The Receptors

Adam C. Errington
Giuseppe Di Giovanni
Vincenzo Crunelli *Editors*

Extrasynaptic $GABA_A$ Receptors

Humana Press

The Receptors

Volume 27

Series Editor
Giuseppe di Giovanni
Department of Physiology & Biochemistry Faculty of Medicine and Surgery,
University of Malta, Malta, Italy

The Receptors book Series, founded in the 1980's, is a broad-based and well-respected series on all aspects of receptor neurophysiology. The series presents published volumes that comprehensively review neural receptors for a specific hormone or neurotransmitter by invited leading specialists. Particular attention is paid to in-depth studies of receptors' role in health and neuropathological processes. Recent volumes in the series cover chemical, physical, modeling, biological, pharmacological, anatomical aspects and drug discovery regarding different receptors. All books in this series have, with a rigorous editing, a strong reference value and provide essential up-to-date resources for neuroscience researchers, lecturers, students and pharmaceutical research.

More information about this series at http://www.springer.com/series/7668

Adam C. Errington • Giuseppe Di Giovanni
Vincenzo Crunelli
Editors

Extrasynaptic $GABA_A$ Receptors

Springer

Editors
Adam C. Errington
Neuroscience and Mental Health
Research Institute
Institute of Psychological Medicine
and Clinical Neuroscience
School of Medicine
Cardiff University
Cardiff
United Kingdom

Giuseppe Di Giovanni
Dept. of Physiology & Biochemistry
University of Malta
Msida MSD
Malta

Vincenzo Crunelli
School of Biosciences
Cardiff University
Cardiff
United Kingdom

ISBN 978-1-4939-1425-8 ISBN 978-1-4939-1426-5 (eBook)
DOI 10.1007/978-1-4939-1426-5
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2014944741

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

Contributors

Justo Aguilar Department of Physiology, Biophysics and Neuroscience, Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), Mexico City, Mexico

Carmen Andrés Department of Physiology, Biophysics and Neuroscience, Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), Mexico City, Mexico

Emmanuel J. Botzolakis Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, PA, USA

Stephen Brickley Biophysics Section, Department of Life Sciences, Imperial College London, London, UK

Damian Bright Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK

Anna Cavaccini Neuroscience Division, Cardiff School of Biosciences Cardiff University, Cardiff, UK

Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Genoa, Italy

Mary Chebib Department of Pharmacy A15, University of Sydney, Sydney, NSW, Australia

Andrew N. Clarkson Departments of Anatomy and Psychology, University of Otago, Dunedin, New Zealand

William M. Connelly Neuroscience Division, Cardiff School of Biosciences, Cardiff University, Cardiff, UK

Vincenzo Crunelli Neuroscience Division, Cardiff School of Biosciences, Cardiff University, Cardiff, UK

Rodolfo Delgado-Lezama Department of Physiology, Biophysics and Neuroscience, Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), Mexico City, Mexico

Giuseppe Di Giovanni Department of Physiology and Biochemistry, Faculty of Medicine, Malta University, Msida, Malta

Adam C. Errington Neuroscience and Mental Health Research Institute, Institute of Psychological Medicine and Clinical Neuroscience, School of Medicine, Cardiff University, Cardiff, UK

Ricardo Felix Department of Cell Biology, Cinvestav-IPN, Mexico City, Mexico

Jean-Marc Fritschy Institute of Pharmacology and Toxicology, University of Zurich, Zürich, Zürich, Switzerland

Catriona Houston Biophysics Section, Department of Life Sciences, Imperial College London, London, UK

Molly M. Huntsman Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Department of Pediatrics, School of Medicine, University of Colorado, Aurora, CO, USA

Emanuel Loeza-Alcocer Department of Physiology, Biophysics and Neuroscience, Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), Mexico City, Mexico

Robert L. Macdonald Department of Neurology, Vanderbilt University Medical Center, Nashville, TN, USA

Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN, USA

Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA

Jamie Maguire Department of Neuroscience, Tufts University School of Medicine, Boston, MA, USA

Brandon S. Martin Children's National Medical Center, Washington, DC, USA

Patrizia Panzanelli Department of Neuroscience Rita Levi Montalcini, University of Turin, Turin, Italy

H. Rheinallt Parri School of Life and Health Sciences, Aston University, Birmingham, UK

Ivan Pavlov Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, UK

Keith A Wafford Eli Lilly UK, Windlesham, Surrey, UK

Matthew C. Walker Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, UK

Josue G. Yagüe Neuroscience Division, Cardiff School of Biosciences Cardiff University, Cardiff, UK

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK

Chapter 1
Extrasynaptic $GABA_A$ Receptors

A Brief Introduction to Extrasynaptic $GABA_A$ Receptors and 'Tonic' $GABA_A$ Receptor-Mediated Inhibition in Physiology and Disease

Adam C. Errington

Abstract γ-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the brain and its actions are mediated by two diverse families of neurotransmitter receptors, the ionotropic receptors, known as $GABA_A$ receptors, and metabotropic receptors that are classified as $GABA_B$ receptors. The classical phasic inhibitory postsynaptic potential (IPSP) is mediated by $GABA_A$ receptors that are located in the postsynaptic membrane. However, GABA also produces tonic inhibition through activation of $GABA_A$ receptors that are located outside the synapse. These extrasynaptic $GABA_A$ receptors respond to low concentrations of GABA to provide more spatially and temporally diffuse inhibition compared to their synaptic counterparts. This book covers the most current knowledge of extrasynaptic $GABA_A$ receptor structure, function, cellular distribution and pharmacology and the roles of tonic inhibition in neuronal excitability, physiology and pathophysiology.

Keywords γ-aminobutyric acid · Extrasynaptic $GABA_A$ receptor · Tonic GABA inhibition · δ subunit · α_5 subunit · Inhibitory neurotransmission · Inhibitory postsynaptic potential (IPSP)

γ-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the brain and its actions are mediated largely by a family of ubiquitously expressed ligand-gated ion channels known as $GABA_A$ receptors (Farrant and Nusser 2005; Brickley and Mody 2012). $GABA_A$ receptors are pentameric assemblies comprising several distinct subunits which open upon GABA binding leading to an increase in membrane permeability to both chloride and bicarbonate ions (Chebib and Johnston 2000). Typically, this occurs when GABA is released from presynaptic terminals causing a transient rise in GABA concentration within the synaptic cleft and activation of postsynaptic receptors. The resulting brief change in membrane conductance

A. C. Errington (✉)
Neuroscience and Mental Health Research Institute, Institute of Psychological Medicine and Clinical Neuroscience, School of Medicine, Cardiff University, Cardiff, UK
e-mail: erringtonac@cardiff.ac.uk

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_1

underlies 'phasic' $GABA_A$ergic inhibition and generation of the 'classical' inhibitory postsynaptic potential (IPSP). However, it has come to light relatively recently that $GABA_A$ receptor activation can occur in a much more spatially and temporally diffuse manner (Farrant and Nusser 2005; Brickley and Mody 2012). It has been demonstrated in several brain regions including granule cells of the cerebellum (Brickley et al. 1996), layer II/III and V cortical pyramidal neurons (Semyanov et al. 2003; Yamada et al. 2007), medium spiny neurons of the striatum (Ade et al. 2008), hippocampal CA1 and CA3 pyramidal neurons and dentate gyrus granule cells (Nusser and Mody 2002; Scimemi et al. 2005; Mortensen and Smart 2006) and thalamocortical (TC) neurons of the ventrobasal, dorsal lateral geniculate and medial geniculate nuclei of thalamus (Cope et al. 2005; Jia et al. 2005; Belelli et al. 2005; Richardson et al. 2011, 2013) that very low (nM) concentrations of GABA, which typically occur in the cerebrospinal fluid (CSF) of the extracellular space, can persistently activate a population of non-synaptic $GABA_A$ receptors resulting in a 'tonic' increase in membrane conductance (Fig. 1.1; Brickley and Mody 2012). In fact, it has been demonstrated in dentate gyrus granule cells that GABA may not even be necessary for this form of inhibition since GABA-independent channel openings, as demonstrated by resistance to the GABA receptor antagonist SR95531 (gabazine) but not the channel blocker picrotoxin, maintain the level of tonic current under low extracellular GABA concentrations (Wlodarczyk et al. 2013). This novel form of $GABA_A$ receptor-mediated signalling has become widely known as tonic GABAergic inhibition and for the first time this book brings together much of the most current knowledge of extrasynaptic $GABA_A$ receptor ($eGABA_AR$) structure, function, cellular distribution and pharmacology and the roles of tonic inhibition in neuronal excitability, physiology and pathophysiology. Unlike the classical 'point-to-point' signalling that occurs at chemical and electrical synapses, for example, those that mediate phasic GABA IPSPs, this form of GABAergic cellular communication is more similar to the type of volume transmission observed with monoamine neurotransmitters like serotonin, dopamine and histamine (Agnati et al. 2010).

As detailed by Fritschy and Panzanelli in Chap. 2, these peri- or extrasynaptic $GABA_A$ receptors differ substantially from their synaptic counterparts in terms of subunit composition and distribution. These differences impart major changes in functional receptor properties including a significantly higher affinity for GABA and markedly slower rates of receptor desensitization compared to synaptic receptors (Farrant and Nusser 2005; Bright et al. 2011). The divergence in properties of synaptic $GABA_ARs$ versus $eGABA_ARs$ is largely conferred by receptor subunit composition. In particular, the inclusion of a δ subunit (typically in place of the γ subunit found in synaptic receptors—Fig. 1.1) is necessary for generation of tonic GABA currents in dentate gyrus granule cells, cerebellar granule cells, TC neurons and layer II/III cortical neurons (Ade et al. 2008; Drasbek and Jensen 2006; Pirker et al. 2000; Nusser et al. 1998) whilst α_5 subunits, usually as $\alpha_5\beta\gamma_2$ receptors, appear to mediate most of the tonic current in pyramidal cells of the hippocampal CA1 and CA3 regions and cortical layer V (Caraiscos et al. 2004; Glykys and Mody 2006, 2007; Pavlov et al. 2009; Semyanov et al. 2004; Sperk et al. 1997; Yamada et al. 2007). In total, nineteen $GABA_A$ receptor subunits have been cloned from the

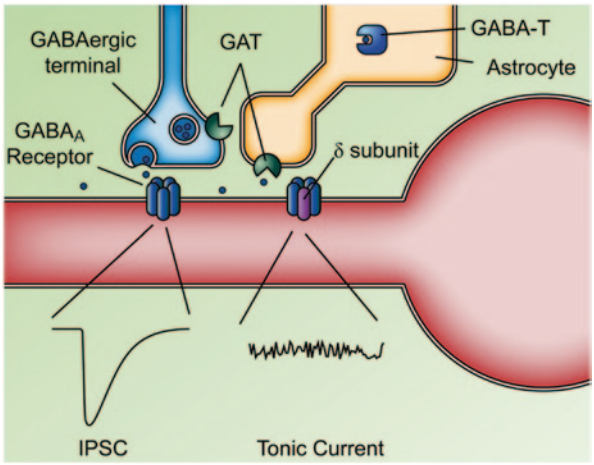

Fig. 1.1 Phasic and tonic $GABA_A$ergic inhibition. Release of GABA from inhibitory neurons into the synaptic cleft results in activation of postsynaptic $GABA_A$ receptors that open transiently to increase the permeability of the postsynaptic membrane to chloride (and bicarbonate) ions. These receptors quickly deactivate and undergo rapid desensitization to GABA. This results in the classical inhibitory postsynaptic current (IPSC). Extrasynaptic $GABA_A$ receptors sense very low concentrations of GABA in the extracellular space surrounding the synapse and are desensitized less rapidly than their synaptic counterparts. Extrasynaptic receptors are tonically activated by GABA released in an activity-dependent fashion from inhibitory GABAergic neurons and also from glia, such as astrocytes. The magnitude of tonic inhibition is regulated by the ambient GABA concentration which is determined by the rate of GABA release and the rate of uptake by the GABA transporters (GAT) which are expressed in both neurons and glia

mammalian CNS (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, θ, π, ρ_{1-3}) offering the potential for an enormous hetereogeneity in functional $GABA_A$ receptor assembly (Olsen and Sieghart 2008, 2009; Sieghart and Sperk 2002). In reality, however, only about 20–30 of the potential combinations have been shown to exist in the brain. The most commonly expressed subunit combination is α_1, β_2 and γ_2 (with stoichiometry of 2α and 2β subunits and a single γ subunit) whilst other common arrangements include $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_3\gamma_2$. Significantly, light microscopic immunofluorescence and electron microscopic (EM) immunogold methods have established that the postsynaptic densities of GABAergic synapses are highly enriched with receptors including α_{1-3}, α_6, β_{2-3} and γ_2 subunits suggesting that these subunits form the $GABA_A$ receptors responsible for classical 'phasic' inhibition. However, in contrast to the aforementioned subunits which are enriched in the postsynaptic density but also abundant at extrasynaptic locations, some $GABA_A$ receptor subunits, especially δ, are not found in the synapse and are exclusively peri- or extrasynaptically located. Extrasynaptic receptors containing the δ subunit are commonly found to co-assemble with α_4 or α_6 subunits ($\alpha_4/\alpha_6\beta_X\delta$) whilst α_5-containing receptors are also mostly extrasynaptic despite usually containing the typically synaptically located γ_2 subunit ($\alpha_5\beta_X\gamma_2$). As a consequence of these differences in receptor subunit composition, extrasynaptic receptors can have markedly different biophysical properties, such as different

channel opening times, compared to their synaptic counterparts and the differences in properties of receptors containing δ and γ subunits are explored in detail in Chap. 3 by Macdonald and Botzolakis.

Another key feature that defines eGABA$_A$Rs and differentiates them from their synaptic counterparts is their unique pharmacology. Receptors targeted to synaptic locations typically contain γ subunits that form an important part of the GABA$_A$ receptor benzodiazepine-binding site. The inclusion of these subunits makes these receptors responsive to the action of benzodiazepine compounds such as diazepam. In contrast, extrasynaptic receptors that often lack γ subunits are mostly, although not completely, insensitive to the actions of benzodiazepines. Extrasynaptic receptors containing δ subunits have unique pharmacological profiles and are targeted selectively (Brown et al. 2002) by the hypnotic agonist 4,5,6,7-tetrahydoisoxazolo[5,4-c] pyridin-3(2H)-one (THIP or Gaboxadol; Wafford and Ebert 2006) at low concentrations (~500 nM, Mortensen et al. 2010). The effects of THIP on extrasynaptic GABA receptors are, in fact, thought to account for its ability to promote both slow-wave (non-REM) and REM sleep since in animals lacking δ subunits the hypnotic effects of the drug are lost (Winsky-Sommerer et al. 2007). Furthermore, recent evidence suggests that eGABA$_A$Rs may also be the preferential high-affinity target of the naturally occurring GABA precursor γ-hydroxybutyric acid (GHB), a depressant drug that is also used recreationally. However, this observation remains controversial since the $\alpha_4\beta_1\delta$ subunit combination expressed in *Xenopus* oocytes in this study are believed to exist only in very low numbers, if at all, in the mammalian brain. In Chap. 4, Wafford provides a comprehensive overview of the pharmacology of synaptic and extrasynaptic receptors highlighting the major similarities and differences in their pharmacological profile and the structural properties that underlie these variations. In addition, extrasynaptic GABA$_A$ receptors are also high-affinity targets for the actions of a range of endogenous and exogenous ligands including naturally occurring neurosteroids (Stell et al. 2003; Belelli and Lambert 2005), a number of anaesthetic agents (Meera et al. 2009; Houston et al. 2012; Orser et al. 1994) and alcohols (Wei et al. 2004; Jia et al. 2008; Yamashita et al. 2006). The actions of neurosteroids at GABA$_A$ receptors are now believed to play an important role in both normal and pathological stress responses as well as during the oestrous cycle and parturition (Maguire et al. 2005; Maguire and Mody 2008; see also Chap. 12). Several of these ligands including 3α,5α-tetrahydrodeoxycorticosterone (3α,5α-THDOC), which are synthesised de novo in the central nervous system, appear to have greater affinity for extrasynaptic δ-subunit-containing GABA receptors compared to γ-containing receptors and may therefore preferentially target these receptors at physiological neurosteroid concentrations. In Chap. 5, Connelly addresses the synthesis of neurosteroids and the evidence demonstrating their effects at GABA receptors in the brain. Indeed, the actions of steroids at GABA$_A$ receptors have been appreciated for a long time (Harrison and Simmonds 1984) and the steroid alphaxalone, which was used clinically as an anaesthetic for several years, has been shown to potentiate the effect of GABA upon GABA$_A$ receptors. In fact, more recent studies show that alphaxalone is more potent at GABA$_A$ receptors that contain δ subunits indicating that its anaesthetic action may be mediated largely

through extrasynaptic receptors. Other anaesthetic agents, for example, propofol, have also been demonstrated to act at extrasynaptic receptors and enhance tonic inhibition in hippocampus (Bai et al. 2001), thalamus (Jia et al. 2008) and cortex (Drasbek et al. 2007). This compound, a widely used intravenous anaesthetic, is a positive allosteric modulator of both α_5- and δ-subunit-containing $eGABA_ARs$.

As previously discussed, $eGABA_ARs$ are activated by GABA that accumulates in the extrasynaptic space—but how does this GABA build-up occur? The neurons that release GABA as their principal neurotransmitter are extraordinarily diverse and often highly specialised (Freund and Buzsaki 1996, Klausberger and Somogyi 2008); for example, interneurons of the dorsal lateral geniculate nucleus of the thalamus release GABA through both axonal and dendritic release sites (Cox and Sherman 1998, 2000). Thus, do common release mechanisms exist that are involved in introducing GABA to the extracellular space and what are the uptake mechanisms that regulate GABA concentration? When compared to their synaptic counterparts, $eGABA_ARs$ have a significantly greater affinity for GABA and can be easily activated by the relatively low levels (nM range) of GABA that are typically found in the extracellular space. Evidence suggests that the release of GABA required to permit such modest extracellular accumulation occurs not only via classical action potential-dependent vesicular release in neurons (Wall and Usowicz 1997; Bright et al. 2007) but also through non-vesicular release mechanisms, such as the Bestrophin-1 channel (Lee et al. 2010), in both neurons and glial cells (particularly astrocytes). Additionally, the GABA concentration in the extracellular space is tightly regulated through reuptake of GABA by GABA transporters (GAT 1 and 3) that are expressed in both neurons and glia (Minelli et al. 1995, 1996; Conti et al. 1998a, b; Jin et al. 2011; and only glia in some areas such as the thalamus, De Biasi et al. 1998) and intracellular breakdown by the enzyme GABA transaminase (GABA-T). Additionally, the local extracellular concentration is determined by the physical morphology surrounding GABA releases site which can encourage or limit the diffusion of GABA, for example, in the glomerular structures found in the thalamus and cerebellum (Wall and Usowicz 1997; Rossi et al. 2003; Errington et al. 2011). The source(s) of GABA that accumulates in the extracellular space and mechanisms underpinning vesicular and non-vesicular GABA release as well as GABA uptake and degradation processes are key determinants of tonic $GABA_A$ receptor currents in all brain areas and these are discussed in detail herein by Bright et al. (Chap. 6).

Like their synaptic counterparts and some other ligand-gated neurotransmitter receptors, evidence is emerging that highlights the functional modulation of $eGABA_ARs$ by a range of metabotropic receptors and their related effector molecules. For example, in the thalamus, dentate gyrus and cerebellum, it has been shown that $eGABA_ARs$ are postsynaptically modulated by metabotropic $GABA_B$ receptors through a mechanism involving adenylyl cyclase and cAMP-dependent kinase (protein kinase A, PKA) (Connelly et al. 2013; Tao et al. 2013). In the thalamus, in particular, modulation of $eGABA_ARs$ by $GABA_B$ receptors appears to occur even at ambient GABA levels since application of the antagonist CGP 55845 significantly reduces gabazine-sensitive tonic currents. Indeed, as demonstrated by elegant dynamic clamp experiments in brain slices, the modulatory effects of $GABA_B$

receptors upon eGABA$_A$Rs have a significant effect upon excitability of thalamic neurons (Connelly et al. 2013). The activation of eGABA$_A$Rs is highly sensitive to the extracellular GABA concentration which in turn is determined by activity-dependent release and reuptake (Rossi and Hamann 1998). Although not a direct postsynaptic effect on the magnitude of neuronal tonic currents, as described previously, presynaptic modulation of GABA release via metabotropic receptors also plays an important role in dynamically regulating tonic GABA inhibition in some brain regions (Errington et al. 2011). In Chap. 7, Di Giovanni et al. cover the most recent data demonstrating direct and indirect modulation of eGABA$_A$Rs and their function by metabotropic receptors such as GABA$_B$, serotonin, dopamine, noradrenalin and metabotropic glutamate receptors.

Despite these advances in understanding of the cellular distribution, regulation and pharmacology of eGABA$_A$Rs, our knowledge of the physiological roles of these receptors remains limited, a factor that should continue to stimulate research in this area for many years to come. However, we know that these receptors do have important physiological roles in several brain regions. For example, in TC neurons, it is thought that eGABA$_A$Rs can regulate action potential firing probability (Cope et al. 2005; Connelly et al. 2013), help to determine firing mode and modulate the temporal precision of burst firing (Bright et al. 2007). In addition, eGABA$_A$Rs have been demonstrated to have potentially important roles in cognitive processes such as the control of hippocampal gamma oscillations (Mann and Mody 2010) and in neuronal computational processes. In hippocampal CA1 pyramidal cells eGABA$_A$Rs mediate outwardly rectifying tonic GABA currents that have the effect of shifting the offset of the neuronal input–output curve without changing the gain, a process which is thought to stabilise information encoding in the hippocampus in response to dynamically changing input levels (Pavlov et al. 2009). Expression of eGABA$_A$Rs is not restricted only to the brain and eGABA$_A$Rs and tonic GABA inhibiton has also found in the spinal cord. A role for GABA in the spinal cord has been known since the late 1950s and early 1960s where the work of Curtis, Eccles and colleagues demonstrated the depression of excitatory potentials in motor neurons in response to GABA release from local interneurons onto GABA$_A$Rs on primary afferent terminals (Curtis et al. 1959; Curtis 1969; Eccles et al. 1963). Subsequently, in situ hybridization, reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry have been used to detect the presence of numerous GABA$_A$ receptors subunits in the spinal cord including α, β, γ, θ and ε types. Moreover, it has now been demonstrated in several spinal cord neuron types that these subunits form receptors that can mediate tonic GABA currents. In Chap. 8, Loeza-Alcocer et al. describe in detail the expression of GABA$_A$Rs in spinal cord and the evidence for the presence of tonic GABAergic signalling, and explore potential roles for these receptors in spinal physiology including their putative role in pain responses and motor control.

In the final part of this book, the role of eGABA$_A$Rs and tonic GABAergic inhibition in a range of pathological conditions including epilepsy, anxiety, stroke and neurodevelopmental disorders are covered. Changes in eGABA$_A$Rs expression and

function have been observed in many of these conditions and these are discussed in detail herein as well as the potential exploitation of $eGABA_A$Rs signalling for therapeutic purposes.

Stroke is a leading cause of mortality and morbidity in the developed world and despite heavy investment in development of prophylactic (to treat those deemed high risk) and restorative (to regain function post infarct) therapeutic agents most of the currently available treatments remain unsatisfactory. In fact, there are no currently available compounds used clinically to encourage functional recovery after ischaemic damage. As a result, stroke-induced loss of sensory and motor limb function results in almost one third of surviving stroke patients being unable to return to work and becoming institutionalized (Dobkin 2004, 2008; Lai et al. 2002; Ng et al. 2007). It has been suggested that the post-infarct brain has the capacity to recover and remodel in order to regain function. Neurons in the peri-infarct region display increased plasticity allowing re-mapping of sensorimotor function. However, this plasticity is dependent upon the balance of excitatory and inhibitory neurotransmission and excessive inhibitory function can act to impair the necessary post-infarct plasticity required to achieve functional recovery. A recent study demonstrated that excessive tonic $GABA_A$ergic inhibition is a key feature of neurons in the cortex surrounding the infarct site (peri-infarct) after induction of stroke in experimental models (Clarkson et al. 2010). The pathological increase in tonic inhibition was mediated by a reduction in expression of the GAT-3/4 GABA transporter. In the motor cortex, where the stroke was induced, $eGABA_A$Rs largely contain α_5 and δ subunits and the authors of this study showed that the α_5-subunit-selective benzodiazepine inverse agonist, L655–708, not only produced a significant reduction in the tonic current amplitude in peri-infarct neurons of slices from post-stroke animals but also improved the performance of animals in an in vivo motor task (Clarkson et al. 2010). These data bring hope that $eGABA_A$Rs may be effective targets for agents that could help facilitate plastic changes in the cortex post stroke and may be valuable in aiding recovery of sensorimotor function in patients. In Chap. 9, Clarkson and Chebib present these findings, amongst others, and discuss the potential of $eGABA_A$Rs becoming a target of choice for drug design in stroke therapy.

Epilepsy is a term used to describe a collection of neurological conditions of widely varying aetiology, symptoms and severity. Focal epilepsy can result from a range of causes including genetic mutations, brain tumours and malformations during cortical development or acute brain trauma including head injury and stroke. The common pathophysiological hallmark of all epilepsy syndromes, the seizure, is the result of aberrant synchronous and rhythmic discharges of populations of neurons that can occur across various brain regions. For several decades, it has been considered that a major factor in many types of epilepsy is the hyperexcitability of neurons caused by a loss of GABAergic inhibition (Schwarzkroin and Prince 1977; Gutnick et al. 1982; Connors 1984). The conventional view that seizures are caused by a loss of synaptic $GABA_A$ergic inhibition has arisen from several lines of evidence. In particular, a decrease in synaptic $GABA_A$ receptors has been observed in animal models of temporal lobe epilepsy and in vitro seizure-like activity in rodent brain slices can be induced by application of $GABA_A$ receptor antagonists such

as bicuculline. However, in recent years, this rather simplistic view of the role of GAB_AR-mediated inhibition in seizure genesis and epilepsy has been reappraised, not least owing to increased understanding of $eGABA_AR$-mediated tonic inhibition. In Chap. 10, Walker and Pavlov discuss a new theory for the role of $GABA_A$ergic inhibition in seizure genesis in acquired focal epilepsies that emphasises the importance of a shift in the balance of phasic and tonic GABA currents. Despite the apparent loss of phasic inhibition and a reduction in GABA release, experimental evidence from animal models of status epilepticus indicates that levels of tonic GABA inhibition are maintained or increased in hippocampal CA1 pyramidal neurons, stratum radiatum interneurons and dentate gyrus granule cells (Scimemi et al. 2005; Naylor et al. 2005; Zhang et al. 2007; Goodkin et al. 2008; Zhan and Nadler 2009; Rajasekaran et al. 2010; Pavlov et al. 2011). Walker and colleagues have proposed that the shift in $GABA_A$ inhibition from phasic to tonic results in neuronal networks that become inherently more unstable and that these networks have smaller operating windows in which inhibition can adequately compensate for significant changes in cellular excitation (Pavlov and Walker 2012). Loss of phasic inhibition could have a significant effect upon the gain of the neuronal input–output relationship and result in even small changes in excitability causing a large number of neurons to fire and recurrent hyperexcitability. Whilst a concurrent increase in $eGABA_AR$-mediated tonic inhibition may help to reduce these effects by shifting the offset of the input–output function, the inability of tonic inhibition to affect the gain (Pavlov et al. 2009) means it will be unable to prevent the transition to a seizure state once threshold is reached. It is thought that this might explain one of the common, yet paradoxical, observations that animals or patients suffering from severe epileptic seizures are able to spend the majority of their time seizure free.

Typical absence epilepsy is characterised by the regular occurrence of non-convulsive seizures that result in periods of sudden and brief (average ≈10 s, range ≈4–40 s) loss of consciousness. In the electroencephalogram (EEG), human absence seizures are typified by the appearance of generalized, synchronous and bilateral ‘spike (or polyspike) and slow wave discharges’ (SWD) occurring at frequencies between 2.5 and 4 Hz (Avoli et al. 2001; Crunelli and Leresche 2002). Although typical absence seizures are significant clinical features of many generalized idiopathic epilepsies (IGEs), as defined by the classification of the International League Against Epilespy (ILAE; ILAE 1989), they are the only neurological symptom presented in childhood absence epilepsy (CAE). In this pure absence epilepsy phenotype, there is a consensus, based upon older invasive studies and more recent imaging investigations, that seizure genesis and propagation occurs as a result of aberrant electrical activity in reciprocally connected thalamic and cortical regions (i.e. TC circuits) without significant involvement of other brain areas including hippocampus and limbic regions which are often associated with convulsive seizures (Holmes et al. 2004; Hamandi et al. 2006; Westmijse et al. 2009; Bai et al. 2010; Szaflarski et al. 2010). Recent work has demonstrated a pivotal role for $eGABA_ARs$ in TC neurons in the expression of spike-and-wave discharges in a number of genetic animal models of absence seizures as well as in the classical pharmacological model using GHB (Cope

et al. 2009). These new findings show that the generation and propagation of spike-and-wave discharges appear to be dependent upon a pathological increase in tonic GABA inhibition in TC neurons that results from reduced GABA uptake by GAT-1, which in thalamus is exclusively expressed by astrocytes (De Biasi et al. 1998). Whether this change in tonic inhibition in the thalamus produces hyper-synchronous firing or reduced TC neuron activity (and impaired relay of sensory information) remains to be seen. It is clear, however, animals missing δ-subunit-containing $eGABA_A$Rs are resistant to the induction of spike-and-wave discharges by THIP or GHB which highlights the importance of these receptors in this form of epilepsy (Cope et al. 2009). In Chap. 11, we summarise these data in detail.

$GABA_A$ receptors have long been known to play a significant role in stress and anxiety disorders. In fact, benzodiazepines that act at γ-subunit-containing synaptic $GABA_A$ receptors are commonly used clinically as anxiolytics despite having poor side-effect profiles. It is now becoming increasingly clear that $eGABA_A$Rs also play an important part in stress and anxiety through their ability to influence and control the hypothalamic–pituitary–adrenal (HPA) axis which mediates the body's physiological stress response. This is due to the fact that most of the key hypothalamic neurons that comprise the HPA receive powerful GABAergic inhibition (50% of synapses in hypothalamus are thought to be GABAergic) from a number of important brain regions including the hippocampus, prefrontal cortex, bed nucleus of the stria terminalis (BNST) and amygdala. For example, parvocellular secretary neurons of the hypothalamus release corticotrophin-releasing hormone (CRH) to regulate the output of the HPA axis and are themselves, in turn, influenced by robust GABAergic inhibition as a result of dense GABAergic inhibition (Miklos and Kovacs 2002). Evidence exists that the majority of CRH neurons express both the α_5 and δ $eGABA_A$Rs on their cell bodies (Pirker et al. 2000), and despite a lack of functional evidence this strongly indicates that these neurons are regulated by not only phasic but also tonic $GABA_A$ inhibition. In Chap. 12, Maguire discusses in detail the anatomy and physiology of the HPA, the expression and function of GABA receptors in its key neurons and the role that they play in stress and anxiety.

As well as playing potentially significant roles in the pathology of stroke, convulsive and non-convulsive epilepsies, stress and anxiety, growing evidence also suggests that $eGABA_A$Rs dysfunction may contribute to the cellular hyperexcitability underlying some neurodevelopmental disorders. In Chap. 13, Martin and Huntsman discuss the potential role of aberrant tonic GABAergic signalling in the neurodevelopmental disorder Fragile X Syndrome (FXS). This disorder, which results from the expansion of an unstable CGG trinucleotide repeat in the regulatory region of the *Fmr1* gene located in the X-chromosome, is a major cause of genetic intellectual disability and one of the leading causes of autism (Hagerman et al. 2009; Belmonte and Bourgeron 2006). Furthermore, FXS is also linked with other disorders including epilepsy and anxiety in which disrupted tonic $GABA_A$ inhibition or $eGABA_A$Rs function have been described. The genetic silencing of the *Fmr1* gene results in the absence of a protein, named Fragile X Mental Retardation Protein (FMRP), which is broadly expressed in GABAergic interneurons and

thought to play a role in normal interneuron development and function. As a result of the loss of FMRP, interneuron development is impaired and in particular the presynaptic machinery required for GABA release is affected. This has the effect of modifying not only phasic $GABA_A$-mediated inhibition but also producing a reduction on tonic GABA signalling as the result of chronic lowering of the GABA concentration in the extracellular space.

Thus, tonic GABAergic inhibition and $eGABA_ARs$ are now known to play a pivotal role in physiological and pathophysiological process throughout several brain regions. The distinct subunit composition of $eGABA_ARs$ gives them unique biophysical properties and pharmacological profiles that make them attractive novel targets in the treatment of a range of diseases. We hope that this book provides an insightful summary of the currently available data regarding $eGABA_ARs$ and tonic GABA inhibition for academics and students alike and that this might stimulate future research in this area, revealing new and exciting roles for these receptors.

References

Ade KK, Janssen MJ, Ortinski PI, Vicini S (2008) Differential tonic GABA conductances in striatal medium spiny neurons. J Neurosci 28(5):1185–1197

Agnati LF, Guidolin D, Guescini M, Genedani S, Fuxe K (2010) Understanding wiring and volume transmission. Brain Res Rev 64:137–159

Avoli M, Rogawski MA, Avanzini G (2001) Generalized epileptic disorders: an update. Epilepsia 42:445–457

Bai D, Zhu G, Pennefather P, Jackson MF, MacDonald JF, Orser BA. (2001) Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by gamma-aminobutyric acid(A) receptors in hippocampal neurons. Mol Pharmacol 59:814–824

Bai X, Vestal M, Berman R, Negishi M, Spann M, Vega C, Desalvo M, Novotny EJ, Constable RT, Blumenfeld H (2010) Dynamic time course of typical childhood absence seizures: EEG behaviour, and functional magnetic resonance imaging. J Neurosci 30:5884–5893

Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 6:565–575

Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ (2005) Extrasynaptic $GABA_A$ receptors of thalamocortical neurons: a molecular target for hypnotics. J Neurosci 25:11513–11520

Belmonte MK, Bourgeron T (2006) Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat Neurosci 9:1221–1225

Brickley SG, Mody I (2012) Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron 73:23–34

Brickley SG, Cull-Candy SG, Farrant M (1996) Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of $GABA_A$ receptors. J Physiol 497(Pt 3):753–759

Bright DP, Aller MI, Brickley SG (2007) Synaptic release generates a tonic GABA(A) receptor-mediated conductance that modulates burst precision in thalamic relay neurons. J Neurosci 27:2560–2569

Bright DP, Renzi M, Bartram J, McGee TP, Mackenzie G, Hosie AM, Farrant M, Brickley SG (2011) Profound desensitization by ambient GABA limits activation of delta-containing $GABA_A$ receptors during spillover. J Neurosci 31:753–763

Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA (2002). Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. Br J Pharmacol 136:965–974

Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA (2004) Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. Proc Natl Acad Sci U S A 101:3662–3667

Chebib M, Johnston GAR (2000) GABA-activated ligand gated ion channels: medicinal chemistry and molecular biology. J Med Chem 43:1427–1447

Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature 468:305–309

Commission on Classification and Terminology of the International League Against Epilepsy (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 30:10

Connelly WM, Fyson SJ, Errington AC, McCafferty CP, Cope DW, Di Giovanni G, Crunelli V (2013). $GABA_B$ receptors regulate extrasynaptic $GABA_A$ receptors. J Neurosci 33:3780–3785

Connors BW (1984) Initiation of synchronized neuronal bursting in neocortex. Nature 310:685–687

Conti F, DeBiasi S, Minelli A, Rothstein JD, Melone M (1998a) EAAC1, a high-affinity glutamate tranporter, is localized to astrocytes and gabaergic neurons besides pyramidal cells in the rat cerebral cortex. Cereb Cortex 8:108–116

Conti F, Melone M, De BS, Minelli A, Brecha NC, Ducati A (1998b) 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

Cope DW, Hughes SW, Crunelli V (2005) $GABA_A$ receptor-mediated tonic inhibition in thalamic neurons. J Neurosci 25:11553–11563

Cope DW, Di Giovanni G, Fyson S, Orbán G, Errington AC, Lörincz ML, Gould TM, Carter DA, Crunelli V (2009) Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. Nat Med 15:1392–1398

Cox CL, Sherman SM (1998) Glutamate locally activates dendritic outputs of thalamic interneurons. Nature 394:478–482

Cox CL, Sherman SM (2000) Control of dendritic outputs of inhibitory interneurons in the lateral geniculate nucleus. Neuron 27:597–610

Crunelli V, Leresche N (2002) Childhood absence epilepsy: genes, channels, neurons and networks. Nat Rev Neurosci 3:371–382

Curtis DR (1969) The pharmacology of spinal postsynaptic inhibition. Prog Brain Res 31:171–189

Curtis DR, Phillis JW, Watkins JC (1959) The depression of spinal neurones by gamma-amino-n-butyric acid and beta-alanine. J Physiol 146:185–203

De Biasi S, Vitellaro-Zuccarello L, Brecha NC (1998) Immunoreactivity for the GABA transporter-1 and GABA transporter-3 is restricted to astrocytes in the rat thalamus. A light and electron-microscopic immunolocalization. Neuroscience 83:815–828

Dobkin BH (2004) Strategies for stroke rehabilitation. Lancet Neurol 3(9):528–536

Dobkin BH (2008) Training and exercise to drive poststroke recovery. Nat Clin Pract Neurol 4(2):76–85

Drasbek KR, Jensen K (2006) THIP, a hypnotic and antinociceptive drug, enhances an extrasynaptic $GABA_A$ receptor-mediated conductance in mouse neocortex. Cereb Cortex 16:1134–1141

Drasbek KR, Hoestgaard-Jensen K, Jensen K (2007) Modulation of extrasynaptic THIP conductances by $GABA_A$ receptor modulators in mouse neocortex. J Neurophysiol 97:2293–2300

Eccles JC, Schmidt R, Willis WD (1963) Pharmacological studies on presynaptic inhibition. J Physiol 168:500–530

Errington AC, Di GG, Crunelli V, Cope DW (2011) mGluR control of interneuron output regulates feedforward tonic $GABA_A$ inhibition in the visual thalamus. J Neurosci 31:8669–8680

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 6:215–229

Freund TF, Buzsaki G (1996) Interneurons of the hippocampus. Hippocampus 6(4):347–470
Glykys J, Mody I (2006) Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABA A receptor alpha5 subunit-deficient mice. J Neurophysiol 95(5):2796–2807
Glykys J, Mody I (2007) The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. J Physiol 582.3:1163–1173
Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J (2008) Subunit-specific trafficking of GABA(A) receptors during status epilepticus. J Neurosci 28:2527–2538
Gutnick MJ, Connors BW, Prince DA (1982). Mechanisms of neocortical epileptogenesis in vitro. J Neurophysiol 48:1321–1335
Hagerman RJ, Berry-Kravis E, Kaufmann WE, Ono MY, Tartaglia N, Lachiewicz A, Kronk R, Delahunty C, Hessl D, Visootsak J, Picker J, Gane L, Tranfaglia M (2009) Advances in the treatment of fragile X syndrome. Pediatrics 123:378–390
Hamandi K, Salek-Haddadi A, Laufs H, Liston A, Friston K, Fish DR, Duncan JS, Lemieux L (2006) EEG-fMRI of idiopathic an secondarily generalized epilepsies. Neuroimage 31:1700–1710
Harrison NL, Simmonds MA (1984) Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res 323:287–292
Holmes MD, Brown M, Tucker DM (2004) Are "generalized" seizures truly generalized? Evidence of localized mesial frontal and frontopolar discharges in absence. Epilepsia 45:1568–1579
Houston CM, McGee TP, MacKenzie G, Troyano-Cuturi K, Rodriguez PM, Kutsarova E, Diamanti E, Hosie AM, Franks NP, Brickley SG (2012) Are extrasynaptic $GABA_A$ receptors important targets for sedative/hypnotic drugs? J Neurosci 32(11):3887–3897
Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, Goldstein PA (2005) An extrasynaptic $GABA_A$ receptor mediates tonic inhibition in thalamic VB neurons. J Neurophysiol 94:4491–4501
Jia F, Yue M, Chandra D, Homanics GE, Goldstein PA, Harrison NL (2008) Isoflurane is a potent modulator of extrasynaptic $GABA_A$ receptors in the thalamus. J Pharmacol Exp Ther 324:1127–1135
Jin XT, Pare JF, Smith Y (2011) Differential localization and function of GABA transporters, GAT-1 and GAT-3, in the rat globus pallidus. Eur J Neurosci 33:1504–1518
Klausberger T, Somogyi P (2008) Neuronal diversity and temporal dynamics: the unit of hippocampal cicuit operations. Science 321:53–57
Lai SM, Studenski S, Duncan PW, Perera S (2002) Persisting consequences of stroke measured by the Stroke Impact Scale. Stroke 33(7):1840–1844
Lee S, Yoon BE, Berglund K, Oh SJ, Park H, Shin HS, Augustine GJ, Lee CJ (2010) Channel-mediated tonic GABA release from glia. Science 330:790–796
Maguire J, Mody I (2008) $GABA_A$R plasticity during pregnancy: relevance to postpartum depression. Neuron 59:207–213
Maguire JL, Stell BM, Rafizadeh M, Mody I (2005). Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci 8:797–804
Mann EO, Mody I (2010) Control of hippocampal gamma oscillation frequency by tonic inhibtion and excitation of interneurons. Nat Neurosci 13(2):205–212
Meera P, Olsen RW, Otis TS, Wallner M (2009) Etomidate, propofol and the neurosteroid THDOC increase the GABA efficacy of recombinant α4β3δ and α4β3 $GABA_A$ receptors expressed in HEK cells. Neuropharmacology 56(1):155–160
Miklos IH, Kovacs KJ (2002) GABAergic innervation of corticotropin-releasing hormone (CRH)-secreting parvocellular neurons and its plasticity as demonstrated by quantitative immunoelectron microscopy. Neuroscience 113:581–592
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
Minelli A, DeBiasi S, Brecha NC, 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. J Neurosci 16:6255–6264

Mortensen M, Smart TG (2006) Extrasynaptic alphabeta subunit $GABA_A$ receptors on rat hippocampal pyramidal neurons. J Physiol 577: 841–856.
Mortensen M, Ebert B, Wafford K, Smart TG (2010) Distinct activities of GABA agonists at synaptic- and extrasynaptic-type $GABA_A$ receptors. J Physiol 588:1251–1268
Naylor DE, Liu H, Wasterlain CG (2005) Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. J Neurosci 25: 7724–7733.
Ng YS, Stein J, Ning M, Black-Schaffer RM (2007) Comparison of clinical characteristics and functional outcomes of ischemic stroke in different vascular territories. Stroke 38(8):2309–2314
Nusser Z, Mody I (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J Neurophysiol 87:2624–2628
Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693–1703
Olsen RW, Sieghart W (2008) International union of pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology and function. Update. Pharmacol Rev 60:243–260
Olsen RW, Sieghart W (2009). GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56(1):141–148
Orser BA, Wang LY, Pennefather PS, MacDonald JF (1994) Propofol modulates activation and desensitization of $GABA_A$ receptors in cultured murine hippocampal neurons. J Neurosci 14:7747–7760
Pavlov I, Walker MC (2012) Tonic GABA(A) receptor-mediated signalling in temporal lobe epilepsy. Neuropharmacology 69:55–61
Pavlov I, Savtchenko LP, Kullmann DM, Semyanov A, Walker MC (2009) Outwardly rectifying tonically active $GABA_A$ receptors in pyramidal cells modulate neuronal offset, not gain. J Neurosci 29:15341–15350
Pavlov I, Huusko N, Drexel M, Kirchmair E, Sperk G, Pitkänen A, Walker MC (2011). Progressive loss of phasic, but not tonic, GABA(A) receptor-mediated inhibition in dentate granule cells in a model of post-traumatic epilepsy in rats. Neuroscience 194:208–219
Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815–850
Rajasekaran K, Joshi S, Sun C, Mtchedlishvilli Z, Kapur J (2010). Receptors with low affinity for neurosteroids and GABA contribute to tonic inhibition of granule cells in epileptic animals. Neurobiol Dis 40:490–501
Richardson BD, Ling LL, Uteshev VV, Caspary DM (2011) Extrasynaptic GABA(A) receptors and tonic inhibition in rat auditory thalamus. PLoS ONE 6(1):e16508
Richardson BD, Ling LL, Uteshev VV, Caspary DM (2013) Reduced GABA(A) receptor-mediated tonic inhibition in aged rat auditory thalamus. J Neurosci 33(3):1218–1227
Rossi DJ, Hamann M (1998) Spillover-mediated transmission at inhibitory synapses promoted by high affinity alpha6 subunit GABA(A) receptors and glomerular geometry. Neuron 20:783–795
Rossi DJ, Hamann M, Attwell D (2003) Multiple modes of GABAergic inhibition of rat cerebellar granule cells. J Physiol 548:97–110
Schwartzkroin PA, Prince DA (1977). Penicillin-induced epileptilorm activity in the hippocampal in vitro preparation. Ann Neurol 1:463–469
Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker M (2005) Multiple and plastic receptors mediate tonic $GABA_A$ receptor currents in the hippocampus. J Neurosci 15:10016–10024
Semyanov A, Walker MC, Kullmann DM (2003) GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nat Neurosci 6:484–490
Semyanov A, Walker MC, Kullmann DM, Silver RA (2004) Tonically active GABA A receptors: modulating gain and maintaining the tone. Trends Neurosci 27:262–269
Sieghart W, Sperk G (2002) Subunit composition, distribution and function of $GABA_A$ receptor subtypes. Curr Top Med Chem 2:795–816
Sperk G, Schwarzer C, Tsunashima K, Fuchs K, Sieghart W (1997) $GABA_A$ receptor subunits in the rat hippocampus I: immunocytochemical distribution of 13 subunits. Neuroscience 80:987–1000

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing $GABA_A$ receptors. Proc Nat Acad Sci U S A 100:14439–14444

Szaflarski JP, Difrancesco M, Hirschauer T, Banks C, Privitera MD, Gotman J, Holland SK (2010) Cortical and subcortical contributions to absence seizure onset examined with EEG/fMRI. Epilepsy Behav 18(4):404–413

Tao W, Higgs MH, Spain WJ, Ransom CB (2013). Postsynaptic $GABA_B$ receptors nnhance extrasynaptic $GABA_A$ receptor function in dentate gyrus granule cells. J Neurosci 33:3738–3743

Wafford KA, Ebert B (2006) Gaboxadol—a new awakening in sleep. Curr Opin Pharmacol 6:30–36

Wall MJ, Usowicz MM (1997) Development of action potential-dependent and independent spontaneous $GABA_A$ receptor-mediated currents in granule cells of postnatal rat cerebellum. Eur J Neurosci 9:533–548

Wei W, Faria LC, Mody I (2004) Low ethanol concentrations selectively augment the tonic inhibition mediated by delta subunit-containing $GABA_A$ receptors in hippocampal neurons. J Neurosci 24:8379–8382

Westmijse I, Ossenblok P, Gunning B, van Luijtelaar G (2009) Onset and propagation of spike and slow wave discharges in human absence epilepsy: a MEG study. Epilepsia 50:2538–2548

Winsky-Sommerer R, Vyazovskiy VV, Homanics GE, Tobler I (2007) The EEG effects of THIP (Gaboxadol) on sleep and waking are mediated by the GABA(A)delta-subunit-containing receptors. Eur J Neurosci 25:1893–1899

Wlodarczyk A, Sylantyev S, Herd MB, Kersanté F, Lambert JJ, Rusakov DA, Linthorst ACE, Semyanov A, Belelli D, Pavlov I, Walker MC (2013). GABA-independent $GABA_A$ receptor openings maintain tonic currents. J Neurosci 33(9):3905–3914

Yamada J, Furukawa T, Ueno S, Yamamoto S, Fukuda A (2007) Molecular basis for the $GABA_A$ receptor-mediated tonic inhibition in rat somatosensory cortex. Cereb Cortex 17:1782–1787

Yamashita M, Marszalec W, Yeh JZ, Narahashi T (2006) Effects of ethanol on tonic GABA currents in cerebellar granule cells and mammalian cells recombinantly expressing $GABA_A$ receptors. J Pharmacol Exp Ther 319:431–438

Zhan R-Z, Nadler JV (2009). Enhanced tonic GABA current in normotopic and hilar ectopic dentate granule cells after pilocarpine-induced status epilepticus. J Neurophysiol 102:670–681

Zhang N, Wei W, Mody I, Houser CR (2007). Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. J Neurosci 27:7520–7531

Chapter 2
Extrasynaptic $GABA_A$ Receptors: Subunit Composition, Distribution, and Regulation

Jean-Marc Fritschy and Patrizia Panzanelli

Abstract Among the multiple $GABA_A$ receptor ($GABA_AR$) subtypes that are assembled from the 18 subunits encoded in the mammalian genome, several of them, notably those containing the subunits α4, α5, α6, and δ are preferentially located outside of the postsynaptic density and are activated tonically by GABA. Owing to their subunit composition, these extrasynaptic $GABA_AR$ have specific functional properties and pharmacological profiles that distinguish them from "postsynaptic" receptors. In this chapter, we summarize anatomical, developmental, and cell biological evidence that extrasynaptic $GABA_AR$ represent a specialized subset of $GABA_AR$ with specific functional roles and regulation mechanisms. Anatomically, the regional and cellular distribution of the subunits forming extrasynaptic $GABA_AR$ explains their enrichment in cerebellum, thalamus, hippocampus, dentate gyrus, striatum, olfactory bulb, and possibly spinal cord. Little is known about the ontogeny of these receptors during central nervous system (CNS) development and neurogenesis, but it is likely that extrasynaptic receptors on progenitor cells and differentiating neurons differ from their adult counterparts, notably owing to late onset of α6 and δ subunit expression. On the cell biological level, the extrasynaptic localization of receptors containing the α4, α5, and δ subunits depends on structural motifs that either prevent interaction with the postsynaptic scaffolding molecule gephyrin or allow interaction with radixin, a member of the ezrin/radixin/moesin family of actin-interacting proteins. Importantly, there is evidence indicating that regulation of $GABA_AR$ trafficking and cell-surface expression, mediated in part by protein phosphorylation mechanisms, allows for rapid changes in the ratio of extrasynaptic/postsynaptic receptors upon physiological and pathophysiological stimuli. A better understanding of the subcellular localization and regulation of extrasynaptic $GABA_AR$ will be required to unravel their multiple contributions to the control of neuronal network activity and behavior.

J.-M. Fritschy (✉)
Institute of Pharmacology and Toxicology, University of Zurich,
Winterthurerstrasse 190, 8057 Zürich, Switzerland
e-mail: fritschy@pharma.uzh.ch

P. Panzanelli
Department of Neuroscience Rita Levi Montalcini,
University of Turin, 10126 Turin, Italy

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_2

Keywords Postsynaptic density · Gephyrin · Hippocampal formation · Thalamus · Cerebellum · Immunoelectron microscopy · Immunofluorescence

2.1 Introduction

Extrasynaptic $GABA_AR$ mediate tonic inhibition upon activation by GABA spillover from synaptic sites as well as by ambient GABA in the extracellular space (Farrant and Nusser 2005; Glykys and Mody 2007; Brickley and Mody 2012). They regulate network excitability and synaptic plasticity, and are selectively modulated by neurosteroids and hypnotic/sedative drugs, such as 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP) and ethanol, as well as by several GABA super-agonists (Caraiscos et al. 2004a; Belelli and Lambert 2005; Belelli et al. 2005; Hosie et al. 2006; Mortensen et al. 2010). Although defined by their localization outside synaptic sites, extrasynaptic $GABA_AR$ are identified by their subunit composition (and hence functional and pharmacological properties) and are subject to multiple regulation mechanisms allowing for rapid changes in tonic inhibition of neurons upon activation by physiological and pathophysiological stimuli (Brickley and Mody 2012).

