interneurons produce $GABA_B$R-IPSPs, although the particular cell class remains to be identified (Thomson & Destexhe, 1999). Recently, it was reported that fast-spiking basket neurons, thought to produce exclusively $GABA_A$R-mediated unitary IPSPs, can elicit $GABA_B$R-IPSPs (Oswald et al., 2009), although only if the interneuron is stimulated at high frequency (80 Hz) (Oswald et al., 2009).

C. Presynaptic $GABA_B$R Effects

As described in Section III.A., $GABA_B$Rs are localized in presynaptic nerve terminals at both GABA and glutamate synapses. At inhibitory

synapses, presynaptic $GABA_BRs$ may downregulate GABA release when activated by GABA released from the same nerve terminal—in other words, acting as autoreceptors (Fig. 2D). Alternatively, presynaptic $GABA_BRs$ can be activated by GABA released from adjacent GABA synapses or by ambient GABA. The activation of presynaptic $GABA_BRs$ at glutamate synapses must depend on heterosynaptic effects, given that glutamate synapses do not release GABA. An exception is the case of the mossy fiber synapses in the immature hippocampus, which are glutamatergic and also release GABA. Presynaptic effects of GABA at such synapses are mostly $GABA_AR$-mediated (Alle & Geiger, 2007), but $GABA_BRs$ may contribute as well (Safiulina & Cherubini, 2009).

Presynaptic and postsynaptic effects of $GABA_BR$ activation appear to be mediated by different mechanisms (Deisz et al., 1997). For instance, presynaptic $GABA_BR$ effects are intact in synapses of Kir K^+ channel knock-out mice that lack postsynaptic $GABA_BR$ effects (Luscher et al., 1997). Several studies have actually shown that $GABA_BRs$ inhibit voltage-dependent Ca^{2+} channels, an effect that may mediate presynaptic regulation of transmitter release by $GABA_BRs$ (Mintz & Bean, 1993; Takahashi et al., 1998; Thompson et al., 1993). At some GABA synapses, however, presynaptic $GABA_B$ autoreceptors inhibit transmitter release but do not regulate action potential-evoked presynaptic Ca^{2+} transients (Price et al., 2008).

Autoreceptor effects at GABA synapses are demonstrated by stimulating the synapses repetitively so that GABA released by a preceding stimulus regulates subsequent release (Fig. 2D). If presynaptic $GABA_BRs$ inhibit transmitter release, then the size of subsequent responses relative to preceding ones should increase when $GABA_BRs$ are blocked (Fig. 2D). Such an effect was demonstrated using extracellular stimulation of GABAergic axons eliciting $GABA_AR$-mediated IPSCs in hippocampal pyramidal cells (Cobb et al., 1999; Davies & Collingridge, 1993; Davies et al., 1990). $GABA_BR$ antagonists increase the amplitude of subsequent unitary IPSPs/IPSCs produced by repetitive stimulation of neurogliaform cells, consistent with downregulation of release via autoreceptors (Olah et al., 2009; Price et al., 2005, 2008). In contrast, $GABA_BR$ autoreceptor effects are absent at connections made by fast-spiking basket neurons (Olah et al., 2009).

Presynaptic $GABA_BRs$ may also regulate GABA release when activated tonically—that is, independent of the effects of GABA released by a recently preceding stimulus. The sources of GABA for tonic effects are difficult to evaluate, but may include GABA released by other GABA synapses, ambient GABA, or eventually GABA released homosynaptically at low frequency (tonic autoreceptor effects). In the last case, the effects of GABA released by a previous stimulus must persist until the next stimulus arrives, even if the inter-stimulus interval is relatively long during low-frequency stimulation. Some studies showed that $GABA_BR$ antagonists enhance $GABA_AR$-mediated

IPSCs evoked with low-frequency stimulation (Buhl et al., 1994; Buhl et al., 1995; Gonzalez-Burgos et al., 2009; Jensen et al., 2003; Lei & McBain, 2003; Price et al., 2008). However, in other cases $GABA_BR$ antagonists failed to modulate $GABA_AR$-IPSCs evoked at low frequency, even if the $GABA_AR$-IPSCs are strongly depressed by presynaptic effects of the $GABA_BR$ agonist baclofen (Neu et al., 2007). These results suggest that tonic activation of presynaptic $GABA_BRs$ by endogenous GABA is synapse type-specific and is not observed in some synapses at which presynaptic $GABA_BRs$ are nevertheless readily activated by exogenous agonists.

As described in Section III.A., $GABA_BRs$ are also localized in glutamate nerve terminals, where $GABA_BRs$ may produce downregulation of glutamate release (Davies & Collingridge, 1996; Isaacson et al., 1993; Lei & McBain, 2003; Pan et al., 2009; Porter & Nieves, 2004). Glutamate release may be actually modulated by presynaptic $GABA_BRs$ activated by endogenous GABA. For instance, burst stimulation of inhibitory inputs just prior to stimulation of glutamate inputs produces a depression of glutamate transmission that is reversed by a $GABA_BR$ antagonist (Davies & Collingridge, 1996; Isaacson et al., 1993). In the absence of prior stimulation of inhibitory inputs, $GABA_BR$ antagonists do not affect EPSCs, showing absence of tonic activation of presynaptic $GABA_BRs$ at glutamate terminals (Lei & McBain, 2003). Whereas various sources may provide endogenous GABA to activate presynaptic $GABA_BRs$ at glutamate synapses, a recent study showed that GABA released by neurogliaform cells is a prominent source of GABA for such presynaptic effects (Olah et al., 2009). More specifically, properly timed stimulation of GABA release by neurogliaform cells depressed, via presynaptic $GABA_BRs$, excitatory transmission between pyramidal neurons or between pyramidal cells and interneurons (Fig. 3B) (Olah et al., 2009).

V. GAT1 Activity and $GABA_BR$ Activation

The functional relevance of GAT1-mediated GABA transport has been assessed by either pharmacological blockade of GAT1 activity or in genetically-engineered GAT1-deficient mice. Such mice have significant behavioral alterations (not reviewed here), which suggest that in cortical circuits GAT1 plays a crucial role that cannot be compensated by other GABA transporters (Cai et al., 2006; Chiu et al., 2005; Gong et al., 2009). Below, I review the evidence from studies addressing the functional significance of GAT1 activity for GABA-mediated inhibition in cortical circuits. The effects of GAT1-mediated transport on $GABA_AR$-IPSCs are reviewed first, since they are crucial to understand the role of GAT1 for $GABA_BR$ activation.

A. GAT1-Mediated Uptake Prevents GABA Spillover onto Synaptic $GABA_A$Rs

A functional role typically assigned to GABA transporter-mediated uptake is the termination of the postsynaptic effect of GABA following presynaptic transmitter release. However, $GABA_A$R-IPSCs produced at single synapses are not altered in time-course or amplitude after GAT1 blockade with NO711 or in GAT1-deficient mice (Bragina et al., 2008; Gonzalez-Burgos et al., 2009; Jensen et al., 2003; Overstreet & Westbrook, 2003). These results are opposed to the prediction that if GAT1 normally controls the time-course and magnitude of $GABA_A$R activation then GAT1 blockade would enhance and prolong IPSCs.