Although considerable attention has been given to extrasynaptic $GABA_AR$ containing the α4/δ subunits in the thalamus, striatum, and dentate gyrus, as well as α5 subunit in the hippocampus and α6 subunit in the cerebellum, there are still numerous aspects of their function, regulation, and physiological relevance remaining to be uncovered. Furthermore, the role of extrasynaptic $GABA_AR$ in the rest of the brain, as well as their developmental regulation during ontogeny and adult neurogenesis, remain largely undefined. Likewise, little is known about their specific cellular and subcellular distribution, and about the molecular mechanisms underlying their regulation and intracellular trafficking. The aim of this chapter is to provide an overview of the anatomical features and regulation of extrasynaptic $GABA_AR$ in the mammalian central nervous system (CNS).

2.2 Subunit Composition, Regional and Cellular Distribution of Extrasynaptic $GABA_AR$

Among the 18 $GABA_AR$ subunits encoded in the mammalian genome (Barnard et al. 1998), the α4, α5, and α6 subunits are generally considered to be assembled in receptor complexes localized extrasynaptically, along with a β subunit variant (often β2) and either the δ or the γ2 subunit. Almost nothing is known about the π and τ subunits, whereas the occurrence and distribution of the ρ1–3 subunits, which form the so-called $GABA_C$ receptors, are only poorly characterized in the brain and spinal cord (Schlicker et al. 2009) and have not been well studied in relation to post- and extrasynaptic sites. In the retina, ρ1 and ρ2 subunits have been reported to be localized postsynaptically (Enz et al. 1996), although there is functional evidence for $GABA_C$ receptor-mediated tonic inhibition (Jones and Palmer 2009).

The α1, α2, and α3 subunits along with β1–3 and γ2 form receptors that are for the most part postsynaptically localized (Sassoè-Pognetto et al. 2000), where they mediate phasic responses in response to vesicular GABA release, and therefore mediate most of the synaptic actions of GABA in the CNS. As discussed below, a fraction of these receptors might be localized extrasynaptically, for instance, due to their lateral diffusion in the membrane. Furthermore, a differential subcellular distribution of these receptors has been reported among various neuron subtypes, with the occurrence of extrasynaptic α3-$GABA_A$R, for example, in the amygdala and inferior olivary nucleus (Devor et al. 2001; Marowsky et al. 2012), and extrasynaptic α1-$GABA_A$R in a subset of cortical interneurons (Glykys et al. 2007; Schneider Gasser et al. 2007). In fact, the α1 subunit is rather promiscuous and contributes to a large variety of $GABA_A$R subtypes, along with the α3, α5, α6, and δ subunits, in particular. In the latter case, the corresponding receptors are composed of the subunits α1/β2/δ and they mediate tonic inhibition in cortical interneurons (Glykys et al. 2007). Additional extrasynaptic α1/δ-$GABA_A$R are formed in cerebellar granule cells, typically along with the α6 subunit (Jones et al. 1997). Studies of recombinant receptors containing the δ subunit unexpectedly revealed that its position within the pentameric complex is variable, being located either between α and β subunits or between two α subunits (Kaur et al. 2009). This finding stands in contrast to receptors containing the γ2 subunit, where its location next to an α subunit contributes to form the benzodiazepine-binding site (Gunther et al. 1995; Balic et al. 2009).

On the regional level, the distribution of the α4, α5, α6, and δ subunits in the rodent brain has been described in detail in numerous original publications (Fritschy and Mohler 1995; Pirker et al. 2000; Schwarzer et al. 2001), and verified with the corresponding knockout mice (Homanics et al. 1997a; Peng et al. 2002; Caraiscos et al. 2004b; Chandra et al. 2006). Each of them has a unique distribution pattern, ranging from being restricted to a single cell type (mature cerebellar and cochlear granule cells for the α6 subunit; Baude et al. 1992; Gutierrez et al. 1996) to being predominant in the forebrain (α4) or widely distributed across the entire neuraxis (α5). There is considerable overlap between α4 and δ, notably in the thalamus, striatum, and dentate gyrus (Benke et al. 1997; Sur et al. 1999; Peng et al. 2002; Sun et al. 2004), and also between α5 and δ (striatum, olfactory bulb; Fritschy and Mohler 1995; Ade et al. 2008). Taken together, these four subunits as well as α1 account for the multiple distinct $GABA_A$R subtypes having in common their extrasynaptic localization. The most prevalent of these receptors include α1/α6 and α1/α6/δ in the granule cell layer of the cerebellum (Bahn et al. 1996; Nusser et al. 1996a); α4/δ in the thalamus, striatum, and hippocampal formation (Sun et al. 2004; Jia et al. 2005; Mann and Mody 2010); α1/α5 or α5 only in the hippocampal formation (Crestani et al. 2002; Prenosil et al. 2006; Bonin et al. 2007; Glykys et al. 2008; Balic et al. 2009), deep cortical layers, olfactory bulb, and most of the brainstem and spinal cord (Serwanski et al. 2006); and α1, a3 and a5/δ in the striatum (Luscher et al. 1997; Santhakumar et al. 2010), olfactory bulb (Fritschy and Mohler 1995), and amygdala (Marowsky et al. 2004). Overall, these features can be appreciated best from the comparison of the regional distribution patterns of the α1, α4, α5, and

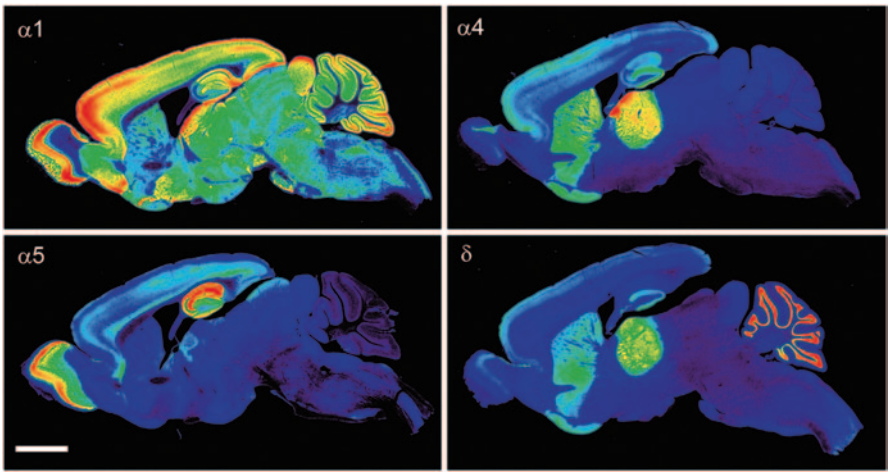

Fig. 2.1 Comparative regional distribution of four subunits (α1, α4, α5, δ) contributing to predominant extrasynaptic $GABA_AR$ subtypes in the brain of adult mice, displayed in false-color images of immunoperoxidase-stained parasagittal sections. Warm colors (*red, orange*) represent high relative staining intensity; cold colors (*violet, blue*) represent low staining intensity. Scale bar, 2 mm. (The materials illustrated are taken from Zheng et al. 2009)

δ subunits (Fig. 2.1). In the spinal cord, the α4 and δ subunits are virtually absent (Paul et al. 2012); evidence for the presence of extrasynaptic $GABA_ARs$ is mainly indirect and derived from pharmacological enhancement of GABAergic transmission by benzodiazepines and neurosteroids in the dorsal horn (Mitchell et al. 2007; Muller et al. 2008; Maeda et al. 2010; Nishikawa et al. 2011), which suggest that extrasynaptic $GABA_AR$ might contribute to the hypnotic and analgesic actions of these drugs, respectively. However, their subunit composition and cellular distribution are not fully determined, although the α2 and α3 subunits are most abundant in the substantia gelatinosa and the α5 subunit in Rexed laminae III and IV (Bohlhalter et al. 1996; Paul et al. 2012).

Note that all extrasynaptic $GABA_AR$ also contain at least one β subunit variant, which has not been identified in most cases, except for α4/β2/δ $GABA_AR$ in the dentate gyrus (Herd et al. 2008) and presumably the thalamus. The presence of a δ or γ subunit in extrasynaptic receptors is not mandatory; for instance, receptors assembled by α/β subunits only, which are highly sensitive to Zn^{2+} and form low conductance channels, have been characterized in hippocampal neurons (Mortensen and Smart 2006). Almost nothing is known about extrasynaptic $GABA_AR$ in the human brain, as the distribution of the α4, α5, and δ subunit proteins have not been mapped so far, to our knowledge.

It is generally considered that for $GABA_AR$ to mediate phasic inhibition upon vesicular GABA release, they have to be concentrated in the postsynaptic density (PSD) by means of interactions with scaffolding proteins, in particular gephyrin, the main scaffolding protein of GABAergic and glycinergic synapses (Fritschy et al. 2008). It should be noted, however, that such receptors can diffuse laterally

in the plasma membrane and participate in a constitutive cycle of externalization and endocytosis (Thomas et al. 2005; Bannai et al. 2009; Mukherjee et al. 2011), thereby blurring the distinction between postsynaptic and extrasynaptic receptors based solely on subcellular localization. However, the contribution of such receptors to tonic inhibition is not known, and their definition as "postsynaptic" receptors mainly reflects their ability to interact with specific proteins of the GABAergic PSD and to aggregate postsynaptically (Sassoè-Pognetto et al. 2000; Fritschy et al. 2012). There is considerable evidence that these properties are imparted to a large degree by a γ subunit variant (notably γ2 or γ3) in the pentameric complex (Gunther et al. 1995; Baer et al. 1999; Schweizer et al. 2003), although the presence of these subunits does not necessarily imply that the receptor is aggregated postsynaptically. It is even likely that a majority of γ2 subunit-containing receptors are located extrasynaptically in most neurons, along with α1, α3, and α5 subunits. Recent work also revealed that the various α subunit variants contain sequence motifs determining the extra- or postsynaptic localization of the pentameric complex (Wu et al. 2012), as well as their binding affinity to gephyrin (Tretter et al. 2008, 2011; Mukherjee et al. 2011).

In light microscopy studies, the localization at pre- and postsynaptic sites can only be inferred from immunostaining of proteins known to be restricted to these compartments. For GABAergic synapses, and hence for assessing "postsynaptic" $GABA_AR$, markers of choice include the vesicular GABA transporter (VGAT) or glutamic acid decarboxylase (GAD) for presynaptic terminals, and gephyrin or neuroligin-2 (NL2) for postsynaptic sites. Both of these proteins appear to be selectively clustered within the PSD of GABAergic synapses (and glycinergic synapses in the case of gephyrin), as confirmed ultrastructurally (Sassoè-Pognetto et al. 2000). Therefore, apposition between $GABA_AR$ subunit immunofluorescence staining and VGAT-positive terminals, as well as colocalization between $GABA_AR$ subunit staining and gephyrin or NL2, is typically taken as an evidence for a postsynaptic localization (Brünig et al. 2002b; Panzanelli et al. 2011; Fritschy et al. 2012). While confocal microscopy analysis allows distinguishing between apposition and colocalization, such results need to be interpreted with caution, as the size of synapses is below the resolution of this technique. Furthermore, there is no proof that gephyrin is present at every GABAergic synapse, and consequently, the absence of colocalization between a $GABA_AR$ subunit and gephyrin is only indicative of a possible extrasynaptic localization (Panzanelli et al. 2004; Lagier et al. 2007). Nevertheless, the validity of these approaches by light microscopy has been confirmed in studies using targeted deletion of $GABA_AR$ subunits (Kralic et al. 2006; Pallotto et al. 2012).

Beyond the dichotomy postsynaptic–extrasynaptic $GABA_AR$, based on their localization relative to the PSD and on their role in mediating phasic and tonic GABAergic currents, there also are $GABA_AR$ located on axons and presynaptic terminals (Grasshoff et al. 2007; Trigo et al. 2008; Long et al. 2009; Paul et al. 2012). Those on the axon initial segment, typically clustered in rows of prominent synapses innervated by axo-axonic interneurons, can be considered bona fide "postsynaptic receptors" (Nusser et al. 1996b; Fritschy et al. 1998), whereas those on distal axons and terminals, often undetectable by immunohistochemistry, would

be classified as extrasynaptic, although their roles presumably include control of action potential transmission, constraining plasticity, neuronal synchronization, regulation of transmitter release, and mediation of presynaptic afferent depolarization. However, because they are largely undetectable morphologically, their subunit composition is unknown. We have recently characterized a population of $GABA_AR$ on primary afferent terminals in the spinal cord that fulfill the criteria for "postsynaptic receptors," i.e., apposition to a GABAergic terminal immunopositive for VGAT and colocalization with gephyrin clusters (Paul et al. 2012). These examples illustrate the functional heterogeneity of $GABA_AR$ and the importance of precisely establishing their subcellular localization to decipher their contribution to the regulation of neuronal activity.

Extrasynaptic $GABA_AR$ containing the δ subunit are the targets of neurosteroids (Mihalek et al. 1999; Porcello et al. 2003; Herd et al. 2007; Smith et al. 2007) and can be activated by low concentrations of drugs acting as super-agonists at the GABA-binding sites, as well as taurine and γ-hydroxybutyrate (Jia et al. 2008; Halonen et al. 2009; Herd et al. 2009; Wafford et al. 2009; Absalom et al. 2014). Therefore, they can be identified pharmacologically and/or their distribution mapped by autoradiography (Halonen et al. 2009; Absalom et al. 2014). However, since such autoradiographic maps do not match exactly the distribution of any of the 18 $GABA_AR$ subunits, it is generally assumed that a combination of several subunits defines pharmacologically distinct $GABA_AR$. In addition, extrasynaptic $GABA_AR$ containing the α4 and α6 subunits are insensitive to the classical benzodiazepine-binding-site ligands, such as diazepam, but have a high affinity for the inverse agonist Ro-154513. These properties are imparted by the presence of an arginine residue at the canonical position 101 (of the α1 subunit) instead of a histidine found in subunits forming diazepam-sensitive $GABA_AR$ (Wieland et al. 1992; Benson et al. 1998). This pharmacological profile again provides the means for autoradiographic mapping of diazepam-insensitive $GABA_AR$. Such maps readily demonstrate that the highest concentration of these receptors, which correspond to a major subset of extrasynaptic $GABA_AR$, is found in the cerebellar granule cell layer, followed by the dentate gyrus, thalamus, and rest of the brain (Rudolph et al. 1999). While such comparisons of the relative abundance of $GABA_AR$ subunits are not possible from immunohistochemical staining (where differential affinity of antibodies for their target preclude direct quantitative comparisons; see Fig. 2.1), these data are in line with estimates of the relative abundance of subunit proteins determined by immunoprecipitation and Western blotting (Benke et al. 1991, 1997; Sieghart and Sperk 2002). Taken together, the abundance of extrasynaptic $GABA_AR$ with an atypical pharmacological profile is low compared to the total number of $GABA_ARs$ in the CNS. However, because the tonic conductance they mediate represents most of the current charge transferred by $GABA_AR$, their functional relevance cannot be overstated (Glykys and Mody 2007; Brickley and Mody 2012).

On the cellular level, it is probably fair to say that no single neuron population expresses only extrasynaptic $GABA_AR$. Rather, one or several $GABA_AR$ subtypes mediating phasic inhibition are also present in any case, such as α1/β2/γ2 receptors in the thalamic relay neurons expressing high levels of α4/δ-$GABA_AR$ (Kralic et al.

2006; Peden et al. 2008); α1- and α2-$GABA_AR$ in the hippocampus and dentate gyrus (Panzanelli et al. 2011); α1-, α2-, and α3-$GABA_AR$ in the cerebral cortex and olfactory bulb (where α4, α5, and δ subunits are expressed); and α2-$GABA_AR$ in spinal cord motoneurons (also expressing α5 subunit; Fritschy and Mohler 1995). Conversely, however, there are neurons, such as Purkinje cells in the cerebellum, in which no GABAergic tonic inhibition can be detected electrophysiologically, and which appear to express a single α subunit variant, suggesting that they lack "extrasynaptic" $GABA_AR$ (Fritschy et al. 2006; Patrizi et al. 2008). Overall, this situation raises the issue how multiple $GABA_AR$ subtypes are assembled, segregated, and regulated in neurons. We will address these questions in Sect. 2.4 of this chapter.

2.3 Ultrastructural Studies of the Subcellular Distribution of Extrasynaptic $GABA_AR$

There are only a few studies reporting the ultrastructural distribution of $GABA_AR$ subunits. In part, the underlying reason is that preembedding techniques fail to detect a majority of epitopes located at postsynaptic sites (Somogyi et al. 1989; Soltesz et al. 1990). Therefore, postembedding techniques, e.g., using Lowicryl-embedded tissue, are required for studying postsynaptic $GABA_AR$ (Baude et al. 1992; Nusser and Somogyi 1994; Somogyi et al. 1996). In this case, however, the majority of antibodies available lack sensitivity and, therefore, information is available for the α1, α2, α4, α5, α6, β2, 3, δ, and γ2 subunits only in selected brain areas (such as cerebral cortex, hippocampus, olfactory bulb, pallidum, thalamus, cerebellum, and spinal cord) (Nusser and Somogyi 1994; Nusser et al. 1995; Somogyi et al. 1996; Nusser et al. 1998, 1999; Hajos et al. 2000; Tretter et al. 2001; Wei et al. 2003; Panzanelli et al. 2004; Peng et al. 2004; Fritschy et al. 2006; Serwanski et al. 2006). Due to the low sensitivity of the method, a quantitative analysis of the fraction of postsynaptic versus extrasynaptic receptors was not readily feasible (Nusser and Somogyi 1994), and most studies focused on post- and perisynaptic receptors. These reports confirmed light microscopy analyses that α4 and δ subunits are not concentrated postsynaptically, but are located peri- and extrasynaptically under basal conditions in wild-type mice (Peng et al. 2002; Wei et al. 2003). Evidence for regulated distribution of these subunits came from the analysis of a model of temporal lobe epilepsy, in which a decrease in δ subunit at perisynaptic sites was compensated by an increase in α4 and γ2 subunits at these locations, at the expense of postsynaptic γ2 subunit localization (Peng et al. 2004).

In contrast to the α4 and δ subunits, the α5 subunit, which shows no obvious colocalization with gephyrin in the hippocampal formation (Brünig et al. 2002a), has been reported to be present in a subset of GABAergic synapses by postembedding electron microscopic (EM) analysis (Serwanski et al. 2006). This result is in line with electrophysiological studies, demonstrating the existence of postsynaptic $GABA_AR$ insensitive to zolpidem (a benzodiazepine site ligand devoid of affinity for α5-$GABA_AR$; Thomson and Jovanovic 2010) and the contribution of α5-$GABA_AR$ in the CA1 area

of the hippocampus to "slow" inhibitory postsynaptic potentials (IPSPs) (Prenosil et al. 2006; Zarnowska et al. 2009; Vargas-Caballero et al. 2010), generated by a specialized subset of interneurons (Ivy and neurogliaform cells; Armstrong et al. 2012).

More recently, immunolabeling of membrane proteins in freeze-fracture replica after sodium dodecyl sulfate (SDS) digestion provided unprecedented sensitivity for the detection of $GABA_AR$ subunits and their spatial relationships relative to postsynaptic sites (Kasugai et al. 2010). These studies revealed that while the density of immunogold labeling for the α1, α2, and β2,3 subunits is much higher postsynaptically than extrasynaptically, the majority of these subunits nevertheless are located extrasynaptically in dendritic and somatic membranes of CA1 pyramidal cells. As noted above, it is not known which fraction of these extrasynaptic receptors is functionally mediating tonic inhibition.

2.4 Regulation of Extrasynaptic $GABA_AR$ Expression During Ontogeny and Neurogenesis

$GABA_ARs$ are expressed at the early stages of fetal brain development by neural precursor cells and during neuronal differentiation, and have been proposed to contribute to the regulation of cell proliferation, migration, and differentiation, possibly involving Ca^{2+}-mediated signals activated by neuronal depolarization (Loturco et al. 1995; Serafini et al. 1998a; Behar et al. 2000; Maric et al. 2001; Ben-Ari 2002). The specific contribution of any $GABA_AR$ subtypes of a defined subunit composition has not been established, and the likelihood of functional redundancy is rather high, since none of the $GABA_AR$ subunit knockouts analyzed so far (α1–α6, β2, β3, δ, γ2) exhibit detectable deficits in brain general architecture at birth; even β3-ko mice, which have cleft palate and a severe behavioral phenotype, show no obvious brain anomaly (Homanics et al. 1997b; Ferguson et al. 2007). Likewise, the contribution of postsynaptic versus extrasynaptic $GABA_AR$ to brain developmental mechanisms or to initial formation of neuronal networks has remained elusive. However, it is well established that the subunit composition of the predominant $GABA_AR$ subtypes changes during the period of synaptogenesis, accounting in part for the distinct functional and pharmacological properties of $GABA_AR$ in neonatal and mature brain (Fritschy et al. 1994; Paysan et al. 1997; Hutcheon et al. 2000; Bosman et al. 2002; Fagiolini et al. 2004; Peden et al. 2008).

Overall, there is little information available on the developmental expression pattern of the subunits that contribute to the main extrasynaptic $GABA_AR$ of the adult brain (Paysan et al. 1997; Smith et al. 2009; Santhakumar et al. 2010), except for classical in situ hybridization and biochemical studies pointing to early expression of the α4 and α5 subunits at late fetal stages, along with the α3 subunit (Laurie et al. 1992; Poulter et al. 1992; Serafini et al. 1998b). In contrast, the α1 and δ subunits appear mainly postnatally during the period of synaptogenesis (second–third postnatal week in rodents; Paysan et al. 1994; Luscher et al. 1997). Furthermore, downregulation of the α5 subunit in layer 4 of the neocortex and its replacement by

the α1 subunit has been shown to be dependent on the integrity of thalamocortical projection (Paysan et al. 1997). In cerebellum, the α6 subunit also appears at late stages of granule cell differentiation (Varecka et al. 1994; Tia et al. 1996), along with the δ subunit (Muller et al. 1994). Accordingly, it is undetectable in the outer granule cell layer and forms a distinct outside-in gradient in the inner granule cell layer (Mellor et al. 1998) that disappears when migration of granule cells is complete. In human neocortex, the GABAergic system develops during the second half of pregnancy and infancy (Xu et al. 2011), and the expression of $GABA_AR$ subunit genes appears to be coordinated based on their respective chromosomal localization (Fillman et al. 2010), with distinct up- and downregulation patterns suggestive of differential expression of specific $GABA_AR$ subtypes.

Adult neurogenesis, taking place in the subventricular zone of the lateral ventricles and in the subgranular zone of the dentate gyrus, offers an attractive paradigm for investigating the contribution of $GABA_AR$, extrasynaptic and postsynaptic, to the regulation of major steps in the formation of new neurons, from proliferation to functional integration into preexisting synaptic circuits (Wang et al. 2003; Liu et al. 2005; Cuzon et al. 2006; Lledo et al. 2006; Duveau et al. 2011; Nissant and Pallotto 2011). Using full or Cre recombinase-mediated conditional deletion of specific $GABA_AR$ subunits, we have unraveled distinct contributions of extrasynaptic and postsynaptic $GABA_AR$ subtypes to these processes. Thus, neuroprogenitor cell proliferation, as measured by pulse-chasing with BrdU, is increased in α4-ko mice (Duveau et al. 2011), in line with a negative feedback function of ambient GABA in the neurogenic niche mediated by extrasynaptic $GABA_AR$ (Wang et al. 2003). Additionally, adult-born neurons in α4-ko mice failed to complete their migration into the granule cell layer and exhibited reduced dendritic growth that persisted at the completion of maturation. Electrophysiological recordings in 14–20-day-old mutant neurons revealed no bicucculine or picrotoxin-sensitive tonic currents (Duveau et al. 2011), suggesting a major role of the α4 subunit for assembly of extrasynaptic $GABA_AR$ in immature adult-born neurons. Accordingly, none of these phenotypes was observed in δ-ko mice, in line with the delayed onset of this subunit during ontogenic development. In contrast, targeted deletion of the α2 subunit affected the maturation of dendrites and the formation of glutamatergic synapses at advanced stages of differentiation in both dentate gyrus and olfactory bulb granule cells (Duveau et al. 2011; Pallotto et al. 2012). In the latter, abolition of postsynaptic GABAergic currents by the *Gabra2* deletion precluded any activity-dependent morphological plasticity in adult-born granule cells (Pallotto et al. 2012), pointing to a major role of GABAergic synaptic inhibition in this process.

Taken together, these observations suggest that $GABA_AR$ expressed by neural precursor cells and differentiating neurons likely differ in subunit composition (and functional properties) compared to those emerging at the end of maturation, when synaptogenesis is already well advanced. The former presumably play a key role in regulating proliferation, migration, and early steps of neuronal differentiation, whereas the latter regulate excitability and signal integration in synaptically interconnected neurons.

2.5 Trafficking and Synaptic Targeting of Extrasynaptic $GABA_AR$

Despite the promiscuity of $GABA_AR$ subtypes within individual neurons, several lines of evidence suggest that they are not functionally interchangeable and are therefore mediating specific roles at precise subcellular sites. For instance, thalamic relay neurons in the ventrobasal complex of adult mice express α1/β2/γ2-$GABA_AR$ at postsynaptic sites, colocalized with gephyrin, and mediate fast phasic spontaneous and evoked GABAergic currents (Peden et al. 2008). These cells also express α4/β2/δ-$GABA_AR$ that are not colocalized with gephyrin (Kralic et al. 2006), but are responsible for tonic inhibition in these cells, as shown by targeted deletion of the α4 subunit (Suryanarayanan et al. 2011). In α1-ko mice, miniature GABAergic inhibitory postsynaptic currents (IPSCs) are completely abolished in mature relay cells and gephyrin postsynaptic clustering is disrupted, with gephyrin forming large intracellular aggregates at nonsynaptic sites (Kralic et al. 2006; Peden et al. 2008). Tonic inhibition is enhanced, pointing to compensatory adaptations, but staining for the α4 subunit revealed no association with gephyrin and no enrichment at postsynaptic sites. These observations suggest that interaction with gephyrin or other proteins of the GABAergic PSD is a prerequisite for postsynaptic clustering of $GABA_AR$, which is lacking in α4/β2/δ-$GABA_A$Rs in these neurons. In turn, gephyrin also appears to depend on such interactions for being able to form a postsynaptic cluster in GABAergic synapses. While the underlying molecular mechanisms are not yet clarified, these interactions likely involve neuroligins and collybistin, a guanine nucleotide exchange factor belonging to the Dbl family and selectively activating cdc-42 (Poulopoulos et al. 2009; Fritschy et al. 2012).

In neurons, such as the hippocampal pyramidal cells, where α5-$GABA_AR$ mediate tonic inhibition, postsynaptic receptors are assembled by the α1 and/or α2 subunits (Panzanelli et al. 2011). There again, targeted deletion of the α2-subunit partially disrupts gephyrin clustering, notably on the soma and proximal dendrites of principal cells, and is not compensated for by an increased formation of postsynaptic α5-$GABA_AR$, despite a compensatory increase in α5-subunit-immunoreactivity in these cells (Panzanelli et al. 2011). This observation underscores the complex regulation of $GABA_AR$ subtypes, suggesting rules for synapse-specific targeting, in view of the fact that a subset of α5-$GABA_AR$ are postsynaptic in hippocampal pyramidal cells. The factors underlying this specificity in various types of synapses are not yet identified.

However, cell-surface expression and function of extrasynaptic $GABA_AR$ is known to be regulated in a subtype-specific manner by phosphorylation-dependent mechanisms (Harney et al. 2003; Abramian et al. 2010). In particular, α5-$GABA_AR$ selectively interact with activated radixin (Loebrich et al. 2006), a member of the ezrin–radixin–moesin family, known to link the actin cytoskeleton and plasma membrane proteins and/or to act as signal transducers in cellular responses involving cytoskeletal remodeling. Additional mechanisms for stabilization of $GABA_AR$ at extrasynaptic sites might involve direct interaction with other transmembrane receptors,

as reported in the spinal cord, where cell-surface dynamics of $GABA_AR$ are modulated via direct interactions with purinergic $P2X_2$ receptors (Shrivastava et al. 2011). These examples, and notably the need for phosphorylation-dependent activation of radixin for binding to the α5 subunit, underscore the fact that plasma membrane localization of extrasynaptic $GABA_AR$ is likely a tightly regulated process.

Overall, it will be of major importance to unravel the mechanisms underlying regulation and synaptic targeting of post- and extrasynaptic $GABA_AR$, notably in view of the fact that ethanol, neurosteroids and stress hormones cause profound changes in the balance between phasic and tonic inhibition by altering $GABA_AR$ trafficking and cell-surface expression (Maguire and Mody 2008; Gunn et al. 2011; Hines et al. 2012; Suryanarayanan et al. 2011). Therefore, abnormal regulation of extrasynaptic $GABA_AR$ function or trafficking might either contribute to the symptoms of pathophysiological states in which neurosteroids and stress hormones are implicated, or occur upstream of these pathological states and therefore represent an attractive target for disease-modifying therapy.

Conclusions

This brief overview highlights how most of the knowledge available to date on extrasynaptic $GABA_AR$ is derived from pharmacological and functional studies, whereas much remains to be done to elucidate their precise cellular and subcellular localization, their contribution to CNS development, and the mechanisms underlying their regulation under physiological and pathophysiological conditions.

References

Abramian AM, Comenencia-Ortiz E, Vithlani M, Tretter EV, Sieghart W, Davies PA, Moss SJ (2010) Protein kinase C phosphorylation regulates membrane insertion of $GABA_A$ receptor subtypes that mediate tonic inhibition. J Biol Chem 285:41795–41805

Absalom N, Eghorn LF, Villumsen IS, Karim N, Bay T, Olsen JV, Knudsen GM, Bräuner-Osborne H, Frølund B, Clausen RP, Chebib M, Wellendorph P (2014) α4βδ $GABA_A$ receptors are high-affinity targets for γ-hydroxybutyric acid (GHB). Proc Natl Acad Sci U S A 87(2):220–228

Ade KK, Janssen MJ, Ortinski PI, Vicini S (2008) Differential tonic GABA conductances in striatal medium spiny neurons. J Neurosci 30:1185–1197

Armstrong CM, Krook-Magnuson E, Soltesz I (2012) Neurogliaform and Ivy cells: a major family of nNOS expressing GABAergic neurons. Front Neural Circuits 6:23

Baer K, Essrich C, Benson JA, Benke D, Bluethmann H, Fritschy JM, Luscher B (1999) Postsynaptic clustering of γ-aminobutyric acid type A receptors by the γ3 subunit in vivo. Proc Natl Acad Sci U S A 96:12860–12865

Bahn S, Harvey RJ, Darlison MG, Wisden W (1996) Conservation of γ-aminobutyric acid type A receptor α6 subunit gene expression in cerebellar granule cells. J Neurochem 66:1810–1818

Balic E, Rudolph U, Fritschy JM, Mohler H, Benke D (2009) The α5(H105R) mutation impairs α5 selective binding properties by altered positioning of the α5 subunit in $GABA_A$ receptors containing two distinct types of α subunits. J Neurochem 110:244–254

Bannai H, Lévi S, Schweizer C, Inoue T, Launey T, Racine V, Sibarita JB, Mikoshiba K, Triller A (2009) Activity-dependent tuning of inhibitory neurotransmission based on $GABA_A$R diffusion dynamics. Neuron 62:670–682

Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ (1998) International Union of Pharmacology. XV. Subtypes of γ-aminobutyric $acid_A$ receptors: classification on the basis of subunit structure and function. Pharmacol Rev 50:291–313

Baude A, Sequier JM, McKernan RM, Oliver KR, Somogyi P (1992) Differential subcellular distribution of the alpha6 subunit versus the alpha1 and beta2/3 subunits of the $GABA_A$/benzodiazepine receptor complex in granule cells of the cerebellar cortex. Neuroscience 51:739–748

Behar TN, Schaffner AE, Scott CA, Greene CL, Barker JL (2000) $GABA_A$ receptor antagonists modulate postmitotic cell migration in slice cultures of embryonic rat cortex. Cereb Cortex 10:899–909

Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the $GABA_A$ receptor. Nat Rev Neurosci 6:565–575

Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ (2005) Extrasynaptic $GABA_A$ receptors of thalamocortical neurons: a molecular target for hypnotics. J Neurosci 25:11513–11520

Ben-Ari Y (2002) Excitatory actions of GABA during development: the nature of the nurture. Nat Rev Neurosci 3:728–739

Benke D, Mertens S, Trzeciak A, Gillessen D, Mohler H (1991) Identification and immunohistochemical mapping of $GABA_A$ receptor subtypes containing the δ-subunit in rat brain. FEBS Lett 283:145–149

Benke D, Michel C, Mohler H (1997) $GABA_A$ receptors containing the α4-subunit: prevalence, distribution, pharmacology, and subunit architecture in situ. J Neurochem 69:806–814

Benson JA, Low K, Keist R, Mohler H, Rudolph U (1998) Pharmacology of recombinant gamma-aminobutyric $acid_A$ receptors rendered diazepam-insensitive by point-mutated α-subunits. FEBS Lett 431:400–404

Bohlhalter S, Weinmann O, Mohler H, Fritschy JM (1996) Laminar compartmentalization of $GABA_A$-receptor subtypes in the spinal cord. J Neurosci 16:283–297

Bonin RP, Martin LJ, Macdonald JF, Orser BA (2007) α5–$GABA_A$receptors regulate the intrinsic excitability of mouse hippocampal pyramidal neurons. J Neurophysiol 98:2244–2254

Bosman LWJ, Rosahl TW, Brussaard AB (2002) Neonatal development of the rat visual cortex: synaptic function of $GABA_A$ receptor α subunits. J Physiol 545:169–181

Brickley SG, Mody I (2012) Extrasynaptic $GABA_A$ receptors: their function in the CNS and implications for disease. Neuron 12:23–34

Brünig I, Scotti E, Sidler C, Fritschy JM (2002a) Intact sorting, targeting, and clustering of γ-aminobutyric acid A receptor subtypes in hippocampal neurons in vitro. J Comp Neurol 443:43–45

Brünig I, Suter A, Knuesel I, Luscher B, Fritschy JM (2002b) GABAergic presynaptic terminals are required for postsynaptic clustering of dystrophin, but not of $GABA_A$ receptors and gephyrin. J Neurosci 22:4805–4813

Caraiscos VB, Newell JG, You-Ten KE, Elliott EM, Rosahl TW, Wafford KA, MacDonald JF, Orser BA (2004a) Selective enhancement on tonic GABAergic inhibition in murine hippocampal neurons by low concentrations of the volatile anesthetic isoflurane. J Neurosci 24:8454–8458

Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA (2004b) Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by α5 subunit-containing γ-aminobutyric acid type A receptors. Proc Natl Acad Sci U S A 101:3662–3667

Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, Homanics GE (2006) $GABA_A$ receptor α4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. Proc Natl Acad Sci U S A 103:15230–15235

Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Bluethmann H, Mohler H, Rudolph U (2002) Trace fear conditioning involves hippocampal α5 $GABA_A$ receptors. Proc Natl Acad Sci U S A 99:8980–8985

Cuzon VC, Yeh PW, Cheng Q, Yeh HH (2006) Ambient GABA promotes cortical entry of tangentially migrating cells derived from the medial ganglionic eminence. Cereb Cortex 16:1377–1388

Devor A, Fritschy JM, Yarom Y (2001) Spatial distribution and subunit composition of $GABA_A$ receptors in the inferior olivary nucleus. J Neurophysiol 85:1686–1696

Duveau V, Laustela S, Barth L, Gianolini F, Vogt KE, Keist R, Chandra D, Homanics GE, Rudolph U, Fritschy JM (2011) Spatiotemporal specificity of $GABA_A$ receptor-mediated regulation of adult hippocampal neurogenesis. Eur J Neurosci 34:362–373

Enz R, Brandstatter JH, Wassle H, Bormann J (1996) Immunocytochemical localization of the GABAC receptor rho subunits in the mammalian retina. J Neurosci 16:4479–4490

Fagiolini M, Fritschy JM, Löw K, Möhler H, Rudolph U, Hensch TK (2004) Specific $GABA_A$ circuits for visual cortical plasticity. Science 303:1681–1683

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. Nat Rev Neurosci 6:215–229

Ferguson C, Hardy SL, Werner DF, Hileman SM, Delorey TM, Homanics GE (2007) New insight into the role of the β3 subunit of the $GABA_A$-R in development, behavior, body weight regulation, and anesthesia revealed by conditional gene knockout. BMC Neuroscience 8:85

Fillman SG, Duncan CE, Webster MJ, Elashoff M, Weickert CS (2010) Developmental co-regulation of the β and γ $GABA_A$ receptor subunits with distinct α subunits in the human dorsolateral prefrontal cortex. Int J Dev Neurosci 28:513–519

Fritschy JM, Mohler H (1995) $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359:154–194

Fritschy JM, Paysan J, Enna A, Mohler H (1994) Switch in the expression of rat $GABA_A$-receptor subtypes during postnatal development: an immunohistochemical study. J Neurosci 14:5302–5324

Fritschy JM, Weinmann O, Wenzel A, Benke D (1998) Synapse-specific localization of NMDA- and $GABA_A$-receptor subunits revealed by antigen-retrieval immunohistochemistry. J Comp Neurol 390:194–210

Fritschy JM, Panzanelli P, Kralic JE, Vogt KE, Sassoè-Pognetto M (2006) Differential dependence of axo-dendritic and axo-somatic GABAergic synapses on $GABA_A$ receptors containing the α1 subunit in Purkinje cells. J Neurosci 26:3245–3255

Fritschy JM, Harvey RJ, Schwarz G (2008) Gephyrin, where do we stand, where do we go? Trends Neurosci 31:257–264

Fritschy JM, Panzanelli P, Tyagarajan SK (2012) Molecular and functional heterogeneity of GABAergic synapses. Cell Mol Life Sci 69:2485–2499

Glykys J, Mody I (2007) The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. J Physiol 582:1163–1178

Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I (2007) A new naturally occurring $GABA_A$ receptor subunit partnership with high sensitivity to ethanol. Nat Neurosci 10:40–48

Glykys J, Mann EO, Mody I (2008) Which $GABA_A$ receptor subunits are necessary for tonic inhibition in the hippocampus? J Neurosci 28:1421–1426

Grasshoff C, Jurd R, Rudolph U, Antkowiak B (2007) Modulation of presynaptic β3-containing $GABA_A$ receptors limits the immobilizing actions of GABAergic anesthetics. Mol Pharmacol 72:780–787

Gunn BG, Brown AR, Lambert JJ, Belelli D (2011) Neurosteroids and $GABA_A$ receptor interactions: a focus on stress. Front Neurosci 5:131

Gunther U, Benson J, Benke D, Fritschy JM, Reyes GH, Knoflach F, Crestani F, Aguzzi A, Arigoni M, Lang Y, Bluethmann H, Mohler H, Luscher B (1995) Benzodiazepine-insensitive mice generated by targeted disruption of the γ2-subunit gene of γ-aminobutyric acid type A receptors. Proc Natl Acad Sci U S A 92:7749–7753

Gutierrez A, Khan ZU, Deblas AL (1996) Immunocytochemical localization of the alpha6 subunit of the gamma-aminobutyric acidA receptor in the rat nervous system. J Comp Neurol 365:504–510

Hajos N, Nusser Z, Rancz EA, Freund TF, Mody I (2000) Cell type-and synapse-specific variability in synaptic $GABA_A$ receptor occupancy. Eur J Neurosci 12:810–818

Halonen LM, Sinkkonen ST, Chandra D, Homanics GE, Korpi ER (2009) Brain regional distribution of $GABA_A$ receptors exhibiting atypical GABA agonism: roles of receptor subunits. Neurochem Int 55:389–396

Harney SC, Frenguelli BG, Lambert JJ (2003) Phosphorylation influences neurosteroid modulation of synaptic $GABA_A$ receptors in rat CA1 and dentate gyrus neurons. Neuropharmacology 45:873–883

Herd MB, Belelli D, Lambert JJ (2007) Neurosteroid modulation of synaptic and extrasynaptic $GABA_A$ receptors. Pharmacol Ther 116:20–34

Herd MB, Haythornthwaite AR, Rosahl TW, Wafford KA, Homanics GE, Lambert JJ, Belelli D (2008) The expression of $GABA_A$ β subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. J Physiol (London) 586:989–1004

Herd MB, Foister N, Chandra D, Peden DR, Homanics GE, Brown VJ, Balfour DJ, Lambert JJ, Belelli D (2009) Inhibition of thalamic excitability by 4,5,6,7-tetrahydroisoxazolo[4,5-c] pyridine-3-ol: a selective role for delta-$GABA_A$ receptors. Eur J Neurosci 29:1177–1187

Hines RM, Davies PA, Moss SJ, Maguire J (2012) Functional regulation of $GABA_A$ receptors in nervous system pathologies. Cur Opin Neurobiol 22(3):552–558

Homanics GE, Ferguson C, Quinlan JJ, Daggett J, Snyder K, Lagenaur C, Mi ZP, Wang XH, Grayson DR, Firestone LL (1997a) Gene knockout of the α6 subunit of the γ-aminobutyric acid type A receptor: lack of effect on responses to ethanol, pentobarbital, and general anesthetics. Mol Pharmacol 51:588–596

Homanics GE, Delorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison NL, Krasowski MD, Rick CEM, Korpi ER, Makela R, Brilliant MH, Hagiwara N, Ferguson C, Snyder K, Olsen RW (1997b) Mice devoid of γ-aminobutyrate type A receptor β3 subunit have epilepsy, cleft palate, and hypersensitive behavior. Proc Natl Acad Sci U S A 94:4143–4148

Hosie AM, Wilkins ME, da Silva HM, Smart TG (2006) Endogenous neurosteroids regulate $GABA_A$ receptors through two discrete transmembrane sites. Nature 444:486–489

Hutcheon B, Morley P, Poulter MO (2000) Developmental change in $GABA_A$ receptor desensitization kinetics and its role in synapse function in rat cortical neurons. J Physiol 522:3–17

Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, Goldstein PA (2005) An extrasynaptic $GABA_A$ receptor mediates tonic inhibition in thalamic VB neurons. J Neurophysiol 94:4491–4501

Jia F, Yue M, Chandra D, Keramidas A, Goldstein PA, Homanics GE, Harrison NL (2008) Taurine is a potent activator of extrasynaptic $GABA_A$ receptors in the thalamus. J Neurosci 28:106–115

Jones A, Korpi ER, McKernan RM, Pelz R, Nusser Z, Makela R, Mellor JR, Pollard S, Bahn S, Stephenson FA, Randall AD, Sieghart W, Somogyi P, Smith AJH, Wisden W (1997) Ligand-gated ion channel subunit partnerships: $GABA_A$ receptor α6 subunit gene inactivation inhibits δ subunit expression. J Neurosci 17:1350–1362

Jones SM, Palmer MJ (2009) Activation of the tonic $GABA_C$ receptor current in retinal bipolar cell terminals by nonvesicular GABA release. J Neurophysiol 102:691–699

Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, Somogyi P (2010) Quantitative localisation of synaptic and extrasynaptic $GABA_A$ receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. Eur J Neurosci 32:1868–1888

Kaur KH, Baur R, Sigel E (2009) Unanticipated structural and functional properties of δ-subunit-containing $GABA_A$ receptors. J Biol Chem 284:7889–7896

Kralic JE, Sidler C, Parpan F, Homanics G, Morrow AL, Fritschy JM (2006) Compensatory alteration of inhibitory synaptic circuits in thalamus and cerebellum of $GABA_A$ receptor α1 subunit knockout mice. J Comp Neurol 495:408–421

Lagier S, Panzanelli P, Russo RE, Nissant A, Bathellier B, Sassoè-Pognetto M, Fritschy JM, Lledo PM (2007) GABAergic inhibition at dendrodendritic synapses tunes γ oscillations in the olfactory bulb. Proc Natl Acad Sci U S A 104:7259–7264

Laurie DJ, Wisden W, Seeburg PH (1992) The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci 12:4151–4172

Liu X, Wang Q, Haydar TF, Bordey A (2005) Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation on GFAP-expressing progenitors. Nat Neurosci 8(9):1179–1187

Lledo PM, Alonso A, Grubb MS (2006) Adult neurogenesis and fuctional plasticity in neuronal circuits. Nat Rev Neurosci 7:179–193

Loebrich S, Bahring R, Katsuno T, Tsukita S, Kneussel M (2006) Activated radixin is essential for $GABA_A$ receptor α5 subunit anchoring at the actin cytoskeleton. EMBO J 25:987–999

Long P, Mercer A, Begum R, Stephens GJ, Sihra TS, Jovanovic JN (2009) Nerve terminal $GABA_A$ receptors activate Ca^{2+}/calmodulin-dependent signaling to inhibit voltage-gated Ca^{2+} influx and glutamate release. J Biol Chem 284:8726–8737

Loturco JJ, Owens DF, Heath MJS, Davis MBE, Kriegstein AR (1995) GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 15:1287–1298

Luscher B, Hauselman R, Leitgeb S, Rulicke T, Fritschy JM (1997) Neuronal subtype-specific expression directed by the $GABA_A$-receptor δ-subunit gene promoter in transgenic mice and in cultured cells. Mol Brain Res 51:197–211

Maeda A, Katafuchi T, Oba Y, Shiokawa H, Yoshimura M (2010) Enhancement of GABAergic tonic currents by midazolam and noradrenaline in rat substantia gelatinosa neurons in vitro. Anesthesiology 113:429–437

Maguire J, Mody I (2008) $GABA_A$R plasticity during pregnancy: relevance to postpartum depression. Neuron 59:207–213

Mann EO, Mody I (2010) Control of hippocampal gamma oscillation freIquency by tonic inhibition and excitation of interneurons. Nat Neurosci 13:205–212

Maric D, Liu QY, Maric I, Chaudry S, Chang YH, Smith SV, Sieghart W, Fritschy JM, Barker JL (2001) GABA expression dominates neuronal lineage progression in the embryonic rat neocortex and facilitates neurite outgrowth via $GABA_A$ autoreceptor/Cl^- channels. J Neurosci 21:2343–2360

Marowsky A, Fritschy JM, Vogt KE (2004) Functional mapping of $GABA_A$ receptor subtypes in the amygdala. Eur J Neurosci 20:1280–1289

Marowsky A, Rudolph U, Fritschy JM, Arand M (2012) Tonic inhibition in principal cells of the amygdala: a central role for α3 subunit-containing $GABA_A$ receptors. J Neurosci 32:8611–8619

Mellor JR, Merlo D, Jones A, Wisden W, Randall AD (1998) Mouse cerebellar granule cell differentiation: electrical activity regulates the $GABA_A$ receptor α6 subunit gene. J Neurosci 18:2822–2833

Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ (1999) Attenuated sensitivity to neuroactive steroids in γ-aminobutyrate type A receptor δ subunit knockout mice. Proc Natl Acad Sci U S A 96:12905–12910

Mitchell EA, Gentet LJ, Dempster J, Belelli D (2007) $GABA_A$ and glycine receptor-mediated transmission in rat lamina II neurones: relevance to the analgesic actions of neuroactive steroids. J Physiol 583:1021–1040

Mortensen M, Smart TG (2006) Extrasynaptic αβ subunit $GABA_A$ receptors on rat hippocampal pyramidal neurons. J Physiol 577:841–856

Mortensen M, Ebert B, Wafford K, Smart TG (2010) Distinct activities of GABA agonists at synaptic- and extrasynaptic-type $GABA_A$ receptors. J Physiol 588:1251–1268

Mukherjee J, Kretschmannova K, Gouzer G, Maric HM, Ramsden SL, Tretter V, Harvey K, Davies PA, Triller A, Schindelin H, Moss SJ (2011) The residence time of $GABA_A$Rs at inhibitory synapses is determined by direct binding of the receptor α1 subunit to gephyrin. J Neurosci 31:14677–14687

Muller E, Le-Corronc H, Legendre P (2008) Extrasynaptic and postsynaptic receptors in glycinergic and GABAergic neurotransmission: a division of labor? Front Mol Neurosci 1:3

Muller T, Fritschy JM, Grosche J, Pratt GD, Mohler H, Kettenmann H (1994) Developmental regulation of voltage-gated K^+-channel and $GABA_A$-receptor expression in Bergmann glial cells. J Neurosci 14:2503–2514

Nishikawa K, Kubo K, Obata H, Yanagawa Y, Saito S (2011) The influence of manipulations to alter ambient GABA concentrations on the hypnotic and immobilizing actions produced by sevoflurane, propofol, and midazolam. Neuropharmacol 61:172–180

Nissant A, Pallotto M (2011) Integration and maturation of newborn neurons in the adult olfactory bulb—from synapses to function. Eur J Neurosci 33:1069–1077

Nusser Z, Somogyi P (1994) Compartmentalised distribution of immunoreactivity for $GABA_A$ receptors on the surface of neurons in relation to single or multiple sources of GABAergic synapses. J Neurochem 63(Suppl. 1):S39

Nusser Z, Roberts JDB, Baude A, Richards JG, Sieghart W, Somogyi P (1995) Immunocytochemical localization of the α1 and β2/3 subunits of the $GABA_A$ receptor in relation to specific GABAergic synapses in the dentate gyrus. Eur J Neurosci 7:630–646

Nusser Z, Sieghart W, Stephenson FA, Somogyi P (1996a) The α6 subunit of the $GABA_A$ receptor is concentrated in both inhibitory and excitatory synapses on cerebellar granule cells. J Neurosci 16:103–114

Nusser Z, Sieghart W, Benke D, Fritschy JM, Somogyi P (1996b) Differential synaptic localization of two major γ-aminobutyric acid type A receptor α subunits on hippocampal pyramidal neurons. Proc Natl Acad Sci U S A 93:11939–11944

Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693–1703

Nusser Z, Ahmad Z, Tretter V, Fuchs K, Wisden W, Sieghart W, Somogyi P (1999) Alterations in the expression of $GABA_A$ receptor subunits in cerebellar granule cells after the disruption of the α6 subunit gene. Eur J Neurosci 11:1685–1697

Pallotto M, Nissant A, J-M F, Rudolph U, Sassoè-Pognetto M, Panzanelli P, Lledo PM (2012) Early formation of GABAergic synapses governs the development of adult-born neurons in the olfactory bulb. J Neurosci 32: 9103–9115

Panzanelli P, Homanics GE, Ottersen OP, Fritschy JM, Sassoè-Pognetto M (2004) Pre- and postsynaptic $GABA_A$ receptors at reciprocal dendrodendritic synapses in the olfactory bulb. Eur J Neurosci 20:2945–2952

Panzanelli P, Gunn BG, Schlatter MC, Benke D, Tyagarajan SK, Scheiffele P, Belelli D, Lambert JJ, Rudolph U, Fritschy JM (2011) Distinct mechanisms regulate $GABA_A$ receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. J Physiol 589:4959–4980

Patrizi A, Scelfo B, Viltono L, Briatore F, Fukaya M, Watanabe M, Strata P, Varoqueaux F, Brose N, Fritschy JM, Sassoè-Pognetto M (2008) Synapse formation and clustering of neuroligin-2 in the absence of $GABA_A$ receptors. Proc Natl Acad Sci U S A 105:13151–13156

Paul J, Zeilhofer HU, Fritschy JM (2012) Selective distribution of $GABA_A$ receptor subtypes in mouse spinal dorsal horn neurons and primary afferents. J Comp Neurol 520(17):3895–3911. doi:10.1002/cne.23129

Paysan J, Bolz J, Mohler H, Fritschy JM (1994) The $GABA_A$-receptor alpha1-subunit: an early marker of cortical parcellation. J Comp Neurol 350:133–149

Paysan J, Kossel A, Bolz J, Fritschy JM (1997) Area-specific regulation of γ-aminobutyric acid A receptor subtypes by thalamic afferents in developing rat neocortex. Proc Natl Acad Sci USA 94:6995–7000

Peden DR, Petitjean CM, Herd MB, Durakoglugil M, Rosahl TW, Wafford K, Homanics GE, Belelli D, Fritschy JM, Lambert JJ (2008) Developmental maturation of synaptic and extrasynaptic $GABA_A$ receptors in mouse thalamic ventrobasal neurones. J Physiol 586:965–987

Peng Z, Hauer B, Mihalek RM, Homanics GE, Sieghart W, Olsen RW, Houser CR (2002) $GABA_A$ receptor changes in δ subunit-deficient mice: altered expression of α4 and γ2 subunits in the forebrain. J Comp Neurol 446:179–197

Peng ZC, Huang CS, Stell BM, Mody I, Houser CR (2004) Altered expression of the d subunit of the $GABA_A$ receptor in a mouse model of temporal lobe epilepsy. J Neurosci 24:8629–8639

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815–850

Porcello DM, Huntsman MM, Mihalek RM, Homanics GE, Huguenard JR (2003) Intact synaptic GABAergic inhibition and altered neurosteroid modulation of thalamic relay neurons in mice lacking δ subunit. J Neurophysiol 89:1378–1386

Poulopoulos A, Aramuni G, Meyer G, Soykan T, Hoon M, Papadopoulos T, Zhang M, Paarmann I, Fuchs C, Harvey K, Jedlicka P, Schwarzacher SW, Betz H, Harvey RJ, Brose N, Zhang W, Varoqueaux F (2009) Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. Neuron 63:628–642

Poulter MO, Barker JL, O'Carroll AM, Lolait SJ, Mahan LC (1992) Differential and transient expression of $GABA_A$ receptor alpha-subunit mRNAs in the developing rat CNS. J Neurosci 12:2888–2900

Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE (2006) Specific subtypes of $GABA_A$ receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. J Neurophysiol 96:846–857

Rudolph U, Crestani F, Benke D, Brünig I, Benson J, Fritschy JM, Martin JR, Bluethmann H, Mohler H (1999) Benzodiazepine actions mediated by specific γ-aminobutyric $acid_A$ receptor subtypes. Nature 401:796–800

Santhakumar V, Jones RT, Mody I (2010) Developmental regulation and neuroprotective effects of striatal tonic $GABA_A$ currents. Neuroscience 167:644–655

Sassoè-Pognetto M, Panzanelli P, Sieghart W, Fritschy JM (2000) Co-localization of multiple $GABA_A$ receptor subtypes with gephyrin at postsynaptic sites. J Comp Neurol 420:481–498

Schlicker K, McCall MA, Schmidt M (2009) GABAC receptor-mediated inhibition is altered but not eliminated in the superior colliculus of $GABA_C\rho 1$ knockout mice. J Neurophysiol 101:2974–2983

Schneider Gasser EM, Duveau V, Prenosil GA, Fritschy JM (2007) Reorganization of GABAergic circuits maintains $GABA_A$ receptor-mediated transmission onto CA1 interneurons in α1ℜsubunit-null mice. Eur J Neurosci 25:3287–3304

Schwarzer C, Berresheim U, Pirker S, Wieselthaler A, Fuchs K, Sieghart W, Sperk G (2001) Distribution of the major γ-aminobutyric acid A receptor subunits in the basal ganglia and associated limbic brain areas of the adult rat. J Comp Neurol 433:526–549

Schweizer C, Balsiger S, Bluethmann H, Mansuy IM, Fritschy JM, Mohler H, Luscher B (2003) The γ2 subunit of $GABA_A$ receptors is required for maintenance of receptors at mature synapses. Mol Cell Neurosci 24:442–450

Serafini R, Ma W, Maric D, Maric I, Lahjouji F, Sieghart W, Barker JL (1998a) Initially expressed early rat embryonic $GABA_A$ receptor Cl^- ion channels exhibit heterogeneous channel properties. Eur J Neurosci 10:1771–1783

Serafini R, Maric D, Maric I, Ma W, Fritschy JM, Zhang L, Barker JL (1998b) Dominant $GABA_A$ receptor/Cl^- channel kinetics correlate with the relative expressions of α2, α3, α5 and β3 subunits in embryonic rat neurons. Eur J Neurosci 10:334–349

Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, De Blas AL (2006) Synaptic and nonsynaptic localization of $GABA_A$ receptors containing the α5 subunit in the rat brain. J Comp Neurol 499:458–470

Shrivastava AN, Triller A, Sieghart W, Sarto-Jackson I (2011) Regulation of $GABA_A$ receptor dynamics by interaction with purinergic P2X(2) receptors. J Biol Chem 286:14455–14468

Sieghart W, Sperk G (2002) Subunit composition, distribution and function of $GABA_A$ receptor subtypes. Curr Top Med Chem 2:795–816

Smith SS, Shen H, Gong QH, Zhou X (2007) Neurosteroid regulation of $GABA_A$ receptors: focus on the α4 and subunits. Pharmacol Ther 116:58–76

Smith SS, Aoki C, Shen H (2009) Puberty, steroids and $GABA_A$ receptor plasticity. Psychoneuroendocrinology 34:S91–S103

Soltesz I, Roberts JDB, Takagi H, Richards JG, Mohler H, Somogyi P (1990) Synaptic and nonsynaptic localization of benzodiazepine/$GABA_A$ receptors/Cl^- channel complex using monoclonal antibodies in the dorsal lateral geniculate nucleus of the cat. Eur J Neurosci 2:414–429

Somogyi P, Takagi H, Richards JG, Mohler H (1989) Subcellular localization of benzodiazepine/$GABA_A$ receptors in the cerebellum of rat, cat, and monkey using monoclonal antibodies. J Neurosci 9:2197–2209

Somogyi P, Fritschy JM, Benke D, Roberts JDB, Sieghart W (1996) The γ2 subunit of the $GABA_A$-receptor is concentrated in synaptic junctions containing the α1 and β2/3 subunits in hippocampus, cerebellum and globus pallidus. Neuropharmacology 35:1425–1444

Sun C, Sieghart W, Kapur J (2004) Distribution of α1, α4, γ2, and δ subunits of $GABA_A$ receptors in hippocampal granule cells. Brain Res 1029:207–216.

Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, McKernan RM (1999) Preferential coassembly of α4 and δ subunits of the γ-aminobutyric $acid_A$ receptor in rat thalamus. Mol Pharmacol 56:110–115

Suryanarayanan A, Liang J, Meyer EM, Lindemeyer AK, Chandra D, Homanics GE, Sieghart W, Olsen RW, Spigelman I (2011) Subunit compensation and plasticity of synaptic $GABA_A$ receptors induced by ethanol in α4 subunit knockout mice. Front Neurosci 5:110

Thomas P, Mortensen M, Hosie AM, Smart TG (2005) Dynamic mobility of functional $GABA_A$ receptors at inhibitory synapses. Nat Neurosci 8:889–897

Thomson AM, Jovanovic JN (2010) Mechanisms underlying synapse-specific clustering of $GABA_A$ receptors. Eur J Neurosci 31:2193–2203

Tia S, Wang JF, Kotchabhakdi N, Vicini S (1996) Developmental changes of inhibitory synaptic currents in cerebellar granule neurons—role of $GABA_A$ receptor α6 subunit. J Neurosci 16:3630–3640

Tretter V, Hauer B, Nusser Z, Mihalek RM, Hoger H, Homanics GE, Somogyi P, Sieghart W (2001) Targeted disruption of the $GABA_A$ receptor δ subunit gene leads to an up-regulation of γ2 subunit-containing receptors in cerebellar granule cells. J Biol Chem 276:10532–10538

Tretter V, Jacob TC, Mukherjee J, Fritschy JM, Pangalos MN, Moss SJ (2008) The clustering of $GABA_A$ receptor subtypes at inhibitory synapses is facilitated via the direct binding of receptor α2 subunits to gephyrin. J Neurosci 28:1356–1365

Tretter V, Kerschner B, Milenkovic I, Ramsden SL, Ramerstorfer J, Saiepour L, Maric HM, Moss SJ, Schindelin H, Harvey RJ, Sieghart W, Harvey K (2011) Molecular basis of the $GABA_A$ receptor α3 subunit interaction with gephyrin. J Biol Chem 286:42105–42114

Trigo FF, Marty A, Stell BM (2008) Axonal $GABA_A$ receptors. Eur J Neurosci 28:841–848

Varecka L, Wu CH, Rotter A, Frostholm A (1994) $GABA_A$-benzodiazepine receptor α6 subunit mRNA in granule cells of the cerebellar cortex and cochlear nuclei: expression in developing and mutant mice. J Comp Neurol 339:341–352

Vargas-Caballero M, Martin LJ, Salter MW, Orser BA, Paulsen O (2010) α5 Subunit-containing $GABA_A$ receptors mediate a slowly decaying inhibitory synaptic current in CA1 pyramidal neurons following Schaffer collateral activation. Neuropharmacology 58:668–675

Wafford KA, van Niel MB, Ma QP, Horridge E, Herd MB, Peden DR, Belelli D, Lambert JJ (2009) Novel compounds selectively enhance delta subunit containing $GABA_A$ receptors and increase tonic currents in thalamus. Neuropharmacology 56:182–189

Wang DD, Krueger DD, Bordey A (2003) GABA depolarizes neuronal progenitors of the postnatal subventricular zone via $GABA_A$ receptor activation. J Physiol 550:785–800

Wei ZW, Zhang N, Peng ZC, Houser CR, Mody I (2003) Perisynaptic localization of δ subunit-containing $GABA_A$ receptors and their activation by GABA spillover in the mouse dentate gyrus. J Neurosci 23:10650–10661

Wieland HA, Luddens H, Seeburg PH (1992) A single histidine in $GABA_A$ receptors is essential for benzodiazepine agonist binding. J Biol Chem 267:1426–1429

Wu X, Wu Z, Ning G, Guo Y, Ali R, Macdonald R, De Blas A, Luscher B, Chen G (2012) γ-Aminobutyric acid type A ($GABA_A$) receptor α subunits play a direct role in synaptic versus extrasynaptic targeting. J Biol Chem 287(33):27417–27430

Xu G, Broadbelt KG, Haynes RL, Folkerth RD, Borenstein NS, Belliveau RA, Trachtenberg FL, Volpe JJ, Kinney HC (2011) Late development of the GABAergic system in the human cerebral cortex and white matter. J Neurophatol Exp Neurol 70:841–858

Zarnowska ED, Keist R, Rudolph U, Pearce RA (2009) $GABA_A$ receptor α5 subunits contribute to GABA, slow synaptic inhibition in mouse hippocampus. J Neurophysiol 101:1179–1191

Zheng F, Adelsberger H, Müller MR, Fritschy JM, Werner S, Alzheimer C (2009) Activin tunes GABAergic neurotransmission and modulates anxiety-like behavior. Mol Psychiatry 14:332–346

Chapter 3
Biophysical Properties of Recombinant γ2- and δ-subunit Containing $GABA_A$ Receptors

Robert L. Macdonald and Emmanuel J. Botzolakis

Abstract $GABA_A$ receptors mediate the majority of GABAergic signaling in the mammalian brain and are thus primarily responsible for maintaining inhibitory tone. $GABA_A$ receptors mediate two modes of inhibitory neurotransmission, phasic synaptic inhibition and tonic extra- or perisynaptic inhibition. Phasic inhibition is mediated primarily by activation of subsynaptic αβγ2 receptors, whereas tonic inhibition is mediated primarily by extrasynaptic or perisynaptic αβδ $GABA_A$ receptors. The biophysical properties of γ2 and δ subunit-containing receptors are complex yet quite different, consistent with the unique demands of phasic and tonic inhibitory systems. Mutations in $GABA_A$ receptor genes (*GABRs*) have been associated with genetic epilepsies in humans. However, genetic epilepsies are more commonly associated with γ-aminobutyric acid-A receptor γ2 (*GABRG2*) mutations than γ-aminobutyric acid-A receptor δ (*GABRD*) mutations, suggesting considerable differences in the role of αβγ2 and αβδ receptors in regulating thalamocortical excitability.

Keywords $GABA_A$ receptors · Kinetic properties · Phasic inhibition · Tonic inhibition · GABRG2 · GABRD

R. L. Macdonald (✉)
Department of Neurology, Vanderbilt University Medical Center,
1211 Medical Center Dr, Nashville, TN 37212, USA
e-mail: Robert.macdonald@vanderbilt.edu

Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, 1211 Medical Center Dr, Nashville, TN 37212, USA

Department of Pharmacology, Vanderbilt University Medical Center,
1211 Medical Center Dr, Nashville, TN 37212, USA

E. J. Botzolakis
Department of Radiology, Hospital of the University of Pennsylvania,
3400 Spruce Street, Philadelphia, PA 19104, USA

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_3

3.1 $GABA_A$ Receptor Subunit Families, Subtypes, and Splice Variants

Like other members of the Cys-loop superfamily of ligand-gated ion channels (LGICs), which includes the nicotinic acetylcholine receptor (nAChR; Noda et al. 1983), the 5-hydroxytryptamine type 3 (5-HT3) serotonin receptor (Maricq et al. 1991), the zinc-activated channel (ZAC; Davies et al. 2003), and the glycine receptor (Langosch et al. 1988), γ-aminobutyric acid type A ($GABA_A$) receptors are heteropentameric and are assembled from a large panel of homologous subunits (Schofield et al. 1987; Mamalaki et al. 1989; Nayeem et al. 1994; Knight et al. 1998; Barrera et al. 2008a, b). There are eight subunit families (α, β, γ, δ, ε, θ, π, and ρ), thus providing enormous potential for receptor heterogeneity. Several of these subunit families are composed of multiple subtypes (α1–6, β1–3, γ1–3, ρ1–3), splice variants (e.g., β2S and β2L; β3-v1 and β3-v2; γ2S and γ2L), and alternatively edited transcripts (e.g., α3I and α3M), further increasing the potential for heterogeneity (Schofield et al. 1987; Levitan et al. 1988; Pritchett et al. 1989; Shivers et al. 1989; Ymer et al. 1989a, b; Whiting et al. 1990; Herb et al. 1992; Kirkness and Fraser 1993; Davies et al. 1997; Hedblom and Kirkness 1997; Bonnert et al. 1999; Simon et al. 2004; Ohlson et al. 2007). Sequence homology is ~60–80% among members of the same $GABA_A$ receptor family, ~20–40% among members of different families, and ~10–20% among members of the $GABA_A$ receptor and other Cys-loop receptor families (Olsen and Tobin 1990).

3.2 Assembly of αβγ and αβδ $GABA_A$ Receptors

All Cys-loop receptors, including $GABA_A$ receptors, are assembled in a complex, multistep process that occurs in the endoplasmic reticulum (ER). Assembly is thought to be relatively inefficient and slow (Green and Millar 1995) and to be assisted by ER luminal and cytoplasmic chaperones (Bollan et al. 2003; Wanamaker and Green 2007; Sarto-Jackson and Sieghart 2008). Working in concert, these proteins facilitate the folding and oligomerization of $GABA_A$ receptor subunits and provide stringent quality control to eliminate misfolded subunits or misassembled receptors in the ER (Ellgaard and Frickel 2003; Bollan et al. 2003).

In addition to these ER quality control requirements, there are also specific subunit requirements for $GABA_A$ receptor assembly that result in preferential oligomerization between specific subunit subtypes, thus limiting receptor heterogeneity (Angelotti et al. 1993). Although most subunit combinations appear capable of oligomerization under some conditions and in some heterologous expression systems (Connolly et al. 1996), sucrose density centrifugation studies of CNS $GABA_A$ receptors indicate that only a small subset of subunit combinations can form receptor form pentamers, a prerequisite for receptor function and surface expression (Angelotti et al. 1993; Gorrie et al. 1997; Connolly et al. 1996, 1999; Taylor et al.

2000; Klausberger et al. 2001; Bollan et al. 2003; Lo et al. 2008; Sarto-Jackson and Sieghart 2008). For example, co-expression of α1 and β2 or β3 subunits formed pentamers, as did co-expression of α1, β2 or β3, and γ2 subunits (Gorrie et al. 1997; Tretter et al. 1997; Connolly et al. 1999; Taylor et al. 2000; Klausberger et al. 2001; Lo et al. 2008; Sarto-Jackson and Sieghart 2008). In contrast, when α1, β2, or γ2 subunits were expressed individually in heterologous cells, primarily monomers and dimers were formed (Gorrie et al. 1997; Connolly et al. 1999; Taylor et al. 1999; Lo et al. 2008; Sarto-Jackson and Sieghart 2008). Similarly, co-expression of either α1 or β3 subunits with γ2 subunits yielded primarily dimers and trimers (Tretter et al. 1997; Sarto-Jackson and Sieghart 2008). Thus, the presence of both α and β subunits is required for pentameric assembly of functional $GABA_A$ receptor channels (Angelotti et al. 1993). Known exceptions are the β3 and ρ1 subunits, which efficiently form homopentameric receptor channels (Taylor et al. 1999; Pan et al. 2006).

Ternary receptors, however, appear to assemble with higher efficiency than binary receptors (Angelotti et al. 1993), suggesting the existence of hierarchical assembly within the subunit family that further limits receptor heterogeneity. For example, co-expression of α and β subunits with γ, δ, ε, or π subunits yields relatively homogeneous receptor populations with kinetic properties distinct from those of receptors formed following co-expression of only α and β subunits (Angelotti and Macdonald 1993; Saxena and Macdonald 1994; Fisher and Macdonald 1997a; Haas and Macdonald 1999; Neelands et al. 1999; Neelands and Macdonald 1999; Lagrange et al. 2007). Thus, the majority of native receptors are likely composed of ternary subunit combinations (McKernan and Whiting 1996; Olsen and Sieghart 2009), with the most widely expressed ternary $GABA_A$ receptors being the αβγ and αβδ isoforms.

For αβγ receptors, a subunit stoichiometry of 2α:2β:1γ (Chang et al. 1996; Tretter et al. 1997; Farrar et al. 1999) and a counterclockwise subunit arrangement of γ-β-α-β-α when viewed top–down from the synaptic cleft has been proposed (Tretter et al. 1997; Baumann et al. 2001, 2002; Baur et al. 2006). Studies using atomic force microscopy suggested a similar stoichiometry and arrangement for αβδ receptors, with the δ subunit replacing the γ subunit in the pentamer, thus yielding a stoichiometry of 2α:2β:1δ and a counterclockwise subunit arrangement of δ-β-α-β-α (Barrera et al. 2008). There is, however, some evidence for alternate patterns of assembly. For example, multiple γ subunit subtypes have been reported in a subset of native (Quirk et al. 1994; Khan et al. 1994; Benke et al. 1996) and recombinant (Backus et al. 1993) receptors. Although it is unclear if multiple δ subunits can also be incorporated in the same pentamer, recent evidence using concatenated subunits suggests that at least two additional subunit arrangements for αβδ receptors are theoretically possible: δ-α-β-β-α (GABA-gated) and δ-α-β-α-β (THDOC-gated) (Kaur et al. 2009).

3.3 Modes of GABAergic Inhibition

$GABA_A$ receptors mediate three modes of inhibitory neurotransmission: phasic, spillover, and tonic inhibition. Phasic inhibition involves transient activation of postsynaptic $GABA_A$ receptors by nearly saturating concentrations of GABA released from presynaptic vesicles. This produces rapidly activating (rise times of ~1 ms or less) but slowly decaying (time constants of 10 to 100 s of ms) inhibitory postsynaptic currents (IPSCs; Maconochie et al. 1994; Jones and Westbrook 1995). While both pre- and postsynaptic factors influence IPSC shape, it is generally accepted that postsynaptic factors are the primary determinants. Indeed, the GABA transient reaches nearly saturating concentrations in <100 μs and decays in <1 ms due to a combination of diffusion and reuptake (Clements 1996; Glavinovic 1999; Ventriglia and Di 2003). Thus, IPSCs significantly outlast the presence of GABA in the synaptic cleft, suggesting that IPSCs are shaped primarily by the intrinsic properties of the postsynaptic $GABA_A$ receptors. Supporting this assertion, application of ultrabrief pulses of saturating GABA to membrane patches excised from cells expressing recombinant $GABA_A$ receptors gives rise to currents with transient kinetic properties resembling those of IPSCs (Jones and Westbrook 1995; Haas and Macdonald 1999).

In addition to mediating fast synaptic inhibition, $GABA_A$ receptors also mediate spillover (Brickley et al. 2001) and tonic (Farrant and Nusser 2005) inhibition. Spillover inhibition refers to activation of receptors in peri-, extra-, or presynaptic locations, as well as at adjacent synapses, by GABA that diffuses from the synaptic cleft (Bright et al. 2011). Tonic inhibition is mediated by peri- and extrasynaptic $GABA_A$ receptors that are activated by persistent subsaturating concentrations of ambient GABA (Bright et al. 2011). Although the source of ambient GABA is not entirely clear, it is generally believed that GABA concentrations of ~1 μM are typically present (Attwell et al. 1993; Nusser et al. 1998; Bach-y-Rita 2001; Farrant and Nusser 2005; Bright et al. 2011). It has been suggested that phasic spillover and tonic inhibition reflect activation of different, spatially distinct $GABA_A$ receptor populations (Bright et al. 2011). Of note, while spillover and tonic currents are relatively small in amplitude compared to phasic currents, their contribution to overall inhibitory tone (net charge transfer) may actually be greater than the summed charge transfer of phasic currents (Brickley et al. 1996; Hamann et al. 2002).

Phasic and tonic inhibition are differentially modulated by various pharmacological agents (Feng and Macdonald 2004; Feng et al. 2004, 2008) and play distinct roles in the pathogenesis of neurological disorders such as epilepsy (Dibbens et al. 2004; Feng et al. 2006; Eugene et al. 2007). This reflects the fact that tonic currents are mediated by different receptor isoforms with kinetic properties distinct from those mediating phasic currents. Specifically, while α1βγ2, α2βγ2, or α3βγ2 receptor isoforms are thought to be the primary mediators of phasic inhibition (Jones and Westbrook 1995; Haas and Macdonald 1999; Lagrange et al. 2007), α4βxδ and α6βxδ receptor isoforms are thought to be the primary mediators of tonic inhibition (Brickley et al. 2001; Farrant and Nusser 2005). That said, α1βxδ and α5βxγ2

receptors may also play a role in mediating tonic inhibition in the hippocampus (Glykys et al. 2008).

3.4 Biophysical and Kinetic Properties of αβγ and αβδ $GABA_A$ Receptor Channels

$GABA_A$ receptor channels are highly permeable to anions including Cl^- and HCO_3^- but are relatively impermeant to cations (permeability ratio of K^+ to Cl^- <0.05) (Bormann et al. 1987). The channels are considerably more permeable to Cl^- than to HCO_3^- (Cl^-:HCO_3^- permeability is ~5:1), and thus, the majority of charge transfer following channel activation is Cl^- mediated. However, HCO_3^- flux through the channel has been hypothesized to play a role following collapse of the Cl^- gradient (Grover et al. 1993; Perkins and Wong 1997; Dallwig et al. 1999; Kim et al. 2009).

The kinetic properties of $GABA_A$ receptors are highly dependent on receptor subunit composition, thereby providing a mechanism for neurons to regulate their sensitivity to GABA (Angelotti and Macdonald 1993; Saxena and Macdonald 1994; Burgard et al. 1996; Fisher and Macdonald 1997a, b; Fisher et al. 1997; Neelands et al. 1999; Neelands and Macdonald 1999; Haas and Macdonald 1999; Bianchi et al. 2002a; Feng and Macdonald 2004; Feng et al. 2004; Barberis et al. 2007; Bianchi et al. 2007; Lagrange et al. 2007; Picton and Fisher 2007; Rula et al. 2008; Keramidas and Harrison 2008; Mortensen et al. 2004a). Indeed, $GABA_A$ receptor αβγ2 and αβδ isoforms have substantially different biophysical properties, which make them more effective for mediating phasic and tonic inhibition, respectively (Fig. 3.1).

$GABA_A$ receptor α1βxγ2 single channels open to a main conductance level of 26–30 pS and to two less frequently occurring subconductance levels (~17–19 and 10–15 pS) (Bormann et al. 1987; Macdonald et al. 1989; Twyman et al. 1990; Newland et al. 1991; Fisher and Macdonald 1997a; Haas and Macdonald 1999; Burkat et al. 2001; Mortensen et al. 2004b). To fully activate the receptor, binding of two molecules of GABA is required. Once GABA is bound, the channel exhibits a complex pattern of opening and closing (Fig. 3.1a). Detailed kinetic analysis of native (Macdonald et al. 1989; Twyman et al. 1990; Newland et al. 1991) and recombinant (Fisher and Macdonald 1997; Haas and Macdonald 1999; Burkat et al. 2001; Mortensen et al. 2004b; Keramidas and Harrison 2008) $GABA_A$ receptor channels demonstrated the existence of multiple open (two to three) and closed (four to five) states (based on exponential fitting of open and closed time distributions) (Fig. 3.1b). When activated by a saturating concentration of GABA (1 mM), $GABA_A$ receptor channel total mean open time was 2.1 ms and total mean closed time was 21.0 ms, and the channels opened to three open states (referred to as O1, O2, and O3), with individual mean open times of 0.3, 2.0, and 3.5 ms and relative frequencies of occurrence of 24, 48, and 28%, respectively (Haas and Macdonald 1999).

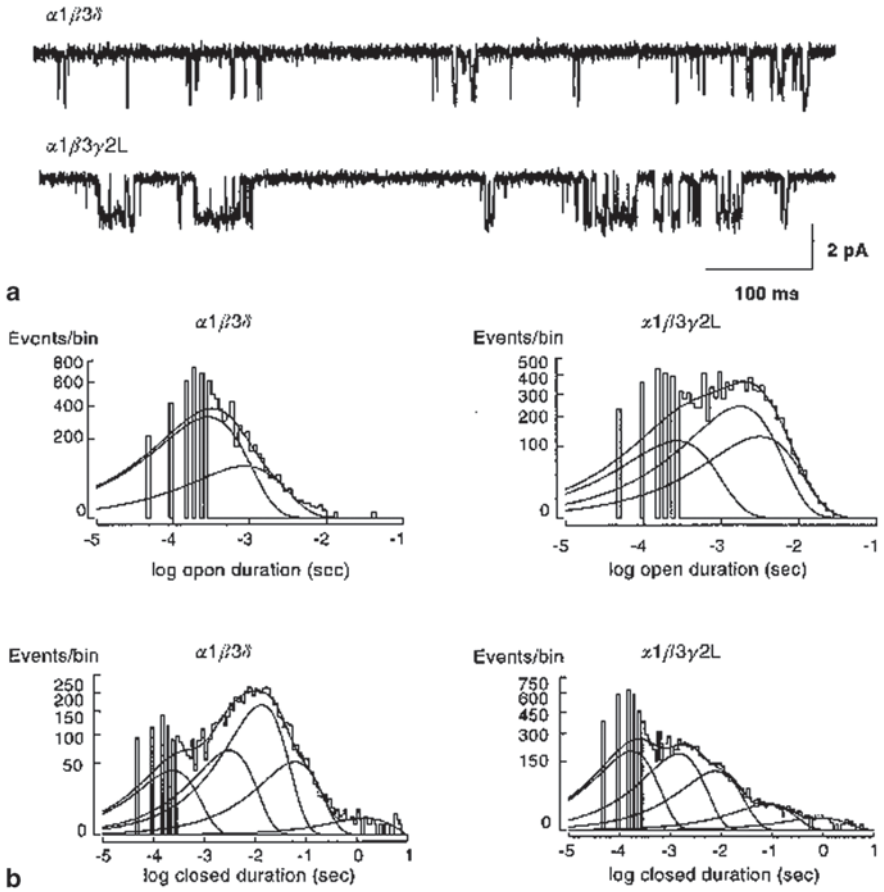

Fig. 3.1 Properties of *α1β3γ2L* and *α1β3δ* $GABA_A$ receptor microscopic single channel currents. **a** In transfected HEK293T cells, single channel $GABA_A$ receptor currents were recorded from outside-out membrane patches voltage-clamped at −75 mV and bathed in 1 mM GABA. Traces shown were continuous 700 ms recordings and channel openings are downwards. **b** Duration histograms of open and closed intervals were obtained from steady-state single-channel data. Open duration histograms for *α1β3γ2L* $GABA_A$ receptors were fitted best with three exponential components, while *α1β3δ* open duration histograms were fitted best with only two components. Closed interval histograms were fitted best by five components in both cases. (Reproduced from Haas and Macdonald 1999)

Channel openings occurred in bursts (a series of openings separated by brief closures that are flanked by closures longer than a specific burst termination duration) of three types (referred to as B1, B2, and B3), each containing a single type of opening (i.e., either O1, O2, or O3) (Twyman et al. 1990). Activating receptors with subsaturating GABA concentrations increased the relative contribution of O1 openings at the expense of both O2 and O3 openings without altering individual mean open times (though the overall total mean open time was decreased since

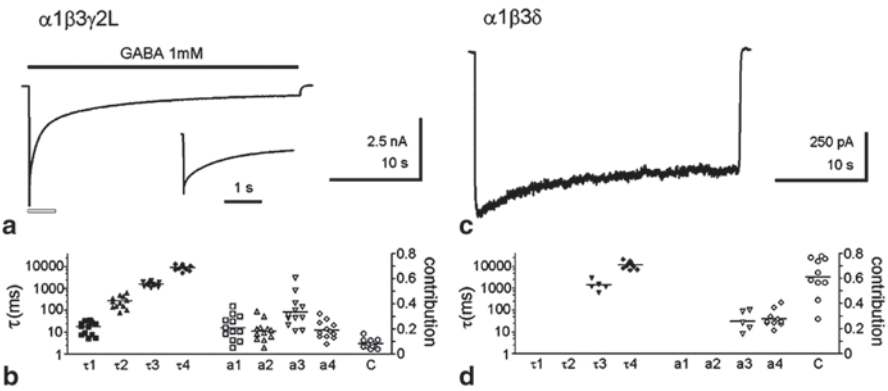

Fig. 3.2 Properties of *α1β3γ2L* and *α1β3δ* $GABA_A$ receptor macroscopic rapid *GABA* application whole cell currents. **a** Response of *α1β3γ2L* receptors transiently expressed in HEK293T cells to a 28 s concentration jump using 1 mM *GABA* (filled bar). The inset shows the first 3 s on an expanded time scale. **c** Response of *α1β3δ* receptors to the same protocol as in Panel **a**. The parameters used to fit *α1β3γ2L* and *α1β3δ* currents are shown as scatter plots in Panels **b** and **d**, respectively. The left ordinate indicates the time constants (τ1–τ4; note the logarithmic scale), and the right ordinate indicates the relative contribution of the corresponding time constants (a1–a4), as well as the constant term to account for incomplete desensitization. For each parameter, a horizontal line is drawn through the mean. (Reproduced from Bianchi and Macdonald 2002)

the lifetime of O1 is short). Considering that receptors have minimal spontaneous openings, this finding has been interpreted to mean that O1 and O2/O3 open states represent receptor sojourns in mono- and di-liganded open states, respectively. It is unclear, however, why O1 openings were detected at all when activated by a saturating GABA concentration, since this should have driven receptor occupancy in mono-liganded states effectively to near zero. One possibility is that two brief open states exist with similar mean open times, one mono-liganded and the other di-liganded, raising the possibility that there may actually be four open states, two of which are simply indistinguishable with classical exponential fitting of open time distributions (Lagrange et al. 2007).

Given the complexity of single channel kinetic properties, it is not at all surprising that $GABA_A$ receptor whole cell current properties (i.e., the ensemble response of hundreds to thousands of channels) are also quite complex (Fig. 3.2; Haas and Macdonald 1999; Bianchi and Macdonald 2001a, b; Mozrzymas et al. 2003; Lagrange et al. 2007). Rapid application of a saturating concentration of GABA to excised outside-out patches from hippocampal neurons or HEK293T cells transiently expressing α1βxγ2 subunits evokes currents that activate in sub-millisecond times (Fig. 3.2a). With prolonged GABA application, these currents undergo substantial multiphasic desensitization, typically with three to four time components of exponential decay with time constants ranging from <10 ms to >1000 ms (Celentano and Wong 1994; Haas and Macdonald 1999; Bianchi and Macdonald 2002; Lagrange et al. 2007). This multiphasic desensitization is an intrinsic property of the channel and reflects the progressive accumulation of receptors into long-lived

nonconducting desensitized states (Celentano and Wong 1994; Bianchi and Macdonald 2002). Desensitization has a steep concentration dependence (Haas and Macdonald 1999; Bianchi et al. 2007), and although this has been suggested to reflect concentration-dependent entry rate into the microscopic desensitized state, multiple modeling studies have demonstrated that this gating structure is not required (Haas and Macdonald 1999; Bianchi et al. 2007). The lack of macroscopic desensitization of whole cell currents with application of low concentrations of GABA is most likely caused by failure to synchronously activate receptors, thus masking the otherwise concentration-independent desensitization process (much like slow application of agonist; Jones and Westbrook 1995; Bianchi and Macdonald 2002). The basis for this conclusion comes from detailed kinetic analysis of desensitization of whole cell currents, which shows that while the relative contribution of each desensitization exponential component changes with GABA concentration, the actual desensitization time constants do not change (Haas and Macdonald 1999).

Following washout of GABA, α1β x γ2 currents typically deactivate biphasically (Jones and Westbrook 1995; Haas and Macdonald 1999), though more complex deactivation has been described (Lagrange et al. 2007). Deactivation time course varies with the duration of GABA application prior to washout, with longer applications being associated with slower deactivation (Jones and Westbrook 1995; Haas and Macdonald 1999; Bianchi et al. 2007; Botzolakis et al. 2008). This relationship between the deactivation rate and the duration of GABA application reflects the inability of GABA to unbind from receptors in desensitized states, which represent an increasing fraction of receptors with longer GABA applications (Bianchi et al. 2007). In other words, the long lifetimes of desensitized states provide channels with the opportunity to reopen long after GABA washout, resulting in slowing of whole cell current deactivation (Jones and Westbrook 1995, 1996). As a result, desensitization and deactivation are commonly referred to as being "coupled" (Jones and Westbrook 1995; Bianchi et al. 2001).

Like γ subunit containing receptors, those containing the δ subunit also require two molecules of GABA for full activation, and are also thought to access multiple open and closed states. However, in contrast to α1β2γ2 receptors, α1β2δ receptor currents exhibit a less complex pattern of opening and closing (Fig. 3.1a). α1β2δ single channels have only two open states (O1 and O2), both of which are relatively short-lived with individual mean open times of 0.3 and 1.0 ms, respectively (Fig. 3.1b). The briefer O1 state openings account for 80% of all openings, thus yielding a short overall mean open time of only 0.4 ms (Fisher and Macdonald 1997; Haas and Macdonald 1999). In addition, α1β2δ receptors have a longer overall mean closed time (~36 ms); reflecting their increased likelihood of entering long-lived closed states. The combination of decreased mean open time and increased mean closed time results in an open probability that is much lower than that of α1β2γ2 receptors (~0.02 vs. ~0.1, respectively). As a result of these differences in microscopic kinetic properties, macroscopic α1β2δ receptor currents (Fig. 3.2c, d) have kinetic properties that are different from those evoked from α1β2γ2 receptors (Fig. 3.2a, b). Whole cell α1β2δ receptor currents are typically small, slowly activating, minimally desensitizing, and rapidly deactivating (Saxena

and Macdonald 1996; Fisher and Macdonald 1997; Haas and Macdonald 1999; Bianchi and Macdonald 2002). These properties are consistent with the known role of αβδ receptors in tonic inhibition. Indeed, because they desensitize more slowly and less extensively than αβγ receptor currents and have a lower GABA EC_{50}, they are better suited for the extrasynaptic environment, where they must respond to very low concentrations of GABA for extended periods of time (Saxena and Macdonald 1996; Haas and Macdonald 1999; Lagrange et al. 2007).

3.5 Mathematical Modeling of $GABA_A$ Receptor Function

Multiple mathematical approaches have been used to understand the microscopic kinetic behavior of ion channels. It is generally agreed, however, that Markov models with multiple, reversibly connected states, each corresponding to a distinct receptor conformation (i.e., open, closed, or desensitized), provide the best description of single channel data (Korn and Horn 1988; McManus et al. 1988; Sansom et al. 1989). Application of Markov models to ion channels involves several assumptions, including that state transitions are random (stochastic) and independent of previous channel activity (memoryless). Markov models have been very useful for describing the single channel kinetic behavior of not only $GABA_A$ receptor channels (Macdonald et al. 1989; Weiss and Magleby 1989; Celentano and Wong 1994; Jones and Westbrook 1995; Haas and Macdonald 1999; Lagrange et al. 2007; Mortensen et al. 2004b; Keramidas and Harrison 2008) but also of numerous other ligand- and voltage-gated ion channels (Horn and Vandenberg 1984; Zagotta et al. 1994; Schoppa and Sigworth 1998; Rothberg and Magleby 2000; Sigg and Bezanilla 2003; Burzomato et al. 2004; Chakrapani et al. 2004; Lape et al. 2008). In addition, Markov models have provided the dominant conceptual framework for interpreting effects of disease-causing mutations and a variety of modulators (Twyman and Macdonald 1992; Twyman et al. 1989a, b; Bianchi and Macdonald 2001a; Feng et al. 2004; Mercik et al. 2006; Plested et al. 2007).

For models to have any practical use, however, they should describe both microscopic and macroscopic behavior of any given channel. Indeed, evaluating receptor channel currents under both microscopic and macroscopic conditions is critical as neither can independently provide enough information for a unique reaction scheme to be generated with certainty. Macroscopic currents, for example, constrain gating schemes by providing nonequilibrium kinetic data, which is typically unavailable from near-equilibrium, steady-state single channel (or microscopic) studies. Single channel data, in contrast, provides direct information regarding channel open and closed states, including the relative frequency of occurrence of each, their connectivity, and in the case of the open states, their approximate mean lifetimes.

The first comprehensive model of micro- and macroscopic $GABA_A$ receptor currents was developed by Haas and Macdonald (1999) and was based on an earlier model by Twyman et al. (1990) that described the behavior of single α1β2γ2

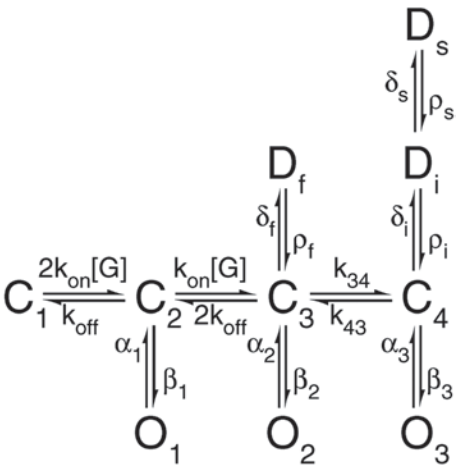

Fig. 3.3 Kinetic models of ligand-gated ion channel function. **a** Comprehensive kinetic model for the α1β3γ2L receptor (Haas and Macdonald 1999) illustrating reversibly connected closed (*C*), open (*O*), and desensitized (D_f, "fast"; D_i, "intermediate"; D_s, "slow") states. For simplicity, the two distal "intraburst" *C* states connected to each *O* state were omitted. The microscopic transitions associated with agonist binding were labeled k_{on} and k_{off} for association and dissociation rate constants, respectively. Each agonist binding step was taken to be equivalent and independent (the first binding and unbinding rates were therefore multiplied by 2). [G], concentration of GABA. **b** Simple 4-state kinetic model in "linear" arrangement. A single binding step connects C_1 (the unbound closed state) and C_2 (the ligand-bound closed state). The *O* state is accessed from C_2 and is arranged in series with the *D* state. **c** Simple 4-state kinetic model in "branched" arrangement. *D* and *O* states are arranged in parallel, each being directly accessible from C_2. (Reproduced from Bianchi et al. 2007)

$GABA_A$ receptor channels (Fig. 3.3). This model contained two GABA binding steps and a total of 16 states – 3 open and 13 closed. Six of the states (the same two closed states for each of three open states) were brief closed states that branch off each open state (not shown in Fig. 3.3 to reduce complexity). The basis for the two closed states distal to each open state is unclear but likely due to random channel closures that are not actually related to channel gating (Macdonald et al. 1989; Twyman et al. 1990). In addition, three of the closed states were given the special designation of "desensitized" states, as they allowed for the macroscopic phenomenon of desensitization to occur. Although this model has been updated recently to take into account several additional macroscopic and microscopic observations, its core gating structure has not significantly changed (Lagrange et al. 2007).

3.6 Involvement of αβγ and αβδ $GABA_A$ Receptors in Epilepsy

Epilepsy is associated with abnormal hypersynchronous activation of neurons in specific neuronal networks. Although the mechanistic bases for partial and generalized forms of epilepsy are uncertain, there is considerable evidence that impaired GABAergic inhibition underlies several types of epilepsy. It has long been known that blockade of GABAergic inhibition with $GABA_A$ receptor antagonists such as penicillin, picrotoxin, or bicuculline produces paroxysmal bursting in isolated neurons and partial seizures in experimental animals (Schwartzkroin and Prince 1980). However, conclusive evidence that loss of GABAergic inhibition was involved in the pathogenesis of human epilepsy syndromes came from the discovery of mutations in genes encoding $GABA_A$ receptor subunits that were associated with genetic epilepsies (Baulac et al. 2001; Wallace et al. 2001; Macdonald et al. 2006). To date, multiple epilepsy mutations have been identified in γ-aminobutyric acid-A receptor γ2 (*GABRG2*), and epilepsy susceptibility variants have been identified in γ-aminobutyric acid-A receptor δ (*GABRD*).

A family with generalized epilepsy with febrile seizures plus (GEFS+) was found to have a γ2 subunit mutation, K328M, located in the extracellular M2–M3 linker (Baulac et al. 2001), a region implicated in transduction of ligand binding to channel gating (Kash et al. 2003). Consistent with the known importance of this protein domain to channel function, recordings from HEK293T cells co-expressing α1β2γ2(K328M) subunits were found to have defective channel gating (shortened mean open times) and accelerated deactivation (Bianchi et al. 2002b). A family with childhood absence epilepsy (CAE) and febrile seizures (FS) had a γ2 subunit mutation, R82Q, located in the benzodiazepine (BDZ)-binding domain of the extracellular N-terminal domain (Wallace et al. 2001). This reduced peak current amplitudes without altering channel kinetics (Bianchi et al. 2002b), the result of reduced surface expression of $GABA_A$ receptors due to retention in the ER (Kang and Macdonald 2004; Sancar and Czajkowski 2004) following disruption of intersubunit contacts at the γ–β subunit interface (Hales et al. 2005). However, γ2(R82Q) subunits may assemble with α3 subunits (Frugier et al. 2007), suggesting that the effects of the mutation may depend on the specific α subunit subtype involved. The γ2 subunit mutation, R177G, altered BDZ sensitivity and accelerated current desensitization (Audenaert et al. 2006). The γ2 subunit mutation, Q390X, which introduced a premature translation-termination codon (PTC) in the cytoplasmic M3–M4 linker was identified in a family with GEFS+ (Harkin et al. 2002). Mutant receptors had no GABA sensitivity when expressed in oocytes, probably due to ER retention. A γ2 subunit splice donor site mutation, IVS6+2T-G, was identified in a family with CAE and FSs (Kananura et al. 2002) that caused activation of a cryptic splice donor site in intron 6, retention of a portion of intron 6, a frame shift and production of a PTC. The resultant truncated γ2 subunit was stable but nonfunctional and led to decreased GABA-evoked current when transfected with α1 and β2 subunits in HEK293T cells (Tian and Macdonald 2012). A γ2 subunit, mutation Q40X, that introduced a PTC at the N-terminus of the mature peptide was identified in a family

with Dravet syndrome (Kanaumi et al. 2004), which triggered nonsense mediated mRNA decay, thus preventing production of even a signal peptide (Huang et al. 2012).

In contrast to epilepsy mutations found in *GABRG2,* no epilepsy mutations have been reported in *GABRD.* However, the *GABRD* variants, E177A and R220H, have been associated with GEFS+ (Dibbens et al. 2004), suggesting that *GABRD* may be a susceptibility gene for generalized epilepsy. These findings in humans suggest that defects in phasic inhibition are more pathogenic for genetic epilepsies than defects in tonic inhibition. Indeed, an increase rather than a decrease in tonic inhibition has been reported in animal models of absence epilepsy (Cope et al. 2009).

References

Angelotti TP, Macdonald RL (1993) Assembly of $GABA_A$ receptor subunits: alpha 1 beta 1 and alpha 1 beta 1 gamma 2S subunits produce unique ion channels with dissimilar single-channel properties. J Neurosci 13:1429–1440

Angelotti TP, Uhler MD, Macdonald RL (1993) Assembly of $GABA_A$ receptor subunits: analysis of transient single cell expression utilizing a fluorescent substrate/marker gene combination. J Neurosci 13:1418–1428

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

Audenaert D, Schwartz E, Claeys KG, Claes L, Deprez L, Suls A, Van Dyck T, Lagae L, Van Broeckhoven C, Macdonald RL, De Jonghe P (2006) The GABRG2 gene and febrile seizures: extension of the mutational spectrum. Neurology 67:687–690

Bach-y-Rita P (2001) Nonsynaptic diffusion neurotransmission in the brain: functional considerations. Neurochem Res 26:871–873

Backus KH, Arigoni M, Drescher U, Scheurer L, Malherbe P, Mohler H, Benson JA (1993) Stoichiometry of a recombinant $GABA_A$ receptor deduced from mutation-induced rectification. Neuroreport 5:285–288

Barberis A, Mozrzymas JW, Ortinski PI, Vicini S (2007) Desensitization and binding properties determine distinct alpha1beta2gamma2 and alpha3beta2gamma2 GABA(A) receptor-channel kinetic behavior. Eur J Neurosci 25:2726–2740

Barrera NP, Henderson RM, Edwardson JM (2008a) Determination of the architecture of ionotropic receptors using AFM imaging. Pflugers Arch 456:199–209

Barrera NP, Betts J, You H, Henderson RM, Martin IL, Dunn SM, Edwardson JM (2008b) Atomic force microscopy reveals the stoichiometry and subunit arrangement of the alpha4beta3delta GABA(A) receptor. Mol Pharmacol 73:960–967

Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, Baulac M, Brice A, Bruzzone R, LeGuern E (2001) First genetic evidence of $GABA_A$ receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat Genet 28:46–48

Baumann SW, Baur R, Sigel E (2001) Subunit arrangement of gamma-aminobutyric acid type A receptors. J Biol Chem 276:36275–36280

Baumann SW, Baur R, Sigel E (2002) Forced subunit assembly in alpha1beta2gamma2 $GABA_A$ receptors Insight into the absolute arrangement. J Biol Chem 277:46020–46025

Baur R, Minier F, Sigel E (2006) A GABA(A) receptor of defined subunit composition and positioning: concatenation of five subunits. FEBS Lett 580:1616–1620

Benke D, Honer M, Michel C, Mohler H (1996) $GABA_A$ receptor subtypes differentiated by their gamma-subunit variants: prevalence, pharmacology and subunit architecture. Neuropharmacology 35:1413–1423

Bianchi MT, Macdonald RL (2001a) Mutation of the 9′ leucine in the GABA(A) receptor gamma2L subunit produces an apparent decrease in desensitization by stabilizing open states without altering desensitized states. Neuropharmacology 41:737–744

Bianchi MT, Macdonald RL (2001b) Agonist trapping by $GABA_A$ receptor channels. J Neurosci 21:9083–9091

Bianchi MT, Macdonald RL (2002) Slow phases of GABA(A) receptor desensitization: structural determinants and possible relevance for synaptic function. J Physiol 544:3–18

Bianchi MT, Haas KF, Macdonald RL (2001) Structural determinants of fast desensitization and desensitization-deactivation coupling in $GABA_A$ receptors. J Neurosci 21:1127–1136

Bianchi MT, Haas KF, Macdonald RL (2002a) Alpha1 and alpha6 subunits specify distinct desensitization, deactivation and neurosteroid modulation of GABA(A) receptors containing the delta subunit. Neuropharmacology 43:492–502

Bianchi MT, Song L, Zhang H, Macdonald RL (2002b) Two different mechanisms of disinhibition produced by $GABA_A$ receptor mutations linked to epilepsy in humans. J Neurosci 22:5321–5327

Bianchi MT, Botzolakis EJ, Haas KF, Fisher JL, Macdonald RL (2007) Microscopic kinetic determinants of macroscopic currents: insights from coupling and uncoupling of $GABA_A$ receptor desensitization and deactivation. J Physiol 584:769–787

Bollan K, Robertson LA, Tang H, Connolly CN (2003) Multiple assembly signals in gamma-aminobutyric acid (type A) receptor subunits combine to drive receptor construction and composition. Bio Chem Soc Trans 31:875–879

Bonnert TP, McKernan RM, Farrar S, le Bourdelles B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Brown N, Wafford KA, Whiting PJ (1999) Theta, a novel gamma-aminobutyric acid type A receptor subunit. Proc Natl Acad Sci U S A 96:9891–9896

Bormann J, Hamill OP, Sakmann B (1987) Mechanism of anion permeation through channels gated by glycine and gamma-aminobutyric acid in mouse cultured spinal neurones. J Physiol 385:243–286

Botzolakis EJ, Maheshwari A, Feng HJ, Lagrange AH, Shaver JF, Kassebaum J, Venkataraman R, Baudenbacher FJ, Macdonald RL (2008) Achieving synaptically relevant pulses of neurotransmitter using PDMS microfluidics. J Neurosci Methods 177:294–302

Brickley SG, Cull-Candy SG, Farrant M (1996) Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of $GABA_A$ receptors. J Physiol 497(Pt 3):753–759

Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M (2001) Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. Nature 409:88–92

Bright DP, Renzi M, Bartram J, McGee TP, Mackenzi G, Hosie AM, Farrant J, Brickley SG (2011) Profound desensitization by ambient GABA limits activation of δ-containing $GABA_A$ receptors during spillover. J Neurosci 31:753–763

Burgard EC, Tietz EI, Neelands TR, Macdonald RL (1996) Properties of recombinant gamma-aminobutyric acid A receptor isoforms containing the alpha 5 subunit subtype. Mol Pharmacol 50:119–127

Burkat PM, Yang J, Gingrich KJ (2001) Dominant gating governing transient GABA(A) receptor activity: a first latency and Po/o analysis. J Neurosci 21:7026–7036

Burzomato V, Beato M, Groot-Kormelink PJ, Colquhoun D, Sivilotti LG (2004) Single-channel behavior of heteromeric alpha1beta glycine receptors: an attempt to detect a conformational change before the channel opens. J Neurosci 24:10924–10940

Celentano JJ, Wong RK (1994) Multiphasic desensitization of the $GABA_A$ receptor in outside-out patches. Biophys J 66:1039–1050

Chakrapani S, Bailey TD, Auerbach A (2004) Gating dynamics of the acetylcholine receptor extracellular domain. J Gen Physiol 123:341–356

Chang Y, Wang R, Barot S, Weiss DS (1996) Stoichiometry of a recombinant $GABA_A$ receptor. J Neurosci 16:5415–5424

Clements JD (1996) Transmitter timecourse in the synaptic cleft: its role in central synaptic function. Trends Neurosci 19:163–171

Connolly CN, Krishek BJ, McDonald BJ, Smart TG, Moss SJ (1996) Assembly and cell surface expression of heteromeric and homomeric gamma-aminobutyric acid type A receptors. J Biol Chem 271:89–96

Connolly CN, Kittler JT, Thomas P, Uren JM, Brandon NJ, Smart TG, Moss SJ (1999) Cell surface stability of gamma-aminobutyric acid type A receptors Dependence on protein kinase C activity and subunit composition. J Biol Chem 274:36565–36572

Cope DW, Di Giovanni G, Fyson SJ, Orban G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V (2009) Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. Nat Med 15:1392–1398

Dallwig R, Deitmer JW, Backus KH (1999) On the mechanism of GABA-induced currents in cultured rat cortical neurons. Pflugers Arch 437:289–297

Davies PA, Hanna MC, Hales TG, Kirkness EF (1997) Insensitivity to anaesthetic agents conferred by a class of GABA(A) receptor subunit. Nature 385:820–823

Davies PA, Wang W, Hales TG, Kirkness EF (2003) A novel class of ligand-gated ion channel is activated by Zn2+. J Biol Chem 278:712–717