Unitary IPSCs produced by individual fast-spiking GABA neurons typically are not altered by GAT1 blockade (Overstreet & Westbrook, 2003; Szabadics et al., 2007). However, if a fast-spiking GABA neuron unitary IPSC is specifically mediated by adjacent synapses, it is significantly prolonged by GAT1 blockade (Fig. 3A), suggesting that proximity of the synapses involved is a critical determinant of GAT1 effects (Overstreet & Westbrook, 2003). Interestingly, $GABA_A$R-IPSCs/IPSPs evoked by extracellular stimulation, which activates multiple axons (and therefore multiple synapses) together, are prolonged by GAT1 blockade or in GAT1-deficient mice (Bragina et al., 2008; Caillard et al., 1998; Gonzalez-Burgos et al., 2009; Keros & Hablitz, 2005; Overstreet & Westbrook, 2003; Thompson & Gahwiler, 1992). Importantly, GAT1 blockade or knock-out increase the duration of $GABA_A$R-IPSCs evoked by multiple-synapse stimulation, but do not increase their amplitude (Fig. 3A) (Bragina et al., 2008; Gonzalez-Burgos et al., 2009; Overstreet & Westbrook, 2003).

The absence of GAT1 effects on single-synapse responses is consistent with the idea that GAT1 does not regulate the time-course of synaptic $GABA_A$R activation following GABA release into an individual synaptic cleft. Possibly, within-synapse GABA diffusion and $GABA_A$R channel gating are significantly faster than the relatively slow speed of GABA uptake by GAT1 (see Section II). Via high-affinity binding, GAT1 potentially could rapidly buffer GABA molecules released into the synaptic cleft independent of a slow translocation rate. However, ultrastructural studies suggest an extra- or peri-synaptic localization of GAT1 (Figs. 1B and 3A) which may preclude effective GABA buffering by binding.

The prolongation by GAT1 blockade of $GABA_A$R-IPSC duration when multiple adjacent synapses are stimulated together may be the consequence of GAT1-controlled GABA diffusion between synapses, or spillover (Fig. 3A). Possibly, GABA spillover prolongs the $GABA_A$R-IPSCs but does not increase their peak amplitude because IPSC amplitude is determined by rapid within-synapse GABA diffusion (Farrant & Kaila, 2007; Mozrzymas, 2004). In contrast, diffusion of GABA between adjacent synapses when

GAT1 is blocked, probably is not rapid enough to contribute to the IPSC peak (Barbour, 2001), thus only increasing the IPSC decay time.

Overall, GAT1 may help preventing the effects of GABA spillover, thus preserving synapse independence—that is, the temporal and spatial specificity of fast $GABA_AR$-mediated transmission. In addition, GAT1 may prevent the densensitization of synaptic $GABA_ARs$ that in the absence of GAT1 activity may be exposed to ambient GABA. For groups of GABA synapses that are sufficiently distant, spillover is unlikely and $GABA_AR$-IPSCs

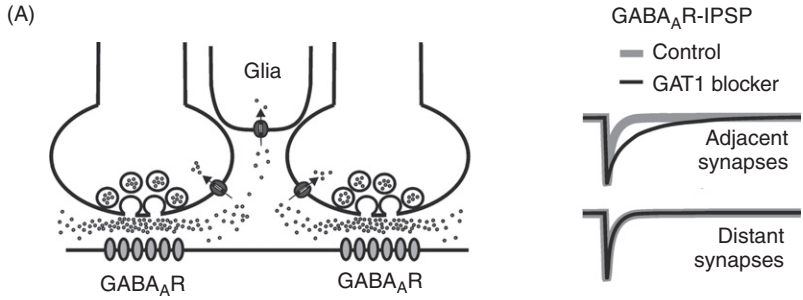


FIGURE 3 (A) Left, A scheme showing the effects of GAT1-mediated uptake on $GABA_AR$ activation when GABA is released by two adjacent synapses. Note that, under this model, at each synapse $GABA_ARs$ are readily activated by GABA released into the synaptic cleft by release sites directly opposed to the synaptic receptors. GAT1 localized in the nerve terminals, and possibly in the glia as well, uptakes most of the GABA that otherwise may diffuse and reach the adjacent synapse. This model suggests that the main function of GAT1 is controlling the effects on synaptic $GABA_ARs$ of GABA diffusing between synapses. (A) Right, Example traces showing the effect of GAT1 blockade on IPSPs produced by stimulation of adjacent synapses. Note that GAT1 blockade prolongs the decay time of the IPSPs when the synapses are adjacent. However, if the stimulated synapses are distant, the likelihood of spillover effects is very low and the IPSPs are insensitive to GAT1 blockade. Note that the duration, measured in control conditions, of IPSPs produced by adjacent synapses (thus prolonged by GAT1 blockade) is identical to the duration of IPSPs produced by distant synapses (that are insensitive to GAT1 blockade). (B) Left, A model for $GABA_BR$ activation by synaptically-released GABA based on data suggesting that $GABA_BR$ activation is mainly produced by GABA released by dendrite-targeting neurogliaform cells. These cells have been actually suggested to activate $GABA_BRs$ and $GABA_ARs$ via GABA spillover given that most of their terminals do not form actual synaptic junctions (see text). Such absence of synaptic junctions is represented in the scheme as a wider space separating release sites and the receptors activated by GABA. Note that GAT1 localized in the neurogliaform cell terminals, in terminals of other GABA neurons as well (not shown in the scheme) and in glia, significantly regulates activation of GABA receptors by GABA released by neurogliaform cells. Such GABA may also reach, under GAT1 control, presynaptic $GABA_BRs$ localized in excitatory glutamatergic synapses. (B) Right, Example traces showing that, under GAT1 control, neurogliaform cell-released GABA produces slow IPSPs by activation of $GABA_BRs$, as well as downregulation of EPSPs via presynaptic $GABA_BR$ activation. Note that, as suggested by recent data, when a neurogliaform cell is activated shortly before EPSPs are elicited, the release of GABA mostly affects, via presynaptic $GABA_BR$ activation, subsequent EPSPs but not the initial EPSP. This suggests very specific mechanisms of action that have not been yet investigated.

(Continued)

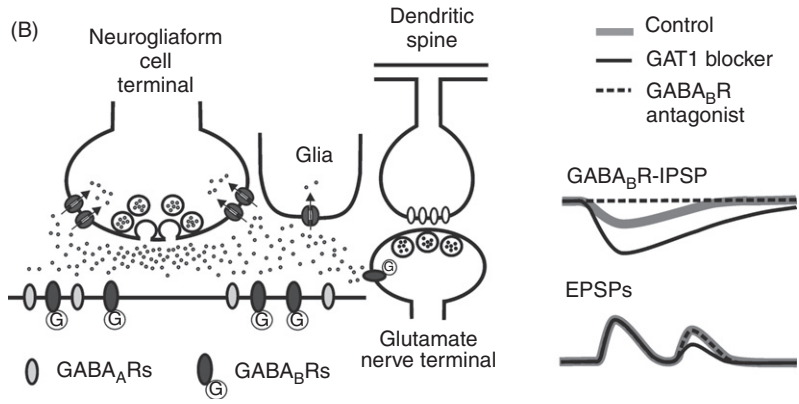


FIGURE 3 (*Continued*).

produced by activation of such synapses are not affected by GAT1 blockade (Fig. 3A). Conversely, GAT1-mediated uptake may be critical to prevent the effects of GABA spillover and $GABA_A$R-IPSCs mediated by adjacent synapses are thus prolonged by GAT1 blockade (Fig. 3A). Interestingly, we have recently reported that GAT1-mediated uptake may completely prevent the effects of spillover on $GABA_A$R-IPSPs (Gonzalez-Burgos et al., 2009). For instance, we found that IPSPs that are sensitive to GAT1 blockade (thus probably mediated by adjacent synapses susceptible of spillover), when measured with GAT1 activity intact, have similar duration compared with IPSPs that are GAT1 blockade-insensitive (thus probably mediated by distant synapses not susceptible to spillover).