Dibbens LM, Feng HJ, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC (2004) GABRD encoding a protein for extra- or peri-synaptic $GABA_A$ receptors is a susceptibility locus for generalized epilepsies. Hum Mol Genet 13:1315–1319

Ellgaard L, Frickel EM (2003) Calnexin, calreticulin, and ERp57: teammates in glycoprotein folding. Cell Biochem Biophys 39:223–247

Eugene E, Depienne C, Baulac S, Baulac M, Fritschy JM, Le Guern E, Miles R, Poncer JC (2007) GABA(A) receptor gamma 2 subunit mutations linked to human epileptic syndromes differentially affect phasic and tonic inhibition. J Neurosci 27:14108–14116

Farrar SJ, Whiting PJ, Bonnert TP, McKernan RM (1999) Stoichiometry of a ligand-gated ion channel determined by fluorescence energy transfer. J Biol Chem 274:10100–10104

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 6:215–229

Feng HJ, Macdonald RL (2004) Multiple actions of propofol on alphabetagamma and alphabetadelta $GABA_A$ receptors. Mol Pharmacol 66:1517–1524

Feng HJ, Bianchi MT, Macdonald RL (2004) Pentobarbital differentially modulates alpha1beta3delta and alpha1beta3gamma2L $GABA_A$ receptor currents. Mol Pharmacol 66:988–1003

Feng HJ, Kang JQ, Song L, Dibbens L, Mulley J, Macdonald RL (2006) Delta subunit susceptibility variants E177A and R220H associated with complex epilepsy alter channel gating and surface expression of alpha4beta2delta $GABA_A$ receptors. J Neurosci 26:1499–1506

Feng HJ, Mathews GC, Kao C, Macdonald RL (2008) Alterations of GABA A-receptor function and allosteric modulation during development of status epilepticus. J Neurophysiol 99:1285–1293

Fisher JL, Macdonald RL (1997a) Single channel properties of recombinant $GABA_A$ receptors containing gamma 2 or delta subtypes expressed with alpha 1 and beta 3 subtypes in mouse L929 cells. J Physiol 505(Pt 2):283–297

Fisher JL, Macdonald RL (1997b) Functional properties of recombinant GABA(A) receptors composed of single or multiple beta subunit subtypes. Neuropharmacology 36:1601–1610

Fisher JL, Zhang J, Macdonald RL (1997) The role of alpha1 and alpha6 subtype amino-terminal domains in allosteric regulation of gamma-aminobutyric acida receptors. Mol Pharmacol 52:714–724

Frugier G, Coussen F, Giraud MF, Odessa MF, Emerit MB, Boue-Grabot E, Garret M (2007) A gamma 2(R43Q) mutation, linked to epilepsy in humans, alters $GABA_A$ receptor assembly and modifies subunit composition on the cell surface. J Biol Chem 282:3819–3828

Glavinovic MI (1999) Monte carlo simulation of vesicular release, spatiotemporal distribution of glutamate in synaptic cleft and generation of postsynaptic currents. Pflugers Arch 437:462–470

Glykys J, Mann EO, Mody I (2008) Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? J Neurosci 28:1421–1426

Gorrie GH, Vallis Y, Stephenson A, Whitfield J, Browning B, Smart TG, Moss SJ (1997) Assembly of $GABA_A$ receptors composed of alpha1 and beta2 subunits in both cultured neurons and fibroblasts. J Neurosci 17:6587–6596

Green WN, Millar NS (1995) Ion-channel assembly. Trends Neurosci 18:280–287

Grover LM, Lambert NA, Schwartzkroin PA, Teyler TJ (1993) Role of HCO3- ions in depolarizing $GABA_A$ receptor-mediated responses in pyramidal cells of rat hippocampus. J Neurophysiol 69:1541–1555

Haas KF, Macdonald RL (1999) $GABA_A$ receptor subunit gamma2 and delta subtypes confer unique kinetic properties on recombinant $GABA_A$ receptor currents in mouse fibroblasts. J Physiol 514(Pt 1):27–45

Hales TG, Tang H, Bollan KA, Johnson SJ, King DP, McDonald NA, Cheng A, Connolly CN (2005) The epilepsy mutation, gamma2(R43Q) disrupts a highly conserved inter-subunit contact site, perturbing the biogenesis of $GABA_A$ receptors. Mol Cell Neurosci 29:120–127

Hamann M, Rossi DJ, Attwell D (2002) Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. Neuron 33:625–633

Harkin LA, Bowser DN, Dibbens LM, Singh R, Phillips F, Wallace RH, Richards MC, Williams DA, Mulley JC, Berkovic SF, Scheffer IE, Petrou S (2002) Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. Am J Hum Genet 70:530–536

Hedblom E, Kirkness EF (1997) A novel class of $GABA_A$ receptor subunit in tissues of the reproductive system. J Biol Chem 272:15346–15350

Herb A, Wisden W, Luddens H, Puia G, Vicini S, Seeburg PH (1992) The third gamma subunit of the gamma-aminobutyric acid type A receptor family. Proc Natl Acad Sci U S A 89:1433–1437

Horn R, Vandenberg CA (1984) Statistical properties of single sodium channels. J Gen Physiol 84:505–534

Huang X, Tian M, Hernandez C, Hu N, Macdonald RL (2012) The Dravet syndrome-associated GABRG2 nonsense mutation, Q40X, activated NMD and generated a truncated subunit that was partially rescued by aminoglycoside-induced stop codon read-through. Neurobiol Dis 48:115–123

Jones MV, Westbrook GL (1995) Desensitized states prolong $GABA_A$ channel responses to brief agonist pulses. Neuron 15:181–191

Jones MV, Westbrook GL (1996) The impact of receptor desensitization on fast synaptic transmission. Trends Neurosci 19:96–101

Kanaumi GF, Ueno S, Ishi A, Haga Y, Hamachi A, Yonetani M, Itoh M, Takashima S, Kaneko S, Mitsudome A, Hirose S (2004) Possible pathogenesis of severe myoclonic epilepsy in infancy: a novel nonsense mutation of GABRG2 leading to aggregation of $GABA_A$ receptors in neurons. Neurology Asia 9 (Suppl 1):151

Kananura C, Haug K, Sander T et al. (2002) A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. Arch Neurol 59:1137–1141

Kang JQ, Macdonald RL (2004) The $GABA_A$ receptor gamma2 subunit R43Q mutation linked to childhood absence epilepsy and febrile seizures causes retention of alpha1beta2gamma2S receptors in the endoplasmic reticulum. J Neurosci 24:8672–8677

Kash TL, Jenkins A, Kelley JC, Trudell JR, Harrison NL (2003) Coupling of agonist binding to channel gating in the GABA(A) receptor. Nature 421:272–275

Kaur KH, Baur R, Sigel E (2009) Unanticipated structural and functional properties of delta subunit containing $GABA_A$ receptors. J Biol Chem 284:7889–7896

Keramidas A, Harrison NL (2008) Agonist-dependent single channel current and gating in alpha4beta2delta and alpha1beta2gamma2S $GABA_A$ receptors. J Gen Physiol 131:163–181

Khan ZU, Gutierrez A, De Blas AL (1994) Short and long form gamma 2 subunits of the $GABA_A$/benzodiazepine receptors. J Neurochem 63:1466–1476

Kim DY, Fenoglio KA, Kerrigan JF, Rho JM (2009) Bicarbonate contributes to $GABA_A$ receptor-mediated neuronal excitation in surgically resected human hypothalamic hamartomas. Epilepsy Res 83:89–93

Kirkness EF, Fraser CM (1993) A strong promoter element is located between alternative exons of a gene encoding the human gamma-aminobutyric acid-type A receptor beta 3 subunit (GABRB3). J Biol Chem 268:4420–4428

Klausberger T, Ehya N, Fuchs K, Fuchs T, Ebert V, Sarto I, Sieghart W (2001) Detection and binding properties of GABA(A) receptor assembly intermediates. J Biol Chem 276:16024–16032

Knight AR, Hartnett C, Marks C, Brown M, Gallager D, Tallman J, Ramabhadran TV (1998) Molecular size of recombinant alpha1beta1 and alpha1beta1gamma2 $GABA_A$ receptors expressed in Sf9 cells. Receptors Channels 6:1–18

Korn SJ, Horn R (1988) Statistical discrimination of fractal and Markov models of single-channel gating. Biophys J 54:871–877

Lagrange AH, Botzolakis EJ, Macdonald RL (2007) Enhanced macroscopic desensitization shapes the response of alpha4 subtype-containing $GABA_A$ receptors to synaptic and extrasynaptic GABA. J Physiol 578:655–676

Langosch D, Thomas L, Betz H (1988) Conserved quaternary structure of ligand-gated ion channels: the postsynaptic glycine receptor is a pentamer. Proc Natl Acad Sci U S A 85:7394–7398

Lape R, Colquhoun D, Sivilotti LG (2008) On the nature of partial agonism in the nicotinic receptor superfamily. Nature 454:722–727

Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Kohler M, Fujita N, Rodriguez HF, Stephenson A, Darlison MG (1988) Structural and functional basis for $GABA_A$ receptor heterogeneity. Nature 335:76–79

Lo WY, Botzolakis EJ, Tang X, Macdonald RL (2008) A conserved Cys-loop receptor aspartate residue in the M3-M4 cytoplasmic loop is required for $GABA_A$ receptor assembly. J Biol Chem 283:29740–29752

Macdonald RL, Rogers CJ, Twyman RE (1989) Kinetic properties of the $GABA_A$ receptor main conductance state of mouse spinal cord neurones in culture. J Physiol 410:479–499

Macdonald RL, Kang JQ, Gallagher MJ, Feng HJ (2006) GABA(A) receptor mutations associated with generalized epilepsies. Adv Pharmacol 54:147–169

Maconochie DJ, Zempel JM, Steinbach JH (1994) How quickly can $GABA_A$ receptors open? Neuron 12:61–71

Mamalaki C, Barnard EA, Stephenson FA (1989) Molecular size of the gamma-aminobutyric acid A receptor purified from mammalian cerebral cortex. J Neurochem 52:124–134

Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D (1991) Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science 254:432–437

McKernan RM, Whiting PJ (1996) Which $GABA_A$-receptor subtypes really occur in the brain? Trends Neurosci 19:139–143

McManus OB, Weiss DS, Spivak CE, Blatz AL, Magleby KL (1988) Fractal models are inadequate for the kinetics of four different ion channels. Biophys J 54:859–870

Mercik K, Pytel M, Cherubini E, Mozrzymas JW (2006) Effect of extracellular pH on recombinant alpha1beta2gamma2 and alpha1beta2 $GABA_A$ receptors. Neuropharmacology 51:305–314

Mortensen M, Ebert B, Wafford K, Smart TG (2004a) Distinct activities of agonists at synaptic and extrasynaptic type $GABA_A$ receptors. J Physiol 558:1251–1268

Mortensen M, Kristiansen U, Ebert B, Frølund B, Krogsgaard-Larsen P, Smart TG (2004b) Activation of single heteromeric GABA(A) receptor ion channels by full and partial agonists. J Physiol 557:389–413

Mozrzymas JW, Barberis A, Mercik K, Zarnowska ED (2003) Binding sites, singly bound states, and conformation coupling shape GABA-evoked currents. J Neurophysiol 89:871–883

Nayeem N, Green TP, Martin IL, Barnard EA (1994) Quaternary structure of the native $GABA_A$ receptor determined by electron microscopic image analysis. J Neurochem 62:815–818

Neelands TR, Macdonald RL (1999) Incorporation of the pi subunit into functional gamma-aminobutyric Acid(A) receptors. Mol Pharmacol 56:598–610

Neelands TR, Fisher JL, Bianchi MT, Macdonald RL (1999) Spontaneous and gamma-aminobutyric acid (GABA)-activated GABA(A) receptor channels formed by epsilon subunit-containing isoforms. Mol Pharmacol 55:168–178

Newland CF, Colquhoun D, Cull-Candy SG (1991) Single channels activated by high concentrations of GABA in superior cervical ganglion neurones of the rat. J Physiol 432:203–233

Noda M, Takahashi H, Tanabe T, Toyosato M, Kikyotani S, Furutani Y, Hirose T, Takashima H, Inayama S, Miyata T, Numa S (1983) Structural homology of *Torpedo californica* acetylcholine receptor subunits. Nature 302:528–532

Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693–1703

Ohlson J, Pedersen JS, Haussler D, Ohman M (2007) Editing modifies the GABA(A) receptor subunit alpha3. RNA 13:698–703

Olsen RW, Sieghart W (2009) GABA(A) receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56:141–148

Olsen RW, Tobin AJ (1990) Molecular biology of $GABA_A$ receptors. FASEB J 4:1469–1480

Pan Y, Ripps H, Qian H (2006) Random assembly of GABA rho1 and rho2 subunits in the formation of heteromeric GABA(C) receptors. Cell Mol Neurobiol 26:289–305

Perkins KL, Wong RK (1997) The depolarizing GABA response. Can J Physiol Pharmacol 75:516–519

Picton AJ, Fisher JL (2007) Effect of the alpha subunit subtype on the macroscopic kinetic properties of recombinant GABA(A) receptors. Brain Res 1165:40–49

Plested AJ, Groot-Kormelink PJ, Colquhoun D, Sivilotti LG (2007) Single-channel study of the spasmodic mutation alpha1A52S in recombinant rat glycine receptors. J Physiol 581:51–73

Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, Seeburg PH (1989) Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338:582–585

Quirk K, Gillard NP, Ragan CI, Whiting PJ, McKernan RM (1994) gamma-Aminobutyric acid type A receptors in the rat brain can contain both gamma 2 and gamma 3 subunits, but gamma 1 does not exist in combination with another gamma subunit. Mol Pharmacol 45:1061–1070

Rothberg BS, Magleby KL (2000) Voltage and Ca2+ activation of single large-conductance Ca2+-activated K+ channels described by a two-tiered allosteric gating mechanism. J Gen Physiol 116:75–99

Rula EY, Lagrange AH, Jacobs MM, Hu N, Macdonald RL, Emeson RB (2008) Developmental modulation of GABA(A) receptor function by RNA editing. J Neurosci 28:6196–6201

Sancar F, Czajkowski C (2004) A $GABA_A$ receptor mutation linked to human epilepsy (gamma2R43Q) impairs cell surface expression of alphabetagamma receptors. J Biol Chem 279:47034–47039

Sansom MS, Ball FG, Kerry CJ, McGee R, Ramsey RL, Usherwood PN (1989) Markov, fractal, diffusion, and related models of ion channel gating A comparison with experimental data from two ion channels. Biophys J 56:1229–1243

Sarto-Jackson I, Sieghart W (2008) Assembly of GABA(A) receptors (review). Mol Membr Biol 25:302–310

Saxena NC, Macdonald RL (1994) Assembly of $GABA_A$ receptor subunits: role of the delta subunit. J Neurosci 14:7077–7086

Saxena NC, Macdonald RL (1996) Properties of putative cerebellar gamma-aminobutyric acid A receptor isoforms. Mol Pharmacol 49:567–579

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA (1987) Sequence and functional expression of the GABA A receptor shows a ligand-gated receptor super-family. Nature 328:221–227

Schoppa NE, Sigworth FJ (1998) Activation of Shaker potassium channels III An activation gating model for wild-type and V2 mutant channels. J Gen Physiol 111:313–342

Schwartzkroin PA, Prince DA (1980) Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity. Brain Res 183:61–76

Shivers BD, Killisch I, Sprengel R, Sontheimer H, Kohler M, Schofield PR, Seeburg PH (1989) Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. Neuron 3:327–337

Sigg D, Bezanilla F (2003) A physical model of potassium channel activation: from energy landscape to gating kinetics. Biophys J 84:3703–3716

Simon J, Wakimoto H, Fujita N, Lalande M, Barnard EA (2004) Analysis of the set of GABA(A) receptor genes in the human genome. J Biol Chem 279:41422–41435

Taylor PM, Thomas P, Gorrie GH, Connolly CN, Smart TG, Moss SJ (1999) Identification of amino acid residues within GABA(A) receptor beta subunits that mediate both homomeric and heteromeric receptor expression. J Neurosci 19:6360–6371

Taylor PM, Connolly CN, Kittler JT, Gorrie GH, Hosie A, Smart TG, Moss SJ (2000) Identification of residues within GABA(A) receptor alpha subunits that mediate specific assembly with receptor beta subunits. J Neurosci 20:1297–1306

Tian M, Macdonald RL (2012) The intronic GABRG2 mutation, IVS6+2TàG, associated with CAE altered γ2 subunit mRNA intron splicing and activated nonsense-mediated mRNA decay and altered splicing. J Neurosci 32:5937–5952

Tretter V, Ehya N, Fuchs K, Sieghart W (1997) Stoichiometry and assembly of a recombinant $GABA_A$ receptor subtype. J Neurosci 17:2728–2737

Twyman RE, Macdonald RL (1992) Neurosteroid regulation of $GABA_A$ receptor single-channel kinetic properties of mouse spinal cord neurons in culture. J Physiol 456:215–245

Twyman RE, Rogers CJ, Macdonald RL (1989a) Differential regulation of gamma-aminobutyric acid receptor channels by diazepam and phenobarbital. Ann Neurol 25:213–220

Twyman RE, Rogers CJ, Macdonald RL (1989b) Pentobarbital and picrotoxin have reciprocal actions on single $GABA_A$ receptor channels. Neurosci Lett 96:89–95

Twyman RE, Rogers CJ, Macdonald RL (1990) Intraburst kinetic properties of the $GABA_A$ receptor main conductance state of mouse spinal cord neurones in culture. J Physiol 423:193–220

Ventriglia F, Di MV (2003) Stochastic fluctuations of the quantal EPSC amplitude in computer simulated excitatory synapses of hippocampus. Biosystems 71:195–204

Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF (2001) Mutant $GABA_A$ receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 28:49–52

Wanamaker CP, Green WN (2007) Endoplasmic reticulum chaperones stabilize nicotinic receptor subunits and regulate receptor assembly. J Biol Chem 282:31113–31123

Weiss DS, Magleby KL (1989) Gating scheme for single GABA-activated Cl- channels determined from stability plots, dwell-time distributions, and adjacent-interval durations. J Neurosci 9:1314–1324

Whiting P, McKernan RM, Iversen LL (1990) Another mechanism for creating diversity in gamma-aminobutyrate type A receptors: RNA splicing directs expression of two forms of gamma 2 phosphorylation site. Proc Natl Acad Sci U S A 87:9966–9970

Ymer S, Schofield PR, Draguhn A, Werner P, Kohler M, Seeburg PH (1989a) $GABA_A$ receptor beta subunit heterogeneity: functional expression of cloned cDNAs. EMBO J 8:1665–1670

Ymer S, Schofield PR, Shivers BD, Pritchett DB, Luddens H, Kohler M, Werner P, Sontheimer H, Kettenmann H, Seeburg PH (1989b) Molecular studies of the $GABA_A$ receptor. J Protein Chem 8:352–355

Zagotta WN, Hoshi T, Aldrich RW (1994) Shaker potassium channel gating III: evaluation of kinetic models for activation. J Gen Physiol 103:321–362

Chapter 4
The Pharmacology of Extrasynaptic $GABA_A$ Receptors

Keith A Wafford

Abstract The different subunits that make up the family of $GABA_A$ receptor subtypes have unique distributions within the brain and nervous system. Their localization at the neuronal level is in many cases not necessarily associated with synaptic densities, and this has led to the hypothesis that extrasynaptic receptors perform a unique function in controlling excitability. In most cases, the subunits that make up extrasynaptic receptors are different to those on synaptic membranes and hence have their own unique pharmacological profile, both in respect to agonists and allosteric modulators. Here I will review the different receptor subtypes that have been classified as extrasynaptic, as well as those that may serve both roles depending on their location, with a view to illustrating their pharmacological properties, and their impact on neuronal function. The identification of functional differences and allosteric sites for specific modulation of these receptors offers an opportunity to gain more knowledge of the role of receptor subtypes and the potential to develop novel therapeutic agents that should impact a number of psychiatric and neurological disorders where these receptors are implicated.

Keywords δ subunit · α5 subunit · Gaboxadol · Anaesthetic · Allosteric modulator · Benzodiazepine

4.1 Introduction

Healthy and efficient activity of the mammalian brain relies on a careful balance between neuronal excitation and inhibition within the networks that control its activity. A major component of that inhibitory control is contributed via the neurotransmitter γ-aminobutyric acid (GABA) acting on a large family of ligand-gated ion channel (LGIC) receptors and G protein-coupled receptors. The ligand-gated $GABA_A$ receptors have a widespread distribution throughout the brain and until recently were believed to exert their prominent effects, inhibiting neuronal excitation, entirely through their clearly demonstrated presence on postsynaptic membranes of GABAergic synapses. More detailed localization experiments and electrophysiological

K. A. Wafford (✉)
Eli Lilly UK, Erl Wood Manor, GU20 6PH Windlesham, Surrey, UK
e-mail: waffordke@lilly.com

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_4

studies have now shown that in addition to their postsynaptic location, $GABA_A$ receptors are also present outside the synapse both on cell bodies and peripheral to synapses. Thought at first to be non-functional or perhaps receptors in transit, recent evidence has suggested that these receptors play a major role in neuronal excitability and are involved in a number of important functions including sleep, cognition and epilepsy. Moreover, these receptors have physiological properties that differentiate them from their synaptic partners and make them ideally suited for their extrasynaptic role. The pharmacological properties of these receptors also differ in many respects, and offer an opportunity to specifically target them as a route to potential novel therapeutic drugs. This chapter will review the pharmacological properties of those $GABA_A$ receptors which are primarily extrasynaptic and compare their properties to postsynaptic $GABA_A$ receptor subtypes.

4.2 $GABA_A$ Receptor Subtypes

All $GABA_A$ receptors are made up of a pentameric arrangement of heterogeneous subunits which combine to form a pore in the plasma membrane that is selectively permeable to chloride (Cl^-) ions. Activation of the receptor allows chloride to pass through the channel in either direction depending on the driving force on the cell. This change in chloride levels will directly influence the excitability of the neuron and will be inhibitory in most cases but in some instances can elicit an excitatory response. The $GABA_A$ receptor family consists of a number of related subunits including α1–α6, β1–β3, γ1–γ3, δ, ε, θ, ρ1–3. The majority of $GABA_A$ receptors comprise two α subunits, two β subunits and one other, predominantly γ2. ρ–containing subunits are members of the of $GABA_A$ receptor family but form an independent receptor sometimes referred to as $GABA_C$ that has its own unique pharmacology and can assemble as a homomeric receptor. Each GABA receptor subunit has an individual distinctive pattern of expression throughout the central nervous system (CNS) and this determines the nature of receptors expressed in particular brain structures. For example, the most abundant receptor in the brain is the α1β2γ2 subtype which is expressed widely, particularly in the cortex, thalamus, olfactory bulb and some parts of the hippocampus (Pirker et al. 2000). Even in the same brain region, expression of subtypes can be cell specific; the major $GABA_A$ receptor subtype in cerebellar Purkinje neurons is α1β3γ2 but in cerebellar granule cells α6βδ predominates (Pirker et al. 2000). Another clear example is the nucleus reticularis of the thalamus which exclusively expresses the α3-containing subtype (Studer et al. 2006). Differential distribution of subunits has also been shown to be present within an individual cell with different receptors being targeted to different parts of the plasma membrane (Mangan et al. 2005). Different promoters control the regional expression of individual receptor subunits in different brain regions, and there are molecular chaperones which target and traffic the subunits on a cellular basis. There have been a number of these identified for $GABA_A$ receptors, including gephyrin, GABARAP, PLIC, GRIF-1, protocadherin γC5, radixin and GRIP (reviewed in Luscher et al. 2011; Tretter et al. 2012).

Although immunoprecipitation techniques have estimated that there are likely to be 10–12 major receptor subtypes, it is impossible not to preclude any minor combinations that might occur at very low levels and with highly specific regional expression (McKernan and Whiting 1996). The question remains however, which of these receptor subtypes are located postsynaptically, and which are extrasynaptic? Until recently it was thought that the γ2 subunit played a major part in determining postsynaptic clustering since receptors in γ2 subunit-deleted mice did not appear to form proper postsynaptic clusters (Günther et al. 1995), a feature which could be restored by transfecting neurons with γ2 cDNA (Baer et al. 2000). More recently, the role played by the protein gephyrin in determining postsynaptic clustering has been better understood and gephyrin binding sites identified on α1, α2 and α3 receptors, which seem to be more important for localizing receptors at postsynaptic membranes (Tretter et al. 2012; Wu et al. 2012). Co-immunolabelling with the GABAergic synaptic marker neuroligin-2 demonstrated that α1, α2 and β3 in CA1 hippocampal membranes are primarily expressed in synaptic membranes compared to extrasynaptic membranes (Kasugai et al. 2010). In contrast, similar experiments studying the δ subunit showed that receptors containing this subunit were excluded from synaptic locations revealing an extrasynaptic location (Nusser et al. 1998). α5 subunit-containing receptors are interesting in this regard since they are primarily thought to be extrasynaptic (Brunig et al. 2002). There is some evidence however, for α5 contributing to postsynaptic responses in the hippocampus (Zarnowska et al. 2009; Serwanski et al. 2006). Recent work has revealed that α5-containing receptors are able to form clusters on the neuronal cell-body membrane associated with the actin-binding protein radixin (Loebrich et al. 2006), and these authors speculate that α5-containing receptors might form specialised neuron–glia contacts. It is likely then that the majority of α1, α2 and α3 plus γ2-containing receptors are primarily postsynaptic in nature, while α4, α5 and α6 appear to be predominantly extrasynaptically located. Alpha 4 and α6 are preferentially co-expressed with the δsubunit, forming receptors with unique biophysical characteristics that enable them to perform quite different functions to postsynaptic receptors, however there is also a population of α4 and α6βγ2 receptors. The α5 subunit co-assembles with a β and γ2 subunit with similar properties to α1, α2 and α3. This receptor is highly sensitive to GABA, and with both extrasynaptic and synaptic locations appears to mediate both tonic extrasynaptic conductance and phasic inhibitory synaptic activity.

4.3 Different Biophysical Properties of Synaptic and Extrasynaptic Receptors

The fact that $GABA_A$ receptors are found outside of the synapse has been known for many years. However, the precise nature of these receptors and identification of a functional role has only been uncovered fairly recently. Postsynaptic receptors clearly respond to activation by presynaptically released GABA, usually at relatively high localised concentrations. As the released GABA is cleared via reuptake mechanisms

and diffusion, the response diminishes rapidly. The majority of postsynaptic receptors will also rapidly desensitize following agonist exposure. Postsynaptic $GABA_A$ receptors usually have a relatively low sensitivity for GABA, typically in the 10–50 μM range. This may be related to the high concentrations of GABA usually found in the synaptic cleft. Having low sensitivity for GABA and rapid desensitization means that these types of receptors located on the neuronal membrane will not be activated following exposure to low concentrations of GABA. The delta subunit which co-assembles primarily with α4 and α6 is exclusively located extrasynaptically and confers several properties that differentiate these receptors from α1, α2 and α3 containing synaptic receptors. Firstly, these receptors possess a significantly higher sensitivity to agonists such as GABA; they are usually activated at concentrations of less than 1 μM GABA (Brown et al. 2002; Saxena and MacDonald 1996; Mortensen et al. 2011). Secondly, they exhibit a lower degree of receptor desensitization (Saxena and MacDonald 1996; Mortensen et al. 2010) to enable sustained or tonic levels of activation, and thirdly a lower open-channel probability, restricting the maximum level of receptor activation (Mortensen et al. 2010). These properties allow receptors to be activated by low levels of extracellular GABA in a continuous manner providing dynamic regulation of the resting membrane potential and a gain control on neuronal output (Semyanov et al. 2004; Cope et al. 2005; Duguid et al. 2012). The presence of a native tonic GABAergic current was first demonstrated in cerebellar granule neurons, which primarily express α6βδ receptors (Brickley et al. 1996). The same type of current was also later identified in various brain regions expressing the α4βδ subtype, such as dentate gyrus of the hippocampus (Stell and Mody 2002; Yeung et al. 2003) and thalamic relay neurons (Jia et al. 2005; Herd et al. 2009; Cope et al. 2005). In addition, δ-containing receptors are expressed at low levels in cerebral cortex and are localised on neurogliaform interneurons (Capogna 2011), which can release GABA to produce self-inhibition and inhibition of surrounding cells expressing δ-containing $GABA_A$ receptors (Oláh et al. 2009). Tonic currents have been demonstrated not only in brain slice preparations but also *in vivo*, for example in the cerebellum (Chadderton et al. 2004). The presence of a tonically activated GABA channel will directly influence the level of intracellular chloride and this in turn is dependent on the presence of chloride transporters such as KCC2 producing a dynamic balance. Interestingly, this has now come full circle, in that the level of intracellular chloride itself has recently been shown to regulate the expression of GABA receptor subunits (Succol et al. 2012). The source of GABA eliciting tonic currents is thought to result from either spillover at synapses or release from surrounding glia (Rossi et al. 2003)(see Chap. 6). Block of GABA uptake by drugs such as tiagabine or NO-711 can enhance tonic currents and in the cerebellum for example, one source of GABA is thought to be via release from astrocytes through bestrophin-1 anion channels (Lee et al. 2010). This group demonstrated that silencing bestrophin-1 could completely abolish tonic currents in granule cells (Lee et al. 2010) and that the levels of astrocytic GABA were generally higher in brain regions identified as expressing GABA-mediated tonic currents, suggesting that release from astrocytes may be a primary source of ambient GABA in these areas (Yoon et al. 2011).

4.4 δ Subunit-Containing Receptors

The δ subunit is highly expressed in cerebellar granule cells where it assembles with α6 and β2/3, and in hippocampal dentate gyrus and the ventrobasal thalamus where it assembles with α4 and β2/3 (Saxena and Macdonald 1996; Brickley et al. 1996; Jia et al. 2005; Stell and Mody 2002). Lower levels of δ subunit expression have been reported in cortex and striatum where it may co-assamble with other α subunits such as α1 (Drasbek and Jensen 2006; Santhakumar et al. 2010). The δ subunit substitutes for the γ subunit resulting in a number of effects on the pharmacology of these subtypes. Firstly, the γ2 subunit contributes a major part to the binding site for benzodiazepines (BZ), and substitution with the δ subunit ablates this binding site making these receptors insensitive to modulation by BZ (Quirk et al. 1995; Saxena and Macdonald 1996; Brown et al. 2002). This change to the binding site is not exclusively down to the δ subunit. The α4 and α6 subunits also co-assemble with βγ2 forming a modified binding site which can bind some BZ site ligands, particularly the imidazobenzodiazepines such as bretazenil and flumazenil, with reasonably high affinity. However, given the different nature of the binding site, the functional efficacy of these drugs is modified. For example, the BZ ligand Ro15-4513 behaves as a partial inverse agonist at α1β3γ2 but has full agonist efficacy at α4β3γ2 and α6β3γ2 (Brown et al. 2002). This change is due to a single histidine to arginine change between the α1 and α4/6 subunits (Wieland et al. 1992). The amino acids contributing to the BZ site are now well explored and pharmacophore modelling has revealed a number of residues important for the binding of BZs (Sigel and Luscher 2011). It is not just the binding site residues that make a difference in functional modulation however, and chimeric subunit studies using γ2 and δ have identified functionally relevant residues Tyr235, Phe236, Thr237 in transmembrane domain 1 (TM1) and Ser280, Thr281, Ile282 in TM2. The TM2–TM3 loop also forms a pocket responsible for transducing BZ signalling to potentiate channel opening in γ2 containing receptors, a region which is different in δ-containing receptors (Jones-Davis et al. 2005). It is currently unknown whether this binding pocket plays a role in the action of other allosteric modulators that can affect δ-containing receptors.

$GABA_A$ receptors are also known to be modulated by some types of steroid, many of which are endogenous, for example, 5α-pregnan-3α-ol-20-one which is a metabolite of progesterone, and 5α-pregnan-3α,21-diol-20-one a metabolite of deoxycorticosterone (Belelli and Lambert 2005)(see Chap. 5). These neurosteroid type modulators are produced at levels which may influence inhibitory synaptic transmission, but given that they also affect tonically active, extrasynaptic receptors, they may actually exert a much more profound effect by modulating these types of receptors (Stell et al. 2003). The potentiating effect of neurosteroids was reported to be much greater at δ-containing receptors than other γ2 containing subtypes (Wohlfarth et al. 2002; Brown et al. 2002). Subsequent exploration of this mechanism however suggests that the apparent increase in neurosteroid efficacy is more likely due to the low efficacy of GABA itself at δ-containing receptors,

resulting in a greater potential for modulation (Bianchi and MacDonald 2003; Shu et al. 2012). The $\delta^{-/-}$ mouse displays a reduced sensitivity to neurosteroids *in vivo*, suggesting that δ-containing receptors are an important target for these modulators (Mihalek et al. 1999). δ subunit expression can also be regulated by the levels of endogenous steroid in females (Maguire and Mody 2009) and these receptors may play a role in the pathology of some types of menstrual disorder such as anxiety associated with premenstrual dysphoria, catamenial epilepsy and postpartum depression (Maguire and Mody 2008; Brickley and Mody 2012)(see Chap. 12). Several residues on the α-subunit have been identified to play a role in conferring the allosteric modulation by neurosteroids (Hosie et al. 2006) and indeed the binding site is conserved in α4β3δ receptors suggesting that the δ subunit does not form part of this site (Hosie et al. 2009). An additional complicating factor with neurosteroid modulation of $GABA_A$ receptors is that it appears to be somewhat dependent on the phosphorylation state of the receptor and reduction of protein kinase C- (PKC) or protein kinase A- (PKA) dependent phosphorylation reduces the degree of potentiation by neurosteroids (Harney et al. 2003). This factor is also likely to influence steroid effects on extrasynaptic δ–containing receptors, since the α4 and β3 subunits also contain sites for phosphorylation and these receptors are regulated by protein kinases (Abramian et al. 2010; Saliba et al. 2012).

Another important pharmacological property of $GABA_A$ receptors is their sensitivity to anaesthetics and it is believed that this mechanism underlies the *in vivo* activity of many commonly used anaesthetics such as propofol and etomidate, as well as the volatile anaesthetics such as halothane and isoflurane (Franks 2008). Like neurosteroids, the majority of $GABA_A$ receptor subtypes have binding sites for anaesthetics and robust enhancement of GABA activity is observed with these agents (Forman and Miller 2011). Site-directed mutagenesis studies and photolabelling using an analogue of etomidate have identified a potential pocket in the transmembrane domain incorporating residues from TM1 and TM3 where anaesthetics can bind (Jenkins et al. 2001; Li et al. 2006; Chiara et al. 2012). GABA responses at both α4βδ and α6βδ subtypes are enhanced by anaesthetic agents, for example propofol (Brown et al. 2002), etomidate and isoflurane (Lees and Edwards 1998; Jia et al. 2008a). In $\delta^{-/-}$ mice, the sleep time produced by propofol, pentobarbital, etomidate and ketamine were not different to wild-type mice and loss of righting reflex to halothane was unchanged suggesting that δ-containing receptors do not play a major role in mediating anaesthetic activity of these agents (Mihalek et al. 1999). While loss of righting reflex and degree of immobility with isoflurane were unaffected in the α4−/− mouse, amnesic effects were reduced, suggesting a potential role for α4-containing receptors in anaesthetic mediated cognitive deficits (Rau et al. 2009). Additional studies have suggested agent-specific differential effects on synaptic and extrasynaptic receptors when comparing thiopental and isoflurane on hippocampal brain slice activity (Bieda et al. 2009) and propofol effects on tonic and phasic GABA currents in supraoptic magnocellular neurons (Jeong et al. 2011). While the gene knockout data would suggest that with the exception of neurosteroids, anaesthetics exert their effects primarily through synaptic receptors, the α4βδ receptors in thalamus have been demonstrated to play a role in hypnotic

activity, and more specifically in the generation of slow-wave sleep with GABA agonists (see later section). Recent work using α6βδ receptors and cerebellar granule cells has also demonstrated that the level of enhancement of tonic GABA-mediated currents by anaesthetic agents is also dependent on the ambient GABA concentrations which will differ from one brain region to the other (Houston et al. 2012). These authors demonstrate that at higher ambient GABA concentrations, propofol enhancement was reduced, whereas other types of modulator such as neurosteroids maintained their efficacy, perhaps accounting for the differences observed in the $\delta^{-/-}$ mouse.

$GABA_A$ receptors are known to be sensitive to alcohols and these molecules are believed to bind in the same region as anaesthetics (McCracken et al. 2010). There have been several papers published demonstrating that δ-containing receptors are particularly sensitive to low concentrations of ethanol (Wallner et al. 2003; Sundstrom-Poromaa et al. 2002) and that there is a binding site conferred by the δ subunit that is also sensitive to the BZ inverse agonist Ro15-4513 (Wallner et al. 2006). In addition, tonic currents in hippocampal recordings are modulated at low concentrations of ethanol (Wei et al. 2004). These findings are however still controversial, as other publications have failed to replicate these effects with low concentrations of ethanol (Borghese et al. 2006; Borghese and Harris 2007; Baur et al. 2009; Yamashita et al. 2006; Shu et al. 2012) or binding of Ro15-4513 (Korpi et al. 2007; Mehta et al. 2007). In addition, for the most part α4−/− and δ−/− mice have normal responses to ethanol (Chandra et al. 2008; Mihalek et al. 2001; Shannon et al. 2004). It is quite possible that similar to the case for neurosteroids, there are other factors that can influence receptor sensitivity to ethanol, for example, PKC isoforms (Werner et al. 2011; Choi et al. 2008; Qi et al. 2007) which are not controlled for in these studies. A recent report has identified an interesting sex difference with regard to the α4 dependence of Ro15-1453 and ethanol induced ataxia, where only male α4−/− were resistant to the effects of Ro15-4513 (Iyer et al. 2011). However, this effect is likely to be mediated through α4βγ2 receptors rather than α4βδ (Linden et al. 2011).

While the agonist binding site is thought to be located at the α/β subunit interface, there are very clear effects on GABA agonist pharmacology when the γ2 subunit is replaced by the δ subunit, indicating that the nature of the third subunit can influence agonist-induced gating. δ containing receptors are generally much more sensitive to agonists (Brown et al. 2002; Mortensen et al. 2011; Stórustovu and Ebert 2006) with GABA EC_{50} values usually less than 1 μM. In addition, the amplitude of maximum currents produced by GABA is reduced relative to other subtypes (Mortensen et al. 2010). Despite the presence of the GABA binding site at the α/β interface, there have been several recent reports showing that the δ-subunit can also contribute to the GABA binding site. Using concatenated subunits, Baur et al. showed that the δ-subunit can assume various positions in the pentameric structure replacing the position of an α-subunit and can form part of a GABA-binding site (Baur et al. 2009; Kaur et al. 2009). In addition, mutating the residue Arg218 of the δ-subunit, equivalent to the GABA-binding residue Arg207 of the β2-subunit, reduced the potency of GABA by 670-fold, again suggesting a potential role for GABA binding to the δ-subunit (Karim et al. 2012a).

Receptor desensitization is reduced although not completely abolished, and the steady-state tonic current that remains when activated by ambient GABA is rendered insensitive to transient increases in GABA, for example from spillover at synaptic junctions (Bright et al. 2011). The agonist pharmacology is also somewhat different on δ–containing receptors compared to γ2-containing receptors. For example GABA behaves as a partial agonist relative to other agonists such as the constrained analogue 4,5,6,7-tetrahydoisoxazolo[5,4-c]pyridin-3(2H)-one (THIP or Gaboxadol), as well as other amino acids such as β-alanine and taurine which, like GABA, have increased potency at δ-containing receptors (Brown et al. 2002; Mortensen et al. 2010; Jia et al. 2008b, Bianchi and Macdonald 2003). The potency and efficacy of THIP at δ-containing receptors led to this being classified as a selective extrasynaptic agonist, and when applied to thalamic slices which express α4βδ, THIP can selectively activate tonic currents with no effect on synaptic transients (Belelli et al. 2005; Cope et al. 2005). These properties combined with its favourable brain distribution following oral dosing, supported the discovery that THIP could induce slow-wave activity and increase sleep in both rodents and humans (Lancel and Langebartels 2000; Lankford et al. 2008; Wafford and Ebert 2006). Despite not being approved as a sleep-enhancing agent, THIP has proven to be a useful tool in exploring the role played by δ-subunit containing, extrasynaptic $GABA_A$ receptors, since its sedating effects are lost in both $\delta^{-/-}$ and $\alpha4^{-/-}$ mice (Winsky-Sommerer et al. 2007; Chandra et al. 2006), implicating α4βδ $GABA_A$ receptors in sleep–wake control mechanisms.

Like other $GABA_A$ receptors, δ-containing receptors are also allosterically potentiated by barbiturates such as pentobarbital (Brown et al. 2002). As with other allosteric modulators, the degree of potentiation observed when using GABA as the agonist appears much greater than with other receptor subtypes, however the potency is not affected. This difference can be accounted for by the partial agonist nature of GABA at this subtype (Feng et al. 2004). Chimeric δ/γ2 subunits have been used to demonstrate a key region from the amino-terminus to proline 241 in the TM1 transmembrane domain that is critical for pentobarbital potentiation, as well as being a part of the subunit that confers reduced desensitization (Feng and Macdonald 2010).

Given the very different properties and role of extrasynaptic δ-containing receptors, there has been recent interest in developing allosteric modulators that would be selective for this subtype. The first δ-selective modulator identified by Wafford et al. was 4-chloro-N-[2-(2-thienyl)imidazo[1,2-a]pyridine-3-yl benzamide or DS2. This drug-induced marked potentiation of α4βδ receptor mediated currents with sub-micromolar potency and robust efficacy comparable to that of neurosteroids and was devoid of effects at other subtypes (Wafford et al. 2009). A close analogue, DS1 was also very potent, but had a strong GABA-mimetic effect, directly activating the receptor, while maintaining subtype selectivity. Further work has shown a dependence on the δ-subunit for potent activity of DS2 and suggests a novel binding site exists for these types of allosteric modulators, since mutations known to affect steroids and anaesthetics do not alter modulation by DS2 (Jensen et al. 2013). The compound also enhances tonic currents in thalamic and cerebellar brain slices

where α4βδ and α6βδ generate the extrasynaptic tonic current (Wafford et al. 2009), and these effects are absent in thalamic slices from the $\delta^{-/-}$ mouse (Jensen et al. 2013). Unfortunately, DS2 has very limited brain penetration so is not a useful tool for *in vivo* experiments, however other compounds have been proposed that have δ-subunit activity with some *in vivo* effects. AA29504 is a retigabine analogue with modest activity at KCNQ ion channels that has been shown to potentiate α4βδ receptor responses activated by GABA and gaboxadol. Like other modulators, AA29504 produced a greater maximal effect on α4βδ than α1βγ2, but was not selective for δ-containing receptors since α1β2γ2 receptors were also potentiated at the same concentrations, and synaptic inhibitory postsynaptic currents (IPSCs) were prolonged in cerebellar granule neurons (Hoestgaard-Jensen et al. 2010; Vardya et al. 2012). When administered subcutaneously, the drug produced anxioytic activity and motor impairment, but the mechanism underlying these effects is not yet determined. A set of dihydropyrimidinones related to barbiturates have also been shown to potentiate α1β2δ receptors with some selectivity over other subtypes (Lewis et al. 2010). These compounds are relatively weak with EC_{50} values from between 190 μM for monastrol, and 410 μM for JM-II-43A. Further investigation revealed that potentiation by these drugs was not completely dependent on the δ subunit since GABA currents at α4β2 receptors were also potentiated. No effects were observed on γ2-containing receptors however, suggesting at least some level of receptor selectivity with this class of molecule (Lewis et al. 2010). Interestingly there appeared to be some differences in potentiation between α1β2δ, α4β2δ and α6β2δ receptors, supporting the hypothesis that the effects of these compounds are modulated by δ, but their binding site may be linked more to the α-subunit.

The NMDA antagonist ketamine has recently been demonstrated to be a weak potentiator of $GABA_A$ receptors with some selectivity for the α6β2/3δ receptor subtype. In addition, at concentrations above 100 μM, ketamine could directly activate these receptors (Hevers et al. 2008). This property was not shared by other NMDA channel blockers such as MK-801 or phencyclidine. Ketamine could potentiate tonic currents in cerebellar granule neurons at 30 μM or above suggesting this effect may contribute to the CNS depressant features of this drug *in vivo*.

Another well-characterised GABA-related drug, gamma-hydroxybutyric acid (GHB), has previously been shown not to affect $GABA_A$ receptors, but exert its effects primarily through $GABA_B$ receptors and potentially bind to a specific site which remains to be identified. More recently, the GABA α4 subunit has been proposed as a potential binding protein for [^{3}H]NCS-382, a potent 'GHB-receptor' ligand, and photo-affinity labelling using [^{125}I]BnOPh-GHB which labels high-affinity GHB sites is inhibited by the $GABA_A$ antagonist gabazine (Absalom et al. 2012). These authors also showed that GHB can behave as a partial agonist at α4β1δ receptors with a potency of 140 nM and can be blocked by gabazine. Interestingly, GHB had remarkable β1 selectivity over β2 and β3 containing receptors and had no effect on γ2-containing receptors. This suggests that α4β1δ may represent a high-affinity binding site for GHB, a hypothesis which is supported by reduced [^{3}H] NCS-382 binding in $\alpha4^{-/-}$ mice. This interesting finding needs to be followed up, and several questions remain, particularly around the pharmacological profiling of

other GHB ligands such as NCS-382. In addition α4 and δ primarily associate with β2 and β3, whereas β1 has low abundance in regions expressing α4 (Pirker et al. 2000; Herd et al. 2008; Belelli et al. 2005).

A number of other agents have claimed to have some activity at extrasynaptic δ-containing receptors, however it is as yet unclear what contribution these receptors may be making to the *in vivo* activity of any of these drugs. For example, 3-hydroxy-20-methoxy-6-methylflavone has been reported to enhance GABA-mediated responses at α1, α2, α4 and α6 but not α3 and α5 containing $GABA_A$ receptors, in addition to acting as a direct agonist at α4βδ and α6βδ receptor subtypes (Karim et al. 2012b). The compound is anxiolytic *in vivo*, but the role played by extrasynaptic receptors in this response remains to be elucidated. Biphenolic extracts from magnolia tree bark, magnolol and honokiol which have been shown to be anxiolytic and sleep inducing in humans also potentiated GABA-mediated currents in recombinant receptors, with no particular subtype selectivity, but more marked effects on δ-containing receptors (Alexeev et al. 2012). Some endocannabinoids such as 2-arachidonoyl glycerol (2-AG) have been shown to potentiate $GABA_A$ receptors between 1 and 10 μM. These compounds seem to show a degree of β2 selectivity and some synergistic effects with neurosteroids. It is possible that endogenous 2-AG can modulate $GABA_A$ responses to neurosteroids (Sigel et al. 2011) and these authors suggest that extrasynaptic receptors would be preferentially affected given a larger effect of 2-AG at low GABA concentrations.

An indirect modulator of $GABA_A$ receptors is insulin, which is able to rapidly recruit new receptors to the plasma membrane and consequently increase the amplitude of induced pluripotent stem cells (IPSCs) (Wan et al. 1997). This is thought to be mediated via tyrosine phosphorylation of residues on the β-subunit and is dependent on the $GABA_A$ receptor-associated protein, phospholipase C-related catalytically inactive protein (PRIP) (Fujii et al. 2010). This process also affects extrasynaptic receptors and insulin enhances tonic currents in hippocampal slices, subsequently reducing neuronal excitability (Jin et al. 2011). Given the relatively larger charge transfer produced by extrasynaptic receptors relative to synaptic receptors, this effect may predominate where extrasynaptic receptors are present and functional. While the δ-subunit itself does not appear to be a substrate for PKC, both the β2 and β3 subunits do contain consensus sites for phosphorylation and can modulate the function of extrasynaptic $GABA_A$ receptors (Saliba et al. 2012). In addition, α4 is also phosphorylated by PKC at Ser^{443} (Abramian et al. 2010). Like insulin PKC phosphorylation can increase the number of receptors in the plasma membrane and stabilizes the receptor, preventing run-down of the current over time when α4βδ receptors are expressed in human embryonic kidney (HEK) cells (Abramian et al. 2010). 8-bromo-cAMP has also been shown to enhance currents produced by expression of α4β3δ, implicating the potential for PKA phosphorylation as another modulatory mechanism for these extrasynaptic receptors (Tang et al. 2010).

As mentioned with regard to anaesthetic sensitivity, the contextual nature of extrasynaptic receptors compared to synaptic receptors can also contribute to their pharmacology. For example, the open channel blocker penicillin was reported to selectively block phasic currents in hippocampal neurons, but be relatively inef-

fective at blocking tonic extrasynaptic currents in the same neurons (Yeung et al. 2003). Using directly applied GABA to recombinant receptors, γ2- and δ-containing receptors were both found to be sensitive to block with penicillin, but in both cases only peak currents were reduced, leaving steady-state currents unaffected (Feng et al. 2009). Likewise δ-containing receptors stimulated in a physiological manner, for instance by continuous application of low concentrations of GABA, produced a steady-state current that was insensitive to penicillin, whereas γ2-containing receptors stimulated with brief, high concentrations of GABA were almost completely inhibited. Penicillin may be one example of this type of contextual-dependent selectivity, and potentiators may also give apparent differences when applied in context. For example propofol, gaboxadol and neurosteroids behave differently depending on the ambient concentration of GABA, and this is likely to influence their *in vivo* properties (Houston et al. 2012).

Another potential contributor to the pharmacology of both types of receptors is the possibility of constitutive activity in the absence of agonist. There is still some debate as to whether there are endogenous constitutively active $GABA_A$ channels; however, in recombinant expression systems there are some instances where channel activity can be measured in the absence of GABA (Maksay et al. 2003). There are a few reports of spontaneous activity with δ-containing receptors (Tang et al. 2010), and one recent study has demonstrated a small amount of activity with α4β3δ expressed in *Xenopus* oocytes. This activity, which appeared to be dependent on the β-subunit isoform as it was not seen in α4β2δ expressing cells, could be potentiated by DS2 (Jensen et al. 2013). In other expression systems, however, the spontaneous activity of this combination is not present (Brown et al. 2002), so it is currently unclear if this represents the native situation or just an artefact of the expression system.

4.5 α5 Subunit-Containing Receptors

In adult brain, the α5 subunit has a very limited distribution. Early on in development however there is more widespread expression, hence this receptor may play an important role during brain maturation (Laurie et al. 1992; Sebe et al. 2010). In adults, the most abundant form of this receptor is $\alpha5\beta_x\gamma2$ and its highest level of expression is in the CA1 and CA3 regions of the hippocampus (Pirker et al. 2000), and lower levels of expression is in the cortex.

The α5βγ2 subunit was one of the first $GABA_A$ receptors to be proposed to function in a tonic fashion (Caraiscos et al. 2004; Glykys and Mody 2006) despite containing a γ2 subunit and being sensitive to BZs. More recent studies have revealed an actin-binding protein radixin, co-associated uniquely with α5, which may be responsible for trafficking and clustering α5-containing receptors within the membrane (Loebrich et al. 2006). There is still some debate about the presence of α5-containing receptors at hippocampal synapses, but accumulating evidence now suggests this receptor may contribute to synaptic responses (Collinson et al. 2002;

Prenosil et al. 2006; Zarnowska et al. 2009) and is located at synaptic terminals in addition to existing in a larger extrasynaptic pool (Serwanski et al. 2006). More specifically, $GABA_A$ α5-containing receptors have been shown to contribute to a slow IPSC termed '$GABA_{A\ slow}$' in CA1 dendrites which is large in amplitude, prolonged by diazepam and activated by stimulation of the Schaffer-collateral pathway (Zarnowski et al. 2009; Vargas-Caballero et al. 2010; Capogna and Pearce 2011).

Given the high level of expression of α5βγ2 receptors in the hippocampus, this receptor has been strongly implicated in cognitive processing, and evidence suggests that inhibition or deletion of α5-containing receptors promotes cognitive performance in both rodents and humans (Collinson et al. 2002; Dawson et al. 2006; Nutt et al. 2007). The potential for the treatment of cognitive disorders such as Alzheimer's disease and schizophrenia has led to the development of selective BZ-site targeted molecules with either binding or functional selectivity for α5βγ2 over other receptor subtypes. Several different molecules have been identified and used as tools in this context. L-655,708 was demonstrated to be 50–100-fold more selective for α5βγ2 over other γ2-containing subtypes using [^{3}H]-flumazenil binding, and behaved as a partial inverse agonist at the receptor (Atack et al. 2006). The compound-enhanced long-term potentiation in hippocampal brain slices and improved cognitive performance in the Morris water maze (Atack et al. 2006). Several other molecules were developed with greater efficacy at inhibiting α5βγ2 receptors and it was discovered that compounds could be designed to exhibit functional selective inhibition at this subtypes versus other receptor subtypes. α5IA has potent affinity at all BZ-sensitive receptors but inhibits only α5βγ2, behaving as a neutral antagonist at the other subtypes with no functional consequences, for example no convulsant liability. α5IA improved cognitive performance in rodent hippocampal-dependent tests of learning and memory (Dawson et al. 2006) as well as reversing alcohol induced memory impairment in humans (Nutt et al. 2007). Other examples of selective α5-negative modulators have also been developed (Ballard et al. 2009; Atack 2011), but none have yet been evaluated in cognitively impaired patients. There have been several recent reports that inhibiting $GABA_A$ α5-containing receptors can improve cognitive deficits in the mouse Ts65Dn model of Down's Syndrome (Braudeau et al. 2011; Möhler 2012) and an α5 inverse agonist (RG1662) is now being evaluated in Down's Syndrome patients.

Recent publications have suggested that there may be an age-related component to modulation of α5-containing receptors and cognitive improvement (Koh et al. 2013). Koh et al. report an improvement in cognitive performance in young rats with an α5−selective allosteric inhibitor, but not in older rats. In contrast, in their older cohort they demonstrate an improvement when the animals are injected with positive α5-selective modulators, such as Compound 44 [6,6-dimethyl-3-(3-hydroxypropyl) thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5H)-one] and Compound 6 [methyl 3,5-diphenylpyridazine-4-carboxylate], which are partial positive allosteric modulators at α5βγ2 receptors. They suggest that the hippocampus is overactive in older animals and in conditions such as Alzheimer's disease, and by potentiating α5-containing $GABA_A$ receptors this activity can be normalised (Koh et al. 2013).

Interestingly, another condition that exhibits abnormal hippocampal overactivity is the methylazoxymethanol acetate (MAM) model of schizophrenia (Lodge and Grace 2007) and application of another positive allosteric modulator at α5βγ2 receptors SH-053-2′F-R-CH3 appears to normalise this activity when administered either systemically or directly injected into hippocampus (Gill et al. 2011). This drug also reduced the enhanced locomotor response to amphetamine seen in this model; however, it has not yet been evaluated in cognitively based tests.

As described above, because general anaesthetics interact with most $GABA_A$ receptors, it is likely that $\alpha5\beta_x\gamma2$ receptors are also involved in their *in vivo* effects. It has recently been shown by using the $\alpha5^{-/-}$ mice that this receptor contributes to the memory impairing effects of both etomidate and the volatile anaesthetic isoflurane on anaesthetic recovery (Martin et al. 2009; Zurek et al. 2012). In addition, by blocking α5-containing receptors with L-655,708, the deficits in short-term memory induced by isoflurane could be prevented (Zurek et al. 2012).

It is not currently clear which population of α5-containing receptors are contributing to the *in vivo* effects observed with α5-selective inhibitors. Given the function and significant effects extrasynaptic receptors have on controlling baseline neuronal excitability, it is quite likely that this population are playing a major role in the *in vivo* effects of allosteric modulators, both positive and negative. While $\alpha5^{-/-}$ mice exhibit enhancements in tests of learning and memory (Collinson et al. 2002), further experiments need to be done to understand the role of the tonic current in the hippocampus and its contribution to spatial learning.

While the majority of effort has focused on α5-containing receptors in the hippocampus and their role in cognition, there are some reports of α5-mediated tonic current in other regions, for example a recent report of tonic GABAergic currents in spinal ventral cord interneurons which are sensitive to α5-selective pharmacological agents (Castro et al. 2011). This opens the possibility that α5-selective agents may have some analgesic properties, although this has yet to be reported.

4.6 Other Potential Extrasynaptic Receptor Subtypes

While a major receptor subtype in the ventrobasal thalamus is α4βδ, there is also a high level of expression of γ2 in this region, which by immunoprecipitation co-assembles with α4 and β (Sur et al. 1999). The α4βγ2 subtype can be expressed in recombinant systems and has some unique properties with its modified BZ binding site being sensitive to certain BZ-site molecules such as the imidazobenzodiazepines, Ro15-4513, bretazenil and flumazenil, all of which behave as positive allosteric modulators (Whittemore et al. 1996; Brown et al. 2002). Like $\alpha6\beta_x\gamma2$, the $\alpha4\beta_x\gamma2$ receptor is also more sensitive to blockade by furosemide than other subtypes (Wafford et al. 1996). Despite the immunoprecipitation data, native receptor pharmacology in the ventrobasal thalamus has not actually revealed a receptor with this pharmacology, and

individual subunit deletion experiments suggest just two populations in this region, α4β2δ and α1β2γ2 (Herd et al. 2009; Peden et al. 2008). In the hippocampus, there is some evidence for receptors which exhibit this unique pharmacology appearing transiently following progesterone withdrawal (Gulinello et al. 2002), which results in up-regulation of α4 in this region. In addition, lorazepam becomes inactive, and flumazenil which is normally silent exhibits an anxiolytic-like effect under these conditions, suggesting α4βγ2 may underlie the response in these animals (Gulinello et al. 2002). It is not clear whether this receptor is located at synapses or extrasynaptically, however the miniature IPSC decay time constant is decreased in many neurons, and these become insensitive to the prolongation normally observed with lorazepam, suggesting that at least a proportion are located synaptically (Hsu et al. 2003).

A recent publication studying $GABA_A$ receptors in the amygdala has identified a tonic current present in principal cells of the basolateral nucleus which expresses predominantly α3-containing receptors (Marowsky et al. 2012). The tonic current is sensitive to BZ and is potentiated by the α3-selective potentiator TP003 and hence contains a γ2 subunit. The current is also significantly reduced in the $\alpha3^{-/-}$ mouse suggesting this receptor may play a role in controlling excitability of the basolateral amygdala. The presence of α3 in extrasynaptic receptors is surprising given it has the lowest sensitivity to GABA, but the local concentration of ambient GABA in this region is unknown.

The majority of $GABA_A$ receptors in the CNS appear to be pentamers comprising a preferred arrangement of two α, two β and one other subunit. Evidence does exist however for native receptors containing only α and β subunits, an arrangement which does function in recombinant systems, and will form in the $\gamma2^{-/-}$ mouse (Günther et al. 1995). In a recent report, a low conductance component of the tonic current in hippocampal pyramidal neurons appeared to be highly sensitive to block by zinc, a feature of receptors composed of α and β subunits alone (Mortensen and Smart 2006). Further investigation revealed this to be insensitive to BZs and have a conductance state similar to αβ but not αβγ or αβδ receptors, making up approximately 10 % of the tonic current in these neurons (Mortensen and Smart 2006). This suggests that at least in some parts of the CNS, there may be extrasynaptic receptors composed of just α and β subunits with their own distinct pharmacology, including high sensitivity to zinc and insensitivity to BZs.

There are two additional $GABA_A$ subunits (θ and ε) which are of very low abundance and in at least the case of ε can dramatically alter the pharmacology of receptors when expressed with α and β subunits. θ remains relatively unexplored (Bonnert et al. 1999) but it has been proposed that α3, θ and ε are expressed together in postnatal spinal cord (Pape et al. 2009), as well as in other brain regions, including the locus coeruleus, the amygdala and various nuclei of the hypothalamus (Moragues et al. 2002; Sinkkonen et al. 2000). The native pharmacology of such a receptor has yet to be defined and the contribution of θ to these receptors is still unknown. The ε-subunit is unusual in that when co-expressed with α and β subunits, it can reduce the sensitivity to anaesthetics (Davis et al. 1997; Thompson et al. 2002) and is insensitive to

BZs. More importantly, it can produce constitutively open receptors which can also be activated by GABA and have a level of spontaneous channel activity (Maksay et al. 2003). Clearly this is likely to be an important feature for an extrasynaptic receptor where a level of constitutive or tonic activity can be maintained even in the absence of GABA. Unfortunately, it has been difficult to demonstrate the presence of such a receptor in native neuronal preparations, even those with the highest levels of expression of these subunits. One study reports a BZ-insensitive GABA synaptic current in locus coeruleus, however this did not exhibit spontaneous activity (Belujon et al. 2009). So at the present time, it is still unclear whether these represent a novel subtype of extrasynaptic receptor, or what the pharmacology of these receptors might be.

In addition to the identification of tonic currents elicited by $GABA_A$ receptor subtypes, it has recently been shown that $GABA_C$ or ρ-containing receptors can also be localised extrasynaptically on retinal bipolar neurons (Jones and Palmer 2009). These are thought to be predominantly ρ1 containing due to their insensitivity to the ρ2-selective antagonist cyclothiazide (Jones and Palmer 2011).

4.7 Beta Subunits

Unlike the α, δ and γ2 subunits, the identity of the β subunits in different populations of extrasynaptic and synaptic receptors has had little investigation. One study has utilized $\beta2^{-/-}$ and the point mutant β2N265S transgenic mice to differentiate populations of receptors in the dentate gyrus of the hippocampus. They discovered that the extrasynaptic α4βδ population contained primarily β2 subunits while the synaptic αβγ2 population contained primarily β3 subunits. The delineation of β3 and β2 to synaptic and extrasynaptic receptors is not universal however, since the diazepam sensitive tonic current present in these neurons, made up of α5βγ2, contained primarily β3 subunits and was unaffected by β2 deletion (Herd et al. 2008). In addition, in thalamic relay neurons, both extrasynaptic and synaptic $GABA_A$ receptors contained primarily β2 subunits (Belelli et al. 2005). Another study using cultured hippocampal pyramidal cells identified α1/2β2/3γ2 in clusters in both synaptic membranes and extrasynaptic membranes, and diffusely distributed α4β1δ receptors in extrasynaptic membranes (Mangan et al. 2005). There are few pharmacological tools that exhibit β-subunit selectivity. Loreclezole and etomidate both show a degree of β2/3 selectivity over β1, and PI 24513, a dioxane analogue has been identified which exhibits preferential allosteric potentiation of β1-containing receptors (Sergeeva et al. 2010). These have recently been used to demonstrate the presence of synaptic β1-containing receptors in the tuberomammilary nucleus and their potential involvement in the sedating effects of PI 24513 (Yanovsky et al. 2012). This tool may be useful to identify the potential presence of β1 in other extrasynaptic receptor populations.

Table 4.1 Direct and indirect modulators of extrasynaptic and synaptic $GABA_A$ receptor subtypes

	α1βxγ2	α2βxγ2	α3βxγ2	α5βxγ2	α4βxγ2	α4βxδ	α6βxδ	α1βxδ	αβ	ρ1
Classical BZ	✓✓✓	✓✓✓	✓✓✓	✓✓✓	—	—	—	—	—	—
imidazoBZ	✓✓	✓✓	✓✓	✓✓✓	✓✓✓	—	—	—	—	—
Zolpidem	✓✓✓	✓✓	✓✓	—	—	—	—	—	—	—
THIP	✓	✓	✓	✓	✓	✓✓✓	✓✓✓		✓✓✓	antag.
DS2	—	—	—	—	—	✓✓✓	✓✓✓		—	
TP003	BZ antag.	BZ antag.	✓✓	BZ antag.	—	—	—	—	—	—
steroids	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓✓	✓✓✓	✓✓✓		—
IAα5	BZ antag.	BZ antag.	BZ antag.	✓✓✓	—	—	—	—	—	—
EtOH	✓	✓	✓	✓✓	✓	✓✓?	✓✓?	✓✓?	✓	—
AA29504	✓	✓	✓	✓	✓	✓✓	✓✓		✓	
Dihydrop.	—	—	—	—	✓	✓✓	✓✓		✓	
Propofol/barbs	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓✓	✓✓✓	✓✓✓	✓✓	—
furosemide	✓	✓	✓	✓	✓✓	✓✓	✓✓		✓	
GABA uptake blockers	✓	✓	✓	✓	✓	✓✓	✓✓	✓✓	✓	✓✓

Illustrates predominantly synaptic receptors

Illustrates predominantly extrasynaptic receptors

Illustrates both synaptic and extrasynaptic receptors

Conclusions

The rich and diverse pharmacology of the $GABA_A$ receptor family can now be extended to that of those receptors that are located extrasynaptically and serve a different function to synaptic receptors. Many of the existing allosteric modulators of $GABA_A$ receptors show overlapping pharmacology on these different populations, while others are more specific (see Table 4.1), and while the understanding of the functional contribution of non-synaptic receptors is growing, it is still to be fully appreciated. It is clear now that some populations are insensitive to BZs such as those containing δ, while others such as those containing α5 can still be modulated via this site. It also appears that the level of receptor activation by GABA can affect some allosteric modulators but not others, which will have major consequences to the *in vivo* activity of these types of drugs and will be an important consideration when developing novel pharmacologically selective molecules. Extrasynaptic receptors also appear to be more plastic, and can respond quickly to changes in their environment, for example, in response to steroid exposure, or an epileptic seizure. The therapeutic potential for modulation of extrasynaptic receptors is extremely diverse, with associated changes in many neurological disorders such as epilepsy, depression, menstrual disorders, anxiety, sleep and Alzheimer's disease (Brickley and Mody 2012). Development of pharmacologically selective agents is still in the early stages and it is hoped that novel agents will be forthcoming in the near future that will prove beneficial in one or more of these disorders.

References

Abramian AM, Comenencia-Ortiz E, Vithlani M, Tretter EV, Sieghart W, Davies PA, Moss SJ (2010) Protein kinase C phosphorylation regulates membrane insertion of $GABA_A$ receptor subtypes that mediate tonic inhibition. J Biol Chem 285:41795–41805

Absalom N, Eghorn LF, Villumsen IS, Karim N, Bay T, Olsen JV, Knudsen GM, Bräuner-Osborne H, Frølund B, Clausen RP, Chebib M, Wellendorph P (2012) α4βδ $GABA_A$ receptors are high-affinity targets for γ-hydroxybutyric acid (GHB). Proc Natl Acad Sci U S A 109:13404–13409

Alexeev M, Grosenbaugh DK, Mott DD, Fisher JL (2012) The natural products magnolol and honokiol are positive allosteric modulators of both synaptic and extra-synaptic $GABA_A$ receptors. Neuropharmacology 62:2507–2514

Atack JR (2011) $GABA_A$ receptor subtype-selective modulators. II. α5-selective inverse agonists for cognition enhancement. Curr Top Med Chem 11:1203–1214

Atack JR, Bayley PJ, Seabrook GR, Wafford KA, McKernan RM, Dawson GR (2006) L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for 5-containing $GABA_A$ receptors. Neuropharmacology 51:1023–1029

Baer K, Essrich C, Balsiger S, Wick MJ, Harris RA, Fritschy JM, Lüscher B (2000) Rescue of gamma2 subunit-deficient mice by transgenic overexpression of the $GABA_A$ receptor gamma2S or gamma2L subunit isoforms. Eur J Neurosci 12:2639–2643

Ballard TM, Knoflach F, Prinssen E, Borroni E, Vivian JA, Basile J, Gasser R, Moreau JL, Wettstein JG, Buettelmann B, Knust H, Thomas AW, Trube G, Hernandez MC (2009) RO4938581, a novel cognitive enhancer acting at $GABA_A$ alpha5 subunit-containing receptors. Psychopharmacology (Berl) 202:207–223

Baur R, Kaur KH, Sigel E (2009) Structure of alpha6 beta3 delta $GABA_A$ receptors and their lack of ethanol sensitivity. J Neurochem 111:1172–1181

Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the $GABA_A$ receptor. Nat Rev Neurosci 6:565–575

Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ (2005) Extrasynaptic $GABA_A$ receptors of thalamocortical neurons: a molecular target for hypnotics. J Neurosci 25:11513–11520

Belujon P, Baufreton J, Grandoso L, Boué-Grabot E, Batten TF, Ugedo L, Garret M, Taupignon AI (2009) Inhibitory transmission in locus coeruleus neurons expressing $GABA_A$ receptor epsilon subunit has a number of unique properties. J Neurophysiol 102:2312–2325

Bianchi MT, Macdonald RL (2003) Neurosteroids shift partial agonist activation of $GABA_A$ receptor channels from low- to high-efficacy gating patterns. J Neurosci 23:10934–10943

Bieda MC, Su H, Maciver MB (2009) Anesthetics discriminate between tonic and phasic gamma-aminobutyric acid receptors on hippocampal CA1 neurons. Anesth Analg 108:484–490

Bonnert TP, McKernan RM, Farrar S, le BB, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Brown N, Wafford KA, Whiting PJ (1999) Theta, a novel gamma-aminobutyric acid type A receptor subunit. Proc Natl Acad Sci U S A 96:9891–9896

Borghese CM, Harris RA (2007) Studies of ethanol actions on recombinant delta-containing gamma-aminobutyric acid type A receptors yield contradictory results. Alcohol 41:155–162

Borghese CM, Stórustovu S, Ebert B, Herd MB, Belelli D, Lambert JJ, Marshall G, Wafford KA, Harris RA (2006) The delta subunit of gamma-aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. J Pharmacol Exp Ther 316:1360–1368

Braudeau J, Delatour B, Duchon A, Pereira PL, Dauphinot L, de Chaumont F, Olivo-Marin JC, Dodd RH, Hérault Y, Potier MC (2011) Specific targeting of the GABA-A receptor α5 subtype by a selective inverse agonist restores cognitive deficits in Down syndrome mice. J Psychopharmacol 25:1030–1042

Brickley SG, Mody I (2012) Extrasynaptic $GABA_A$ receptors: their function in the CNS and implications for disease. Neuron 73:23–34

Brickley SG, Cull-Candy SG, Farrant M (1996) Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of $GABA_A$ receptors. J Physiol 497:753–759

Bright DP, Renzi M, Bartram J, McGee TP, MacKenzie G, Hosie AM, Farrant M, Brickley SG (2011) Profound desensitization by ambient GABA limits activation of δ-containing $GABA_A$ receptors during spillover. J Neurosci 31:753–763

Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA (2002) Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta $GABA_A$ receptors. Br J Pharmacol 136:965–974

Brünig I, Scotti E, Sidler C, Fritschy JM (2002) Intact sorting, targeting, and clustering of gamma-aminobutyric acid A receptor subtypes in hippocampal neurons in vitro. J Comp Neurol 443:43–55

Capogna M (2011) Neurogliaform cells and other interneurons of stratum lacunosum-moleculare gate entorhinal-hippocampal dialogue. J Physiol 589:1875–1883

Capogna M, Pearce RA (2011) GABA A, slow: causes and consequences. Trends Neurosci 34:101–112

Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA (2004) Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. Proc Natl Acad Sci U S A 101:3662–3667

Castro A, Aguilar J, González-Ramírez R, Loeza-Alcocer E, Canto-Bustos M, Felix R, Delgado-Lezama R (2011) Tonic inhibition in spinal ventral horn interneurons mediated by α5 subunit-containing $GABA_A$ receptors. Biochem Biophys Res Commun 412:26–31

Chadderton P, Margrie TW, Häusser M (2004) Integration of quanta in cerebellar granule cells during sensory processing. Nature 428:856–860

Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, Homanics GE (2006) $GABA_A$ receptor alpha 4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. Proc Natl Acad Sci U S A 103:15230–15235

Chandra D, Werner DF, Liang J, Suryanarayanan A, Harrison NL, Spigelman I, Olsen RW, Homanics GE (2008) Normal acute behavioral responses to moderate/high dose ethanol in $GABA_A$ receptor alpha 4 subunit knockout mice. Alcohol Clin Exp Res 32:10–18

Chiara DC, Dostalova Z, Jayakar SS, Zhou X, Miller KW, Cohen JB (2012) Mapping general anesthetic binding site(s) in human α1β3 γ-aminobutyric acid type A receptors with [^{3}H]TDBzl-etomidate, a photoreactive etomidate analogue. BioChemistry 51:836–847

Choi DS, Wei W, Deitchman JK, Kharazia VN, Lesscher HM, McMahon T, Wang D, Qi ZH, Sieghart W, Zhang C, Shokat KM, Mody I, Messing RO (2008) Protein kinase C delta regulates ethanol intoxication and enhancement of GABA-stimulated tonic current. J Neurosci 28:11890–11899

Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW (2002) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. J Neurosci 22:5572–5580

Cope DW, Hughes SW, Crunelli V (2005) $GABA_A$ receptor-mediated tonic inhibition in thalamic neurons. J Neurosci 25:11553–11563

Davies PA, Hanna MC, Hales TG, Kirkness EF (1997) Insensitivity to anaesthetic agents conferred by a class of $GABA_A$ receptor subunit. Nature 385:820–823

Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, Choudhury HI, McDonald LM, Pillai G, Rycroft W, Smith AJ, Sternfeld F, Tattersall FD, Wafford KA, Reynolds DS, Seabrook GR, Atack JR (2006) An inverse agonist selective for alpha5 subunit-containing $GABA_A$ receptors enhances cognition. J Pharmacol Exp Ther 316:1335–1345

Drasbek KR, Jensen K (2006) THIP, a hypnotic and antinociceptive drug, enhances an extrasynaptic $GABA_A$ receptor-mediated conductance in mouse neocortex. Cereb Cortex 16:1134–1141

Duguid I, Branco T, London M, Chadderton P, Häusser M (2012) Tonic inhibition enhances fidelity of sensory information transmission in the cerebellar cortex. J Neurosci 32:11132–11143

Feng HJ, Bianchi MT, Macdonald RL (2004) Pentobarbital differentially modulates alpha1beta3delta and alpha1beta3gamma2L $GABA_A$ receptor currents. Mol Pharmacol 66:988–1003

Feng HJ, Botzolakis EJ, Macdonald RL (2009) Context-dependent modulation of alphabetagamma and alphabetadelta $GABA_A$ receptors by penicillin: implications for phasic and tonic inhibition. Neuropharmacology 56:161–173

Feng HJ, Macdonald RL (2010) Barbiturates require the N terminus and first transmembrane domain of the delta subunit for enhancement of alpha1beta3delta $GABA_A$ receptor currents. J Biol Chem 285:23614–23621

Forman SA, Miller KW (2011) Anesthetic sites and allosteric mechanisms of action on Cys-loop ligand-gated ion channels. Can J Anaesth 58:191–205

Franks NP (2008) General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 9:370–386

Fujii M, Kanematsu T, Ishibashi H, Fukami K, Takenawa T, Nakayama KI, Moss SJ, Nabekura J, Hirata M (2010) Phospholipase C-related but catalytically inactive protein is required for insulin-induced cell surface expression of gamma-aminobutyric acid type A receptors. J Biol Chem 285:4837–4846

Gill KM, Lodge DJ, Cook JM, Aras S, Grace AA (2011) A novel α5 $GABA_A$R-positive allosteric modulator reverses hyperactivation of the dopamine system in the MAM model of schizophrenia. Neuropsychopharmacology 36:1903–1911

Glykys J, Mody I (2006) Hippocampal network hyperactivity after selective reduction of tonic inhibition in $GABA_A$ receptor alpha5 subunit-deficient mice. J Neurophysiol 95:2796–2807

Gulinello M, Gong QH, Smith SS (2002) Progesterone withdrawal increases the alpha4 subunit of the $GABA_A$ receptor in male rats in association with anxiety and altered pharmacology—a comparison with female rats. Neuropharmacology 43:701–714

Günther U, Benson J, Benke D, Fritschy JM, Reyes G, Knoflach F, Crestani F, Aguzzi A, Arigoni M, Lang Y (1995) Benzodiazepine-insensitive mice generated by targeted disruption of the gamma 2 subunit gene of gamma-aminobutyric acid type A receptors. Proc Natl Acad Sci U S A 92:7749–7753

Harney SC, Frenguelli BG, Lambert JJ (2003) Phosphorylation influences neurosteroid modulation of synaptic $GABA_A$ receptors in rat CA1 and dentate gyrus neurones. Neuropharmacology 45:873–883

Herd MB, Haythornthwaite AR, Rosahl TW, Wafford KA, Homanics GE, Lambert JJ, Belelli D (2008) The expression of $GABA_A$ beta subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. J Physiol 586:989–1004

Herd MB, Foister N, Chandra D, Peden DR, Homanics GE, Brown VJ, Balfour DJ, Lambert JJ, Belelli D (2009) Inhibition of thalamic excitability by 4,5,6,7-tetrahydroisoxazolo[4,5-c] pyridine-3-ol: a selective role for delta-$GABA_A$ receptors. Eur J Neurosci 29:1177–1187

Hevers W, Hadley SH, Lüddens H, Amin J (2008) Ketamine, but not phencyclidine, selectively modulates cerebellar $GABA_A$ receptors containing alpha6 and delta subunits. J Neurosci 28:5383–5393

Hoestgaard-Jensen K, Dalby NO, Wolinsky TD, Murphey C, Jones KA, Rottländer M, Frederiksen K, Watson WP, Jensen K, Ebert B (2010) Pharmacological characterization of a novel positive modulator at alpha 4 beta 3 delta-containing extrasynaptic $GABA_A$ receptors. Neuropharmacology 58:702–711

Hosie AM, Wilkins ME, da Silva HM, Smart TG (2006) Endogenous neurosteroids regulate $GABA_A$ receptors through two discrete transmembrane sites. Nature 444:486–489

Hosie AM, Clarke L, da Silva H, Smart TG (2009) Conserved site for neurosteroid modulation of $GABA_A$ receptors. Neuropharmacology 56:149–154

Houston CM, McGee TP, Mackenzie G, Troyano-Cuturi K, Rodriguez PM, Kutsarova E, Diamanti E, Hosie AM, Franks NP, Brickley SG (2012) Are extrasynaptic $GABA_A$ receptors important targets for sedative/hypnotic drugs? J Neurosci 32:3887–3897

Hsu FC, Waldeck R, Faber DS, Smith SS (2003) Neurosteroid effects on GABAergic synaptic plasticity in hippocampus. J Neurophysiol 89:1929–1940

Iyer SV, Benavides RA, Chandra D, Cook JM, Rallapalli S, June HL, Homanics GE (2011) α4-Containing $GABA_A$ Receptors are Required for Antagonism of Ethanol-Induced Motor Incoordination and Hypnosis by the Imidazobenzodiazepine Ro15-4513. Front Pharmacol 2:18

Jenkins A, Greenblatt EP, Faulkner HJ, Bertaccini E, Light A, Lin A, Andreasen A, Viner A, Trudell JR, Harrison NL (2001) Evidence for a common binding cavity for three general anesthetics within the $GABA_A$ receptor. J Neurosci 21:RC136

Jensen ML, Wafford KA, Brown AR, Belelli D, Lambert JJ, Mirza NR (2013) The delta selective compound 2 (DS2): a detailed study of subunit selectivity, mechanism and site of action utilising human recombinant and rodent native $GABA_A$ receptors. Br J Pharmacol 168:1118–1132

Jeong JA, Kim EJ, Jo JY, Song JG, Lee KS, Kim HW, Lee SD, Jeon BH, Lee JU, Park JB (2011) Major role of $GABA_A$-receptor mediated tonic inhibition in propofol suppression of supraoptic magnocellular neurons. Neurosci Lett 494:119–123

Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, Goldstein PA (2005) An extrasynaptic $GABA_A$ receptor mediates tonic inhibition in thalamic VB neurons. J Neurophysiol 94:4491–4501

Jia F, Yue M, Chandra D, Homanics GE, Goldstein PA, Harrison NL (2008a) Isoflurane is a potent modulator of extrasynaptic $GABA_A$ receptors in the thalamus. J Pharmacol Exp Ther 324:1127–1135

Jia F, Yue M, Chandra D, Keramidas A, Goldstein PA, Homanics GE, Harrison NL (2008b) Taurine is a potent activator of extrasynaptic $GABA_A$ receptors in the thalamus. J Neurosci 28:106–115

Jin Z, Jin Y, Kumar-Mendu S, Degerman E, Groop L, Birnir B (2011) Insulin reduces neuronal excitability by turning on $GABA_A$ channels that generate tonic current. PLoS ONE 6:e16188

Jones SM, Palmer MJ (2009) Activation of the tonic $GABA_C$ receptor current in retinal bipolar cell terminals by nonvesicular GABA release. J Neurophysiol 102:691–699

Jones SM, Palmer MJ (2011) Pharmacological analysis of the activation and receptor properties of the tonic $GABA_C$R current in retinal bipolar cell terminals. PLoS ONE 6:e24892

Jones-Davis DM, Song L, Gallagher MJ, Macdonald RL (2005) Structural determinants of benzodiazepine allosteric regulation of $GABA_A$ receptor currents. J Neurosci 25:8056–8065

Karim N, Wellendorph P, Absalom N, Bang LH, Jensen ML, Hansen MM, Lee HJ, Johnston GA, Hanrahan JR, Chebib M (2012a) Low nanomolar GABA effects at extrasynaptic α4β1/β3δ $GABA_A$ receptor subtypes indicate a different binding mode for GABA at these receptors. Biochem Pharmacol 84:549–557

Karim N, Curmi J, Gavande N, Johnston GA, Hanrahan JR, Tierney ML, Chebib M (2012b) 2(-Methoxy-6-methylflavone: a novel anxiolytic and sedative with subtype selective activating and modulating actions at $GABA_A$ receptors. Br J Pharmacol 165:880–896

Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, Somogyi P (2010) Quantitative localisation of synaptic and extrasynaptic $GABA_A$ receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. Eur J Neurosci 32:1868–1888

Kaur KH, Baur R, Sigel E (2009) Unanticipated structural and functional properties of delta-subunit-containing $GABA_A$ receptors. J Biol Chem 284:7889–7896

Koh MT, Rosenzweig-Lipson S, Gallagher M (2013) Selective $GABA_A$ α5 positive allosteric modulators improve cognitive function in aged rats with memory impairment. Neuropharmacology 64:145–152

Korpi ER, Debus F, Linden AM, Malécot C, Leppä E, Vekovischeva O, Rabe H, Böhme I, Aller MI, Wisden W, Lüddens H (2007) Does ethanol act preferentially via selected brain $GABA_A$ receptor subtypes? The current evidence is ambiguous. Alcohol 41:163–176

Lancel M, Langebartels A (2000) gamma-aminobutyric Acid(A) ($GABA_A$) agonist 4,5, 6, 7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol persistently increases sleep maintenance and intensity during chronic administration to rats. J Pharmacol Exp Ther 293:1084–1090

Lankford DA, Corser BC, Zheng YP, Li Z, Snavely DB, Lines CR, Deacon S (2008) Effect of gaboxadol on sleep in adult and elderly patients with primary insomnia: results from two randomized, placebo-controlled, 30-night polysomnography studies. Sleep 31:1359–1370

Laurie DJ, Wisden W, Seeburg PH (1992) The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci 12:4151–4172

Lee S, Yoon BE, Berglund K, Oh SJ, Park H, Shin HS, Augustine GJ, Lee CJ (2010) Channel-mediated tonic GABA release from glia. Science 330:790–796

Lees G, Edwards MD (1998) Modulation of recombination human gamma-aminobutyric acid A receptors by isoflurane: influence of the delta subunit. Anesthesiology 88:206–217

Lewis RW, Mabry J, Polisar JG, Eagen KP, Ganem B, Hess GP (2010) Dihydropyrimidinone positive modulation of delta-subunit-containing gamma-aminobutyric acid type A receptors, including an epilepsy-linked mutant variant. Biochemistry 49:4841–4851

Li GD, Chiara DC, Sawyer GW, Husain SS, Olsen RW, Cohen JB (2006) Identification of a $GABA_A$ receptor anesthetic binding site at subunit interfaces by photolabeling with an etomidate analog. J Neurosci 26:11599–11605

Linden AM, Schmitt U, Leppä E, Wulff P, Wisden W, Lüddens H, Korpi ER (2011) Ro 15-4513 Antagonizes Alcohol-Induced Sedation in Mice Through αβγ2-type $GABA_A$ Receptors. Front Neurosci 5:3

Lodge DJ, Grace AA (2007) Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia. J Neurosci 27:11424–11430

Loebrich S, Bähring R, Katsuno T, Tsukita S, Kneussel M (2006) Activated radixin is essential for $GABA_A$ receptor alpha5 subunit anchoring at the actin cytoskeleton. EMBO J 25:987–999

Luscher B, Fuchs T, Kilpatrick CL (2011) $GABA_A$ receptor trafficking-mediated plasticity of inhibitory synapses. Neuron 70:385–409

Maguire J, Mody I (2008) $GABA_AR$ plasticity during pregnancy: relevance to postpartum depression. Neuron 59:207–213

Maguire J, Mody I (2009) Steroid hormone fluctuations and $GABA_AR$ plasticity. Psychoneuroendocrinology 34(Suppl 1):S84–S90

Maksay G, Thompson SA, Wafford KA (2003) The pharmacology of spontaneously open alpha 1 beta 3 epsilon $GABA_A$ receptor-ionophores. Neuropharmacology 44:994–1002

Mangan PS, Sun C, Carpenter M, Goodkin HP, Sieghart W, Kapur J (2005) Cultured Hippocampal Pyramidal Neurons Express Two Kinds of $GABA_A$ Receptors. Mol Pharmacol 67:775–788

Marowsky A, Rudolph U, Fritschy JM, Arand M (2012) Tonic inhibition in principal cells of the amygdala: a central role for α3 subunit-containing $GABA_A$ receptors. J Neurosci 32:8611–8619

Martin LJ, Oh GH, Orser BA (2009) Etomidate targets alpha5 gamma-aminobutyric acid subtype A receptors to regulate synaptic plasticity and memory blockade. Anesthesiology 111:1025–1035

McCracken ML, Borghese CM, Trudell JR, Harris RA (2010) A transmembrane amino acid in the $GABA_A$ receptor β2 subunit critical for the actions of alcohols and anesthetics. J Pharmacol Exp Ther 335:600–606

McKernan RM, Whiting PJ (1996) Which $GABA_A$-receptor subtypes really occur in the brain? Trends Neurosci 19:139–143

Mehta AK, Marutha Ravindran CR, Ticku MK (2007) Low concentrations of ethanol do not affect radioligand binding to the delta-subunit-containing $GABA_A$ receptors in the rat brain. Brain Res 1165:15–20

Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE (1999) Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. Proc Natl Acad Sci U S A 96:12905–12910

Mihalek RM, Bowers BJ, Wehner JM, Kralic JE, VanDoren MJ, Morrow AL, Homanics GE (2001) $GABA_A$-receptor delta subunit knockout mice have multiple defects in behavioral responses to ethanol. Alcohol Clin Exp Res 25:1708–1718

Möhler H (2012) Cognitive enhancement by pharmacological and behavioral interventions: the murine Down syndrome model. Biochem Pharmacol 84:994–999

Moragues N, Ciofi P, Tramu G, Garret M (2002) Localisation of $GABA_A$ receptor epsilon-subunit in cholinergic and aminergic neurones and evidence for co-distribution with the theta-subunit in rat brain. Neuroscience 111:657–669

Mortensen M, Smart TG (2006) Extrasynaptic alphabeta subunit $GABA_A$ receptors on rat hippocampal pyramidal neurons. J Physiol 577:841–856

Mortensen M, Ebert B, Wafford K, Smart TG (2010) Distinct activities of GABA agonists at synaptic- and extrasynaptic-type $GABA_A$ receptors. J Physiol 588:1251–1268

Mortensen M, Patel B, Smart TG (2011) GABA Potency at $GABA_A$ Receptors Found in Synaptic and Extrasynaptic Zones. Front Cell Neurosci 6:1

Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693–16703

Nutt DJ, Besson M, Wilson SJ, Dawson GR, Lingford-Hughes AR (2007) Blockade of alcohol's amnestic activity in humans by an alpha5 subtype benzodiazepine receptor inverse agonist. Neuropharmacology 53:810–820

Oláh S, Füle M, Komlósi G, Varga C, Báldi R, Barzó P, Tamás G (2009) Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. Nature 461:1278–1281

Pape JR, Bertrand SS, Lafon P, Odessa MF, Chaigniau M, Stiles JK, Garret M (2009) Expression of $GABA_A$ receptor alpha3-, theta-, and epsilon-subunit mRNAs during rat CNS development and immunolocalization of the epsilon subunit in developing postnatal spinal cord. Neuroscience 160:85–96

Peden DR, Petitjean CM, Herd MB, Durakoglugil MS, Rosahl TW, Wafford K, Homanics GE, Belelli D, Fritschy JM, Lambert JJ (2008) Developmental maturation of synaptic and extrasynaptic $GABA_A$ receptors in mouse thalamic ventrobasal neurones. J Physiol 586:965–987

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815–850

Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE (2006) Specific subtypes of $GABA_A$ receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. J Neurophysiol 96:846–857

Qi ZH, Song M, Wallace MJ, Wang D, Newton PM, McMahon T, Chou WH, Zhang C, Shokat KM, Messing RO (2007) Protein kinase C epsilon regulates gamma-aminobutyrate type A receptor sensitivity to ethanol and benzodiazepines through phosphorylation of gamma2 subunits. J Biol Chem 282:33052–33063

Quirk K, Whiting PJ, Ragan CI, McKernan RM (1995) Characterisation of delta-subunit containing $GABA_A$ receptors from rat brain. Eur J Pharmacol 290:175–181

Rau V, Iyer SV, Oh I, Chandra D, Harrison N, Eger EI 2nd, Fanselow MS, Homanics GE, Sonner JM (2009) Gamma-aminobutyric acid type A receptor alpha 4 subunit knockout mice are resistant to the amnestic effect of isoflurane. Anesth Analg 109:1816–1822

Rossi DJ, Hamann M, Attwell D (2003) Multiple modes of GABAergic inhibition of rat cerebellar granule cells. J Physiol 548:97–110

Saliba RS, Kretschmannova K, Moss SJ (2012) Activity-dependent phosphorylation of $GABA_A$ receptors regulates receptor insertion and tonic current. EMBO J 31:2937–2951

Santhakumar V, Jones RT, Mody I (2010) Developmental regulation and neuroprotective effects of striatal tonic $GABA_A$ currents. Neuroscience 167:644–655

Saxena NC, Macdonald RL (1996) Properties of putative cerebellar gamma-aminobutyric acid A receptor isoforms. Mol Pharmacol 49:567–579

Sebe JY, Looke-Stewart EC, Estrada RC, Baraban SC (2010) Robust tonic GABA currents can inhibit cell firing in mouse newborn neocortical pyramidal cells. Eur J Neurosci 32:1310–1318

Semyanov A, Walker MC, Kullmann DM, Silver RA (2004) Tonically active $GABA_A$ receptors: modulating gain and maintaining the tone. Trends Neurosci 27:262–269

Sergeeva OA, Kletke O, Kragler A, Poppek A, Fleischer W, Schubring SR, Görg B, Haas HL, Zhu XR, Lübbert H, Gisselmann G, Hatt H (2010) Fragrant dioxane derivatives identify beta1-subunit-containing $GABA_A$ receptors. J Biol Chem 285:23985–23993

Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, De Blas AL (2006) Synaptic and nonsynaptic localization of $GABA_A$ receptors containing the alpha5 subunit in the rat brain. J Comp Neurol 499:458–470

Shannon EE, Shelton KL, Vivian JA, Yount I, Morgan AR, Homanics GE, Grant KA (2004) Discriminative stimulus effects of ethanol in mice lacking the gamma-aminobutyric acid type A receptor delta subunit. Alcohol Clin Exp Res 28:906–913

Shu HJ, Bracamontes J, Taylor A, Wu K, Eaton MM, Akk G, Manion B, Evers AS, Krishnan K, Covey DF, Zorumski CF, Steinbach JH, Mennerick S (2012) Characteristics of concatemeric

$GABA_A$ receptors containing α4/δ subunits expressed in *Xenopus* oocytes. Br J Pharmacol 165:2228–2243

Sigel E, Lüscher BP (2011) A closer look at the high affinity benzodiazepine binding site on $GABA_A$ receptors. Curr Top Med Chem 11:241–246

Sigel E, Baur R, Rácz I, Marazzi J, Smart TG, Zimmer A, Gertsch J (2011) The major central endocannabinoid directly acts at $GABA_A$ receptors. Proc Natl Acad Sci U S A 108:18150–18155

Sinkkonen ST, Hanna MC, Kirkness EF, Korpi ER (2000) $GABA_A$ receptor epsilon and theta subunits display unusual structural variation between species and are enriched in the rat locus ceruleus. J Neurosci 20:3588–3595

Stell BM, Mody I (2002) Receptors with different affinities mediate phasic and tonic $GABA_A$ conductances in hippocampal neurons. J Neurosci 22:RC223

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing $GABA_A$ receptors. Proc Natl Acad Sci U S A 100:14439–14444

Stórustovu SI, Ebert B (2006) Pharmacological characterization of agonists at delta-containing $GABA_A$ receptors: Functional selectivity for extrasynaptic receptors is dependent on the absence of gamma2. J Pharmacol Exp Ther 316:1351–1359

Studer R, von Boehmer L, Haenggi T, Schweizer C, Benke D, Rudolph U, Fritschy JM (2006) Alteration of GABAergic synapses and gephyrin clusters in the thalamic reticular nucleus of $GABA_A$ receptor alpha3 subunit-null mice. Eur J Neurosci 24:1307–1315

Succol F, Fiumelli H, Benfenati F, Cancedda L, Barberis A (2012) Intracellular chloride concentration influences the $GABA_A$ receptor subunit composition. Nat Commun 3:738

Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, Wiedmann M, Williams K, Smith SS (2002) Hormonally regulated alpha(4)beta(2)delta $GABA_A$ receptors are a target for alcohol. Nat Neurosci 5:721–722

Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, McKernan RM (1999) Preferential coassembly of alpha4 and delta subunits of the gamma-aminobutyric acid A receptor in rat thalamus. Mol Pharmacol 56:110–115

Tang X, Hernandez CC, Macdonald RL (2010) Modulation of spontaneous and GABA-evoked tonic alpha4beta3delta and alpha4beta3gamma2L $GABA_A$ receptor currents by protein kinase A. J Neurophysiol 103:1007–1019

Thompson SA, Bonnert TP, Cagetti E, Whiting PJ, Wafford KA (2002) Overexpression of the $GABA_A$ receptor epsilon subunit results in insensitivity to anaesthetics. Neuropharmacology 43:662–668

Tretter V, Mukherjee J, Maric HM, Schindelin H, Sieghart W, Moss SJ (2012) Gephyrin, the enigmatic organizer at GABAergic synapses. Front Cell Neurosci 6:23. (Epub 15 May 2012)

Vardya I, Hoestgaard-Jensen K, Nieto-Gonzalez JL, Dósa Z, Boddum K, Holm MM, Wolinsky TD, Jones KA, Dalby NO, Ebert B, Jensen K (2012) Positive modulation of δ-subunit containing $GABA_A$ receptors in mouse neurons. Neuropharmacology 63:469–479

Vargas-Caballero M, Martin LJ, Salter MW, Orser BA, Paulsen O (2010) Alpha5 Subunit-containing $GABA_A$ receptors mediate a slowly decaying inhibitory synaptic current in CA1 pyramidal neurons following Schaffer collateral activation. Neuropharmacology 58:668–675

Wafford KA, Thompson SA, Thomas D, Sikela J, Wilcox AS, Whiting PJ (1996) Functional characterization of human gamma-aminobutyric acid A receptors containing the alpha 4 subunit. Mol Pharmacol 50:670–678

Wafford KA, Ebert B (2006) Gaboxadol-a new awakening in sleep. Curr Opin Pharmacol 6:30–36

Wafford KA, van Niel MB, Ma QP, Horridge E, Herd MB, Peden DR, Belelli D, Lambert JJ (2009) Novel compounds selectively enhance delta subunit containing $GABA_A$ receptors and increase tonic currents in thalamus. Neuropharmacology 56:182–189

Wallner M, Hanchar HJ, Olsen RW (2003) Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc Natl Acad Sci U S A 100:15218–15223

Wallner M, Hanchar HJ, Olsen RW (2006) Low-dose alcohol actions on alpha4beta3delta $GABA_A$ receptors are reversed by the behavioural alcohol antagonist Ro15-4513. Proc Natl Acad Sci U S A 103:8540–8545

Wan Q, Xiong ZG, Man HY, Ackerley CA, Braunton J, Lu WY, Becker LE, MacDonald JF, Wang YT (1997) Recruitment of functional $GABA_A$ receptors to postsynaptic domains by insulin. Nature 388:686–690

Wei W, Faria LC, Mody I (2004) Low ethanol concentrations selectively augment the tonic inhibition mediated by delta subunit-containing $GABA_A$ receptors in hippocampal neurons. J Neurosci 24:8379–8382

Werner DF, Kumar S, Criswell HE, Suryanarayanan A, Fetzer JA, Comerford CE, Morrow AL (2011) PKCγ is required for ethanol-induced increases in $GABA_A$ receptor α4 subunit expression in cultured cerebral cortical neurons. J Neurochem 116:554–563

Whittemore ER, Yang W, Drewe JA, Woodward RM (1996) Pharmacology of the human gamma-aminobutyric acid A receptor alpha 4 subunit expressed in *Xenopus laevis* oocytes. Mol Pharmacol 50:1364–1375

Wieland HA, Lüddens H, Seeburg PH (1992) A single histidine in $GABA_A$ receptors is essential for benzodiazepine agonist binding. J Biol Chem 267:1426–1429

Winsky-Sommerer R, Vyazovskiy VV, Homanics GE, Tobler I (2007) The EEG effects of THIP (Gaboxadol) on sleep and waking are mediated by the $GABA_A$ delta-subunit-containing receptors. Eur J Neurosci 25:1893–1899

Wohlfarth KM, Bianchi MT, Macdonald RL (2002) Enhanced neurosteroid potentiation of ternary $GABA_A$ receptors containing the delta subunit. J Neurosci 22:1541–1549

Wu X, Wu Z, Ning G, Guo Y, Ali R, Macdonald RL, De Blas AL, Luscher B, Chen G (2012) γ-Aminobutyric acid type A ($GABA_A$) receptor α subunits play a direct role in synaptic versus extrasynaptic targeting. J Biol Chem 287:27417–27430

Yamashita M, Marszalec W, Yeh JZ, Narahashi T (2006) Effects of ethanol on tonic GABA currents in cerebellar granule cells and mammalian cells recombinantly expressing $GABA_A$ receptors. J Pharmacol Exp Ther 319:431–438

Yanovsky Y, Schubring S, Fleischer W, Gisselmann G, Zhu XR, Lübbert H, Hatt H, Rudolph U, Haas HL, Sergeeva OA (2012) $GABA_A$ receptors involved in sleep and anaesthesia: β1- versus β3-containing assemblies. Pflugers Arch 463:187–199

Yeung JY, Canning KJ, Zhu G, Pennefather P, MacDonald JF, Orser BA (2003) Tonically activated $GABA_A$ receptors in hippocampal neurons are high-affinity, low-conductance sensors for extracellular GABA. Mol Pharmacol 63:2–8

Yoon BE, Jo S, Woo J, Lee JH, Kim T, Kim D, Lee CJ (2011) The amount of astrocytic GABA positively correlates with the degree of tonic inhibition in hippocampal CA1 and cerebellum. Mol Brain 4:42

Zarnowska ED, Keist R, Rudolph U, Pearce RA (2009) $GABA_A$ receptor alpha5 subunits contribute to $GABA_A$ slow synaptic inhibition in mouse hippocampus. J Neurophysiol 101:1179–1191

Zurek AA, Bridgwater EM, Orser BA (2012) Inhibition of α5 γ-aminobutyric acid type A receptors restores recognition memory after general anesthesia. Anesth Analg 114:845–855

Chapter 5
Neurosteroids and Extrasynaptic $GABA_A$ Receptors

William M. Connelly

Abstract Steroid sex hormones are generally thought to act through intracellular receptors, where they regulate gene expression and protein synthesis, an effect that takes minutes to hours to occur. However, steroid metabolites can also directly modulate the activity of numerous ligand-gated ion channels. These metabolites can be synthesised de novo in the CNS, and are termed *neurosteroids*. The action of neurosteroids at ion channels, most notably $GABA_A$ receptors, is implicated in natural and pathophysiological stress responses and neurophysiological changes during the oestrous cycle and parturition. Here, I will review the synthesis, action and the activity of neurosteroids on synaptic and extrasynaptic $GABA_A$ receptors.

Keywords GABA · Neurosteroids · Tonic inhibition · Extrasynaptic

5.1 Metabolism

The notion that steroids could be synthesised de novo in the central nervous system (CNS) was raised by Corpéchot et al. (1981) when dehydroepiandrosterone (DHEA) was shown to be present in the CNS at concentrations 20 times that of plasma. Furthermore, CNS levels of the steroid were not reduced by removal of the testes, adrenal glands or by treatment with dexamethasone, indicating that the steroids were not derived for classical peripheral sources, a result soon repeated for pregnenolone and its sulphate ester (Corpéchot et al. 1983). Thereafter, the mitochondrial cytochrome P450 cholesterol side-chain cleavage (P450scc) enzyme, which catalyses the conversion of cholesterol to the steroid precursor, pregnenolone, was identified in neurons and glial cells (Le Goascogne et al. 1987; Jung-Testas et al. 1989; Zwain and Yen 1999). The subsequent metabolism of active neurosteroids is complex, and a complete discussion of the various biosynthetic pathways is outside the scope of this review. However, a brief outline of the most potent and commonly researched neurosteroids is in order. All neurosteroids can be derived from progesterone (PROG) which is pro-

W. M. Connelly (✉)
Neuroscience Division, Cardiff School of Biosciences, Cardiff University,
The Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX, UK
e-mail: connellywm@cardiff.ac.uk

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_5

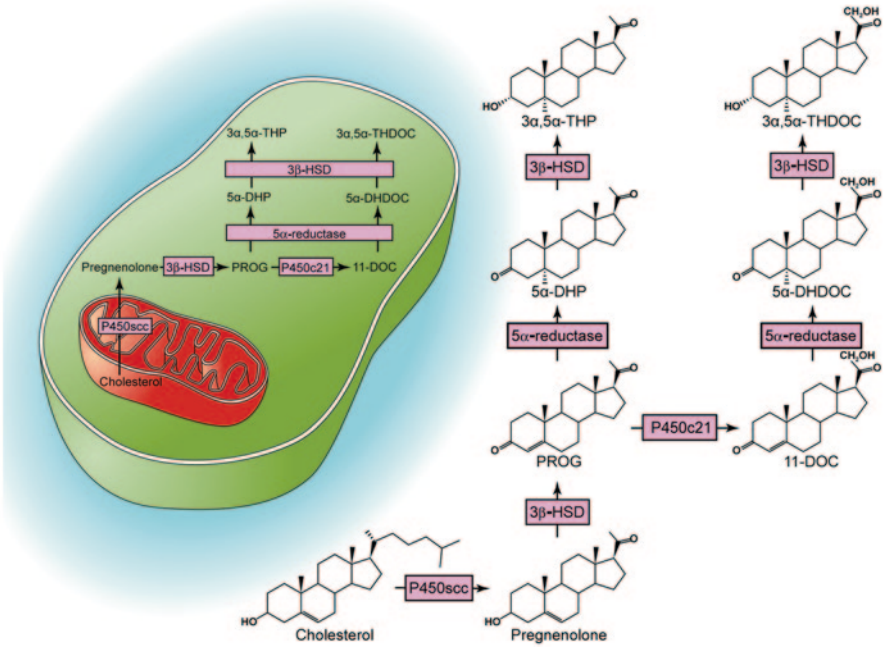

Fig. 5.1 Biosynthesis of neurosteroids. Cholesterol is metabolised by mitochondrial cytochrome P450 cholesterol side-chain cleavage (P450scc) to pregnenolone. 3-β-hydroxysteroid dehydrogenase (3β-HSD), located largely in the endoplasmic reticulum converts pregneolone to progesterone (*PROG*). PROG is converted to 5α-dihydroprogesterone (5α-DHP) by 5α-reductase also located largely in the endoplasmic reticulum, which is then metabolised by 3α-HSD to form 3α,5α-tetrahydroprogesterone (3α,5α-THP). PROG can also be metabolised by P450c21 to 11-deoxycorticosterone (11-DOC). Which in turn is converted to 5α-dihydrodeoxycorticosterone (5α-DHDOC). Tetrahydrodeoxycorticosterone (3α,5α-THDOC) is produced that is metabolised by 3β-HSD. Note, even though P450c21 is represented in this diagram, it is not expressed in the CNS

duced by the action of 3-β-hydroxysteroid dehydrogenase (3β-HSD) on pregnenolone. To produce 3α,5α-tetrahydroprogesterone (3α,5α-THP; allopregnanolone), PROG is converted to 5α-dihydroprogesterone by 5α-reductase, which is then metabolised by 3α-HSD. Alternatively, 3α,5α-tetrahydrodeoxycorticosterone (3α,5α-THDOC) is produced when PROG is metabolised by P450c21 to 11-deoxycorticosterone (11-DOC), and then acted on by 5α-reductase to produce 5α-dihydrodeoxycorticosterone which is subsequently metabolised by 3α-HSD (Fig. 5.1). There are numerous other end pathways and products which are reviewed in detail by Compagnone and Mellon (2000). As with the previously discussed pathways, all of these alternative biosynthetic enzymes, apart from P450c21, can be found in the CNS (reviewed by Mellon et al. 2001).