B. GAT1 Activity and $GABA_B$R-Mediated IPSPs

The data reviewed in Section V.A suggest that GAT1 activity prevents the activation of synaptic $GABA_A$Rs by GABA released at heterologous nearby synapses. Although GAT1 may completely prevent the heterosynaptic effects of GABA diffusion (Gonzalez-Burgos et al., 2009), it is possible that GAT1 does not completely decrease GABA spillover, but reduces it to levels below those necessary to activate synaptic $GABA_A$Rs, which have low affinity for GABA (Mozrzymas, 2004). If so, a basal level of GABA diffusion out of the synaptic cleft may normally occur with GAT1 activity intact, and could activate high-affinity receptors such as the extrasynaptic $GABA_A$Rs (Glykys & Mody, 2007) and the $GABA_B$Rs (Bowery & Enna, 2000).

Consistent with such possibility is the finding that tonic $GABA_AR$-mediated currents, typically mediated by extrasynaptic $GABA_ARs$, are measurable with GAT1 activity intact and, moreover, are increased by GAT1 blockade or in GAT1-deficient mice (Bragina et al., 2008; Glykys & Mody, 2007; Jensen et al., 2003). Surprisingly, $GABA_BRs$ do not seem to produce tonic currents either with GAT1 activity intact or when this activity is reduced in GAT1-deficient mice (Jensen et al., 2003).

Considering the role of GAT1 in regulating $GABA_AR$ activation, a crucial question is whether similar mechanisms regulate $GABA_BR$ activation. As reviewed in Section IV, $GABA_BR$-IPSPs have much slower decay time-course (decay time >>100 ms) than $GABA_AR$-IPSPs (decay time < 20 ms). Therefore, $GABA_BR$-IPSPs are less likely to contribute to temporally precise fast synaptic transmission and GAT1-mediated control of $GABA_BR$-IPSP decay may be less critical for the rapid flow of neural activity in cortical circuits.

Production of fast $GABA_AR$-IPSCs requires rapid gating of the $GABA_ARs$ which in synapses is achieved by exposure of the receptors to a brief and high concentration transient of GABA in the synaptic cleft (Farrant & Kaila, 2007; Mozrzymas, 2004). However, the high affinity of $GABA_BRs$ and the slow kinetics of their postsynaptic effects suggest that production of $GABA_BR$-IPSPs does not require exposure of $GABA_BRs$ to GABA released into the synaptic cleft. Indeed, whereas an ultrastructural study found some $GABA_BRs$ concentrated postsynaptically at symmetric synapses (Gonchar et al., 2001), other studies using three-dimensional quantitative immunogold particle electron microscopy suggest that $GABA_BR$ subunits are localized mainly extra- or peri-synaptically in dendritic shafts and extrasynaptically near glutamate synapses in dendritic spines (Kulik et al., 2003, 2006).

As reviewed in Section IV, neurogliaform cells are the only interneuron subtype thus far identified to produce unitary $GABA_BR$-IPSPs (Price et al., 2005; Tamas et al., 2003). Neurogliaform cells mainly target the dendritic spines and shafts of pyramidal cells (Olah et al., 2009; Szabadics et al., 2007; Tamas et al., 2003), and in addition to producing slow unitary $GABA_BR$-IPSCs, produce unusually slow $GABA_AR$-IPSCs (Olah et al., 2009; Szabadics et al., 2007; Tamas et al., 2003). Such properties of the IPSCs lead others to hypothesize that the GABA receptors mediating neurogliaform cell responses are extra- or peri-synaptic (Fig. 3B) (Szabadics et al., 2007). For instance, neurogliaform cell-mediated unitary IPSCs have pharmacological properties similar to those of extrasynaptic $GABA_ARs$ (Szabadics et al., 2007). In addition, the effects of low-affinity $GABA_AR$ antagonists on neurogliaform cell-mediated unitary IPSCs are consistent with receptor activation by spillover (Szabadics et al., 2007). Notably, both $GABA_AR$- and $GABA_BR$-mediated unitary IPSCs produced by neurogliaform cells are extremely sensitive to GAT1 blockade by NO711 (Szabadics et al., 2007). NO711 application substantially prolongs the

$GABA_B$R-IPSPs and, in addition, significantly increases their amplitude (Gonzalez-Burgos et al., 2009; Isaacson et al., 1993; Szabadics et al., 2007). These finding suggests that, contrasting with $GABA_A$R-IPSPs, GAT1 regulates the strength of the $GABA_B$R-IPSPs, represented by their peak amplitude, possibly by regulating the number of $GABA_B$Rs bound to GABA (Fig. 3B).

Electrophysiological studies therefore suggest that vesicular GABA release from neurogliaform cell terminals produces, under GAT1 control, slow activation of extra- or peri-synaptic $GABA_A$Rs and $GABA_B$Rs by spillover. Only one study thus far examined whether $GABA_B$Rs are postsynaptic to neurogliaform cell release sites, but did not provide detailed quantitative analysis of the receptor localization (Price et al., 2005). Importantly, an ultrastructural analysis of neurogliaform cell nerve terminals showed that most (78%) of the synaptic vesicle-containing varicosities in the neurogliaform cell axon do not form actual synaptic junctions (Olah et al., 2009). Moreover, the nerve terminals apparently connecting individual neurogliaform cells onto postsynaptic neurons to produce slow unitary IPSPs lack clear synaptic junctions (Olah et al., 2009). This observation confirms that neurogliaform cells produce responses in their target cells without involving synaptic junctions and possibly by activation of extra- or peri-synaptic receptors by spillover (Fig. 3B).

An important morphological property of neurogliaform cells described by Santiago Ramon y Cajal more than 100 years ago is that their axonal arborization branches profusely near the cell body (Kawaguchi & Kubota, 1997; Olah et al., 2007, 2009; Povysheva et al., 2007; Zaitsev et al., 2009). Such profuse branching and a high density of boutons per unit of axon length (Olah et al., 2009) increase the probability of GABA spillover between neurogliaform cell terminals, potentially overcoming the GABA uptake system. The fact that GAT1 blockade with NO711 significantly enhances the neurogliaform cell-mediated $GABA_B$R- and $GABA_A$R-IPSPs (Szabadics et al., 2007) demonstrates that GAT1-mediated GABA uptake critically controls the postsynaptic response to GABA released by these neurons.

Whereas neurogliaform cell-mediated GABA release shows rapid exhaustion during repetitive stimulation (Tamas et al., 2003), several studies (see Section IV) showed that repetitive stimulation applied extracellularly or to non-neurogliaform cells is more efficient than single stimuli to produce $GABA_B$R-IPSPs (Gonzalez-Burgos et al., 2009; Isaacson et al., 1993; Oswald et al., 2009; Scanziani, 2000; Thomson & Destexhe, 1999). Moreover, the $GABA_B$R-IPSPs produced by repetitive stimulation are significantly enhanced by GAT1 blockade (Gonzalez-Burgos et al., 2009; Isaacson et al., 1993; Scanziani, 2000). These results support the conclusion that GAT1-mediated uptake strongly regulates $GABA_B$R-IPSPs produced by interneurons of other subtypes in addition to neurogliaform cells. Such conclusion raises the question of whether GAT1 controls the activation, by other interneuron

subtypes, of $GABA_BRs$ that are normally targeted by neurogliaform cell-released GABA (Fig. 3B). For example, some paired recordings studies have shown that single-pulse or repetitive stimulation of an interneuron may not produce $GABA_AR$-IPSPs or $GABA_BR$-IPSPs in a simultaneously recorded neuron, but that subsequent inhibition of GAT1 revealed a substantial $GABA_BR$-IPSP (Scanziani, 2000). Whether such $GABA_BR$ effects observed only after GAT1 is blocked reflect activation of $GABA_BRs$ normally activated by neurogliaform cells remains to be determined.