Once synthesised, it is assumed that neurosteroids leave the cell via diffusion through the plasma membrane. Clearance of neurosteroids is probably mediated by the same enzymes which synthesise them. This view is supported by evidence such

that inhibition of 3α-HSD amplifies the action of exogenously applied 3α,5α-THP (Belelli and Herd 2003).

5.2 Action

In 1941, Selye demonstrated that intraperitoneal delivery of PROG and 11-DOC produced a rapid and reversible anaesthesia. This discovery led to the development of alphaxalone, a short-acting steroid anaesthetic, which for a brief time enjoyed widespread clinical use (Gyermek and Soyka et al. 1975). While these anaesthetic steroids were originally hypothesised to act via non-specific membrane disruption, several results made this suggestion untenable. For instance, alphaxalone, while being similarly non-polar to PROG, is a significantly more potent anaesthetic (see Gyermek and Soyka et al. 1975). Furthermore, the beta-hydroxy isomer of alphaxalone, betaxalone, which is again equally lipophilic, is completely devoid of anaesthetic action (Phillipps 1974). The notion that neuroactive steroids may have a specific receptor was confirmed when Harrison and Simmonds (1984) demonstrated that alphaxalone (but not betaxalone) potentiated the extracellular field response to muscimol in brainstem slices, as well as [^{3}H]muscimol binding to synaptic membranes. Indeed, previous results had shown that alphaxalone slowed the decay of *inhibitory postsynaptic currents* (IPSCs) recorded in cortical brain slices (Scholfield 1980). Further evidence comes from whole-cell recording from cultured bovine chromaffin cells, where low (10 nM–1 μM) concentrations of alphaxalone potentiated the response to GABA, and higher concentrations directly evoked $GABA_A$ receptor-mediated currents (Cottrell et al. 1987). Single-channel studies indicated that neurosteroids acted in a manner similar to barbiturates, increasing the frequency of channel opening and the likelihood that a channel will enter an open state of long duration, without altering the main conductance level (Callachan et al. 1987; Twyman and Macdonald 1992). However, it is not as simple as the notion that all neurosteroids potentiate the $GABA_A$ receptor. Pregnenolone sulphate and DHEA sulphate are well established to block $GABA_A$ receptors at low micromolar concentrations (Majewska et al. 1988; Demirgören et al. 1991; Akk et al. 2001). Furthermore, some 3β-hydroxypregnane steroids such as 3β,5α-THP and 3β,5α-THDOC appear to act as neurosteroid antagonists: inactive at the $GABA_A$ receptor themselves, but able to antagonize the effect of 3α-hydroxypregnane steroids (Wang et al. 2002; Lundgren et al. 2003).

During this era of research, two facts were becoming evident, first, that the $GABA_A$ receptor was made up of a dizzying array of subunits, and that certain drugs only acted at receptors made up of specific subunits. The prototypical class of these are the benzodiazepines. The first evidence of subunit-dependent drug action was revealed when the γ2 subunit was cloned, and it was shown that this subunit was required for benzodiazepine action (Pritchett et al. 1989). This idea was extended to show that benzodiazepine action was dependent on the presence of α1, 2, 3 or 5 subunits. (Sigel et al. 1990; Smith and Olsen 1995). However, such subunit se-

lectivity was not initially forthcoming for steroids. Early work either showed that steroids could bind to a large variety of receptors (e.g. Gee et al. 1988 and Puia et al. 1990) or contradictory results regarding subunit selectivity. For example Korpi and Lüddens (1993) showed that α6βγ2 receptors are highly sensitive to steroids while Puia et al. (1993) reported that α6βγ2 receptors are less sensitive. Similarly, Shingai et al. (1991) reported that α1-containing receptors were the most sensitive to steroids while Lan et al. (1991) reported that α3-containing receptors are more sensitive than α1. Possibly one of the most interesting subunits, when it comes to the action of steroids is the δ subunit. Initial reports suggested that the δ subunit significantly decreased the action of steroids in oocyte expression systems, and likewise, that cultured cerebellar granule cells that expressed γ2 subunits were more sensitive to the action of 3α,5α-THDOC than those that express δ subunits (Zhu et al. 1996). This result was repeated by measuring the decay kinetics of miniature IPSCs by Cooper et al. (1999), where more mature, δ-containing cerebellar granule cells became insensitive to steroid action. However, these results were soon contested when it was reported that, while 3α,5α-THDOC has similar EC50s for potentiating submaximal GABA responses in α1β3γ2L and α6β3γ2L versus α1β3δ and α6β3δ receptors, the neurosteroid produced a far greater magnitude of potentiation in δ-containing receptors when the receptors were exposed to saturating concentrations of GABA (Belelli et al. 2002; Wohlfarth et al. 2002). Likewise, while 3α,5α-THP, alphaxalone and 3α,5α-THDOC all had similar EC50s for potentiating submaximal GABA responses in α4β3γ2 and α4β3δ receptors, the steroids produced a far greater magnitude of potentiation in δ-containing receptors in the presence of saturating concentrations of GABA (Brown et al. 2002). Indeed, these results were foreshadowed by a study using fluorescence resonance energy transfer (FRET) imaging of GABA-evoked depolarization (Adkins et al. 2001). Furthermore, these results were reinforced when it was shown that the tonic current in dentate gyrus and cerebellar granule cells, known to be produced by α4βδ and α6βδ receptors respectively, was sensitive to low concentrations (10–100 nM) of 3α,5α-THDOC, while the sensitivity was lost in $\delta^{-/-}$ animals (Stell et al. 2003). It is important to note that while there is no evidence to support the notion that steroids such as 3α,5α-THDOC are *pharmacologically* selective for δ-containing receptors (that is, they do not have a lower EC50 at these receptors, Lambert et al. 2003), because neurons that use these receptors to mediate extrasynaptic currents generally receive the majority of their inhibitory charge transfer through extrasynaptic receptors, neurosteroid-mediated potentiation of these receptors can be seen as *functionally* selective for δ-containing receptors. That is, the potentiation of inhibition in response to steroids is largely due to the enhancement of charge transfer through δ-containing receptors.

5.3 Anxiety/Stress

The initial observation by Purdy et al. (1991) that mild stress-induced synthesis of neurosteroids has fuelled research into the role of steroids in stress and anxiety. Their experiments reported that swim stress induced a 4–20-fold increase in both

the levels of circulating and CNS neurosteroids, reaching concentrations of approximately 50 nM. Moreover, the increase in central 3α,5α-THP levels preceded that in the plasma, and occurred even in adrenalectomized rats, indicating that the steroid was centrally synthesised. It is now well know that these neurosteroids have anxiolytic actions in humans and experimental animals (Freeman et al. 1993; Bitran et al. 1999), thus, it seems possible that neurosteroid release may act as an endogenous anxiolytic in response to stressful situations. While I am unaware of any evidence that specifically tests this hypothesis, it seems that stress-induced neurosteroid synthesis is responsible for the antiseizure effect of mild stress, as treatment with the 5α-reductase inhibitor, finasteride, prevents the stress-induced increase in plasma THDOC levels, as well as the stress-induced increase in seizure threshold (Reddy and Rogawski 2002; though see Pericić et al. 2000). Indeed, neurosteroids may regulate basal levels of anxiety, as microinjections of finasteride into the amygdale appears to increase anxiety-related behaviour of unstressed rats (Walf et al. 2006). Interestingly, there is some evidence that patients with generalized anxiety disorder (GAD) may have low-circulating levels of pregnenolone sulphate (Semeniuk et al. 2001; Heydari and Le Mellédo 2002).

While the interaction of neurosteroids with the hypothalamic–pituitary–adrenal (HPA) axis is covered elsewhere in this book (Maguire, Chap. 12), one example warrants mention in this chapter. The release of corticotropin-releasing hormone (CRH) from the hypothalamus is instrumental in coordinating the stress response, causes the release of adrenocorticotropic hormone and subsequently corticosterone. As would be expected, THDOC potentiates δ-containing $GABA_A$ receptors on CRH neurons, reducing their output (Sarkar et al. 2011). However, during stress the THDOC-mediated enhancement of GABAergic signalling actually increases the output of CRH neurons due to a shift in Cl-gradient induced by phosphorylation of the K^+/Cl^- co-transporter KCC2. Furthermore, under normal conditions, intraperitoneal THDOC has no effect on circulating corticosterone levels while under stressed conditions THDOC actually enhances the release of corticosterone. Indeed, the physiological release of corticosterone in response to stress is dependent on this neurosteroid-mediated excitement of CRH neurons, as finasteride (which does not directly affect the synthesis of corticosterone) prevents stress-induced increase in corticosterone (Sarkar et al. 2011).

A final and potentially most illustrative example of the interaction between neurosteroids, $GABA_A$ receptors subunits and anxiety comes from studying neurophysiological changes during puberty. Plasma concentrations of 3α,5α-THP have been shown to increase throughout puberty (Fadalti et al. 1999), and this steroid is a well-established anxiolytic. However, seemingly at odds with this, stress responses are increased and anxiety disorders first become apparent during puberty (Modesti et al. 1994; Hayward and Sanborn 2002). This discord is potentially explained by the findings of Shen et al. (2007) who demonstrated that 3α,5α-THP in fact inhibits currents produced by α4β2δ receptors (while still potentiating α4β3δ receptors) by enhancing desensitization. They showed that during puberty, there is an increase of α4 and δ subunits in area CA1 of the hippocampus, an area where they are essentially undetectable before puberty (they did not investigate β subunit expression). Importantly, 3α,5α-THP inhibited the tonic current recorded in CA1 pyramidal

neurons from pubertal animals, whereas in prepubetal animals, it potentiated it. In line with this, while exogenous 3α,5α-THP was anxiolytic in prepubertal animals, it was anxiogenic in pubertal animals. These results show the complexity that the neurosteroid system can have when interacting with the dynamic subunit composition of the $GABA_A$ receptor.

5.4 Oestrous Cycle, Parturition and Puberty

Some of the most striking examples of the potential endogenous role for neurosteroids come from studying the alteration in steroid synthesis during the oestrous cycle and parturition. It is well established that PROG and 3α,5α-THP have antiseizure effects (e.g. Selye 1942; Belelli et al. 1989). Plasma PROG levels peak during the luteal phase of the oestrous cycle, a period during which animals have a decreased seizure susceptibility (Frye and Bayon 1998). Thus, it seems reasonable to hypothesise that the increased PROG and 3α,5α-THP levels seen during the luteal phase may be responsible for the lowered seizure susceptibility during this phase. Indeed, during artificially induced oestrous cycling, finasteride blocks the increase in plasma 3α,5α-THP, as well as the antiseizure effects of oestrous (Frye et al. 1998). Furthermore, during natural oestrous cycling, the level of the δ subunit of the $GABA_A$ receptor increases, coincident with high levels of PROG, and there is in turn an increase in the magnitude of the tonic current recorded in dentate gyrus granule cells. Importantly, the decrease in seizure sensitivity seen during this high PROG, high δ-subunit phase, is removed when the δ-subunit is knocked down by RNA interference (Maguire et al. 2005). Thus, it seems that during the luteal phase, there is both an increase in neurosteroids as well receptors they target.

Pregnancy is another condition where neurosteroids are elevated. In females, plasma and central levels of both PROG and 3α,5α-THP increase massively over the course of pregnancy (Concas et al. 1998; Luisi et al. 2000). In parallel with this, there is an increase in seizure threshold during pregnancy, an effect that is blocked by finasteride (Nau et al. 1985; Fyre and Bayon 1998). However, the subunit changes in the $GABA_A$ receptor that occur during pregnancy remain unclear. The initial report by Maguire and Mody (2008) demonstrated that during pregnancy the level of δ-subunit protein decreases in the hippocampus, along with the magnitude of the tonic current recorded in dentate gyrus granule cells. However, Sanna et al. (2009) reported an increase in the magnitude of the tonic current, increasing the tonic current's sensitivity to 3α,5α-THP and a parallel increase in immunoreactivity for δ-subunit protein in the hippocampus. Subsequently, Maguire and colleagues repeated their results, and implicated the difference in the antibodies used for the different results (Maguire et al. 2009). However, this fails to explain the diametrically opposed results regarding the magnitude of the tonic current during pregnancy. Interestingly, slices from Maguire et al. (2009) are shown to be hyperexcitable when taken from pregnant animals, apparently opposing what is known about the

lowered seizure threshold during pregnancy. As the work of Maguire and colleagues was performed in mice, while that of Sanna and colleagues was performed in rats, perhaps these differences are simply due to the species/strain used.

Conclusion

In the 50 years since Selye's report of the anaesthetic action of neurosteroids, much has been revealed about their action and physiological relevance. However, many important areas remain to be properly covered. Apart from the regulation of CRH release, what roles do neurosteroids play in the physiological response to stress? Is the action of neurosteroids enhanced or decreased in pregnancy? How is neurosteroid synthesis regulated in the CNS? It is still not completely clear which $GABA_A$ receptor subtypes neurosteroids target. There appears to be a dearth of molecular tools to investigate these questions (for instance, a CNS-specific knockout of 5α-reductase) and hopefully the development of tools like this will help elucidate the role of neurosteroids in the healthy and diseased brain.

References

Adkins CE, Pillai GV, Kerby J, Bonnert TP, Haldon C, McKernan RM, Gonzalez JE, Oades K, Whiting PJ, Simpson PB (2001) α4β3δ GABA(A) receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential. J Biol Chem 276:38934–38939

Akk G, Bracamontes J, Steinbach JH (2001) Pregnenolone sulfate block of GABA(A) receptors: mechanism and involvement of a residue in the M2 region of the alpha subunit. J Physiol 532:673–684

Belelli D, Bolger MB, Gee KW (1989) Anticonvulsant profile of the progesterone metabolite 5 alpha-pregnan-3 alpha-ol-20-one. Eur J Pharmacol 166:325–329

Belelli D, Casula A, Ling A, Lambert JJ (2002) The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. Neuropharmacology 43:651–661

Belelli D, Herd MB (2003) The contraceptive agent Provera enhances $GABA_A$ receptor-mediated inhibitory neurotransmission in the rat hippocampus: evidence for endogenous neurosteroids? J Neurosci 23:10013–10020

Bitran D, Dugan M, Renda P, Ellis R, Foley M (1999) Anxiolytic effects of the neuroactive steroid pregnanolone (3alpha-OH-5beta-pregnan-20-one) after microinjection in the dorsal hippocampus and lateral septum. Brain Res 850:217–224

Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA (2002) Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. Br J Pharmacol 136(7):965–967

Callachan H, Cottrell GA, Hather NY, Lambert JJ, Nooney JM, Peters JA (1987) Modulation of the $GABA_A$ receptor by progesterone metabolites. Proc R Soc Lond B Biol Sci 231:359–369

Compagnone NA, Mellon SH (2000) Neurosteroids: biosynthesis and function of these novel neuromodulators. Front Neuroendocrinol 21:1–56

Concas A, Mostallino MC, Porcu P, Follesa P, Barbaccia ML, Trabucchi M, Purdy RH, Grisenti P, Biggio G (1998) Role of brain allopregnanolone in the plasticity of gamma-aminobutyric

acid type A receptor in rat brain during pregnancy and after delivery. Proc Natl Acad Sci U S A 95:13284–13289
Cooper EJ, Johnston GA, Edwards FA (1999) Effects of a naturally occurring neurosteroid on $GABA_A$ IPSCs during development in rat hippocampal or cerebellar slices. J Physiol 521:437–449
Corpéchot C, Robel P, Axelson M, Sjövall J, Baulieu EE (1981) Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. Proc Natl Acad Sci U S A 78:4704–4707
Corpéchot C, Synguelakis M, Talha S, Axelson M, Sjövall J, Vihko R, Baulieu EE, Robel P (1983) Pregnenolone and its sulfate ester in the rat brain. Brain Res 270:119–125
Cottrell GA, Lambert JJ, Peters JA (1987) Modulation of $GABA_A$ receptor activity by alphaxalone. Br J Pharmacol 90:491–500
Demirgören S, Majewska MD, Spivak CE, London ED (1991) Receptor binding and electrophysiological effects of dehydroepiandrosterone sulfate, an antagonist of the $GABA_A$ receptor. Neuroscience 45:127–135
Fadalti M, Petraglia F, Luisi S, Bernardi F, Casarosa E, Ferrari E, Luisi M, Saggese G, Genazzani AR, Bernasconi S (1999) Changes of serum allopregnanolone levels in the first 2 years of life and during pubertal development. Pediatr Res 46:323–327
Freeman EW, Purdy RH, Coutifaris C, Rickels K, Paul SM (1993) Anxiolytic metabolites of progesterone: correlation with mood and performance measures following oral progesterone administration to healthy female volunteers. Neuroendocrinology 58:478–484
Frye CA, Bayon LE (1998) Seizure activity is increased in endocrine states characterized by decline in endogenous levels of the neurosteroid 3α,5α-THP. Neuroendocrinology 68:272–280
Frye CA, Scalise TJ, Bayon LE (1998) Finasteride blocks the reduction in ictal activity produced by exogenous estrous cyclicity. J Neuroendocrinol 10:291–6
Gee KW, Bolger MB, Brinton RE, Coirini H, McEwen BS (1988) Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. J Pharmacol Exp Ther 246:803–812
Gyermek L, Soyka LF (1975) Steroid anesthetics. Anesthesiology 42:331–344
Harrison NL, Simmonds MA (1984) Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res 323:287–292
Hayward C, Sanborn K (2002) Puberty and the emergence of gender differences in psychopathology. J Adolesc Health 30S:49–58
Heydari B, Le Mellédo JM (2002) Low pregnenolone sulphate plasma concentrations in patients with generalized social phobia. Psychol Med 32:929–933
Jung-Testas I, Hu ZY, Baulieu EE, Robel P (1989) Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. Endocrinology 125:2083–2091
Korpi ER, Lüddens H (1993) Regional gamma-aminobutyric acid sensitivity of t-butylbicyclophosphoro[35S]thionate binding depends on gamma-aminobutyric acidA receptor alpha subunit. Mol Pharmacol 44:87–92
Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA (2003) Neurosteroid modulation of $GABA_A$ receptors. Prog Neurobiol 71:67–80
Lan NC, Gee KW, Bolger MB, Chen JS (1991) Differential responses of expressed recombinant human gamma-aminobutyric acidA receptors to neurosteroids. J Neurochem 57:1818–1821
Le Goascogne C, Robel P, Gouézou M, Sananès N, Baulieu EE, Waterman M (1987) Neurosteroids: cytochrome P-450scc in rat brain. Science 237:1212–1215
Luisi S, Petraglia F, Benedetto C, Nappi RE, Bernardi F, Fadalti M, Reis FM, Luisi M, Genazzani AR (2000) Serum allopregnanolone levels in pregnant women: changes during pregnancy, at delivery, and in hypertensive patients. J Clin Endocrinol Metab 85(7):2429–2433
Lundgren P, Strömberg J, Bäckström T, Wang M (2003) Allopregnanolone-stimulated GABA-mediated chloride ion flux is inhibited by 3beta-hydroxy-5alpha-pregnan-20-one (isoallopregnanolone). Brain Res 982:45–53
Maguire J, Mody I (2008) GABA(A)R plasticity during pregnancy: relevance to postpartum depression. Neuron 59:207–213

Maguire JL, Stell BM, Rafizadeh M, Mody I (2005) Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci 8:797–804

Maguire J, Ferando I, Simonsen C, Mody I (2009) Excitability changes related to $GABA_A$ receptor plasticity during pregnancy. J Neurosci 2009

Majewska MD, Mienville JM, Vicini S (1988) Neurosteroid pregnenolone sulfate antagonizes electrophysiological responses to GABA in neurons. Neurosci Lett 90:279–284

Mellon SH, Griffin LD, Compagnone NA (2001) Biosynthesis and action of neurosteroids. Brain Res Brain Res Rev 37:3–12

Modesti PA, Pela I, Cecioni I, Gensini GF, Serneri GG, Bartolozzi G (1994) Changes in blood pressure reactivity and 24-hour blood pressure profile occurring at puberty. Angiology 45:443–450

Nau H, Kuhnz W, Löscher W (1985) Effects of pregnancy on seizure threshold and the disposition and efficacy of antiepileptic drugs in the mouse. Life Sci 36:663–669

Perićić D, Svob D, Jazvinsćak M, Mirković K (2000) Anticonvulsive effect of swim stress in mice. Pharmacol Biochem Behav 66:879–886

Phillipps GH (1974) Structure-activity relationships in steroidal anaesthetics. In: Halsey MJ, Millar RA, Sutton JA (eds) Molecular mechanisms in general anaesthesia. Churchill Livingstone, Edinburgh, pp 32–46

Pritchett DB1, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, Seeburg PH (1989) Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338(6216):582–585

Puia G, Santi MR, Vicini S, Pritchett DB, Purdy RH, Paul SM, Seeburg PH, Costa E (1990) Neurosteroids act on recombinant human $GABA_A$ receptors. Neuron 4:759–765

Puia G, Ducić I, Vicini S, Costa E (1993) Does neurosteroid modulatory efficacy depend on $GABA_A$ receptor subunit composition? Recept Channels 1:135–142

Purdy RH, Morrow AL, Moore PH Jr, Paul SM (1991) Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci U S A 88:4553–4557

Reddy DS, Rogawski MA (2002) Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. J Neurosci 22:3795–3805

Sanna E, Mostallino MC, Murru L, Carta M, Talani G, Zucca S, Mura ML, Maciocco E, Biggio G (2009) Changes in expression and function of extrasynaptic $GABA_A$ receptors in the rat hippocampus during pregnancy and after delivery. J Neurosci 29:1755–1765

Sarkar J, Wakefield S, MacKenzie G, Moss SJ, Maguire J (2011) Neurosteroidogenesis is required for the physiological response to stress: role of neurosteroid-sensitive $GABA_A$ receptors. J Neurosci 31:18198–18210

Scholfield CN (1980) Potentiation of inhibition by general anaesthetics in neurones of the olfactory cortex in vitro. Pflugers Arch 383:249–255

Selye H (1941) Anaesthetic effects of steroid hormones. Proc Soc Exp Biol Med 46:116–121

Selye H (1942) The antagonism between anesthetic steroid hormones and pentamethylenetetrazol (metrazol). J Lab Clin Med 27:1051–1053

Semeniuk T, Jhangri GS, Le Mellédo JM (2001) Neuroactive steroid levels in patients with generalized anxiety disorder. J Neuropsychiatry Clin Neurosci 13:396–398

Sigel E, Baur R, Trube G, Möhler H, Malherbe P (1990) The effect of subunit composition of rat brain $GABA_A$ receptors on channel function. Neuron 5:703–711

Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, Smith SS (2007) Reversal of neurosteroid effects at α4β2δ $GABA_A$ receptors triggers anxiety at puberty Nat Neurosci 10:469–477

Shingai R, Sutherland ML, Barnard EA (1991) Effects of subunit types of the cloned $GABA_A$ receptor on the response to a neurosteroid. Eur J Pharmacol 206:77–80

Smith GB1, Olsen RW (1995) Functional domains of $GABA_A$ receptors. Trends Pharmacol Sci 16(5):162–168

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing $GABA_A$ receptors. Proc Natl Acad Sci U S A 100:14439–14444

Twyman RE, Macdonald RL (1992) Neurosteroid regulation of $GABA_A$ receptor single-channel kinetic properties of mouse spinal cord neurons in culture. J Physiol 456:215–245

Walf AA, Sumida K, Frye CA (2006) Inhibiting 5alpha-reductase in the amygdala attenuates antianxiety and antidepressive behavior of naturally receptive and hormone-primed ovariectomized rats. Psychopharmacology 186:302–311

Wang M, He Y, Eisenman LN, Fields C, Zeng CM, Mathews J, Benz A, Fu T, Zorumski E, Steinbach JH, Covey DF, Zorumski CF, Mennerick S (2002) 3beta -hydroxypregnane steroids are pregnenolone sulfate-like GABA(A) receptor antagonists. J Neurosci 22:3366–3375

Wohlfarth KM, Bianchi MT, Macdonald RL (2002) Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. J Neurosci 22:1541–1549

Zhu WJ, Wang JF, Krueger KE, Vicini S (1996) Delta subunit inhibits neurosteroid modulation of $GABA_A$ receptors. J Neurosci 16:6648–6656

Zwain IH, Yen SS (1999) Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. Endocrinology 140:3843–3852

Chapter 6
Sources of GABA That Activate Extrasynaptic $GABA_A$ Receptors

Damian Bright, Catriona Houston and Stephen Brickley

Abstract Extrasynaptic γ-aminobutyric acid type A ($GABA_A$) receptors in the brain are activated by GABA molecules present in the extracellular space, leading to the generation of a tonic conductance that has been shown to influence neuronal excitability (see other chapters). In the current chapter, we explore the many factors that can influence the ambient GABA concentration paying particular attention to how GABA synthesis, release, diffusion and uptake both provides and controls extracellular GABA levels. Evidence in support of both vesicular and non-vesicular sources of GABA is presented. We also consider how the physical environment of the extracellular space influences ambient GABA concentrations by shaping transmitter diffusion and regulating GABA availability due to the complex spatial distribution of GABA-binding proteins. Measurements of GABA levels in the brain are reviewed including some discussion of how ambient GABA concentrations are reported to change in pathological conditions.

Keywords GABA · Vesicular · Non-vesicular · GABA transporter · Extrasynaptic · Tonic inhibition

6.1 Introduction

Tonic inhibition is provided by various populations of extrasynaptic γ-aminobutyric acid type A ($GABA_A$) receptors within the brain. Specific extrasynaptic $GABA_A$ receptor populations are formed from defined subunit combinations (normally containing either the δ subunit or the α5 subunit). It is the high GABA affinity of these extrasynaptic $GABA_A$ receptor populations that results in their continuous activation by the low ambient GABA concentrations present in the extracellular space (ECS). These tonic $GABA_A$ receptor-mediated conductances have been

S. Brickley (✉) · C. Houston
Biophysics Section, Department of Life Sciences, Imperial College London,
South Kensington Campus, London SW7 2AZ, UK
e-mail: s.brickley@imperial.ac.uk

D. Bright
Department of Neuroscience, Physiology and Pharmacology, University College London,
London WC1E 6BT, UK

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_6

identified in various neuronal populations within all major brain areas, including cortex, hippocampus, thalamus, hypothalamus and brain stem (Farrant and Nusser 2005; Brickley and Mody 2012). Several studies have suggested an important role for tonic conductances in the control of neuronal excitability, with distinct physiological roles from the more well-studied phasic inhibition (Brickley et al. 1996; Mitchell and Silver 2003; Chadderton et al. 2004; Pavlov et al. 2009). However, a critical factor in understanding the functional role of tonic inhibition is to appreciate how these $GABA_A$ receptor-mediated conductances can be modulated. Therefore, it is important to identify the sources of GABA that give rise to tonic receptor activation and to understand the various mechanisms that might control the concentration of GABA within the ECS.

We will review how GABA is synthesized and stored and our current knowledge about the sources of GABA responsible for the various tonic $GABA_A$ receptor-mediated conductances that have been identified. We will then discuss factors that control the extracellular GABA concentration under various conditions and how these are likely to influence tonic inhibition. Finally, we will review measurements of extracellular GABA concentration in brain tissue, including some discussion of how GABA levels are determined by behaviour, pharmacology and pathophysiology.

6.2 Synthesis and Sequestration of GABA

6.2.1 GABA in Neurons

GABA is taken up into neurons by specific membrane-bound GABA transporters (GATs, see Sect. 6.4.3). However, as illustrated in Fig. 6.1 most GABA within inhibitory neurons appears to be derived from *de novo* synthesis (Martin and Tobin 2000). The principal enzyme responsible for the conversion of glutamate to GABA (Roberts and Frankel 1950) is glutamate decarboxylase (GAD). GAD exists in two isoforms (GAD65 and GAD67), which as well as differing in molecular weight have different catalytic and kinetic properties (Battaglioli et al. 2003; Fenalti et al. 2007), and variant cellular and subcellular distributions (Kaufman et al. 1991; Esclapez et al. 1994) with GAD65 apparently preferentially targeted to axon terminals, compared with a more uniform distribution throughout the cytoplasm for GAD67 (Kaufman et al. 1991).

Since glutamate is the principal substrate for GABA synthesis, control of the availability and uptake of this excitatory amino acid is known to have an impact upon inhibitory GABAergic transmission. Neuronal glutamate uptake is mediated by specific excitatory amino acid carriers (EAACs; also known as transporters, EAATs), of which EAAC1 appears to be the major carrier expressed at presynaptic inhibitory terminals (Rothstein et al. 1994; Conti et al. 1998a). Knockdown of EAAC1 leads to a downregulation of GABA synthesis and a concomitant reduction in total hippocampal GABA levels of about 50%, subsequently resulting in

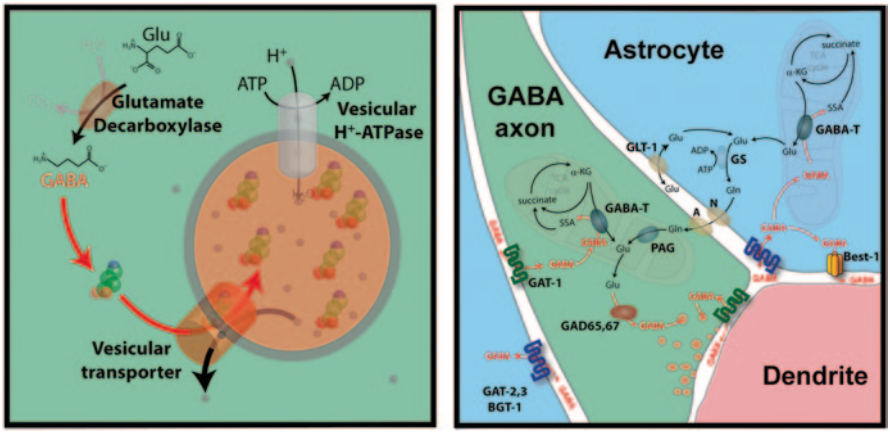

Fig. 6.1 Illustration of the synthesis, sequestration, release and transport of γ-aminobutyric acid (*GABA*) within the complex environment surrounding the synapse. *Axon terminals* are depicted in *green* and *astrocytes* are blue. The *postsynaptic dendrite* is shown in *red* and the extracellular space is shown in *white*. Lipid bilayers are drawn in *grey* with various cytoplasmic and membrane-bound proteins indicated where necessary. Scale and anatomical location are not represented in these cartoons. The *left-hand panel* concentrates on the synthesis and packaging of *GABA* into vesicles within the axon terminal. *GABA* molecules are shown being synthesized by the enzyme *glutamate decarboxylase* (*GAD*). The *GAD65* isoform is reported to be concentrated in nerve terminals whereas *GAD67* may be distributed more uniformly in neurons. *GABA* is then moved into vesicles via the vesicular *GABA* transporter (*VGAT*) also known as the vesicular inhibitory amino acid transporter (*VIAAT*; since it also transports glycine). Vesicular transport is driven by a proton gradient that has been established across the vesicular membrane by the vesicular *H+-ATPase*. The *right-hand panel* gives a broad overview of GABA synthesis within *astrocytes* and *axon terminals* and the interplay between these two cell compartments. Briefly, the *GABA* precursor glutamate (*glu*) is synthesized from glutamine (*Gln*) by phosphate-activated glutaminase (*PAG*) and from α-ketoglutarate (*α-KG*) by several enzymes, including GABA transaminase (*GABA-T*). *GABA* is degraded in mitochondria in both neurons and astrocytes by *GABA-T*, which produces succinic semialdehyde (*SSA*); the *SSA* is then converted to succinate. Succinate and *α-KG* are both intermediates of the tricarboxylic acid (*TCA*) cycle. Within *astrocytes*, extracellular glutamate is taken up by the action of the astrocyte-specific glutamate transporter-1 (*GLT-1*) where it is converted into glutamine by glutamine synthetase (*GS*) and exported back to neurons. Glutamine is released from astrocytes by system N transporters (*N*) and taken up into neurons by system A transporters (*A*). It has been suggested that GABA release through the anion channel bestrophin-1 (*Best-1*) may also take place.

epileptiform activity (Sepkuty et al. 2002). It has also been shown that increasing extracellular glutamate levels drives an increase in GABA synthesis and enhances inhibitory synaptic transmission (Mathews and Diamond 2003). Hence, control of glutamate uptake may also represent an important regulator of extracellular GABA levels and thus modulate tonic inhibition.

As shown in Fig. 6.1, glutamate levels within the CNS are critically determined by the availability of the amino acid, glutamine. Extracellular glutamate is taken up into glial cells by the action of the astrocyte-specific glutamate transporter-1 (GLT-1) where it is converted into glutamine by glutamine synthetase (Pines et al.

1992; Arriza et al. 1994). Glutamine is then released from astrocytes by system N transporters into the ECS where levels can reach hundreds of micromoles (μM; Kanamori and Ross 2004). Uptake of glutamine into neurons is mediated by system A transporters (SNATs, Chaudhry et al. 2002) where it can then be converted into glutamate by glutaminase. This glutamate–glutamine cycle has been shown to provide an important source of glutamate for conversion into GABA within inhibitory neurons, with perturbations of this cycle leading to altered GABAergic transmission (Roth and Draguhn 2012). For instance, blockade of neuronal glutamine uptake within the hippocampus has been shown to reduce GABA levels (Rae et al. 2003) and inhibit evoked GABAergic synaptic transmission (Liang et al. 2006). In thalamus, disruption of the glutamate–glutamine cycle by blockade of neuronal glutamine uptake or by selective ablation of glial cells was also shown to impair inhibitory synaptic transmission and resulted in attenuated intra-thalamic rhythmic activity (Yang and Cox 2011). Given the impact on GABA synthesis of the glutamate–glutamine cycle, it appears likely that regulation of this system will also be important for extrasynaptic $GABA_A$ receptor activation.

Within axon terminals, GABA is concentrated in vesicles by the actions of the vesicular GABA transporter (VGAT; also known as the vesicular inhibitory amino acid transporter, VIAAT, since it can also transport glycine). This vesicular GABA can then be released into the synaptic cleft by a Ca^{2+}-dependent exocytotic process triggered by an action potential. It has been suggested that this vesicular GABA may be synthesized largely by GAD65; firstly, due to the localisation of this enzyme within terminals and also due to a functional coupling between GAD65 and VGAT (Jin et al. 2003). Although GABA is highly enriched in the vesicles compared with the cytosol (about 1000-fold; Edwards 2007), there may still be a significant concentration of GABA in the cytosolic compartment (1–10 mM; Otsuka et al. 1971; Schwartz 1987; Wu et al. 2007). This extravesicular GABA may also be subject to release from the neuron under various conditions which will be discussed below.

6.2.2 GABA in Glial Cells

Uptake of GABA from the ECS into glial cells is mediated by GATs (see Sect. 6.4.3 Madsen et al. 2010). However, glia can also synthesize GABA as particular populations of glial cells express GAD enzymes and can produce GABA from the decarboxylation of glutamate as in neurons (Martinez-Rodriguez et al. 1993). A second pathway is also available since the polyamine putrescine can be converted into GABA by the actions of diamine oxidase (Seiler et al. 1979; Laschet et al. 1992). This pathway may be particularly important during pathological conditions, with production of GABA from putrescine being significantly higher in astrocytes from an epileptic mouse strain (Laschet et al. 1992). Although cytosolic levels of GABA will be reduced by the actions of the enzyme GABA-transaminase (GABA-T), which is highly expressed in astrocytes and converts GABA into glutamate, significant levels of GABA can still be detected in specific populations of glial

cells (Angulo et al. 2008). For instance, Bergmann glia in the cerebellum display significant immunoreactivity for GABA (Benagiano et al. 2000), as do astrocytes in the brainstem (Blomqvist and Broman 1988). The cytosolic GABA concentration is difficult to measure, especially for glial cells where turnover of GABA by uptake, synthesis and catalysis may be particularly high; however, an intracellular GABA concentration of 3.5 mM has been reported for cultured astrocytes (Bardakdjian et al. 1979). Interestingly, it has been shown that astrocytes possess some of the factors necessary for exocytotic glutamate release, including storage vesicles, pumps for accumulating transmitter in the vesicles and machinery to promote fusion of the vesicles with the plasma membrane (reviewed by Hamilton and Attwell 2010). This is consistent with studies demonstrating that astrocytes release glutamate when their internal $[Ca^{2+}]$ is raised. At this moment, there is a paucity of evidence to directly support exocytotic release of glial GABA, although this mechanism does not seem implausible given the situation with glial glutamate. Much less attention has been focussed on GABA release from glial cells but it is becoming clear that glia may represent an important source of GABA within the central nervous system (Angulo et al. 2008; Yoon et al. 2012).

6.3 Release of GABA

Mechanisms for the release of GABA into the ECS can be divided into two broad categories: (1) vesicular release and (2) non-vesicular release. These mechanisms will be addressed in more detail below, including discussion of where identified tonic currents can be attributed to a particular source of GABA.

6.3.1 Vesicular Release

The vesicular release of GABA from the axon terminals of inhibitory interneurons to activate synaptic receptors on the soma and dendrites of adjacent neurons is a well-established concept. Although GABA will be cleared from the synapse and the surrounding area by highly efficient transporters (GATs 1/3, see Sect. 6.4.3), some of this GABA is likely to escape, particularly under conditions of enhanced release (Rossi and Hamann 1998; Wei et al. 2003). This spillover of released transmitter provides a potential source for the GABA that activates extrasynaptic receptors.

It should also be noted that in recent years, other forms of exocytotic GABA release have been identified. For instance, certain neurons are able to release GABA from dendrites to provide a retrograde or lateral inhibition (Koch and Magnusson 2009). A well-characterised example is in the thalamus where local interneurons can release GABA from presynaptic (F2) terminals on their distal dendrites (Sherman 2004; Acuna-Goycolea et al. 2008). Another atypical mode of vesicular GABA release has recently been identified in the cortex (Olah et al. 2009) and in other brain

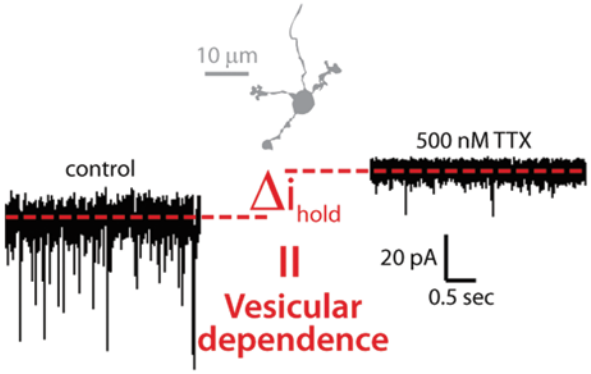

Fig. 6.2 A typical whole-cell voltage-clamp recording from an adult cerebellar granule cell (postnatal day 41) before and after reduction in the rate of vesicular γ-aminobutyric acid (*GABA*) release in the acute slice preparation. A neurobiotin-filled cerebellar granule cell is also shown following a recording of this type. The standing holding current measured at −70 mV is clearly reduced following the application of *500 nM TTX* to the recording solution as is the rate of spontaneous inhibitory post synaptic conductances (IPSCs). This type of experiment represents clear evidence in favour of vesicular GABA release contributing to the generation of a tonic conductance

areas (see Sect. 6.4.2.1). A particular class of interneurons, the neurogliaform cells, produces very slow inhibitory post synaptic conductances (IPSCs) in their target cells. These are thought to be caused by release of a cloud of GABA from the unusually dense axonal arborisation of these interneurons, mostly from release sites not situated at synapses. Extracellular GABA derived from vesicular release is therefore likely to depend upon various characteristics of the parent interneuron population.

The following section will briefly review the contributions of vesicular release in generating the tonic $GABA_A$ receptor-mediated currents that have been identified in various brain areas.

6.3.1.1 Cerebellar Granule Cells

Granule cells in the cerebellum exhibit a tonic inhibition that is generated by high-affinity $GABA_A$ receptors containing the α6 subunit, probably in combination with δ subunits. In a number of studies (see Fig. 6.2), the tonic inhibition appears to be generated by GABA released in response to action potentials, probably in Golgi cells (Kaneda et al. 1995; Brickley et al. 1996; Bright et al. 2011; Houston et al. 2012). However, some investigations report that a component of the tonic conductance is not blocked by tetrodotoxin (TTX) and therefore is not dependent upon action potential-evoked release of GABA (Wall and Usowicz 1997; Rossi et al. 2003). These authors suggested that the GABA must be released from either Golgi cell terminals or astrocytes by a non-vesicular, Ca^{2+} -independent mechanism. It has also recently been suggested that GABA release via the anion channel bestrophin-1

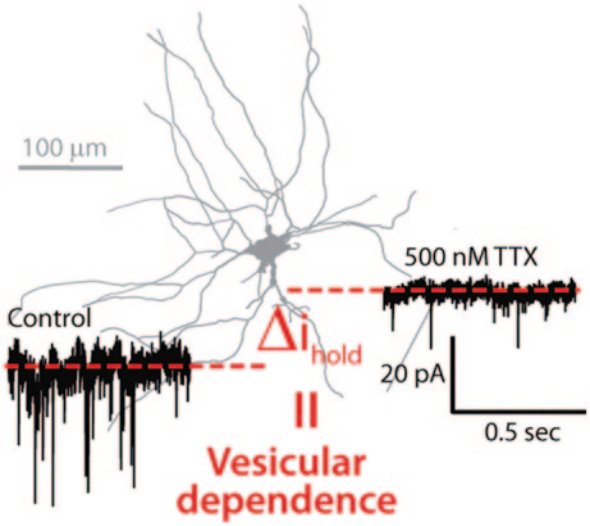

Fig. 6.3 A typical whole-cell voltage-clamp recording from an adult thalamic relay neuron (postnatal day 35) before and after reduction in the rate of vesicular γ-aminobutyric acid (*GABA*) release in the acute slice preparation. A neurobiotin-filled thalamic relay neuron is also shown following a recording of this type. The standing holding current measured at −60 mV is clearly reduced following the application of 500 nM TTX to the recording solution as is the rate of spontaneous inhibitory post synaptic conductances (IPSCs). This type of experiment represents clear evidence in favour of vesicular GABA release contributing to the generation of a tonic conductance

may be responsible for setting the ambient GABA level in the cerebellum (Lee et al. 2010); however, another report has failed to replicate these findings (Diaz et al. 2011; see Sect. 6.3.2.2).

6.3.1.2 Dentate Gyrus Granule Cells

Hippocampal granule cells of the dentate gyrus exhibit a tonic $GABA_A$ conductance that is likely to be mediated primarily by α4δ receptors with a component due to receptors containing the α5 subunit (Glykys et al. 2008). A recent study using mouse hippocampal slices has demonstrated that TTX causes a reduction in both the frequency of IPSCs and in the size of the tonic current, suggesting that about 50% of the tonic conductance is caused by action potential-dependent release of GABA (Holter et al. 2010). These authors suggest that the remaining component of the tonic current is generated by action potential-independent release. Reversed transport is unlikely to contribute since use of the GAT-1-specific antagonist, NO-711, causes a potentiation of the tonic, suggesting that GAT-1 acts as a sink, rather than a source, of GABA (see Sect. 6.3.2.1), consistent with an earlier study in the adult rat (Nusser and Mody 2002; Fig. 6.3).

6.3.1.3 Hippocampal Pyramidal Cells

A significant tonic $GABA_A$ current is present in juvenile hippocampal pyramidal cells but this diminishes with development to become only a small component of the membrane conductance in adult animals (Semyanov et al. 2004). It is thought that this current is mediated by α5βγ, α4βγ and αβ receptors. The relative contributions of these different receptor populations may be determined by the ambient GABA concentration, with one study suggesting that at low GABA concentrations, the tonic conductance is mediated largely by α4βγ $GABA_A$Rs, with increased GABA concentrations leading to recruitment of α5-containing receptors (Scimemi et al. 2005). Glykys and Mody (2007b) have recently demonstrated a strong correlation between phasic inhibition (IPSCs) and tonic inhibition in CA1 pyramidal cells. This is consistent with the majority of the tonic receptor activation in these cells being due to vesicular GABA release.

6.3.1.4 Cortical Pyramidal Cells

Tonic $GABA_A$ receptor-mediated conductances have been identified in both layer 2/3 and layer 5 pyramidal neurons in the somatosensory cortex (Keros and Hablitz 2005; Drasbek and Jensen 2006) as well as in some interneurons (Krook-Magnuson et al. 2008). Although there is no direct evidence to support a vesicular source of GABA, it has been proposed that enhanced GABA release caused by repetitive stimulation of thalamocortical fibres may cause increased tonic inhibition in specific populations of cortical interneurons. This could provide an explanation for the increased ratio of excitation to inhibition observed in layer four barrel cortex excitatory cells under such conditions (Krook-Magnuson et al. 2008).

6.3.1.5 Thalamus

Extrasynaptic $GABA_A$ receptors were first identified immunohistochemically in the thalamus (Soltesz et al. 1990) and these have since been attributed to the expression of high-affinity δ-$GABA_A$Rs (Sur et al. 1999; Jia et al. 2005; Kralic et al. 2006). These receptors underlie the tonic conductance that has been measured in relay neurons of the ventrobasal (VB) thalamus (Jia et al. 2005; Cope et al. 2005; Belelli et al. 2005) and dorsal lateral geniculate nucleus (dLGN; Cope et al. 2005). We have demonstrated that the tonic inhibition exhibited by dLGN relay neurons depends upon the global level of inhibitory activity within the thalamus (Bright et al. 2007). Manipulations that alter vesicular release of GABA across the thalamus, such as blockade of action potential-dependent release with TTX or reduction of release probability by lowering external Ca^{2+}concentration, concomitantly cause a decrease in the tonic conductance. Consistent with this, we have shown more recently that increasing GABA release across thalamic synapses by application of a nitric oxide donor leads to an enhancement of the tonic conductance (Bright and Brickley 2008).

Interestingly, in this study, we also demonstrated a novel activity-dependent mechanism for enhancing GABA release at a limited set of thalamic synapses. However, this up-regulation of local GABA release was not associated with a change in the tonic conductance, suggesting that the underlying receptors will only respond to more global changes in GABA release, reflecting concerted alterations in inhibitory activity. Inhibitory circuitry within the thalamus plays a key role in the generation of oscillatory activity that underlies various physiological and pathophysiological states (McCormick and Bal 1997; Huguenard and McCormick 2007). Hence, tonic inhibition within the thalamus may be dynamically regulated by global changes in GABA release, corresponding to various behavioural states.

6.3.1.6 Striatum

It has recently been shown that a particular subset of medium spiny neurons (MSNs) within the striatum express a significant tonic $GABA_A$ conductance. These neurons are part of the striatal–pallidal pathway and selectively express dopamine D_2 receptors (Ade et al. 2008; Janssen et al. 2009). The tonic conductance is almost completely abolished by application of TTX, suggesting that most of the GABA responsible is derived from action potential-dependent vesicular release.

6.3.1.7 Hypothalamus

Tonic $GABA_A$ receptor-mediated conductances have been identified in two hypothalamic nuclei: the supraoptic nucleus (SON) of the magnocellular neurosecretory system (Park et al. 2006) and the paraventricular nucleus (PVN; Park et al. 2007). With regard to the source of the GABA, the two hypothalamic tonic conductances exhibit somewhat disparate biophysical fingerprints. Park et al. (2007) described a significant positive correlation between basal IPSC frequency and tonic conductance in the PVN, suggesting that synaptically released GABA may contribute to the tonic conductance. In the case of the SON tonic conductance, no such correlation was apparent and furthermore, elevated GABA release caused by application of the K^+ channel blocker 4-AP was ineffective in increasing the tonic conductance (Park et al. 2006). The authors of these two studies speculate that these differences could be due to distinct neuronal-glial microenvironments present in the two nuclei. Specifically, neurons within the SON and their synapses are tightly enveloped by thin glial processes. This arrangement may act as a diffusion barrier, limiting the ability of synaptically released GABA to activate the extrasynaptic receptors that give rise to tonic inhibition. Under conditions of repetitive afferent stimulation, both hypothalamic neuronal populations exhibit enhancement of the tonic conductance. This would suggest that strong synchronous release of GABA can result in elevated levels of neurotransmitter, capable of activating the receptors that underlie tonic inhibition in the hypothalamus.

6.3.1.8 Brainstem

Recently, a tonic $GABA_A$ receptor-mediated conductance has been identified in auditory neurons of the avian nucleus laminaris, an analogue of the mammalian medial superior olive (Tang et al. 2011). Experimental manipulations that reduced the level of synaptic release, including lowering the extracellular Ca^{2+} concentration and activation of presynaptic $GABA_B$ receptors, were shown to significantly reduce the tonic current. Furthermore, it was demonstrated that the magnitude of the tonic inhibition was highly correlated with the magnitude of the phasic inhibition (IPSC frequency and amplitude). Therefore, this study provides convincing evidence that the source of the GABA in this nucleus is vesicular release.

6.3.1.9 Spinal Cord

Ataka and Gu (2006) have shown that neurons in lamina 2 of the adult mouse spinal cord dorsal horn display a tonic $GABA_A$ conductance. This tonic conductance presumably displays a dependence on species and/or age since a similar study in juvenile rats failed to detect a significant basal tonic $GABA_A$ conductance in the same neurons (Mitchell et al. 2007). However, this latter study did demonstrate the presence of a significant population of extrasynaptic receptors, since application of a GABA uptake blocker, and of both endogenous and exogenous neurosteroids, induced large bicuculline-sensitive inward currents. Evidence supporting a vesicular source for the GABA responsible for activating these receptors is provided by the positive correlation between IPSC frequency and the tonic conductance in adult mouse lamina 2 neurons (Ataka and Gu 2006).

6.3.2 Non-vesicular Release of GABA

In Sect. 6.3.1, we reviewed the large body of evidence that suggests that levels of vesicular release alter the magnitude of tonic inhibitory conductances across the brain (see Figs. 6.2–6.3). However, there has been some controversy on the relative role of synaptic release in setting the ambient GABA concentration (Hamann et al. 2002; Rossi et al. 2003). In some cases, abolition of vesicular release causes only a partial reduction in the tonic current, suggesting that other non-vesicular sources must also contribute. Therefore, the potential role of GATs in releasing GABA and other non-vesicular sources must be considered.

6.3.2.1 GATs: A Dynamic Equilibrium

GATs such as GAT-1 undergo a single reaction cycle that will generate coupled translocation of 2 Na^+, one Cl^- ion and one GABA molecule by utilising the energy

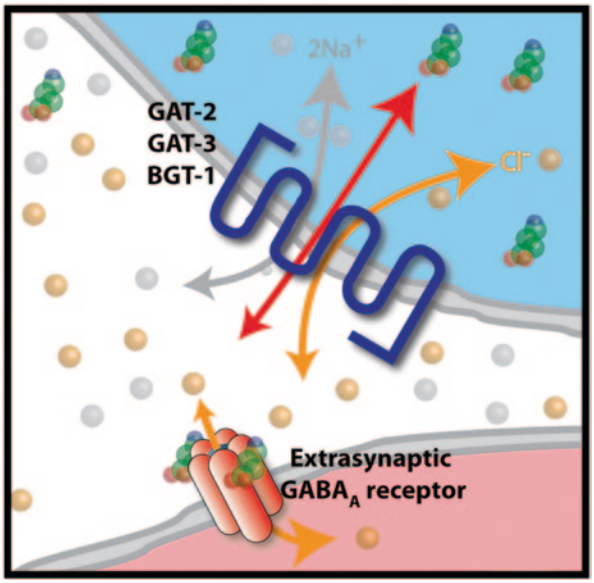

Fig. 6.4 Extracellular *GABA* concentration is determined by the stoichiometry of γ-aminobutyric acid (*GABA*) transporters located within the plasma membrane of neurons and astrocytes. There are believed to be at least four distinct *GABA* transporters (*GAT-1*, *GAT-2*, *GAT-3* and *BGT-1*). *GAT-1* is principally found in neurons whereas *GAT-2*, *GAT-3* and *BGT-1* are believed to be present in astrocytes. Irrespective of the transporter type, the movement of *GABA* depends upon the coupled translocation of two sodium ions ($2Na^+$, *grey*) and one chloride ion (Cl^-, orange). Therefore, uptake of *GABA* results in a net gain of positive charge inside the cell. The direction of *GABA* transport, however, will depend upon the ion gradient and the membrane potential. Therefore, the equilibrium state of the *GABA* transporter will largely determine the resting ambient *GABA* concentration present in the extracellular space. The lowest theoretical estimates of ambient *GABA* concentration (low nM) are believed to be sufficient to bind to high-affinity extrasynaptic $GABA_A$ receptors and cause chloride flux through the open channels.

provided by the inward Na^+ gradient (Fig. 6.4: Lu and Hilgemann 1999; Wu et al. 2007). The transporters act to move GABA towards an equilibrium determined by the internal and external substrate concentrations (Na^+, Cl^- and GABA) and the membrane potential. The theoretical prediction is that GATs have a reversal potential close to the resting potential of neurons acting to reduce GABA to an ambient concentration of 0.1–0.4 μM (Attwell et al. 1993; Wu et al. 2003; Richerson and Wu 2003). The transporter is equally capable of moving in the reverse direction if the ambient GABA concentration is below the transporters equilibrium state. Inhibition of GATs clearly increases the extracellular GABA concentration as detected using a "sniffer patch" of membrane pulled from a cell positioned close to a slice preparation (Isaacson et al. 1993). The theoretical predictions have been confirmed by direct measurement of transporter currents (Cammack et al. 1994; Lu and Hilgemann 1999; Gonzales et al. 2007) which showed a predicted sensitivity to internal and external Na^+ and Cl^- concentrations.

Using a more sensitive approach in which Chinese hamster ovary (CHO) cells were transfected with high-affinity αβδ $GABA_A$ receptors ("sniffer patches") to detect release of GABA from CHO cells expressing GAT-1, it was demonstrated that GATs can reverse under physiological conditions. This was confirmed by paired recordings in hippocampal neurons in culture (Wu et al. 2007). Depolarisation and increased intracellular Na^{2+} concentration, as may occur during an action potential, was shown to lead to GABA transport into the ECS. Transporter currents have also been observed in Bergmann glia in rat cerebellar slices mediated by GAT-1. In these cells, it appeared GAT-1 was acting to import GABA but increased intracellular GABA and Na^{2+} again revealed that reverse transport of GABA was possible (Barakat et al. 2002). Hence, conditions that increase internal Na^{2+} and membrane potential during high-frequency activity or seizures will lead to a change in transporter equilibrium and an increase in ambient GABA concentration (Wu et al. 2001, 2003, 2007). Under physiologically relevant conditions, the equilibrium of transporters may be such that the extracellular GABA concentration is high enough to activate high-affinity extrasynaptic receptors (Brickley et al. 1996; Bright et al. 2011). The GAT equilibrium however is highly dynamic and will be modulated by changes in Na^+, Cl^- and GABA homeostasis, as well as being dependent upon membrane potential.

6.3.2.2 GABA-Permeant Channels as a Source of Ambient GABA

It has also been reported that specific channel types in glial cells may be capable of releasing GABA into the ECS. The bestrophin1 (Best-1) anion channel has been reported to contribute to tonic inhibition in the cerebellum (Lee et al. 2010). Best-1 belongs to a family of volume regulating channels that open in response to changes in the osmolarity of extracellular solutions (Hartzell et al. 2008). These Ca^{2+}-activated anion channels have been identified in astrocytes and are also thought to be permeable to the neurotransmitters glutamate and GABA (Park et al. 2009; Lee et al. 2010). Lee et al. (2010) demonstrated in HEK cells that Best-1 is permeable to GABA and that the Cl^- channel blocker 5-nitro-2-(3-phenylpropyloamino) benzoic acid (NPPB) blocked Best-1 but not $GABA_C$ receptors. They observed a significant reduction in tonic current recorded from cerebellar granule cells after addition of NPPB and other Cl^- channel blockers to the slice. Knock-down of Best-1 with sh-RNA removed the effect of NPPB and reduced tonic current, suggesting that resting Ca^{2+} levels in glial cells could activate Best-1 and allow GABA release, contributing to the extracellular GABA concentration. However, in a previous study in adult rat slices the application of NPBB resulted in an increase in tonic current recorded from cerebellar granule cells (Rossi et al. 2003). In this study, slices were also pre-incubated in hyperosmotic solution to control for potential swelling-activated release of neurotransmitter (Rutledge and Kimelberg 1996; Hartzell et al. 2008; Haskew-Layton et al. 2008) but no significant differences were observed from control. It is possible that release of GABA through Best-1 could be related to slicing conditions and swelling-related activation. In both rat and mouse cerebellum

(Diaz et al. 2011), an increase in tonic current on NPPB application has also been reported whilst in the hippocampus application of NPPB had no effect (Shigetomi et al. 2012). It is not clear at present why there are such conflicting results when using NPPB in acute slice preparations.

6.3.2.3 $GABA_A$ Receptor Currents Evoked by Non-vesicular Release from Glia

There are some clear examples of $GABA_A$ receptor activation by GABA derived from non-vesicular sources such as in the olfactory bulb where slow $GABA_A$ receptor-mediated currents could be observed in both mitral and granule cells, even in the absence of synaptic transmission (Kozlov et al. 2006). These currents could be evoked by mechanical stimulation of astrocytes, but not neurons, and were sensitive to changes in extracellular osmolarity. These observations suggest that GABA released from astrocytes through volume-regulated anion channels is responsible for the slow $GABA_A$currents. A similar mechanism has been proposed to generate slow $GABA_A$ receptor-mediated currents in the rodent VB thalamus, with the further observation that these currents are produced by activation of extrasynaptic δ-$GABA_A$ receptors (Jimenez-Gonzalez et al. 2011). The antiepileptic drug vigabatrin increased the frequency of these currents, but this effect was not modulated by GABA transport blockers, suggesting that astrocytic GABA release may play an important role in the therapeutic effects of this drug. Slow $GABA_A$ receptor-mediated currents have also been identified in principal neurons of the three main hippocampal fields (CA1, CA3 and dentate gyrus) and are likely to be generated by astrocytic GABA release through volume-sensitive anion channels (Le Meur et al. 2012). These findings suggest that astrocytic GABA release via these channels may provide an important mechanism for controlling neuronal excitability.

6.3.3 Tonic Inhibition Without GABA

The majority of experimental evidence suggests that the magnitude of any tonic inhibitory conductance will be primarily determined by the availability of GABA. However, there are reports of GABA-independent $GABA_A$ receptor gating either involving alternative endogenous agonists or spontaneous channel openings. As such, the magnitude of a tonic conductance mediated by such mechanisms would be independent of the extracellular GABA concentration.

6.3.3.1 Constitutive Activity

Channel opening without GABA binding may contribute to the tonic conductance observed in some neurons. Spontaneously opening extrasynaptic channels have been reported in cell-attached patches from CA1 pyramidal neurons in slices

(Birnir et al. 2000; McCartney et al. 2007; Le Meur et al. 2012). A number of $GABA_A$ receptor subtypes have been reported to exhibit spontaneous channel openings without agonist binding in recombinant systems (Neelands et al. 1999; Wagner et al. 2005; Hadley and Amin 2007; Tang et al. 2010) including the δ and ε subunit-containing receptors which are thought to be primarily extrasynaptic (Wagner et al. 2005; Hadley and Amin 2007). Typical synaptic subtypes (αβγ) have also been reported to exhibit spontaneous opening (Knoflach et al. 1996). Given the large number of extrasynaptic $GABA_A$ receptors present on the plasma membrane of neurons, a low spontaneous open probability of these channels could theoretically generate a significant tonic conductance (Kasugai et al. 2010).

6.3.3.2 Other Endogenous Agonists of $GABA_A$ Receptors

Taurine (2-aminoethane sulphonic acid) is an abundant amino acid in the brain and is a known agonist at both glycine and $GABA_A$ receptors, showing higher efficacy for activation of δ-containing receptors than γ-containing receptors (Jia et al. 2008). In the mouse VB thalamus, it has been reported that taurine (10–100 μM) can activate extrasynaptic α4βδ-containing $GABA_A$ receptors and mediate tonic inhibition (Jia et al. 2008). Tonic inhibition was also enhanced by a taurine transporter antagonist suggesting that taurine levels may contribute to extrasynaptic receptor activation in this region. Extracellular taurine has been measured within the range of 1–10 μM by microdialysis but as with GABA, the precise extracellular concentration in any one region *in vivo* is unclear (Sect. 6.5.2; Segovia et al. 1997; Albrecht and Schousboe 2005). Taurine levels are regulated as with GABA by specific taurine transporters (TAUTs) which are expressed across the brain with high levels in the cerebellum and hippocampus (Pow et al. 2002; Sergeeva et al. 2003). Taurine plays a role in cell volume regulation and is known to be released from glia and neurons in response to membrane depolarisation, ischaemia and hypo-osmotic stimulation (Oja and Saransaari 2000; Barakat et al. 2002; Albrecht and Schousboe 2005; Haskew-Layton et al. 2008). Release can occur both by reversed transport (Saransaari and Oja 1999) and through osmo-sensitive anion channels (Hussy et al. 2001; Choe et al. 2012). In addition, there is some evidence that taurine may block GABA transporters GAT-2/3 and could potentially influence ambient GABA levels as a result (Takanaga et al. 2001). Therefore, extracellular taurine may also contribute to extrasynaptic $GABA_A$ receptor activation and the magnitude of tonic inhibition. This may be of particular consideration in pathophysiological situations such as ischaemia or excessive excitatory activity where taurine release may be elevated.

6.4 Regulation of Extracellular GABA Concentration

Once GABA has been released into the ECS, whether by vesicular release at a synapse or by non-vesicular release from extrasynaptic sites on neurons or glia, it will diffuse away from the release site. There are multiple additional factors controlling the extracellular GABA concentration, including the efficacy of release, the physical geometry of the ECS and uptake of GABA by transporters. Here, we will consider how these factors might determine the GABA concentration sensed by extrasynaptic $GABA_A$ receptors.

6.4.1 GABA Content in Vesicles

6.4.1.1 Regulation of GABA Synthesis

Transgenic animals lacking expression of the synthetic enzymes for GABA, GAD65 and GAD67 have been developed and used to study the impact of impaired GABA synthesis. GAD67 (−/−) mice die shortly after birth (Asada et al. 1997) but GAD65 (−/−) mice are viable. Tissue GABA levels in the GAD65 (−/−) mice are reduced by about 50 % compared to wild-type mice (Stork et al. 2000) and consistent with this, tonic inhibition in these mice is also impaired (Kubo et al. 2009; Walls et al. 2010). Regulation of GABA synthesis may also reflect an important homeostatic control of inhibition under pathological conditions. Using a mouse strain exhibiting high anxiety-related behaviour (HAB), it was shown that levels of both GAD65 and GAD67 were elevated in the amygdala, resulting in elevated GABA levels (Tasan et al. 2011). It was hypothesized that this increase in GABA synthesis may result in increased tonic inhibition as a compensatory mechanism to balance the persistent over-stimulation of limbic brain areas during anxiety. This is consistent with the anxiolytic effects of the drug, gabapentin, which, amongst other actions, is known to increase ambient GABA levels via a modulation of GAD activity (Taylor 1997). Other studies have shown changes in GAD expression during schizophrenia (Fatemi et al. 2005), bipolar disorder (Guidotti et al. 2000) and epilepsy (Esclapez and Houser 1999; Freichel et al. 2006) in areas where tonic conductances have been identified.

6.4.1.2 Filling of Synaptic Vesicles

To date, only a single transporter VGAT (or VIAAT) has been identified as being responsible for the filling of vesicles at GABAergic synapses (Wojcik et al. 2006). This transporter is critical for inhibitory synaptic transmission since genetic deletion of VGAT results in almost complete abolition of transmitter release from both GABAergic and glycinergic neurons (Wojcik et al. 2006). Indeed, this deficit leads

to embryonic lethality in these mice. Currently, there is very limited information available on the regulation of VGAT (Gasnier 2004). However, it has been suggested that the antiepileptic drug vigabatrin, which has its main effect via a blockade of GABA-T (see Sect. 6.4.3.7), may cause a paradoxical proconvulsant effect via inhibition of VGAT (Gasnier 2004). This effect is seen in the early hours after drug administration, before the main anticonvulsant effect caused by increased GABA levels takes precedence.

However, another experimental tool is available to alter the extent of vesicular filling. Uptake of GABA into synaptic vesicles is driven by the proton gradient across the vesicular membrane established by the H^+-ATPase (Drose and Altendorf 1997). Therefore, blockade of the H^+ATPase with concanamycin A prevents accumulation of neurotransmitter within vesicles and reduces synaptic transmission (Zhou et al. 2000). Concurrent with this effect, it has also been shown that concanamycin A application reduces tonic inhibition in cerebellum (Rossi et al. 2003), hippocampus (Glykys and Mody 2007b) and in auditory brainstem (Tang et al. 2011), consistent with decreased ambient GABA levels.

6.4.1.3 Control of Exocytosis

The final stage in the process of synaptic release is the fusion of transmitter-containing vesicles with the plasma membrane, followed by exocytosis into the synaptic cleft. This phenomenon is promoted by the formation of a complex of vesicle and plasma membrane SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, which is regulated by a Ca^{2+} sensor (Sudhof and Rizo 2011). In neurons, rapid exocytosis is catalysed when Ca^{2+} (which enters the cells through voltage-gated Ca^{2+} channels) binds to the Ca^{2+} sensor, vesicular synaptotagmin 1, causing this protein to bind to the plasma membrane to initiate fusion. Since vesicular exocytosis is highly dependent upon Ca^{2+} influx, adjustment of external Ca^{2+} concentration is often used to manipulate synaptic release. Hence, this has been used as a tool to establish the vesicular dependence of tonic $GABA_A$ conductances in thalamus (Bright et al. 2007) and auditory brainstem (Tang et al. 2011).

Synaptic release can also be effectively controlled by neuromodulators, either by G-protein-dependent modulation of presynaptic Ca^{2+} channels (Catterall and Few 2008), or by regulation of presynaptic neuronal excitability. The impact of neuromodulatory input on synaptic inhibitory transmission has been well documented but far fewer studies have examined effects on tonic inhibition. Neuromodulatory input is particularly salient for controlling the flow of sensory information through thalamic relay nuclei (Sherman 2005) and effects of neuromodulators on thalamic tonic inhibitory conductances via modulation of synaptic release have been recently described. Application of nitric oxide (NO) donors stimulates increased GABA release on to thalamic relay neurons (Yang and Cox 2007). This effect persists in the presence of TTX, suggesting that suprathreshold excitation of presynaptic neurons is not required and instead implicates an increase in release probability. We

subsequently showed that the increase in GABA release evoked by NO donors also leads to an enhanced tonic conductance in dLGN relay neurons (Bright and Brickley 2008). Tonic inhibition within the dLGN has also been shown to be modulated by metabotropic glutamate receptor (mGluR) activation (Errington et al. 2011a). Activation of group I mGluRs with the selective agonist, dihydroxyphenylglycine (DHPG) leads to increases in both IPSC frequency and tonic GABA current, whilst group II/III mGluR activation results in reduced phasic and tonic inhibition. Interestingly, the mGluR-evoked enhancement in transmission is not blocked in the presence of TTX but is sensitive to the L-type Ca^{2+} channel blocker, nimodipine. These results suggest a selective mGluR-dependent modulation of dendrodendritic GABA release from the presynaptic F2 terminals on interneurons involved in triadic synapses (Sherman 2004).

6.4.2 Physical Factors Controlling Ambient GABA Concentration

6.4.2.1 Density of Release Sites

The density of GABA release sites may be a key determinant of extrasynaptic $GABA_A$ receptor activation since a high density of release is more likely to be capable of overwhelming the effects of uptake and diffusion. The impact of release site density on GABAergic synaptic transmission within the hippocampus has been investigated (Overstreet and Westbrook 2003). In this study, unitary IPSCs (uIPSCs) generated by axo-axonic interneuron input onto dentate gyrus granule cells were shown to be more sensitive to transporter block than those generated by basket cell inhibitory input. This is consistent with a high level of GABA transport, acting to limit spillover between the more closely packed axo-axonic synapses. Indeed, density of inhibitory synaptic connections onto principal hippocampal neurons has been shown to be highly heterogeneous, with a relatively high density of synapses concentrated in the perisomatic region of CA1 pyramidal neurons (Megias et al. 2001).

It has been suggested that particular classes of interneurons may be specialized to generate extrasynaptic $GABA_A$ receptor activation (Klausberger 2009). Neurogliaform cells in the hippocampus, cortex and amygdala and hippocampal ivy cells both have extremely dense axonal arborisations, manifesting a large number of release sites that are probably mostly situated at non-synaptic locations (Tamas et al. 2003; Price et al. 2005, 2008; Olah et al. 2009; Manko et al. 2012). In the case of cortical neurogliaform cells, the density of release sites has been found to be about fivefold to sixfold higher than for adjacent basket cells (Olah et al. 2009). This high density of release appears to promote the generation of unusually slow $GABA_A$-mediated currents, presumably by the asynchronous activation of receptors that are not synaptically apposed. Interestingly, cortical neurogliaform cells express extrasynaptic δ-$GABA_A$ receptors that are capable of being activated by the cloud of GABA generated by their own firing. This suggests that control

of the input–output gain of neurogliaform cells may be achieved autonomously or potentially by neurosteroid modulation (Olah et al. 2009). For these specialized interneurons, action potentials in a single cell might be capable of generating extrasynaptic receptor activation without the synchronous or prolonged activity required in other interneuronal populations (Rossi and Hamann 1998; Wei et al. 2003; Ataka and Gu 2006).

6.4.2.2 Diffusion in the Extracellular Space

Diffusion of GABA may also represent an important factor in the control of extrasynaptic $GABA_A$receptor activation, particularly in cases where specific uptake of GABA is limited (Draguhn and Heinemann 1996). The diffusion of substances within the brain depends upon the diffusion parameters of the ECS, namely the ECS volume fraction α(α = ECS volume/total tissue volume) and the tortuosity γ (γ^2 = free/apparent diffusion coefficient; Sykova 2004). These diffusion parameters differ in various brain regions and also show heterogeneity dependent upon both physiological and pathological states (Sykova and Vargova 2008). Changes in diffusion parameters have been noted in many different states, including aging, neuronal activity, lactation, ischaemia, brain injury, degenerative diseases and tumour growth, in which cell swelling, glial remodelling and extracellular matrix changes may be important regulators of diffusion (Sykova and Vargova 2008). For instance, during transient ischaemia, the ECS volume fraction α is reversibly decreased from a control value of 0.20–0.07 (Zoremba et al. 2008). Such large changes would be expected to have a significant impact upon the diffusion of neuroactive substances, including GABA.

In certain areas of the brain, diffusion of neurotransmitters may be particularly constrained by glial cell ensheathment of synapses. One of the most extensively studied of these so-called glomerular synapses is the Golgi cell to granule cell synapse in the cerebellar cortex (Jakab and Hamori 1988; Jakab 1989). In this case, the glomerulus (glial sheath) is centred on the large, axonal terminal of a glutamatergic mossy fibre afferent, which is surrounded by dendrites from 50 to 60 distinct granule cells. Also encapsulated within the glomerulus are numerous Golgi cell terminals that form GABAergic synapses onto the granule cells dendrites. It has been suggested that GABA entrapment within this structure might lead to enhanced activation of the high-affinity α6δ-$GABA_A$Rs expressed on granule cells. This has been thought to manifest itself in two ways, firstly as slow currents generated by spillover of GABA between adjacent synapses and secondly, as a tonic current generated by the ambient GABA constrained within the glomerulus (Brickley et al. 1996; Rossi and Hamann 1998). Indeed, early in situ hybridisation studies led to the suggestion that high-affinity δ-$GABA_A$ receptors might be predominantly expressed by neurons possessing glomerular synapses, in particular, periglomerular cells in the olfactory bulb, thalamocortical relay neurons and cerebellar granule cells (Shivers et al. 1989). Hence, it has been widely assumed that δ-$GABA_A$ receptors within

glomeruli mediate both spillover and tonic currents (Farrant and Nusser 2005; Glykys and Mody 2007a). We have recently challenged this assumption by showing that δ-$GABA_A$ receptors can be desensitized by low ambient GABA concentrations and are therefore insensitive to brief GABA transients (Bright et al. 2011). This would indicate that, whilst tonic currents are mediated by δ-$GABA_A$ receptor activation, spillover currents are more likely to result from activation of γ-containing receptors within glomeruli.

6.4.2.3 Spatial Distribution of Release Sites, Transporters and Receptors

The relative locations of release sites, transporters and receptors are obviously an important determinant of how much GABA is available to activate extrasynaptic $GABA_A$ receptors. If release sites and receptors were in close proximity, then we would obviously expect greater receptor activation. However, if transporters are also closely intermingled, then the local GABA concentration will be significantly controlled by their action and receptor activation will be constrained. Since it has been suggested that GATs act to clamp the local GABA concentration at a level determined by the transporter reversal potential (Wu et al. 2007; Sect. 6.3.2.1), in areas where extrasynaptic receptors are in close apposition with a high density of transporters, their activation would be largely determined by the transporter equilibrium. Thus, receptors located at a greater distance from transporters might be more independent and capable of greater modulation by changes in ambient GABA. Differences in relative spatial location may be another factor that explains the variable effects of transporter block on tonic currents (see Sect. 6.4.3.3).

At this moment, there is limited information available to quantify the relative spatial distribution of these key components. However, efforts have been made to define the location of individual components in isolation. As discussed briefly in Sect. 6.4.2.1, various studies have attempted to define the distribution of GABAergic synaptic terminals, particularly for interneurons in the hippocampus and cortex (Markram et al. 2004; Klausberger 2009). The localisation of extrasynaptic $GABA_A$ receptors has been studied in various brain areas (Soltesz et al. 1990; Nusser et al. 1998; Wei et al. 2003), as has the distribution of GATs (Borden 1996; Chiu et al. 2002). A major challenge is to draw this information together so that we can begin to address how the relative spatial distribution of systems responsible for GABA release, uptake and detection can influence local GABA concentration.

Computational approaches have been used to produce biologically constrained models of GABA diffusion, uptake and detection for both $GABA_A$ and $GABA_B$ receptors (Destexhe and Sejnowski 1995; Overstreet et al. 2002; Beenhakker and Huguenard 2010). These simulations can provide insight into how factors that affect GABA diffusion and uptake might influence extracellular GABA concentrations and thus modulate GABAergic transmission. For instance, in the study by Overstreet et al. (2002), it was noted that receptors at a distance from release sites are more likely to be desensitized than open, since active uptake tends to produce a low

and slowly rising wavefront of GABA. This correlates with our recent observations that extrasynaptic receptors are likely to be desensitized by low ambient GABA concentrations (Bright et al. 2011). Simulation of $GABA_B$ transmission within the thalamus highlights the importance of the localisation of GATs in controlling the spatiotemporal profile of extracellular GABA (Beenhakker and Huguenard 2010). Specifically, the expression of GAT-3 in distal regions relative to release sites appears to strongly constrain diffusion of GABA into extrasynaptic regions, consistent with the demonstration that GAT-3 blockade has a large impact on tonic $GABA_A$ currents (Cope et al. 2009).

6.4.2.4 Receptor Occupancy

The hypnotic drug 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) exerts some of its physiological actions through δ-$GABA_A$ receptors (Winsky-Sommerer et al. 2007). THIP is an agonist at these receptors with a lower affinity for the receptor than GABA. We have shown that raising ambient GABA leads to attenuation of the enhancement of tonic inhibition by THIP (Houston et al. 2012). In order to observe an enhancement of tonic inhibition on THIP application to the slice, there must be a low number of $GABA_A$ receptors bound with GABA and the availability of spare receptors. We also observed a lack of correlation between surface area of granule cells calculated from 3D reconstructions and the magnitude of tonic conductance, which is predicted if there is a small number of GABA molecules in the extracellular space compared to a large number of extrasynaptic receptors.

6.4.3 Regulation of Uptake and Degradation

6.4.3.1 GABA Transporter Localisation

GATs belong to a larger family of Na^+ and Cl^--dependent transporters that utilise transmembrane ion gradients to drive neurotransmitter transport. Molecular cloning has revealed at least four distinct GATs (GAT-1, GAT-2, GAT-3 and BGT-1; Guastella et al. 1990; Borden et al. 1992, 1995) which have distinct localisation and expression profiles in the brain (Radian et al. 1990; Minelli et al. 1995, 1996; Borden 1996; Conti et al. 1998b; Chiu et al. 2002). GATs are localised on the GABAergic pre-synaptic terminal and on surrounding glial processes. GAT-1 and 3 are the most heavily expressed throughout the brain. In general, GAT-1 is thought to be more strongly expressed in pre-synaptic terminals (Minelli et al. 1995; Conti et al. 1998b) and GAT-3 in astrocytic processes (Minelli et al. 1996; Jin et al. 2011). However, this is not exclusive since GAT-1 expression has also been reported in non-GABAergic neurons and glial cells (Minelli et al. 1995). In addition, GAT-1 density varies at different synapses (Chiu et al. 2002).

6.4.3.2 GABA Transporter Localisation: Shaping IPSCs

The localisation of GATs on the pre-synaptic terminal and the membranes of glia surrounding synapses is consistent with their role in the removal of GABA after synaptic release. Blockade of transporters is known to alter the profile of IPSCs although the effect can be variable (Isaacson et al. 1993; Overstreet et al. 2000; Nusser and Mody 2002; Overstreet and Westbrook 2003; Jensen et al. 2003; Jin et al. 2011). Larger evoked synaptic responses are prolonged on GAT blockade whereas with smaller evoked events and minimal stimulation, blockade has less effect, leading to the proposition that transporters play a greater role in the regulation of synaptic transmission when activity and release levels are high (Rossi and Hamann 1998). The effect on IPSCs of transporter block in hippocampal dentate gyrus granule cells was found to be variable depending on the density of active release sites and repetitive activation. This has led to the view that transporters at the synapse primarily act to prevent spillover to neighbouring synapses and increase specificity of synaptic transmission (Overstreet and Westbrook 2003). Increasing levels of ambient GABA can influence synaptic transmission if levels are high enough to induce desensitization of post-synaptic receptors (Overstreet et al. 2000; Shigetomi et al. 2012). As well as a role in the regulation of synaptic transmission, GATs are also localised to regions at some distance from the synapse. Such transporters would be predicted to play a role in regulating the GABA concentration in regions outside of the synapse and therefore influence the magnitude of tonic inhibition.

6.4.3.3 GABA Transporter Localisation: Pharmacological Evidence

Selective blockade of GATs with specific inhibitors (e.g. GAT-1 with the inhibitor NO711 and GAT-2/3 inhibition with SNAP-5114) has been used to show relative contributions of the different subtypes to the regulation of ambient GABA levels and tonic inhibition in different regions. In the rat neocortex, application of both NO711 and SNAP-5114 was required to alter tonic inhibition whilst individually neither drug had an affect, suggesting that GAT-1 and GAT-3 act synergistically to control ambient GABA levels (Keros and Hablitz 2005). In the rat globus pallidus and in VB thalamic neurons, both GAT-1 and GAT-3 blockade together induced a tonic current larger than either alone (Cope et al. 2009; Jin et al. 2011). In hypoglossal motoneurones blockade of both had no effect but the addition of GABA to the slice was required to activate δ-containing $GABA_A$ receptors and reveal a tonic current (Numata et al. 2012). Similarly, in vagal motoneurones blockade of GAT-1 and 3 was effective only in the presence of added GABA, suggesting that the ambient GABA level in this preparation is low and transporters are only involved when the GABA level is increased (Gao and Smith 2010). Blocking GAT-1 function in the hippocampus reveals tonic inhibition in pyramidal neurons, although in nearby interneurons a tonic conductance is observed without the need to block transporter function (Semyanov et al. 2003). It appears therefore that in different regions of the

brain, the effect of GAT inhibition can be variable. This is likely to reflect differences in the subtype, expression level and localisation of transporters, in addition to perhaps different levels of GABA in distinct slice preparations.

6.4.3.4 GABA Transporters: Dynamic Trafficking and Recycling

The ability of transporters to reduce external GABA to the equilibrium level is dependent on the numbers of transporters available in the plasma membrane. As transporter expression is heterogeneous, local variations in different transporter subtypes and expression level may alter the ambient GABA concentration spatially (Radian et al. 1990; Chiu et al. 2002; Jin et al. 2011). The expression of transporters at the plasma membrane is capable of being dynamically regulated as they are shuttled in and out of the membrane. GAT-1 can be internalised and reinserted into the plasma membrane within vesicles in the order of minutes in parallel with transmitter-containing vesicles. Recycling is calcium dependent and can be modulated by second messengers and interacting proteins such as protein kinase C and syntaxin 1A (Deken et al. 2000, 2003; Wang and Quick 2005). GAT-3 trafficking in hippocampal astrocytes is regulated by astrocytic Ca^{2+} signalling through transient receptor potential A1 (TRPA1) channels. A reduction in astrocytic Ca^{2+} alters GAT-3 expression and leads to reduced GABA transport, an elevated tonic current and reduced mIPSC amplitude in interneurons, consistent with elevated ambient GABA (Shigetomi et al. 2012). Interaction with other G-protein-coupled receptors such as adenosine receptors has been shown to influence GAT activity in astrocytes (Cristovao-Ferreira et al. 2011). GAT trafficking and expression at the plasma membrane is capable of rapid modulation. In addition, other receptors and signalling pathways within astrocytes can potentially modulate transporter function.

6.4.3.5 GABA Transporters in Pathophysiology

In certain pathophysiological situations, GAT function may be compromised. There is some evidence for compromised transporter function in temporal lobe epilepsy (Patrylo et al. 2001) although some reports suggest transporter function remains unaltered (Scimemi et al. 2005). Recent examination of mouse models of absence epilepsy has revealed impairment in GAT-1 function in the thalamus as a common feature. In several mouse models of this form of epilepsy, an increased tonic GABAergic inhibition was observed in the VB thalamus (Cope et al. 2009). Application of GAT inhibitors selective for GAT-1 or GAT-3 revealed that both transporters regulate ambient GABA in this region as both increased the tonic current recorded. In mouse models of epilepsy, the GAT-1 selective blocker had no effect suggesting reduced uptake of GABA by GAT-1 underlies this observed increase in tonic inhibition. Furthermore, it was proposed that the elevated extrasynaptic $GABA_A$ receptor activation resulting from this dysfunctional GABA transport is sufficient to promote seizure generation in absence epilepsy (Cope et al. 2009).

In hippocampal slices, GABA was shown to be released from astrocytes through GAT-2/3 in an *in vitro* model of epilepsy. Glutamate transporter activity in astrocytes, subsequent to increased activity and elevated glutamate release, triggered reversal of the GAT by a proposed change in internal Na^{2+} concentration (Heja et al. 2012). Given potential changes in intracellular GABA (Esclapez and Houser 1999) as well as alterations to membrane potential and internal Na^{+} during seizures, transporter reversal is possible (Wu et al. 2001; Sperk et al. 2003). However, there is no direct evidence of this and the role this potentially plays in epilepsy is unclear (Vivar and Gutierrez 2005; Pavlov and Walker 2012).

The region surrounding stroke-damaged areas of the brain (peri-infarct zone) is crucial for neuroplasticity and recovery from stroke. Tonic inhibition is increased in layer 2/3 pyramidal cells in this region. Application of the GAT-1 and GAT-2/3 (3/4 in the rat) selective blockers (NO-711 and SNAP-5114) suggested impairment to GAT-2/3 function as blockade had no effect in post-stroke neurons. A decreased GAT2/3 protein level was also observed post stroke. Reduction of tonic inhibition with $GABA_A$ receptor selective inhibitors led to better motor recovery after stroke suggesting that increased tonic inhibition in this region may reduce the plasticity required for recovery (Clarkson et al. 2010).

6.4.3.6 GAT Knockouts

The GAT-1 knockout mouse exhibits motor disorders and mild anxiety and there is an observed increase in tonic inhibition in cerebellar granule cells and hippocampal pyramidal cells (Jensen et al. 2003; Chiu et al. 2005). Many of these behavioural features are similar to the clinical side effects of tiagabine (a clinically used GAT-1 inhibitor) suggesting these effects are due to GAT-1 inhibition across the brain (Chiu et al. 2005). Increasing ambient GABA through targeting GATs is of some interest as a potential therapeutic strategy in the treatment of epilepsy (Sills 2003) and is also used in the treatment of generalised anxiety disorders (Schwartz and Nihalani 2006).

6.4.3.7 Regulation of Degradation

GABA that has been taken up into neurons and glial cells is degraded by the actions of GABA-T, an enzyme that is expressed in mitochondria across the brain (Nagai et al. 1983). Blockade of GABA-T using the anticonvulsant drug vigabatrin has been shown to result in an increase in intracellular GABA levels, consistent with impaired breakdown (Jung et al. 1977). Concurrently, there is an increase in basal extracellular GABA levels and an increase in stimulated GABA release (Abdul-Ghani et al. 1981; Neal and Shah 1989; Qume et al. 1995; Qume and Fowler 1997). The anticonvulsant properties of vigabatrin in animal models of temporal lobe epilepsy and human partial epilepsy are thought to arise from the elevation in extracellular GABA levels (Sarup et al. 2003). It has also been shown that the elevated

cytosolic GABA arising from GABA-T blockade can affect the equilibrium potential of GATs and therefore lead to altered/reversed GABA transport which can impact upon tonic inhibition (Wu et al. 2001, 2003).

Another enzyme that is important for control of GABA levels is succinic semialdehyde dehydrogenase (SSADH). Degradation of GABA requires GABA-T to convert GABA to succinic semialdehyde (SSA) and SSA is subsequently oxidized by SSADH to succinate, a constituent of the TCA cycle. Animals deficient in SSADH (SSADH(−/−)) show elevated total GABA levels in the brain (Hogema et al. 2001) as expected from an impairment in the GABA breakdown process. More recently, studies have demonstrated that these animals also exhibit enhanced tonic GABA currents in both cortical pyramidal neurons and VB thalamic relay neurons (Drasbek et al. 2008; Errington et al. 2011b).

6.5 Measurement of Extracellular GABA Concentrations

As discussed in the previous sections, GABA sequestered in both neurons and glial cells can be released by vesicular and non-vesicular mechanisms into the ECS. The local concentration of this extracellular GABA will then be regulated by diffusion and uptake. Many studies have attempted to measure the ambient GABA levels in brain tissue that result from this complex interplay of release, diffusion and uptake. Here, we will discuss measurements of GABA both *in vivo* and *in vitro* and how behavioural state, pathophysiology and pharmacological manipulation can impact upon GABA concentrations within the brain.

6.5.1 In Vitro GABA Levels

Most experimental studies of tonic GABAergic inhibition have been carried out using acute brain slice preparations as this currently represents the best experimental preparation for obtaining information about neuronal function with high spatial and temporal resolution. Tonic currents have been recorded in many different laboratories under many different conditions (Glykys and Mody 2007a). This diversity of recording conditions is likely to impact upon many of the factors responsible for controlling ambient GABA and makes comparisons between different studies difficult (Glykys and Mody 2007a). In most studies, immature animals are used, since slices from younger animals seem to harbour a higher proportion of healthy cells than in adults and the lack of fully developed connective tissue makes it easier to visualise cells within the preparation. However, the use of juvenile animals presents problems, since many of the systems associated with neurotransmitter release, detection and uptake may not be fully developed. Another important experimental variable is likely to be the way in which slices are kept for recording, either in a submerged or an interface chamber. Submerged chambers are generally used for patch-clamp

recordings since the slice is more accessible for recording and fast perfusion of drugs than in interface chambers. However, the disadvantage of submerged slices is that oxygen supply to the tissue appears to be impaired compared to interface chambers and in the intact brain (Hajos and Mody 2009), and neuronal activity that requires a high oxygen consumption, such as high-frequency synchronous oscillation, can only be observed under conditions of enhanced perfusion (Hajos et al. 2009). Hypoxic conditions could impact upon ambient GABA concentrations by causing shrinkage of the ECS (Nicholson and Sykova 1998). Furthermore, hypoxia may cause functional impairments in other molecular components important for ambient GABA control, such as transporters (see below).

It has been observed that GABA can be released from glia in response to swelling and mechanical stress (Kozlov et al. 2006). Cell swelling and changes in cell volume (as could potentially occur in preparation of brain slices) can induce neurotransmitter release (Haskew-Layton et al. 2008). The Best-1 channel is volume sensitive so GABA release may occur through these channels if slices are osmotically challenged or if swelling occurs (Hartzell et al. 2008). Oxygen-glucose deprivation, trauma, axotomy and eplieptogenic injury can shift internal Cl^- concentrations which may in turn alter the equilibrium of the GAT (van den Pol et al. 1996; Nabekura et al. 2002; Dzhala et al. 2010). Overall, it is important to consider that in an *in vitro* preparation there are many factors, which might alter GAT function or lead to the release of neurotransmitter from other non-vesicular sources. In addition, due to a loss of sensory input, overall activity levels and levels of transmitter release are likely to be different in a slice from that observed *in vivo* (Chadderton et al. 2004; Loewenstein et al. 2005). The temperature of experiments *in vitro* should also be considered as this will not only affect release probability but also transporter kinetics (Gonzales et al. 2007). As such, ambient GABA levels in the slice may vary considerably from that observed *in vivo* and from one laboratory to another.

It is worth noting that many investigators add extra GABA or GABA uptake blockers to boost the tonic current amplitude. Although this may represent a way of standardizing conditions to allow comparison between recordings (Glykys and Mody 2007a), it is not clear what the ambient GABA level "should be" (see Sect. 6.5.2 below). Also, using such manipulations to enhance GABA may cause problems since increased GABA levels may recruit additional populations of receptors (Scimemi et al. 2005) as well as desensitizing high-affinity δ-$GABA_A$ receptors (Bright et al. 2011).

6.5.2 In Vivo GABA Levels

The extracellular ambient GABA concentration *in vivo* has been estimated at 30–300 nM by microdialysis (Bianchi et al. 2003; Xi et al. 2003; de Groote and Linthorst 2007) and can be altered according to physiological states in a brain region-specific manner. GABA levels in the pontine reticular formation and the thalamus, for example, are dependent on wakefulness, rapid eye movement (REM) and non-REM

sleep (Kekesi et al. 1997; Vanini et al. 2011). In the ventral hippocampus, GABA levels rise to around 800 nM during active exploration (de Groote and Linthorst 2007). It is noteworthy that the predicted transporter equilibrium concentration is in a range from 10 to 400 nM and that measured ambient GABA concentrations are in the range capable of activating high-affinity δ-$GABA_A$ receptors (de Groote and Linthorst 2007; Bright et al. 2011).

In addition to being modulated by behavioural state, brain GABA levels measured by microdialysis can be also be modulated by various drug treatments (reviewed in Del Arco et al. 2003). Drugs that have been tested include amphetamines, clozapine, haloperidol, lithium and modafinil. GABA levels are modulated in a region-specific manner. For instance, the dopaminergic antagonist, haloperidol, causes an increase in extracellular GABA in the globus pallidus (Osborne et al. 1990) but decreases GABA levels in the prefrontal cortex (Bourdelais and Deutch 1994). As discussed below, investigators often try to ascertain the vesicular dependence of changes in ambient GABA by using classical release criteria such as Ca^{2+} and TTX dependence. In this respect, it is has been found that some drug-evoked changes in GABA levels are clearly dependent or independent of vesicular release, whilst others show mixed modes of release (Del Arco et al. 1998).

Microdialysis has been widely used to measure concentrations of various neurotransmitters, including GABA, in the living brain. However, there is some debate as to the origin of the GABA (and glutamate) that is detectable (Timmerman and Westerink 1997; Del Arco et al. 2003; van der Zeyden et al. 2008). Investigators use various criteria based upon normal neuronal function to try to determine the vesicular dependence of dialysate GABA. Thus, vesicular release from neurons is expected to be sensitive to depolarisation by potassium, sodium channel blockade by TTX, removal of extracellular calcium and depletion of presynaptic vesicles by local administration of the selective neurotoxin α-latrotoxin. When these criteria are applied, it has been found that dialysate GABA levels show only a partial dependence on exocytotic release and that changes in GABA occur very slowly (more than 0.5–2.5 h; Timmerman and Westerink 1997; Del Arco et al. 2003; van der Zeyden et al. 2008). This is in surprising contrast to the supposedly more slow-acting monoamine transmitters, such as dopamine, which appear to be tightly coupled to vesicular release (van der Zeyden et al. 2008). For instance, application of TTX causes the disappearance of dopamine from dialysate samples within about 5 min (Feenstra and Botterblom 1996).

Given the questions surrounding the origin of the GABA, the conditions used for measuring dialysate GABA have recently been critically re-examined (van der Zeyden et al. 2008). It was shown that the most widely used chromatographic method for assay of GABA is extremely sensitive to small changes in analytical conditions, particularly pH, and that unless conditions are optimized, measured GABA levels may be artifactually elevated. Using optimized conditions, these investigators report basal GABA levels of 2–4 nM in various brain regions that are significantly lower than in other studies. It was also noted that under these conditions, dialysate GABA appeared to be more sensitive to infusion of TTX, showing that this optimized analytical approach may be a better way of establishing the vesicular

dependence of GABA levels. Since microdialysis probes are also known to create a disrupted zone of tissue that may well influence local neurotransmitter levels, it was suggested that the development of microsensors to assay GABA might be necessary to fully define extracellular GABA concentrations within the brain.

6.5.2.1 GABA Levels in Pathophysiology

Numerous studies have shown that changes in brain extracellular GABA levels are associated with various pathophysiological conditions, including anxiety, schizophrenia, Parkinson's disease (PD), Alzheimer's disease, epilepsy, Huntington's chorea, as well as in senility (reviewed in Timmerman and Westerink (1997); Del Arco et al. (2003); van der Zeyden et al. (2008)). Much attention has been focused on changes in GABA levels associated with schizophrenia. It has been reported that GAT and GAD expression may be decreased in a subset of interneurons (parvalbumin expressing) in the prefrontal cortex in schizophrenic patients (Woo et al. 1998; Volk et al. 2001; Hashimoto et al. 2003). These neurons are thought to play a critical role in working memory and dysfunction in these neurons is thought to underlie some of the cognitive deficits associated with schizophrenia (Uhlhaas and Singer 2010; Lewis et al. 2012). Magnetic resonance spectroscopy has allowed non-invasive measurement of GABA levels in the sensorimotor cortex of patients during memory tasks (Puts et al. 2011). Patients with schizophrenia had reduced visual cortex GABA levels (~10%) and this reduction in GABA correlated with reduced performance in a specific task thought to rely on GABAergic transmission in the cortex (orientation-specific surround suppression), which is diminished in schizophrenic patients (Yoon et al. 2010). Interestingly, another study reported an increase in GABA levels in the anterior cingulate and parietal occipital cortex in schizophrenia (Ongur et al. 2010), suggesting a region-specific dysregulation within the cortex. Furthermore, expression of a previously identified risk factor for this condition, receptor tyrosine kinase ErbB4, which controls inhibitory circuit development in the cortex, was also found to correlate with GABA levels in the cortex (Marenco et al. 2011). A specific role for tonic inhibition in the pathology of schizophrenia has yet to be established but cortical pyramidal neurons exhibit a tonic inhibitory conductance (Salin and Prince 1996) and express δ-containing $GABA_A$ receptors. These changes, combined with the observed reduction of α4 and δ expression in schizophrenic patients and primate animal models (Maldonado-Aviles et al. 2009), strongly implicate alterations in ambient GABA levels and tonic inhibition in the aetiology of this condition, thus providing a potential therapeutic target.

There is some evidence GABA levels may be altered in PD. Tonic inhibition has been reported in MSNs of the striatum (Sect. 6.3.1.6) and loss of dopaminergic modulation of the excitability of these neurons is thought to underlie some of the motor and cognitive symptoms of PD (Chase and Oh 2000). It has been reported that in patients with mild to moderate PD, GABA levels in the pons and putamen are elevated (Emir et al. 2012). In mouse models of PD, dopaminergic neuron loss is associated with increased GABA in the striatum, an increase that was reduced

to control level by levodopa administration (Chassain et al. 2008, 2010). This is consistent with an early report of increased striatal GABA levels correlated with dopaminergic cell loss in postmortem PD patients (Kish et al. 1986) and a reported increase in GAD65/67 expression in a subset of neurons in the putamen in a PD model in monkeys (Soghomonian and Laprade 1997). The recent reports of tonic inhibition in this region (Ade et al. 2008; Janssen et al. 2009; Santhakumar et al. 2010) and the known expression of δ-containing $GABA_A$ receptors (Wisden et al. 1992), combined with changes in observed GABA levels in PD patients, suggests MSN excitability may be altered through enhanced tonic $GABA_A$ receptor activity in PD. However, the precise role that changes in GABA levels and tonic inhibition play in the progression of PD is as yet unknown.

6.5.2.2 GABA Levels and Pharmacology

Tonic inhibition has emerged as a potential therapeutic target in a number of pathophysiological conditions in recent years (Brickley and Mody 2012). Along with the development of pharmacological agents that selectively act at extrasynaptic $GABA_A$receptor subtypes there is also a need to consider how the ambient GABA concentration influences binding and modulation of these receptors (Houston et al. 2012). We have reported that drugs such as the anaesthetic propofol and the hypnotic THIP enhance tonic inhibition mediated by δ-$GABA_A$ only when ambient GABA levels are low (below 100–250 nM). In conditions of enhanced vesicular GABA release in the slice preparation, the action of these drugs is attenuated. However, neurosteroids enhance tonic inhibition regardless of the GABA level and as such, neurosteroid modulation of tonic inhibition may be a more effective strategy for modulation of tonic inhibition *in vivo*.

Concluding Remarks

GABA is stored in significant quantities in neurons and glial cells throughout the CNS. This GABA can be released into the ECS by vesicular release from neurons (and possibly astrocytes) and by non-vesicular mechanisms from both neurons and glia. The ambient GABA levels generated by these release processes can then activate high-affinity extrasynaptic $GABA_A$ receptors to produce tonic currents in various areas of the brain. As reviewed in Sect. 6.3.1, there is a large body of evidence showing that tonic currents recorded in brain slice preparations exhibit a significant dependence upon GABA derived from vesicular release. Although in some cases it appears likely that there are additional non-vesicular sources of GABA, definitive evidence to pinpoint these is lacking (see Sect. 6.3.2.2 for discussion of the source of GABA in the cerebellum).

Ambient GABA levels within the ECS will be determined by the interplay of a plethora of different factors involved in controlling the release, diffusion, uptake and degradation of this ubiquitous inhibitory neurotransmitter (Sect. 6.4). A key player in the control of extracellular GABA is the family of GATs that utilise transmembrane ion gradients to drive GABA in and out of neurons and glia (Sect. 6.4.3.1). Many studies have investigated the role of GATs in setting the ambient GABA concentration (Sects. 6.3.2 and 6.4.3) and subsequently, in determining the level of tonic $GABA_A$ receptor activity. The function of GATs is dynamically regulated under both normal and pathophysiological conditions supporting their critical role in controlling both phasic and tonic inhibition.

The source and control of extracellular GABA will be crucial for determining the functional efficacy of tonic $GABA_A$ receptor-mediated inhibition within the brain. If GABA derived from vesicular release dominates the local ambient levels detected by extrasynaptic receptors, then tonic inhibition will exhibit a tight coupling with network activity, since spillover from synapses will be driven by excitatory input onto interneurons. We have shown that this is likely to be the case in the thalamus where tonic inhibition is dependent upon action potential-driven release from large populations of interneurons (Bright et al. 2007), but is insensitive to local changes in release (Bright and Brickley 2008). Hence, in areas of the brain which exhibit synchronous activation of arrays of interneurons, such as the thalamus, hippocampus and cortex, a vesicular-dependent tonic inhibition is likely to influence the nature of oscillatory activity (Cope et al. 2005; Maguire et al. 2005; Vida et al. 2006). It is worth noting that in cases where GABA responsible for tonic inhibition is derived from non-vesicular release, there is also likely to be a more indirect coupling with excitatory activity. This is due to the dynamic nature of the equilibrium potential for GATs, which is set by transmembrane gradients for Na^+ and Cl^- ions, as well being dependent upon GABA homeostasis and membrane potential (Sect. 6.3.2.1). Thus, control of extracellular GABA by transport equilibrium is likely to reflect changes in local excitatory activity (Wu et al. 2001, 2003, 2007; Heja et al. 2012).

Extracellular GABA levels have been assayed in many studies and exhibit considerable heterogeneity across different brain regions, behavioural states, pharmacological interventions and pathophysiological conditions (Sect. 6.5). High-affinity extrasynaptic $GABA_A$ receptors are best specialized to respond to these low concentrations of ambient transmitter, and the highly dynamic nature of extracellular GABA concentrations under these various conditions indicates that tonic inhibition is likely to be critical in determining normal brain function. However, more investigation is necessary to elucidate the complex regulation of extrasynaptic $GABA_A$ receptor-mediated transmission, especially in the light of our recent observations that receptor occupancy and desensitization at relatively low concentrations of GABA will be key in determining both basal tonic activity and modulation by drugs (Bright et al. 2011; Houston et al. 2012). This is particularly important if we are to understand the function of tonic inhibition in both normal and pathophysiological conditions and to maximise the therapeutic potential of targeting extrasynaptic $GABA_A$ receptors (Brickley and Mody 2012).