C. GAT1 Activity and Presynaptic $GABA_BR$ Activation

As reviewed in previous sections, some $GABA_BRs$ have been localized to presynaptic nerve terminals at GABA and glutamate synapses, and electrophysiological experiments have demonstrated that presynaptic $GABA_BRs$ regulate transmitter release. Does GAT1-mediated uptake control activation of presynaptic $GABA_BRs$? Some studies have shown that $GABA_BR$ antagonists fail to produce presynaptic effects at GABA synapses, suggesting that the amount of GABA normally accessing presynaptic sites is too small to produce significant autoreceptor activation (Caillard et al., 1998; Gonzalez-Burgos et al., 2009; Neu et al., 2007; Olah et al., 2009). However, GAT1 blockade reveals presynaptic effects of $GABA_BRs$, possibly by increasing the exposure of such presynaptic receptors to GABA (Caillard et al., 1998; Gonzalez-Burgos et al., 2009; Lei & McBain, 2003). Why presynaptic $GABA_BRs$ at some GABA synapses do not produce autoreceptor effects (Caillard et al., 1998; Gonzalez-Burgos et al., 2009; Neu et al., 2007; Olah et al., 2009) but can be activated by exogenous agonists or by endogenous GABA following GAT1 blockade is a puzzling issue that remains to be resolved.

Activation of presynaptic $GABA_BRs$ localized in glutamate synapses requires GABA diffusion for some distance in the extracellular space (Fig. 3B). It is therefore reasonable that GAT1 blockade enhances inhibition of glutamate release by such presynaptic receptors (Isaacson et al., 1993; Lei & McBain, 2003; Olah et al., 2009). An identified source of GABA for activation of presynaptic $GABA_BRs$ localized in glutamate nerve terminals are the neurogliaform cells (Olah et al., 2009). Interestingly, neurogliaform cells elicit presynaptic effects within a volume of tissue closely matching the distribution of their axon, which gives rise to a high density of branches around their soma (Olah et al., 2009). The presynaptic $GABA_BR$-mediated modulation of glutamate release by neurogliaform cells is enhanced by GAT1 blockade with NO711 (Olah et al., 2009). In contrast, NO711 does not reveal presynaptic $GABA_BR$ effects at glutamate synapses by GABA released by fast-spiking basket neurons (Olah et al., 2009).

VI. Conclusions

This chapter reviews studies suggesting that in cortical circuits GAT1-mediated GABA transport is important for the cellular electrophysiological responses produced by $GABA_BR$ activation by endogenous GABA. $GABA_BRs$ produce slow hyperpolarizing IPSPs in pyramidal cells and also regulate GABA and glutamate release via presynaptic effects (Figs. 2 and 3B). These physiological effects of $GABA_BR$ activation are enhanced by pharmacological inhibition of GAT1 activity, showing that GAT1 tightly regulates the amount of GABA available for $GABA_BR$ activation (Fig. 3).

GAT1 critically controls the activation of $GABA_ARs$, playing a "protective" role by preventing the stimulation of $GABA_ARs$ at a given synapse by GABA released at other synapses (Fig. 3A). Does GAT1 also protect $GABA_BRs$ from inter-synaptic cross-talk by GABA diffusion between synapses? If, as indicated by several studies, a majority of the $GABA_BRs$ are actually localized extra- or peri-synaptically, $GABA_BRs$ are therefore normally activated by GABA diffusing far away from the release sites (Fig. 3B). Therefore, instead of playing such a protective role, GAT1-mediated uptake may be a physiological mechanism regulating $GABA_BR$ activation.

The experimental observations reviewed here are consistent with the idea that $GABA_BRs$ signal via volume transmission. Such mechanism operates when a neurotransmitter is released into extracellular space compartments that are not directly opposed to membranes containing postsynaptic receptors (Fuxe et al., 2007). The properties of neurogliaform cell-mediated responses reviewed in this chapter, including their control by GAT1 activity, are actually consistent with $GABA_BR$-mediated volume transmission. Whether additional interneuron subtypes produce $GABA_BR$-mediated responses remains to be established. If so, then GAT1 may play two different functions: first, regulating the strength of physiological $GABA_BR$-mediated responses by controlling the amount of GABA reaching those receptors and, second, preventing the activation of $GABA_BRs$ normally targeted by a particular interneuron subtype, for instance neurogliaform cells, by GABA released by other interneurons (Fig. 3B).

If $GABA_BRs$ exclusively mediate signaling by neurogliaform interneurons, then their general role in cortical circuits is tightly linked to the neurogliaform cell function. Neurogliaform cells are present in various cortical areas and their physiological properties are being characterized in the cortex and hippocampus of rodents (Kawaguchi & Kubota, 1997; Price et al., 2005; Tamas et al., 2003), monkeys (Krimer et al., 2005; Povysheva et al., 2007; Zaitsev et al., 2009), and humans (Olah et al., 2007). Importantly, a main function of interneurons is the production of synchronized oscillations in hippocampal and cortical circuits (Klausberger & Somogyi,

2008). Interneurons involved in the mechanisms of network oscillations may fire at frequencies within the 30–80 Hz range (gamma oscillations) or 4–10 Hz (theta oscillations). Because GABA release from neurogliaform cells strongly depresses during repetitive activity, if these interneurons participate in network oscillations, then their repetitive firing would produce severe depression of GABA release. Since neurogliaform cells actually fire coupled to theta oscillations (Fuentealba et al., 2010), an interesting question is therefore whether during high-frequency firing that depresses GABA release, these interneurons release neuromodulators such as neuropeptide Y (Karagiannis et al., 2009) that could complement the $GABA_BR$-mediated signaling. Importantly, neurogliaform cell activity is decreased by neurosteroids (Olah et al., 2009) and enhanced by norephinephrine (Kawaguchi & Shindou, 1998), and thus can be modulated by the slow paracrine effects of these endogenous substances.

As highlighted elsewhere (Olah et al., 2009), solitary spikes in neurogliaform cells would fill, with a "cloud" of GABA, a volume of tissue closely matching the geometry of their axonal arbor. Following such spike, $GABA_BR$ activation via volume transmission would produce long-lasting $GABA_BR$-IPSPs in a relatively large group of pyramidal neurons and also would decrease excitation between pairs of pyramidal cells connected via synapses located in the same volume of tissue. Interestingly, the time-courses of presynaptic effects of $GABA_BRs$ on excitatory transmission and of $GABA_BR$-IPSPs are very similar (Isaacson et al., 1993). If so, then neurogliaform cell spikes would create a time-window of several hundred milliseconds during which pyramidal cell excitability is reduced by the $GABA_BR$-IPSP and pyramidal cell excitation is reduced by the inhibition of glutamate release by presynaptic $GABA_BRs$. Such an effect may contribute to terminating persistent activity in cortical networks (Mann et al., 2009), an activity pattern that may be essential for cognitive function dependent on working memory. The data reviewed in this chapter show that up- or down-regulating GAT1-mediated uptake could potentiate or weaken the strength and duration of such $GABA_BR$-mediated effects. Furthermore, regulation of GAT1-mediated uptake could potentially expand or contract the effective volume of tissue in which neurogliaform cells produce $GABA_BR$-mediated volume transmission.

Acknowledgments

The author is grateful to Dr David A Lewis and the members of the Translational Neuroscience Program for continuous support. Work by the author and colleagues which is cited in this manuscript was funded by NIH grants MH-45156 and MH-51234.

Conflict of interest: The author declares no conflict of interest.

Non-standard abbreviations

Ca^{2+}	calcium ion
Cl^-	chloride ion
$GABA_AR$	GABA A receptor
$GABA_BR$	GABA B receptor
GAT1	plasma membrane GABA transporter 1
IPSC	inhibitory postsynaptic current
IPSP	inhibitory postsynaptic potential
K^+	potassium ion
Kir	inward-rectifying potassium channel
Na^+	sodium ion

References

Ali, A. B., Bannister, A. P., & Thomson, A. M. (1999). IPSPs elicited in CA1 pyramidal cells by putative basket cells in slices of adult rat hippocampus. *European Journal of Neuroscience*, *11*, 1741–1753.

Ali, A. B., & Thomson, A. M. (2008). Synaptic alpha 5 subunit-containing GABAA receptors mediate IPSPs elicited by dendrite-preferring cells in rat neocortex. *Cerebral Cortex*, *18*, 1260–1271.

Alle, H., & Geiger, J. R. (2007). GABAergic spill-over transmission onto hippocampal mossy fiber boutons. *Journal of Neuroscience*, *27*, 942–950.

Andrasfalvy, B. K., & Mody, I. (2006). Differences between the scaling of miniature IPSCs and EPSCs recorded in the dendrites of CA1 mouse pyramidal neurons. *Journal of Physiology (Paris)*, *576*, 191–196.

Ascoli, G. A., Alonso-Nanclares, L., Anderson, S. A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., et al. (2008). Petilla terminology: Nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nature Reviews. Neuroscience*, *9*, 557–568.

Attwell, D., Barbour, B., & Szatkowski, M. (1993). Nonvesicular release of neurotransmitter. *Neuron*, *11*, 401–407.

Barbour, B. (2001). An evaluation of synapse independence. *Journal of Neuroscience*, *21*, 7969–7984.

Bartos, M., Vida, I., Frotscher, M., Meyer, A., Monyer, H., Geiger, J. R., et al. (2002). Fast synaptic inhibition promotes synchronized gamma oscillations in hippocampal interneuron networks. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 13222–13227.

Bernstein, E. M., & Quick, M. W. (1999). Regulation of gamma-aminobutyric acid (GABA) transporters by extracellular GABA. *Journal of Biological Chemistry*, *274*, 889–895.

Bertrand, S., & Lacaille, J. C. (2001). Unitary synaptic currents between lacunosum-moleculare interneurones and pyramidal cells in rat hippocampus. *Journal of Physiology (Paris)*, *532*, 369–384.

Bicho, A., & Grewer, C. (2005). Rapid substrate-induced charge movements of the GABA transporter GAT1. *Biophysical Journal*, *89*, 211–231.

Borden, L. A. (1996). GABA transporter heterogeneity: Pharmacology and cellular localization. *Neurochemistry International*, *29*, 335–356.

Bowery, N. G., & Enna, S. J. (2000). Gamma-aminobutyric acid(B) receptors: First of the functional metabotropic heterodimers. *Journal of Pharmacology and Experimental Therapeutics*, *292*, 2–7.

Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J., et al. (1980). (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature*, *283*, 92–94.

Bragina, L., Marchionni, I., Omrani, A., Cozzi, A., Pellegrini-Giampietro, D. E., Cherubini, E., et al. (2008). GAT-1 regulates both tonic and phasic GABA(A) receptor-mediated inhibition in the cerebral cortex. *Journal of Neurochemistry*, *105*, 1781–1793.

Buhl, E. H., Cobb, S. R., Halasy, K., & Somogyi, P. (1995). Properties of unitary IPSPs evoked by anatomically identified basket cells in the rat hippocampus. *European Journal of Neuroscience*, *7*, 1989–2004.

Buhl, E. H., Halasy, K., & Somogyi, P. (1994). Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature*, *368*, 823–828.

Cai, Y. Q., Cai, G. Q., Liu, G. X., Cai, Q., Shi, J. H., Shi, J., et al. (2006). Mice with genetically altered GABA transporter subtype I (GAT1) expression show altered behavioral responses to ethanol. *Journal of Neuroscience Research*, *84*, 255–267.

Caillard, O., McLean, H. A., Ben-Ari, Y., & Gaiarsa, J. L. (1998). Ontogenesis of presynaptic GABAB receptor-mediated inhibition in the CA3 region of the rat hippocampus. *Journal of Neurophysiology*, *79*, 1341–1348.

Chen, N. H., Reith, M. E., & Quick, M. W. (2004). Synaptic uptake and beyond: The sodium- and chloride-dependent neurotransmitter transporter family SLC6. *Pflugers Archiv*, *447*, 519–531.

Cherubini, E., & Conti, F. (2001). Generating diversity at GABAergic synapses. *Trends in Neurosciences*, *24*, 155–162.

Chiu, C. S., Brickley, S., Jensen, K., Southwell, A., Mckinney, S., Cull-Candy, S., et al. (2005). GABA transporter deficiency causes tremor, ataxia, nervousness, and increased GABA-induced tonic conductance in cerebellum. *Journal of Neuroscience*, *25*, 3234–3245.

Chiu, C. S., Jensen, K., Sokolova, I., Wang, D., Li, M., Deshpande, P., et al. (2002). Number, density, and surface/cytoplasmic distribution of GABA transporters at presynaptic structures of knock-in mice carrying GABA transporter subtype 1-green fluorescent protein fusions. *Journal of Neuroscience*, *22*, 10251–10266.

Cobb, S. R., Manuel, N. A., Morton, R. A., Gill, C. H., Collingridge, G. L., & Davies, C. H. (1999). Regulation of depolarizing GABA(A) receptor-mediated synaptic potentials by synaptic activation of GABA(B) autoreceptors in the rat hippocampus. *Neuropharmacology*, *38*, 1723–1732.

Connors, B. W., Malenka, R. C., & Silva, L. R. (1988). Two inhibitory postsynaptic potentials, and GABAA and GABAB receptor-mediated responses in neocortex of rat and cat. *Journal of Physiology*, *406*, 443–468.

Conti, F., Melone, M., De Biasi, S., Minelli, A., Brecha, N. C., & 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. *Journal of Comparative Neurology*, *396*, 51–63.

Conti, F., Minelli, A., & Melone, M. (2004). GABA transporters in the mammalian cerebral cortex: Localization, development and pathological implications. *Brain Research. Molecular Brain Research*, *45*, 196–212.

Davies, C. H., & Collingridge, G. L. (1993). The physiological regulation of synaptic inhibition by GABAB autoreceptors in rat hippocampus. *Journal of Physiology (Paris)*, *472*, 245–265.

Davies, C. H., & Collingridge, G. L. (1996). Regulation of EPSPs by the synaptic activation of GABAB autoreceptors in rat hippocampus. *Journal of Physiology (Paris)*, *496*(Pt 2), 451–470.

Davies, C. H., Davies, S. N., & Collingridge, G. L. (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *Journal of Physiology (Paris)*, *424*, 513–531.

Debanne, D., Boudkkazi, S., Campanac, E., Cudmore, R. H., Giraud, P., Fronzaroli-Molinieres, L., et al. (2008). Paired-recordings from synaptically coupled cortical and hippocampal neurons in acute and cultured brain slices. *Nature Protocols*, *3*, 1559–1568.

Deisz, R. A. (1999). The GABA(B) receptor antagonist CGP 55845a reduces presynaptic GABA(B) actions in neocortical neurons of the rat in vitro. *Neuroscience*, *93*, 1241–1249.

Deisz, R. A., Billard, J. M., & Zieglgansberger, W. (1997). Presynaptic and postsynaptic GABAB receptors of neocortical neurons of the rat in vitro: Differences in pharmacology and ionic mechanisms. *Synapse*, *25*, 62–72.

Diamond, J. S., & Jahr, C. E. (1997). Transporters buffer synaptically released glutamate on a submillisecond time scale. *Journal of Neuroscience*, *17*, 4672–4687.

Doischer, D., Hosp, J. A., Yanagawa, Y., Obata, K., Jonas, P., Vida, I., et al. (2008). Postnatal differentiation of basket cells from slow to fast signaling devices. *Journal of Neuroscience*, *28*, 12956–12968.

Dutar, P., & Nicoll, R. A. (1988). A physiological role for GABAB receptors in the central nervous system. *Nature*, *332*, 156–158.

Farrant, M., & Kaila, K. (2007). The cellular, molecular and ionic basis of GABAA receptor signalling. *Progress in Brain Research*, *160*, 59–87.

Frahm, C., Engel, D., & Draguhn, A. (2001). Efficacy of background GABA uptake in rat hippocampal slices. *NeuroReport*, *12*, 1593–1596.

Frahm, C., Haupt, C., Weinandy, F., Siegel, G., Bruehl, C., & Witte, O. W. (2004). Regulation of GABA transporter mRNA and protein after photothrombotic infarct in rat brain. *Journal of Comparative Neurology*, *478*, 176–188.

Freund, T. F., & Katona, I. (2007). Perisomatic inhibition. *Neuron*, *56*, 33–42.

Fuentealba, P., Klausberger, T., Karayannis, T., Suen, W. Y., Huck, J., Tomioka, R., et al. (2010). Expression of COUP-TFII nuclear receptor in restricted GABAergic neuronal populations in the adult rat hippocampus. *Journal of Neuroscience*, *30*, 1595–1609.

Fueta, Y., Vasilets, L. A., Takeda, K., Kawamura, M., & Schwarz, W. (2003). Down-regulation of GABA-transporter function by hippocampal translation products: Its possible role in epilepsy. *Neuroscience*, *118*, 371–378.

Fuxe, K., Dahlstrom, A., Hoistad, M., Marcellino, D., Jansson, A., Rivera, A., et al. (2007). From the golgi-cajal mapping to the transmitter-based characterization of the neuronal networks leading to two modes of brain communication: Wiring and volume transmission. *Brain Research Reviews*, *55*, 17–54.

Galarreta, M., Erdelyi, F., Szabo, G., & Hestrin, S. (2008). Cannabinoid sensitivity and synaptic properties of 2 GABAergic networks in the neocortex. *Cerebral Cortex*, *18*, 2296–2305.

Gether, U., Andersen, P. H., Larsson, O. M., & Schousboe, A. (2006). Neurotransmitter transporters: Molecular function of important drug targets. *Trends in Pharmacological Sciences*, *27*, 375–383.

Glickfeld, L. L., Atallah, B. V., & Scanziani, M. (2008). Complementary modulation of somatic inhibition by opioids and cannabinoids. *Journal of Neuroscience*, *28*, 1824–1832.

Glickfeld, L. L., & Scanziani, M. (2006). Distinct timing in the activity of cannabinoid-sensitive and cannabinoid-insensitive basket cells. *Nature Neuroscience*, *9*, 807–815.

Glykys, J., & Mody, I. (2007). Activation of GABAA receptors: Views from outside the synaptic cleft. *Neuron*, *56*, 763–770.

Gonchar, Y., Pang, L., Malitschek, B., Bettler, B., & Burkhalter, A. (2001). Subcellular localization of GABA(B) receptor subunits in rat visual cortex. *Journal of Comparative Neurology*, *431*, 182–197.

Gong, N., Li, Y., Cai, G. Q., Niu, R. F., Fang, Q., Wu, K., et al. (2009). GABA transporter-1 activity modulates hippocampal theta oscillation and theta burst stimulation-induced long-term potentiation. *Journal of Neuroscience*, *29*, 15836–15845.

Gonzalez-Burgos, G., Krimer, L. S., Povysheva, N. V., Barrionuevo, G., & Lewis, D. A. (2005). Functional properties of fast spiking interneurons and their synaptic connections with pyramidal cells in primate dorsolateral prefrontal cortex. *Journal of Neurophysiology*, *93*, 942–953.

Gonzalez-Burgos, G., Rotaru, D. C., Zaitsev, A. V., Povysheva, N. V., & Lewis, D. A. (2009). GABA transporter GAT1 prevents spillover at proximal and distal GABA synapses onto primate prefrontal cortex neurons. *Journal of Neurophysiology*, *101*, 533–547.

Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M. C., et al. (1990). Cloning and expression of a rat brain GABA transporter. *Science*, *249*, 1303–1306.

Gulledge, A. T., & Stuart, G. J. (2003). Excitatory actions of GABA in the cortex. *Neuron*, *37*, 299–309.

Hefft, S., & Jonas, P. (2005). Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. *Nature Neuroscience*, *8*, 1319–1328.

Hu, J. H., Ma, Y. H., Jiang, J., Yang, N., Duan, S. H., Jiang, Z. H., et al. (2004). Cognitive impairment in mice over-expressing gamma-aminobutyric acid transporter 1 (GAT1. *NeuroReport*, *15*, 9–12.

Hu, J., & Quick, M. W. (2008). Substrate-mediated regulation of gamma-aminobutyric acid transporter 1 in rat brain. *Neuropharmacology*, *54*, 309–318.

Isaacson, J. S., Solis, J. M., & Nicoll, R. A. (1993). Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron*, *10*, 165–175.

Jensen, K., Chiu, C. S., Sokolova, I., Lester, H. A., & Mody, I. (2003). GABA transporter-1 (GAT1)-deficient mice: Differential tonic activation of GABAA versus GABAB receptors in the hippocampus. *Journal of Neurophysiology*, *90*, 2690–2701.

Kapfer, C., Glickfeld, L. L., Atallah, B. V., & Scanziani, M. (2007). Supralinear increase of recurrent inhibition during sparse activity in the somatosensory cortex. *Nature Neuroscience*, *10*, 743–753.

Karagiannis, A., Gallopin, T., David, C., Battaglia, D., Geoffroy, H., Rossier, J., et al. (2009). Classification of NPY-expressing neocortical interneurons. *Journal of Neuroscience*, *29*, 3642–3659.

Kawaguchi, Y., & Kubota, Y. (1997). GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cerebral Cortex*, *7*, 476–486.

Kawaguchi, Y., & Shindou, T. (1998). Noradrenergic excitation and inhibition of GABAergic cell types in rat frontal cortex. *Journal of Neuroscience*, *18*, 6963–6976.

Keros, S., & Hablitz, J. J. (2005). Subtype-specific GABA transporter antagonists synergistically modulate phasic and tonic GABAA conductances in rat neocortex. *Journal of Neurophysiology*, *94*, 2073–2085.

Klausberger, T., & Somogyi, P. (2008). Neuronal diversity and temporal dynamics: The unity of hippocampal circuit operations. *Science*, *321*, 53–57.

Krimer, L. S., Zaitsev, A. V., Czanner, G., Kroner, S., Gonzalez-Burgos, G., Povysheva, N. V., et al. (2005). Cluster analysis-based physiological classification and morphological properties of inhibitory neurons in layers 2-3 of monkey dorsolateral prefrontal cortex. *Journal of Neurophysiology*, *94*, 3009–3022.

Kulik, A., Vida, I., Fukazawa, Y., Guetg, N., Kasugai, Y., Marker, C. L., et al. (2006). Compartment-dependent colocalization of kir3.2-Containing K+ channels and GABAB receptors in hippocampal pyramidal cells. *Journal of Neuroscience*, *26*, 4289–4297.

Kulik, A., Vida, I., Lujan, R., Haas, C. A., Lopez-Bendito, G., Shigemoto, R., et al. (2003). Subcellular localization of metabotropic GABA(B) receptor subunits GABA(B1a/b) and GABA(B2) in the rat hippocampus. *Journal of Neuroscience*, *23*, 11026–11035.

Laroche, S. M. (2007). A new look at the second-generation antiepileptic drugs: A decade of experience. *Neurologist*, *13*, 133–139.

Lee, T. S., Bjornsen, L. P., Paz, C., Kim, J. H., Spencer, S. S., Spencer, D. D., et al. (2006). GAT1 and GAT3 expression are differently localized in the human epileptogenic hippocampus. *Acta Neuropathologica*, *111*, 351–363.

Lei, S., & McBain, C. J. (2003). GABA B receptor modulation of excitatory and inhibitory synaptic transmission onto rat CA3 hippocampal interneurons. *Journal of Physiology (Paris)*, *546*, 439–453.

Lewis, D. A., Hashimoto, T., & Volk, D. W. (2005). Cortical inhibitory neurons and schizophrenia. *Nature Reviews. Neuroscience*, *6*, 312–324.

Liu, G. X., Cai, G. Q., Cai, Y. Q., Sheng, Z. J., Jiang, J., Mei, Z., et al. (2007). Reduced anxiety and depression-like behaviors in mice lacking GABA transporter subtype 1. *Neuropsychopharmacology*, *32*, 1531–1539.

Lopez-Bendito, G., Shigemoto, R., Kulik, A., Paulsen, O., Fairen, A., & Lujan, R. (2002). Expression and distribution of metabotropic GABA receptor subtypes GABABR1 and GABABR2 during rat neocortical development. *European Journal of Neuroscience*, *15*, 1766–1778.

Luscher, C., Jan, L. Y., Stoffel, M., Malenka, R. C., & Nicoll, R. A. (1997). G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. *Neuron*, *19*, 687–695.

Mahendrasingam, S., Wallam, C. A., & Hackney, C. M. (2003). Two approaches to double post-embedding immunogold labeling of freeze-substituted tissue embedded in low temperature lowicryl HM20 resin. *Brain Research. Brain Research Protocols*, *11*, 134–141.

Mann, E. O., Kohl, M. M., & Paulsen, O. (2009). Distinct roles of GABA(A) and GABA(B) receptors in balancing and terminating persistent cortical activity. *Journal of Neuroscience*, *29*, 7513–7518.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., & Wu, C. (2004). Interneurons of the neocortical inhibitory system. *Nature Reviews. Neuroscience*, *5*, 793–807.

McCormick, D. A. (1989). GABA as an inhibitory neurotransmitter in human cerebral cortex. *Journal of Neurophysiology*, *62*, 1018–1027.

Megias, M., Emri, Z., Freund, T. F., & Gulyas, A. I. (2001). Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience*, *102*, 527–540.

Minelli, A., Brecha, N. C., 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. *Journal of Neuroscience*, *15*, 7734–7746.

Minelli, A., DeBiasi, S., Brecha, N. C., Zuccarello, L. V., & Conti, F. (1996). GAT-3, a high-affinity GABA plasma membrane transporter, is localized to astrocytic processes, and it is not confined to the vicinity of GABAergic synapses in the cerebral cortex. *Journal of Neuroscience*, *16*, 6255–6264.

Mintz, I. M., & Bean, B. P. (1993). GABAB receptor inhibition of P-type ca2+ channels in central neurons. *Neuron*, *10*, 889–898.

Mohler, H., & Fritschy, J. M. (1999). GABAB receptors make it to the top–as dimers. *Trends in Pharmacological Sciences*, *20*, 87–89.

Moss, F. J., Imoukhuede, P. I., Scott, K., Hu, J., Jankowsky, J. L., Quick, M. W., et al. (2009). GABA transporter function, oligomerization state, and anchoring: Correlates with subcellularly resolved FRET. *Journal of General Physiology*, *134*, 489–521.

Mozrzymas, J. W. (2004). Dynamism of GABAA receptor activation shapes the "personality" of inhibitory synapses. *Neuropharmacology*, *47*, 945–960.

Murayama, M., Perez-Garci, E., Nevian, T., Bock, T., Senn, W., & Larkum, M. E. (2009). Dendritic encoding of sensory stimuli controlled by deep cortical interneurons. *Nature*, *457*, 1137–1141.

Neu, A., Foldy, C., & Soltesz, I. (2007). Postsynaptic origin of CB1-dependent tonic inhibition of GABA release at cholecystokinin-positive basket cell to pyramidal cell synapses in the CA1 region of the rat hippocampus. *Journal of Physiology (Paris)*, *578*, 233–247.

Nicoll, R. A. (2004). My close encounter with GABA(B) receptors. *Biochemical Pharmacology*, *68*, 1667–1674.

Nurse, S., & Lacaille, J. C. (1997). Do GABAA and GABAB inhibitory postsynaptic responses originate from distinct interneurons in the hippocampus? *Canadian Journal of Physiology and Pharmacology*, *75*, 520–525.

Olah, S., Fule, M., Komlosi, G., Varga, C., Baldi, R., Barzo, P., et al. (2009). Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. *Nature*, *461*, 1278–1281.

Olah, S., Komlosi, G., Szabadics, J., Varga, C., Toth, E., Barzo, P., et al. (2007). Output of neurogliaform cells to various neuron types in the human and rat cerebral cortex. *Frontiers in Neural Circuits*, *1*, 4.

Ong, W. Y., Yeo, T. T., Balcar, V. J., & Garey, L. J. (1998). A light and electron microscopic study of GAT-1-positive cells in the cerebral cortex of man and monkey. *Journal of Neurocytology*, *27*, 719–730.

Ortinski, P. I., Turner, J. R., Barberis, A., Motamedi, G., Yasuda, R. P., Wolfe, B. B., et al. (2006). Deletion of the GABA(A) receptor alpha1 subunit increases tonic GABA(A) receptor current: A role for GABA uptake transporters. *Journal of Neuroscience*, *26*, 9323–9331.

Oswald, A. M., Doiron, B., Rinzel, J., & Reyes, A. D. (2009). Spatial profile and differential recruitment of GABAB modulate oscillatory activity in auditory cortex. *Journal of Neuroscience*, *29*, 10321–10334.

Overstreet, L. S., & Westbrook, G. L. (2003). Synapse density regulates independence at unitary inhibitory synapses. *Journal of Neuroscience*, *23*, 2618–2626.

Pan, B. X., Dong, Y., Ito, W., Yanagawa, Y., Shigemoto, R., & Morozov, A. (2009). Selective gating of glutamatergic inputs to excitatory neurons of amygdala by presynaptic GABAb receptor. *Neuron*, *61*, 917–929.

Papp, E., Leinekugel, X., Henze, D. A., Lee, J., & Buzsaki, G. (2001). The apical shaft of CA1 pyramidal cells is under GABAergic interneuronal control. *Neuroscience*, *102*, 715–721.

Perez-Garci, E., Gassmann, M., Bettler, B., & Larkum, M. E. (2006). The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca2+ spikes in layer 5 somatosensory pyramidal neurons. *Neuron*, *50*, 603–616.

Porter, J. T., & Nieves, D. (2004). Presynaptic GABAB receptors modulate thalamic excitation of inhibitory and excitatory neurons in the mouse barrel cortex. *Journal of Neurophysiology*, *92*, 2762–2770.

Povysheva, N. V., Zaitsev, A. V., Kroener, S., Krimer, O. A., Rotaru, D. C., Gonzalez-Burgos, G., et al. (2007). Electrophysiological differences between neurogliaform cells from monkey and rat prefrontal cortex. *Journal of Neurophysiology*, *97*(2), 1030–1039.

Price, C. J., Cauli, B., Kovacs, E. R., Kulik, A., Lambolez, B., Shigemoto, R., et al. (2005). Neurogliaform neurons form a novel inhibitory network in the hippocampal CA1 area. *Journal of Neuroscience*, *25*, 6775–6786.

Price, C. J., Scott, R., Rusakov, D. A., & Capogna, M. (2008). GABA(B) receptor modulation of feedforward inhibition through hippocampal neurogliaform cells. *Journal of Neuroscience*, *28*, 6974–6982.

Quick, M. W., Hu, J., Wang, D., & Zhang, H. Y. (2004). Regulation of a gamma-aminobutyric acid transporter by reciprocal tyrosine and serine phosphorylation. *Journal of Biological Chemistry*, *279*, 15961–15967.

Safiulina, V. F., & Cherubini, E. (2009). At immature mossy fibers-CA3 connections, activation of presynaptic GABA(B) receptors by endogenously released GABA contributes to synapses silencing. *Frontiers in Cellular Neuroscience*, *3*, 1.

Scanziani, M. (2000). GABA spillover activates postsynaptic GABA(B) receptors to control rhythmic hippocampal activity. *Neuron*, *25*, 673–681.

Schousboe, A., & Waagepetersen, H. S. (2006). Glial modulation of GABAergic and glutamatergic neurotransmission. *Current Topics in Medicinal Chemistry*, *6*, 929–934.

Somogyi, P., Tamas, G., Lujan, R., & Buhl, E. H. (1998). Salient features of synaptic organisation in the cerebral cortex. *Brain Research Reviews*, *26*, 113–135.

Szabadics, J., Tamas, G., & Soltesz, I. (2007). Different transmitter transients underlie presynaptic cell type specificity of GABAA,slow and GABAA,fast. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 14831–14836.

Szabadics, J., Varga, C., Molnar, G., Olah, S., Barzo, P., & Tamas, G. (2006). Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science*, *311*, 233–235.

Takahashi, T., Kajikawa, Y., & Tsujimoto, T. (1998). G-protein-coupled modulation of presynaptic calcium currents and transmitter release by a GABAB receptor. *Journal of Neuroscience*, *18*, 3138–3146.

Tamas, G., Buhl, E. H., & Somogyi, P. (1997). Fast IPSPs elicited via multiple synaptic release sites by different types of GABAergic neurone in the cat visual cortex. *Journal of Physiology (Paris)*, *500*(Pt 3), 715–738.

Tamas, G., Lorincz, A., Simon, A., & Szabadics, J. (2003). Identified sources and targets of slow inhibition in the neocortex. *Science*, *299*, 1902–1905.

Tamas, G., Somogyi, P., & Buhl, E. H. (1998). Differentially interconnected networks of GABAergic interneurons in the visual cortex of the cat. *Journal of Neuroscience*, *18*, 4255–4270.

Thompson, S. M., Capogna, M., & Scanziani, M. (1993). Presynaptic inhibition in the hippocampus. *Trends in Neurosciences*, *16*, 222–227.

Thompson, S. M., & Gahwiler, B. H. (1992). Effects of the GABA uptake inhibitor tiagabine on inhibitory synaptic potentials in rat hippocampal slice cultures. *Journal of Neurophysiology*, *67*, 1698–1701.

Thomson, A. M., Bannister, A. P., Hughes, D. I., & Pawelzik, H. (2000). Differential sensitivity to zolpidem of IPSPs activated by morphologically identified CA1 interneurons in slices of rat hippocampus. *European Journal of Neuroscience*, *12*, 425–436.

Thomson, A. M., & Destexhe, A. (1999). Dual intracellular recordings and computational models of slow inhibitory postsynaptic potentials in rat neocortical and hippocampal slices. *Neuroscience*, *92*, 1193–1215.

Thomson, A. M., West, D. C., Hahn, J., & Deuchars, J. (1996). Single axon IPSPs elicited in pyramidal cells by three classes of interneurones in slices of rat neocortex. *Journal of Physiology*, *496*, 81–102.

Ulrich, D., & Bettler, B. (2007). GABA(B) receptors: Synaptic functions and mechanisms of diversity. *Current Opinion in Neurobiology*, *17*, 293–303.

Vida, I., Halasy, K., Szinyei, C., Somogyi, P., & Buhl, E. H. (1998). Unitary IPSPs evoked by interneurons at the stratum radiatum-stratum lacunosum-moleculare border in the CA1 area of the rat hippocampus in vitro. *Journal of Physiology (Paris)*, *506*(Pt 3), 755–773.

Vigot, R., Barbieri, S., Brauner-Osborne, H., Turecek, R., Shigemoto, R., Zhang, Y. P., et al. (2006). Differential compartmentalization and distinct functions of GABAB receptor variants. *Neuron*, *50*, 589–601.

Vitellaro-Zuccarello, L., Calvaresi, N., & De Biasi, S. (2003). Expression of GABA transporters, GAT-1 and GAT-3, in the cerebral cortex and thalamus of the rat during postnatal development. *Cell and Tissue Research*, *313*, 245–257.

Wang, D., & Quick, M. W. (2005). Trafficking of the plasma membrane gamma-aminobutyric acid transporter GAT1. Size and rates of an acutely recycling pool. *Journal of Biological Chemistry*, *280*, 18703–18709.

Wu, Y., Wang, W., Diez-Sampedro, A., & Richerson, G. B. (2007). Nonvesicular inhibitory neurotransmission via reversal of the GABA transporter GAT-1. *Neuron*, *56*, 851–865.

Yan, X. X., Cariaga, W. A., & Ribak, C. E. (1997). Immunoreactivity for GABA plasma membrane transporter, GAT-1, in the developing rat cerebral cortex: Transient presence in the somata of neocortical and hippocampal neurons. *Brain Research. Developmental Brain Research*, *99*, 1–19.

Zaitsev, A. V., Povysheva, N. V., Gonzalez-Burgos, G., Rotaru, D., Fish, K. N., Krimer, L. S., et al. (2009). Interneuron diversity in layers 2–3 of monkey prefrontal cortex. *Cerebral Cortex*, *19*, 1597–1615.