References

Abdul-Ghani AS, Norris PJ, Smith CC, Bradford HF (1981) Effects of gamma-acetylenic GABA and gamma-vinyl GABA on synaptosomal release and uptake of GABA. Biochem Pharmacol 30:1203–1209

Acuna-Goycolea C, Brenowitz SD, Regehr WG (2008) Active dendritic conductances dynamically regulate GABA release from thalamic interneurons. Neuron 57:420–431

Ade KK, Janssen MJ, Ortinski PI, Vicini S (2008) Differential tonic GABA conductances in striatal medium spiny neurons. J Neurosci 28:1185–1197

Albrecht J, Schousboe A (2005) Taurine interaction with neurotransmitter receptors in the CNS: an update. Neurochem Res 30:1615–1621

Angulo MC, Le MK, Kozlov AS, Charpak S, Audinat E (2008) GABA, a forgotten gliotransmitter. Prog Neurobiol 86:297–303

Arriza JL, Fairman WA, Wadiche JI, Murdoch GH, Kavanaugh MP, Amara SG (1994) Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. J Neurosci 14:5559–5569

Asada H, Kawamura Y, Maruyama K, Kume H, Ding RG, Kanbara N, Kuzume H, Sanbo M, Yagi T, Obata K (1997) Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. Proc Natl Acad Sci U S A 94:6496–6499

Ataka T, Gu JG (2006) Relationship between tonic inhibitory currents and phasic inhibitory activity in the spinal cord lamina II region of adult mice. Mol Pain 2:36

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

Barakat L, Wang D, Bordey A (2002) Carrier-mediated uptake and release of taurine from Bergmann glia in rat cerebellar slices. J Physiol 541:753–767

Bardakdjian J, Tardy M, Pimoule C, Gonnard P (1979) GABA metabolism in cultured glial cells. Neurochem Res 4:517–527

Battaglioli G, Liu H, Martin DL (2003) Kinetic differences between the isoforms of glutamate decarboxylase: implications for the regulation of GABA synthesis. J Neurochem 86:879–887

Beenhakker MP, Huguenard JR (2010) Astrocytes as gatekeepers of $GABA_B$ receptor function. J Neurosci 30:15262–15276

Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ (2005) Extrasynaptic $GABA_A$ receptors of thalamocortical neurons: a molecular target for hypnotics. J Neurosci 25:11513–11520

Benagiano V, Virgintino D, Rizzi A, Flace P, Troccoli V, Bormann J, Monaghan P, Robertson D, Roncali L, Ambrosi G (2000) Glutamic acid decarboxylase-positive neuronal cell bodies and terminals in the human cerebellar cortex. Histochem J 32:557–564

Bianchi L, Ballini C, Colivicchi MA, Della CL, Giovannini MG, Pepeu G (2003) Investigation on acetylcholine, aspartate, glutamate and GABA extracellular levels from ventral hippocampus during repeated exploratory activity in the rat. Neurochem Res 28:565–573

Birnir B, Everitt AB, Lim MS, Gage PW (2000) Spontaneously opening GABA(A) channels in CA1 pyramidal neurones of rat hippocampus. J Membr Biol 174:21–29

Blomqvist A, Broman J (1988) Light and electron microscopic immunohistochemical demonstration of GABA-immunoreactive astrocytes in the brain stem of the rat. J Neurocytol 17:629–637

Borden LA (1996) GABA transporter heterogeneity: pharmacology and cellular localization. Neurochem Int 29:335–356

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

Borden LA, Smith KE, Gustafson EL, Branchek TA, Weinshank RL (1995) Cloning and expression of a betaine/GABA transporter from human brain. J Neurochem 64:977–984

Bourdelais AJ, Deutch AY (1994) The effects of haloperidol and clozapine on extracellular GABA levels in the prefrontal cortex of the rat: an *in vivo* microdialysis study. Cereb Cortex 4:69–77

Brickley SG, Mody I (2012) Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron 73:23–34

Brickley SG, Cull-Candy SG, Farrant M (1996) Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of $GABA_A$ receptors. J Physiol 497(Pt 3):753–759

Bright DP, Brickley SG (2008) Acting locally but sensing globally: impact of GABAergic synaptic plasticity on phasic and tonic inhibition in the thalamus. J Physiol 586:5091–5099

Bright DP, Aller MI, Brickley SG (2007) Synaptic release generates a tonic GABA(A) receptor-mediated conductance that modulates burst precision in thalamic relay neurons. J Neurosci 27:2560–2569

Bright DP, Renzi M, Bartram J, McGee TP, Mackenzie G, Hosie AM, Farrant M, Brickley SG (2011) Profound desensitization by ambient GABA limits activation of delta-containing $GABA_A$ receptors during spillover. J Neurosci 31:753–763

Cammack JN, Rakhilin SV, Schwartz EA (1994) A GABA transporter operates asymmetrically and with variable stoichiometry. Neuron 13:949–960

Catterall WA, Few AP (2008) Calcium channel regulation and presynaptic plasticity. Neuron 59:882–901

Chadderton P, Margrie TW, Hausser M (2004) Integration of quanta in cerebellar granule cells during sensory processing. Nature 428:856–860

Chase TN, Oh JD (2000) Striatal dopamine- and glutamate-mediated dysregulation in experimental Parkinsonism. Trends Neurosci 23:S86–S91

Chassain C, Bielicki G, Durand E, Lolignier S, Essafi F, Traore A, Durif F (2008) Metabolic changes detected by proton magnetic resonance spectroscopy *in vivo* and *in vitro* in a murin model of Parkinson's disease, the MPTP-intoxicated mouse. J Neurochem 105:874–882

Chassain C, Bielicki G, Keller C, Renou JP, Durif F (2010) Metabolic changes detected *in vivo* by 1H MRS in the MPTP-intoxicated mouse. NMR Biomed 23:547–553

Chaudhry FA, Schmitz D, Reimer RJ, Larsson P, Gray AT, Nicoll R, Kavanaugh M, Edwards RH (2002) Glutamine uptake by neurons: interaction of protons with system a transporters. J Neurosci 22:62–72

Chiu CS, Jensen K, Sokolova I, Wang D, Li M, Deshpande P, Davidson N, Mody I, Quick MW, Quake SR, Lester HA (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. J Neurosci 22:10251–10266

Chiu CS, Brickley S, Jensen K, Southwell A, Mckinney S, Cull-Candy S, Mody I, Lester HA (2005) GABA transporter deficiency causes tremor, ataxia, nervousness, and increased GABA-induced tonic conductance in cerebellum. J Neurosci 25:3234–3245

Choe KY, Olson JE, Bourque CW (2012) Taurine release by astrocytes modulates osmosensitive glycine receptor tone and excitability in the adult supraoptic nucleus. J Neurosci 32:12518–12527

Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature 468:305–309

Conti F, DeBiasi S, Minelli A, Rothstein JD, Melone M (1998a) EAAC1, a high-affinity glutamate tranporter, is localized to astrocytes and gabaergic neurons besides pyramidal cells in the rat cerebral cortex. Cereb Cortex 8:108–116

Conti F, Melone M, De BS, Minelli A, Brecha NC, Ducati A (1998b) 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

Cope DW, Hughes SW, Crunelli V (2005) $GABA_A$ receptor-mediated tonic inhibition in thalamic neurons. J Neurosci 25:11553–11563

Cope DW, Di GG, Fyson SJ, Orban G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V (2009) Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. Nat Med 15:1392–1398

Cristovao-Ferreira S, Navarro G, Brugarolas M, Perez-Capote K, Vaz SH, Fattorini G, Conti F, Lluis C, Ribeiro JA, McCormick PJ, Casado V, Franco R, Sebastiao AM (2011) Modulation of GABA transport by adenosine A1R-A2AR heteromers, which are coupled to both Gs- and G(i/o)-proteins. J Neurosci 31:15629–15639

de Groote L, Linthorst AC (2007) Exposure to novelty and forced swimming evoke stressor-dependent changes in extracellular GABA in the rat hippocampus. Neuroscience 148:794–805

Deken SL, Beckman ML, Boos L, Quick MW (2000) Transport rates of GABA transporters: regulation by the N-terminal domain and syntaxin 1A. Nat Neurosci 3:998–1003

Deken SL, Wang D, Quick MW (2003) Plasma membrane GABA transporters reside on distinct vesicles and undergo rapid regulated recycling. J Neurosci 23:1563–1568

Del Arco A, Castaneda TR, Mora F (1998) Amphetamine releases GABA in striatum of the freely moving rat: involvement of calcium and high affinity transporter mechanisms. Neuropharmacology 37:199–205

Del Arco A, Segovia G, Fuxe K, Mora F (2003) Changes in dialysate concentrations of glutamate and GABA in the brain: an index of volume transmission mediated actions? J Neurochem 85:23–33

Destexhe A, Sejnowski TJ (1995) G protein activation kinetics and spillover of gamma-aminobutyric acid may account for differences between inhibitory responses in the hippocampus and thalamus. Proc Natl Acad Sci U S A 92:9515–9519

Diaz MR, Wadleigh A, Hughes BA, Woodward JJ, Valenzuela CF (2011) Bestrophin1 channels are insensitive to ethanol and do not mediate tonic GABAergic currents in cerebellar granule cells. Front Neurosci 5:148

Draguhn A, Heinemann U (1996) Different mechanisms regulate IPSC kinetics in early postnatal and juvenile hippocampal granule cells. J Neurophysiol 76:3983–3993

Drasbek KR, Jensen K (2006) THIP, a hypnotic and antinociceptive drug, enhances an extrasynaptic $GABA_A$ receptor-mediated conductance in mouse neocortex. Cereb Cortex 16:1134–1141

Drasbek KR, Vardya I, Delenclos M, Gibson KM, Jensen K (2008) SSADH deficiency leads to elevated extracellular GABA levels and increased GABAergic neurotransmission in the mouse cerebral cortex. J Inherit Metab Dis 31:662–668

Drose S, Altendorf K (1997) Bafilomycins and concanamycins as inhibitors of V-ATPases and P-ATPases. J Exp Biol 200:1–8

Dzhala VI, Kuchibhotla KV, Glykys JC, Kahle KT, Swiercz WB, Feng G, Kuner T, Augustine GJ, Bacskai BJ, Staley KJ (2010) Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. J Neurosci 30:11745–11761

Edwards RH (2007) The neurotransmitter cycle and quantal size. Neuron 55:835–858

Emir UE, Tuite PJ, Oz G (2012) Elevated pontine and putamenal GABA levels in mild-moderate Parkinson disease detected by 7 T proton MRS. PLoS One 7:e30918

Errington AC, Di GG, Crunelli V, Cope DW (2011a) mGluR control of interneuron output regulates feed forward tonic $GABA_A$ inhibition in the visual thalamus. J Neurosci 31:8669–8680

Errington AC, Gibson KM, Crunelli V, Cope DW (2011b) Aberrant GABA(A) receptor-mediated inhibition in cortico-thalamic networks of succinic semialdehyde dehydrogenase deficient mice. PLoS One 6:e19021

Esclapez M, Houser CR (1999) Up-regulation of GAD65 and GAD67 in remaining hippocampal GABA neurons in a model of temporal lobe epilepsy. J Comp Neurol 412:488–505

Esclapez M, Tillakaratne NJ, Kaufman DL, Tobin AJ, Houser CR (1994) Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in rat brain supports the concept of functional differences between the forms. J Neurosci 14:1834–1855

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 6:215–229

Fatemi SH, Stary JM, Earle JA, Araghi-Niknam M, Eagan E (2005) GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and Reelin proteins in cerebellum. Schizophr Res 72:109–122

Feenstra MG, Botterblom MH (1996) Rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty. Brain Res 742:17–24

Fenalti G, Law RH, Buckle AM, Langendorf C, Tuck K, Rosado CJ, Faux NG, Mahmood K, Hampe CS, Banga JP, Wilce M, Schmidberger J, Rossjohn J, El-Kabbani O, Pike RN, Smith AI, Mackay IR, Rowley MJ, Whisstock JC (2007) GABA production by glutamic acid decarboxylase is regulated by a dynamic catalytic loop. Nat Struct Mol Biol 14:280–286

Freichel C, Potschka H, Ebert U, Brandt C, Loscher W (2006) Acute changes in the neuronal expression of GABA and glutamate decarboxylase isoforms in the rat piriform cortex following status epilepticus. Neuroscience 141:2177–2194

Gao H, Smith BN (2010) Tonic $GABA_A$ receptor-mediated inhibition in the rat dorsal motor nucleus of the vagus. J Neurophysiol 103:904–914

Gasnier B (2004) The SLC32 transporter, a key protein for the synaptic release of inhibitory amino acids. Pflugers Arch 447:756–759

Glykys J, Mody I (2007a) Activation of $GABA_A$ receptors: views from outside the synaptic cleft. Neuron 56:763–770

Glykys J, Mody I (2007b) The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. J Physiol 582:1163–1178

Glykys J, Mann EO, Mody I (2008) Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? J Neurosci 28:1421–1426

Gonzales AL, Lee W, Spencer SR, Oropeza RA, Chapman JV, Ku JY, Eskandari S (2007) Turnover rate of the gamma-aminobutyric acid transporter GAT1. J Membr Biol 220:33–51

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

Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R, Uzunov D, Costa E (2000) Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch Gen Psychiatry 57:1061–1069

Hadley SH, Amin J (2007) Rat alpha6beta2delta $GABA_A$ receptors exhibit two distinct and separable agonist affinities. J Physiol 581:1001–1018

Hajos N, Mody I (2009) Establishing a physiological environment for visualized *in vitro* brain slice recordings by increasing oxygen supply and modifying aCSF content. J Neurosci Methods 183:107–113

Hajos N, Ellender TJ, Zemankovics R, Mann EO, Exley R, Cragg SJ, Freund TF, Paulsen O (2009) Maintaining network activity in submerged hippocampal slices: importance of oxygen supply. Eur J Neurosci 29:319–327

Hamann M, Rossi DJ, Attwell D (2002) Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. Neuron 33:625–633

Hamilton NB, Attwell D (2010) Do astrocytes really exocytose neurotransmitters? Nat Rev Neurosci 11:227–238

Hartzell HC, Qu Z, Yu K, Xiao Q, Chien LT (2008) Molecular physiology of bestrophins: multifunctional membrane proteins linked to best disease and other retinopathies. Physiol Rev 88:639–672

Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA (2003) Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci 23:6315–6326

Haskew-Layton RE, Rudkouskaya A, Jin Y, Feustel PJ, Kimelberg HK, Mongin AA (2008) Two distinct modes of hypoosmotic medium-induced release of excitatory amino acids and taurine in the rat brain *in vivo*. PLoS One 3:e3543

Heja L, Nyitrai G, Kekesi O, Dobolyi A, Szabo P, Fiath R, Ulbert I, Pal-Szenthe B, Palkovits M, Kardos J (2012) Astrocytes convert network excitation to tonic inhibition of neurons. BMC Biol 10:26

Hogema BM, Gupta M, Senephansiri H, Burlingame TG, Taylor M, Jakobs C, Schutgens RB, Froestl W, Snead OC, Diaz-Arrastia R, Bottiglieri T, Grompe M, Gibson KM (2001) Pharmacologic rescue of lethal seizures in mice deficient in succinate semialdehyde dehydrogenase. Nat Genet 29:212–216

Holter NI, Zylla MM, Zuber N, Bruehl C, Draguhn A (2010) Tonic GABAergic control of mouse dentate granule cells during postnatal development. Eur J Neurosci 32:1300–1309

Houston CM, McGee TP, Mackenzie G, Troyano-Cuturi K, Rodriguez PM, Kutsarova E, Diamanti E, Hosie AM, Franks NP, Brickley SG (2012) Are extrasynaptic $GABA_A$ receptors important targets for sedative/hypnotic drugs? J Neurosci 32:3887–3897

Huguenard JR, McCormick DA (2007) Thalamic synchrony and dynamic regulation of global forebrain oscillations. Trends Neurosci 30:350–356

Hussy N, Bres V, Rochette M, Duvoid A, Alonso G, Dayanithi G, Moos FC (2001) Osmoregulation of vasopressin secretion via activation of neurohypophysial nerve terminals glycine receptors by glial taurine. J Neurosci 21:7110–7116

Isaacson JS, Solis JM, Nicoll RA (1993) Local and diffuse synaptic actions of GABA in the hippocampus. Neuron 10:165–175

Jakab RL (1989) Three-dimensional reconstruction and synaptic architecture of cerebellar glomeruli in the rat. Acta Morphol Hung 37:11–20

Jakab RL, Hamori J (1988) Quantitative morphology and synaptology of cerebellar glomeruli in the rat. Anat Embryol (Berl) 179:81–88

Janssen MJ, Ade KK, Fu Z, Vicini S (2009) Dopamine modulation of GABA tonic conductance in striatal output neurons. J Neurosci 29:5116–5126

Jensen K, Chiu CS, Sokolova I, Lester HA, Mody I (2003) GABA transporter-1 (GAT1)-deficient mice: differential tonic activation of $GABA_A$ versus $GABA_B$ receptors in the hippocampus. J Neurophysiol 90:2690–2701

Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, Goldstein PA (2005) An extrasynaptic $GABA_A$ receptor mediates tonic inhibition in thalamic VB neurons. J Neurophysiol 94:4491–4501

Jia F, Yue M, Chandra D, Keramidas A, Goldstein PA, Homanics GE, Harrison NL (2008) Taurine is a potent activator of extrasynaptic GABA(A) receptors in the thalamus. J Neurosci 28:106–115

Jimenez-Gonzalez C, Pirttimaki T, Cope DW, Parri HR (2011) Non-neuronal, slow GABA signalling in the ventrobasal thalamus targets delta-subunit-containing GABA(A) receptors. Eur J Neurosci 33:1471–1482

Jin H, Wu H, Osterhaus G, Wei J, Davis K, Sha D, Floor E, Hsu CC, Kopke RD, Wu JY (2003) Demonstration of functional coupling between gamma -aminobutyric acid (GABA) synthesis and vesicular GABA transport into synaptic vesicles. Proc Natl Acad Sci U S A 100:4293–4298

Jin XT, Pare JF, Smith Y (2011) Differential localization and function of GABA transporters, GAT-1 and GAT-3, in the rat globus pallidus. Eur J Neurosci 33:1504–1518

Jung MJ, Lippert B, Metcalf BW, Bohlen P, Schechter PJ (1977) gamma-Vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T: effects on brain GABA metabolism in mice. J Neurochem 29:797–802

Kanamori K, Ross BD (2004) Quantitative determination of extracellular glutamine concentration in rat brain, and its elevation *in vivo* by system A transport inhibitor, alpha-(methylamino) isobutyrate. J Neurochem 90:203–210

Kaneda M, Farrant M, Cull-Candy SG (1995) Whole-cell and single-channel currents activated by GABA and glycine in granule cells of the rat cerebellum. J Physiol 485(Pt 2):419–435

Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, Somogyi P (2010) Quantitative localisation of synaptic and extrasynaptic $GABA_A$ receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. Eur J Neurosci 32:1868–1888

Kaufman DL, Houser CR, Tobin AJ (1991) Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J Neurochem 56:720–723

Kekesi KA, Dobolyi A, Salfay O, Nyitrai G, Juhasz G (1997) Slow wave sleep is accompanied by release of certain amino acids in the thalamus of cats. Neuroreport 8:1183–1186

Keros S, Hablitz JJ (2005) Subtype-specific GABA transporter antagonists synergistically modulate phasic and tonic $GABA_A$ conductances in rat neocortex. J Neurophysiol 94:2073–2085

Kish SJ, Rajput A, Gilbert J, Rozdilsky B, Chang LJ, Shannak K, Hornykiewicz O (1986) Elevated gamma-aminobutyric acid level in striatal but not extrastriatal brain regions in Parkinson's disease: correlation with striatal dopamine loss. Ann Neurol 20:26–31

Klausberger T (2009) GABAergic interneurons targeting dendrites of pyramidal cells in the CA1 area of the hippocampus. Eur J Neurosci 30:947–957

Knoflach F, Benke D, Wang Y, Scheurer L, Luddens H, Hamilton BJ, Carter DB, Mohler H, Benson JA (1996) Pharmacological modulation of the diazepam-insensitive recombinant gamma-aminobutyric acidA receptors alpha 4 beta 2 gamma 2 and alpha 6 beta 2 gamma 2. Mol Pharmacol 50:1253–1261

Koch U, Magnusson AK (2009) Unconventional GABA release: mechanisms and function. Curr Opin Neurobiol 19:305–310

Kozlov AS, Angulo MC, Audinat E, Charpak S (2006) Target cell-specific modulation of neuronal activity by astrocytes. Proc Natl Acad Sci U S A 103:10058–10063

Kralic JE, Sidler C, Parpan F, Homanics GE, Morrow AL, Fritschy JM (2006) Compensatory alteration of inhibitory synaptic circuits in cerebellum and thalamus of gamma-aminobutyric acid type A receptor alpha1 subunit knockout mice. J Comp Neurol 495:408–421

Krook-Magnuson EI, Li P, Paluszkiewicz SM, Huntsman MM (2008) Tonically active inhibition selectively controls feedforward circuits in mouse barrel cortex. J Neurophysiol 100:932–944

Kubo K, Nishikawa K, Hardy-Yamada M, Ishizeki J, Yanagawa Y, Saito S (2009) Altered responses to propofol, but not ketamine, in mice deficient in the 65-kilodalton isoform of glutamate decarboxylase. J Pharmacol Exp Ther 329:592–599

Laschet J, Grisar T, Bureau M, Guillaume D (1992) Characteristics of putrescine uptake and subsequent GABA formation in primary cultured astrocytes from normal C57BL/6J and epileptic DBA/2J mouse brain cortices. Neuroscience 48:151–157

Le Meur K, Mendizabal-Zubiaga J, Grandes P, Audinat E (2012) GABA release by hippocampal astrocytes. Front Comput Neurosci 6:59

Lee S, Yoon BE, Berglund K, Oh SJ, Park H, Shin HS, Augustine GJ, Lee CJ (2010) Channel-mediated tonic GABA release from glia. Science 330:790–796

Lewis DA, Curley AA, Glausier JR, Volk DW (2012) Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci 35:57–67

Liang SL, Carlson GC, Coulter DA (2006) Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. J Neurosci 26:8537–8548

Loewenstein Y, Mahon S, Chadderton P, Kitamura K, Sompolinsky H, Yarom Y, Hausser M (2005) Bistability of cerebellar Purkinje cells modulated by sensory stimulation. Nat Neurosci 8:202–211

Lu CC, Hilgemann DW (1999) GAT1 (GABA:Na^+:Cl^-) cotransport function. Kinetic studies in giant Xenopus oocyte membrane patches. J Gen Physiol 114:445–457

Madsen KK, White HS, Schousboe A (2010) Neuronal and non-neuronal GABA transporters as targets for antiepileptic drugs. Pharmacol Ther 125:394–401

Maguire JL, Stell BM, Rafizadeh M, Mody I (2005) Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci 8:797–804

Maldonado-Aviles JG, Curley AA, Hashimoto T, Morrow AL, Ramsey AJ, O'Donnell P, Volk DW, Lewis DA (2009) Altered markers of tonic inhibition in the dorsolateral prefrontal cortex of subjects with schizophrenia. Am J Psychiatry 166:450–459

Manko M, Bienvenu TC, Dalezios Y, Capogna M (2012) Neurogliaform cells of amygdala: a source of slow phasic inhibition in the basolateral complex. J Physiol 590(Pt 22):5611–27

Marenco S, Geramita M, van der Veen JW, Barnett AS, Kolachana B, Shen J, Weinberger DR, Law AJ (2011) Genetic association of ErbB4 and human cortical GABA levels *in vivo*. J Neurosci 31:11628–11632

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 5:793–807

Martin DL, Tobin AJ (2000) Mechanisms controlling GABA synthesis and degradation in the brain. In: Martin DL, Olsen RW (eds) GABA in the nervous system: the view at fifty years. Lippincott Williams and Wilkins, Philadelphia, pp 25–41

Martinez-Rodriguez R, Tonda A, Gragera RR, Paz-Doel R, Garcia-Cordovilla R, Fernandez-Fernandez E, Fernandez AM, Gonzalez-Romero F, Lopez-Bravo A (1993) Synaptic and non-synaptic immunolocalization of GABA and glutamate acid decarboxylase (GAD) in cerebellar cortex of rat. Cell Mol Biol (Noisy-le-grand) 39:115–123

Mathews GC, Diamond JS (2003) Neuronal glutamate uptake contributes to GABA synthesis and inhibitory synaptic strength. J Neurosci 23:2040–2048

McCartney MR, Deeb TZ, Henderson TN, Hales TG (2007) Tonically active $GABA_A$ receptors in hippocampal pyramidal neurons exhibit constitutive GABA-independent gating. Mol Pharmacol 71:539–548

McCormick DA, Bal T (1997) Sleep and arousal: thalamocortical mechanisms. Annu Rev Neurosci 20:185–215

Megias M, Emri Z, Freund TF, Gulyas AI (2001) Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. Neuroscience 102:527–540

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

Minelli A, DeBiasi S, Brecha NC, Zuccarello LV, 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. J Neurosci 16:6255–6264

Mitchell EA, Gentet LJ, Dempster J, Belelli D (2007) $GABA_A$ and glycine receptor-mediated transmission in rat lamina II neurones: relevance to the analgesic actions of neuroactive steroids. J Physiol 583:1021–1040

Mitchell SJ, Silver RA (2003) Shunting inhibition modulates neuronal gain during synaptic excitation. Neuron 38:433–445

Nabekura J, Ueno T, Okabe A, Furuta A, Iwaki T, Shimizu-Okabe C, Fukuda A, Akaike N (2002) Reduction of KCC2 expression and $GABA_A$ receptor-mediated excitation after *in vivo* axonal injury. J Neurosci 22:4412–4417

Nagai T, McGeer PL, McGeer EG (1983) Distribution of GABA-T-intensive neurons in the rat forebrain and midbrain. J Comp Neurol 218:220–238

Neal MJ, Shah MA (1989) Baclofen and phaclofen modulate GABA release from slices of rat cerebral cortex and spinal cord but not from retina. Br J Pharmacol 98:105–112

Neelands TR, Fisher JL, Bianchi M, Macdonald RL (1999) Spontaneous and gamma-aminobutyric acid (GABA)-activated GABA(A) receptor channels formed by epsilon subunit-containing isoforms. Mol Pharmacol 55:168–178

Nicholson C, Sykova E (1998) Extracellular space structure revealed by diffusion analysis. Trends Neurosci 21:207–215

Numata JM, van Brederode JF, Berger AJ (2012) Lack of an endogenous $GABA_A$ receptor-mediated tonic current in hypoglossal motoneurons. J Physiol 590:2965–2976

Nusser Z, Mody I (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J Neurophysiol 87:2624–2628

Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693–1703

Oja SS, Saransaari P (2000) Modulation of taurine release by glutamate receptors and nitric oxide. Prog Neurobiol 62:407–425

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

Ongur D, Prescot AP, McCarthy J, Cohen BM, Renshaw PF (2010) Elevated gamma-aminobutyric acid levels in chronic schizophrenia. Biol Psychiatry 68:667–670

Osborne PG, O'Connor WT, Drew KL, Ungerstedt U (1990) An *in vivo* microdialysis characterization of extracellular dopamine and GABA in dorsolateral striatum of awake freely moving and halothane anaesthetised rats. J Neurosci Methods 34:99–105

Otsuka M, Obata K, Miyata Y, Tanaka Y (1971) Measurement of gamma-aminobutyric acid in isolated nerve cells of cat central nervous system. J Neurochem 18:287–295
Overstreet LS, Westbrook GL (2003) Synapse density regulates independence at unitary inhibitory synapses. J Neurosci 23:2618–2626
Overstreet LS, Jones MV, Westbrook GL (2000) Slow desensitization regulates the availability of synaptic GABA(A) receptors. J Neurosci 20:7914–7921
Overstreet LS, Westbrook GL, Jones MV (2002) Measuring and modeling the spatiotemporal profile of GABA at the synapse. In: Quick MW (ed) Transmembrane transporters. Wiley, New York, pp. 259–275
Park JB, Skalska S, Stern JE (2006) Characterization of a novel tonic gamma-aminobutyric acidA receptor-mediated inhibition in magnocellular neurosecretory neurons and its modulation by glia. Endocrinology 147:3746–3760
Park JB, Skalska S, Son S, Stern JE (2007) Dual $GABA_A$ receptor-mediated inhibition in rat presympathetic paraventricular nucleus neurons. J Physiol 582:539–551
Park H, Oh SJ, Han KS, Woo DH, Park H, Mannaioni G, Traynelis SF, Lee CJ (2009) Bestrophin-1 encodes for the Ca2+-activated anion channel in hippocampal astrocytes. J Neurosci 29:13063–13073
Patrylo PR, Spencer DD, Williamson A (2001) GABA uptake and heterotransport are impaired in the dentate gyrus of epileptic rats and humans with temporal lobe sclerosis. J Neurophysiol 85:1533–1542
Pavlov I, Walker MC (2012) Tonic GABA(A) receptor-mediated signalling in temporal lobe epilepsy. Neuropharmacology 69:55–61
Pavlov I, Savtchenko LP, Kullmann DM, Semyanov A, Walker MC (2009) Outwardly rectifying tonically active $GABA_A$ receptors in pyramidal cells modulate neuronal offset, not gain. J Neurosci 29:15341–15350
Pines G, Danbolt NC, Bjoras M, Zhang Y, Bendahan A, Eide L, Koepsell H, Storm-Mathisen J, Seeberg E, Kanner BI (1992) Cloning and expression of a rat brain L-glutamate transporter. Nature 360:464–467
Pow DV, Sullivan R, Reye P, Hermanussen S (2002) Localization of taurine transporters, taurine, and (3)H taurine accumulation in the rat retina, pituitary, and brain. Glia 37:153–168
Price CJ, Cauli B, Kovacs ER, Kulik A, Lambolez B, Shigemoto R, Capogna M (2005) Neurogliaform neurons form a novel inhibitory network in the hippocampal CA1 area. J Neurosci 25:6775–6786
Price CJ, Scott R, Rusakov DA, Capogna M (2008) GABA(B) receptor modulation of feedforward inhibition through hippocampal neurogliaform cells. J Neurosci 28:6974–6982
Puts NA, Edden RA, Evans CJ, McGlone F, McGonigle DJ (2011) Regionally specific human GABA concentration correlates with tactile discrimination thresholds. J Neurosci 31:16556–16560
Qume M, Fowler LJ (1997) Effect of chronic treatment with the GABA transaminase inhibitors gamma-vinyl GABA and ethanolamine O-sulphate on the *in vitro* GABA release from rat hippocampus. Br J Pharmacol 122:539–545
Qume M, Whitton PS, Fowler LJ (1995) The effect of chronic treatment with the GABA transaminase inhibitors gamma-vinyl-GABA and ethanolamine-O-sulphate on the *in vivo* release of GABA from rat hippocampus. J Neurochem 64:2256–2261
Radian R, Ottersen OP, Storm-Mathisen J, Castel M, Kanner BI (1990) Immunocytochemical localization of the GABA transporter in rat brain. J Neurosci 10:1319–1330
Rae C, Hare N, Bubb WA, McEwan SR, Broer A, McQuillan JA, Balcar VJ, Conigrave AD, Broer S (2003) Inhibition of glutamine transport depletes glutamate and GABA neurotransmitter pools: further evidence for metabolic compartmentation. J Neurochem 85:503–514
Richerson GB, Wu Y (2003) Dynamic equilibrium of neurotransmitter transporters: not just for reuptake anymore. J Neurophysiol 90:1363–1374
Roberts E, Frankel S (1950) gamma-aminobutyric acid in brain: its formation from glutamic acid. J Biol Chem 187:55–63

Rossi DJ, Hamann M (1998) Spillover-mediated transmission at inhibitory synapses promoted by high affinity alpha6 subunit GABA(A) receptors and glomerular geometry. Neuron 20:783–795
Rossi DJ, Hamann M, Attwell D (2003) Multiple modes of GABAergic inhibition of rat cerebellar granule cells. J Physiol 548:97–110
Roth FC, Draguhn A (2012) GABA metabolism and transport: effects on synaptic efficacy. Neural Plast 2012:805830
Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N, Kuncl RW (1994) Localization of neuronal and glial glutamate transporters. Neuron 13:713–725
Rutledge EM, Kimelberg HK (1996) Release of [3H]-D-aspartate from primary astrocyte cultures in response to raised external potassium. J Neurosci 16:7803–7811
Salin PA, Prince DA (1996) Spontaneous $GABA_A$ receptor-mediated inhibitory currents in adult rat somatosensory cortex. J Neurophysiol 75:1573–1588
Santhakumar V, Jones RT, Mody I (2010) Developmental regulation and neuroprotective effects of striatal tonic $GABA_A$ currents. Neuroscience 167:644–655
Saransaari P, Oja SS (1999) Characteristics of ischemia-induced taurine release in the developing mouse hippocampus. Neuroscience 94:949–954
Sarup A, Larsson OM, Schousboe A (2003) GABA transporters and GABA-transaminase as drug targets. Curr Drug Targets CNS Neurol Disord 2:269–277
Schwartz EA (1987) Depolarization without calcium can release gamma-aminobutyric acid from a retinal neuron. Science 238:350–355
Schwartz TL, Nihalani N (2006) Tiagabine in anxiety disorders. Expert Opin Pharmacother 7:1977–1987
Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker MC (2005) Multiple and plastic receptors mediate tonic $GABA_A$ receptor currents in the hippocampus. J Neurosci 25:10016–10024
Segovia G, Del AA, Mora F (1997) Endogenous glutamate increases extracellular concentrations of dopamine, GABA, and taurine through NMDA and AMPA/kainate receptors in striatum of the freely moving rat: a microdialysis study. J Neurochem 69:1476–1483
Seiler N, Bink G, Grove J (1979) Regulatory interrelations between GABA and polyamines. I. Brain GABA levels and polyamine metabolism. Neurochem Res 4:425–435
Semyanov A, Walker MC, Kullmann DM (2003) GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nat Neurosci 6:484–490
Semyanov A, Walker MC, Kullmann DM, Silver RA (2004) Tonically active GABA A receptors: modulating gain and maintaining the tone. Trends Neurosci 27:262–269
Sepkuty JP, Cohen AS, Eccles C, Rafiq A, Behar K, Ganel R, Coulter DA, Rothstein JD (2002) A neuronal glutamate transporter contributes to neurotransmitter GABA synthesis and epilepsy. J Neurosci 22:6372–6379
Sergeeva OA, Chepkova AN, Doreulee N, Eriksson KS, Poelchen W, Monnighoff I, Heller-Stilb B, Warskulat U, Haussinger D, Haas HL (2003) Taurine-induced long-lasting enhancement of synaptic transmission in mice: role of transporters. J Physiol 550:911–919
Sherman SM (2004) Interneurons and triadic circuitry of the thalamus. Trends Neurosci 27:670–675
Sherman SM (2005) Thalamic relays and cortical functioning. Prog Brain Res 149:107–126
Shigetomi E, Tong X, Kwan KY, Corey DP, Khakh BS (2012) TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. Nat Neurosci 15:70–80
Shivers BD, Killisch I, Sprengel R, Sontheimer H, Kohler M, Schofield PR, Seeburg PH (1989) Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. Neuron 3:327–337
Sills GJ (2003) Pre-clinical studies with the GABAergic compounds vigabatrin and tiagabine. Epileptic Disord 5:51–56
Soghomonian JJ, Laprade N (1997) Glutamate decarboxylase (GAD67 and GAD65) gene expression is increased in a subpopulation of neurons in the putamen of Parkinsonian monkeys. Synapse 27:122–132
Soltesz I, Roberts JD, Takagi H, Richards JG, Mohler H, Somogyi P (1990) Synaptic and nonsynaptic localization of benzodiazepine/$GABA_A$ receptor/Cl- channel complex using monoclonal antibodies in the dorsal lateral geniculate nucleus of the cat. Eur J Neurosci 2:414–429

Sperk G, Schwarzer C, Heilman J, Furtinger S, Reimer RJ, Edwards RH, Nelson N (2003) Expression of plasma membrane GABA transporters but not of the vesicular GABA transporter in dentate granule cells after kainic acid seizures. Hippocampus 13:806–815

Stork O, Ji FY, Kaneko K, Stork S, Yoshinobu Y, Moriya T, Shibata S, Obata K (2000) Postnatal development of a GABA deficit and disturbance of neural functions in mice lacking GAD65. Brain Res 865:45–58

Sudhof TC, Rizo J (2011) Synaptic vesicle exocytosis. Cold Spring Harb Perspect Biol 3(12):pii a005637. doi: 10.1101/cshperspect.a005637

Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, McKernan RM (1999) Preferential coassembly of alpha4 and delta subunits of the gamma-aminobutyric acidA receptor in rat thalamus. Mol Pharmacol 56:110–115

Sykova E (2004) Diffusion properties of the brain in health and disease. Neurochem Int 45:453–466

Sykova E, Vargova L (2008) Extrasynaptic transmission and the diffusion parameters of the extracellular space. Neurochem Int 52:5–13

Takanaga H, Ohtsuki S, Hosoya K, Terasaki T (2001) GAT2/BGT-1 as a system responsible for the transport of gamma-aminobutyric acid at the mouse blood-brain barrier. J Cereb Blood Flow Metab 21:1232–1239

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

Tang X, Hernandez CC, Macdonald RL (2010) Modulation of spontaneous and GABA-evoked tonic alpha4beta3delta and alpha4beta3gamma2L $GABA_A$ receptor currents by protein kinase A. J Neurophysiol 103:1007–1019

Tang ZQ, Dinh EH, Shi W, Lu Y (2011) Ambient GABA-activated tonic inhibition sharpens auditory coincidence detection via a depolarizing shunting mechanism. J Neurosci 31:6121–6131

Tasan RO, Bukovac A, Peterschmitt YN, Sartori SB, Landgraf R, Singewald N, Sperk G (2011) Altered GABA transmission in a mouse model of increased trait anxiety. Neuroscience 183:71–80

Taylor CP (1997) Mechanisms of action of gabapentin. Rev Neurol (Paris) 153(Suppl 1):S39–S45

Timmerman W, Westerink BH (1997) Brain microdialysis of GABA and glutamate: what does it signify? Synapse 27:242–261

Uhlhaas PJ, Singer W (2010) Abnormal neural oscillations and synchrony in schizophrenia. Nat Rev Neurosci 11:100–113

van den Pol AN, Obrietan K, Chen G (1996) Excitatory actions of GABA after neuronal trauma. J Neurosci 16:4283–4292

van der Zeyden M, Oldenziel WH, Rea K, Cremers TI, Westerink BH (2008) Microdialysis of GABA and glutamate: analysis, interpretation and comparison with microsensors. Pharmacol Biochem Behav 90:135–147

Vanini G, Wathen BL, Lydic R, Baghdoyan HA (2011) Endogenous GABA levels in the pontine reticular formation are greater during wakefulness than during rapid eye movement sleep. J Neurosci 31:2649–2656

Vida I, Bartos M, Jonas P (2006) Shunting inhibition improves robustness of gamma oscillations in hippocampal interneuron networks by homogenizing firing rates. Neuron 49:107–117

Vivar C, Gutierrez R (2005) Blockade of the membranal GABA transporter potentiates GABAergic responses evoked in pyramidal cells by mossy fiber activation after seizures. Hippocampus 15:281–284

Volk D, Austin M, Pierri J, Sampson A, Lewis D (2001) GABA transporter-1 mRNA in the prefrontal cortex in schizophrenia: decreased expression in a subset of neurons. Am J Psychiatry 158:256–265

Wagner DA, Goldschen-Ohm MP, Hales TG, Jones MV (2005) Kinetics and spontaneous open probability conferred by the epsilon subunit of the $GABA_A$ receptor. J Neurosci 25:10462–10468

Wall MJ, Usowicz MM (1997) Development of action potential-dependent and independent spontaneous $GABA_A$ receptor-mediated currents in granule cells of postnatal rat cerebellum. Eur J Neurosci 9:533–548

Walls AB, Nilsen LH, Eyjolfsson EM, Vestergaard HT, Hansen SL, Schousboe A, Sonnewald U, Waagepetersen HS (2010) GAD65 is essential for synthesis of GABA destined for tonic inhibition regulating epileptiform activity. J Neurochem 115:1398–1408

Wang D, Quick MW (2005) Trafficking of the plasma membrane gamma-aminobutyric acid transporter GAT1. Size and rates of an acutely recycling pool. J Biol Chem 280:18703–18709

Wei W, Zhang N, Peng Z, Houser CR, Mody I (2003) Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. J Neurosci 23:10650–10661

Winsky-Sommerer R, Vyazovskiy VV, Homanics GE, Tobler I (2007) The EEG effects of THIP (Gaboxadol) on sleep and waking are mediated by the GABA(A)delta-subunit-containing receptors. Eur J Neurosci 25:1893–1899

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 12:1040–1062

Wojcik SM, Katsurabayashi S, Guillemin I, Friauf E, Rosenmund C, Brose N, Rhee JS (2006) A shared vesicular carrier allows synaptic corelease of GABA and glycine. Neuron 50:575–587

Woo TU, Whitehead RE, Melchitzky DS, Lewis DA (1998) A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. Proc Natl Acad Sci U S A 95:5341–5346

Wu Y, Wang W, Richerson GB (2001) GABA transaminase inhibition induces spontaneous and enhances depolarization-evoked GABA efflux via reversal of the GABA transporter. J Neurosci 21:2630–2639

Wu Y, Wang W, Richerson GB (2003) Vigabatrin induces tonic inhibition via GABA transporter reversal without increasing vesicular GABA release. J Neurophysiol 89:2021–2034

Wu Y, Wang W, Díez-Sampedro A, Richerson GB (2007) Nonvesicular inhibitory neurotransmission via reversal of the GABA transporter GAT-1. Neuron 56:851–865

Xi ZX, Ramamoorthy S, Shen H, Lake R, Samuvel DJ, Kalivas PW (2003) GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. J Neurosci 23:3498–3505

Yang S, Cox CL (2007) Modulation of inhibitory activity by nitric oxide in the thalamus. J Neurophysiol 97:3386–3395

Yang S, Cox CL (2011) Attenuation of inhibitory synaptic transmission by glial dysfunction in rat thalamus. Synapse 65:1298–1308

Yoon JH, Maddock RJ, Rokem A, Silver MA, Minzenberg MJ, Ragland JD, Carter CS (2010) GABA concentration is reduced in visual cortex in schizophrenia and correlates with orientation-specific surround suppression. J Neurosci 30:3777–3781

Yoon BE, Woo J, Justin LC (2012) Astrocytes as GABA-ergic and GABA-ceptive Cells. Neurochem Res 37(11):2474–9

Zhou Q, Petersen CC, Nicoll RA (2000) Effects of reduced vesicular filling on synaptic transmission in rat hippocampal neurones. J Physiol 525(Pt 1):195–206

Zoremba N, Homola A, Slais K, Vorisek I, Rossaint R, Lehmenkuhler A, Sykova E (2008) Extracellular diffusion parameters in the rat somatosensory cortex during recovery from transient global ischemia/hypoxia. J Cereb Blood Flow Metab 28:1665–1673

Chapter 7
GPCR Modulation of Extrasynapitic $GABA_A$ Receptors

William M. Connelly, Adam C. Errington, Josue G. Yagüe, Anna Cavaccini, Vincenzo Crunelli and Giuseppe Di Giovanni

Abstract γ-Aminobutyric acid type A ($GABA_A$) receptors ($GABA_A$Rs), the main inhibitory neurotransmitter-gated ion channels in the central nervous system, are finely tuned by other neurotransmitters and endogenous ligands. The regulation of synaptic $GABA_A$Rs ($sGABA_A$Rs) by G protein-coupled receptors (GPCRs) has been well characterized and is known to occur either through the conventional activation of second-messenger signalling cascades by G proteins or directly by protein–protein coupling. In contrast, research on the modulation of extrasynaptic $GABA_A$R ($eGABA_A$Rs) is still in its infancy and it remains to be determined whether both of the above mechanisms are capable of controlling $eGABA_A$R function. In this chapter, we summarize the available literature on $eGABA_A$R modulation by GPCRs, including $GABA_B$, serotonin (5-HT), dopamine (DA), noradrenaline (NA) and metabotropic glutamate (mGlu) receptors. Although at present these GPCRs-$eGABA_A$Rs cross-talks have been investigated in a limited number of brain areas (i.e., thalamus, cerebellum, hippocampus, striatum), it is already evident that $eGABA_A$Rs show a nucleus- and neuronal type-selective regulation by GPCRs that differs from that of $sGABA_A$Rs. This distinct regulation of $eGABA_A$Rs versus $sGABA_A$Rs by GPCRs provides mechanisms for receptor adaptation in response

V. Crunelli (✉) · W. M. Connelly · J. G. Yagüe · A. Cavaccini · G. Di Giovanni
Neuroscience Division, Cardiff School of Biosciences, Cardiff University, The Sir Martin Evans Building Museum Avenue, Cardiff CF10 3AX, UK
e-mail: crunelli@cardiff.ac.uk

G. Di Giovanni
Department of Physiology and Biochemistry, Faculty of Medicine,
Malta University, Msida, Malta
e-mail: giuseppe.digiovanni@um.edu.mt

J. G. Yagüe
Strathclyde Institute of Pharmacy and Biomedical Sciences,
University of Strathclyde, Glasgow, UK

A. Cavaccini
Department of Neuroscience and Brain Technologies,
Istituto Italiano di Tecnologia, Genoa, Italy

A. C. Errington
Neuroscience and Mental Health Research Institute, Institute of Psychological Medicine and Clinical Neuroscience, School of Medicine, Cardiff University,
Cardiff, UK

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_7

to a variety of physiological stimuli and under different pathophysiological conditions. Further research will advance our understanding of $eGABA_AR$ and GPCR signalling and offer novel targets for the treatment of many neurological and neuropsychiatric disorders where abnormalities in $eGABA_AR$ have been suggested to exist.

Keywords Monoamines · Tonic $GABA_A$ inhibition · Metabotropic receptors · Parkinson's disease · Epilepsy · Receptor Phosphorylation

Abbreviations

5-HT	Serotonin
CT	Carboxyl terminus
DA	Dopamine
dLGN	Dorsal lateral geniculate nucleus
DR	Dorsal raphe
$eGABA_ARs$	Extrasynaptic $GABA_AR$ ()
GABA	γ-Aminobutyric acid
$GABA_ARs$	$GABA_A$ receptors
GAERS	Genetic absence epilepsy rats from strasbourg
GP	Globus pallidus
GPCRs	Protein-coupled receptors
IPSCs	Inhibitory postsynaptic currents
IPSP	Inhibitory postsynaptic potential
MAP	Mitogen-activated protein
MD	Mediodorsal
mGLU	Metabotropic glutamate
mIPSCs	Miniature IPSCs
MR	Median raphe
MSNs	Medium spiny neurons
NA	Noradrenaline
NRT	Nucleus reticularis thalami
PD	Parkinson's Disease
PKA	Protein kinases A
PKC	Protein kinases C
PLC	Phospholipase C
PTK	Protein tyrosine kinase
$sGABA_ARs$	Synaptic $GABA_ARs$
sIPSCs	Spontaneous IPSCs
SNc	Substantia nigra pars compacta
TC	Thalamocortical
TRP	Transient receptor potential
TRPC4	Transient receptor potential channel 4
VTA	Ventral tegmental area

7.1 Introduction

γ-Aminobutyric acid (GABA) type A receptors ($GABA_A$Rs) are ubiquitously expressed throughout the central nervous system (CNS) and represent the principal inhibitory neurotransmitter receptors in the adult mammalian brain (Schwartz 1988). Two main $GABA_A$R populations exist that mediate two distinct forms of inhibition: the so-called phasic inhibition generated by synaptic $GABA_A$Rs (s$GABA_A$Rs) and 'tonic' inhibition mediated by extrasynaptic $GABA_A$Rs (e$GABA_A$Rs) (Farrant and Nusser 2005; Belelli et al. 2009).

The different properties of s$GABA_A$Rs and e$GABA_A$Rs originate from their different subunit composition, particularly the inclusion of the δ subunit in the dentate gyrus and cerebellar granule cells, thalamocortical (TC) and some cortical neurons (Nusser et al. 1998; Pirker et al. 2000; Nusser and Mody 2002; Belelli et al. 2005; Cope et al. 2005; Jia et al. 2005), and the α5 subunit in CA1 and CA3 hippocampal pyramidal cells (Caraiscos et al. 2004; Hortnagl et al. 2013). The δ subunit containing e$GABA_A$Rs co-assemble with two α (α4 or α6) and two β subunits. The α5 subunit containing e$GABA_A$R usually co-assemble with α, β and γ2 subunit. α1 and α2 subunits as well as β3 subunits are also found at extrasynaptic locations on the soma of hippocampus CA1 pyramidal neurons, suggesting that these subunits may also contribute to e$GABA_A$R signalling and their specific pharmacological properties (Kasugai et al. 2010).

GABA released in the synaptic cleft brings about brief changes of membrane conductance due to the activation of s$GABA_A$R which gives rise to phasic inhibition. On the other hand, the very low GABA concentration that is present in the extracellular space can activate e$GABA_A$R-mediated tonic inhibition that occurs in a much more spatially and temporally diffuse manner (Farrant and Nusser 2005). This form of inhibition has been identified in the cerebellum (Brickley et al. 1996), hippocampus (Stell and Mody 2002), striatum (Ade et al. 2008) and thalamus (Cope et al. 2005). Importantly, increasing evidence has uncovered not only some of the physiological roles of e$GABA_A$Rs but also their involvement in diverse neurological and neuropsychiatric disorders (Belelli et al. 2009; Hines et al. 2012), including stroke (Clarkson et al. 2011), epilepsy (Cope et al. 2009; Di Giovanni et al. 2011a), anxiety (Lydiard 2003), depression (Maguire et al. 2005; Luscher et al. 2011), schizophrenia (Guidotti et al. 2005) and autism (Pizzarelli and Cherubini 2011).

GPCRs are single-polypeptide proteins containing seven hydrophobic transmembrane domains that transduce extracellular neurotransmitter signals into the cell interior by interacting with heterotrimeric G proteins (Dohlman et al. 1991; Neer 1995). These, in turn, modulate a diverse array of cellular effectors to produce changes in cellular second-messenger systems and/or ionic conductance and ultimately physiological responses. GPCRs engage many signalling pathways that involve protein kinases A and C (PKA and PKC), protein tyrosine kinase (PTK), through PKC interaction with β-arrestin, Src kinase, and hence the mitogen-activated protein (MAP) kinase cascades (Luttrell and Luttrell 2004).

GPCR modulation of $sGABA_ARs$ via classical phosphorylation has been extensively investigated, and for dopamine Rs (DARs) and $GABA_BRs$ it is also well established that a direct protein–protein interaction exists between each of these GPCRs and $sGABA_ARs$. Instead, the regulation of $eGABA_AR$ by GPCRs is a relatively new area of research, and in this chapter, we review the evidence in support of a modulation of $eGABA_ARs$ by the metabotropic $GABA_B$, serotonin (5-HT), DA, noradrenaline (NA) and metabotropic glutamate (mGlu) receptors in different brain areas.

7.2 $GABA_BRs$—$eGABA_ARs$ Interaction

$GABA_BRs$ are heteromeric complexes composed of a $GABA_{B1}$ and a $GABA_{B2}$ subunit (Kaupmann et al. 1998). Their heterogeneity results from the $GABA_B$ subunit isoforms ($GABA_{B1a-j}$), most prominently $GABA_{B1a}$ and $GABA_{B1b}$, which combine with $GABA_{B2}$ to form functional receptors (Belley et al. 1999; Ulrich et al. 2007). $GABA_BRs$ are found both pre- and postsynaptically: Presynaptic $GABA_BRs$ regulate the release of GABA (autoreceptors) and other neurotransmitters (heteroreceptors), whereas postsynaptically they elicit a slow inhibitory postsynaptic potential (IPSP) (Bettler et al. 2004). These actions are mediated by stimulation of intracellular G-protein signalling cascades that activate inwardly rectifying K^+ channels, inhibit voltage-gated Ca^{2+} channels, and regulate cyclic adenosine monophosphate (cAMP) and PKA (Padgett and Slesinger 2010).

A direct physical interaction between discrete regions of $GABA_{B1}R$ and the $GABA_AR$ γ2S subunit has been described. This association has significant consequences for $GABA_BR$ trafficking and endocytosis, both promoting $GABA_{B1}$ surface expression in the absence of $GABA_{B2}$, as well as enhancing $GABA_{B1}$ endocytosis in the presence of $GABA_{B2}$ (Balasubramanian et al. 2004). Moreover, the physical association between $GABA_ARs$ and $GABA_BRs$ increases the potency of GABA acting at $GABA_ARs$ expressed in oocytes probably due to a conformational change in the $GABA_AR$ complex that enhances its affinity for GABA (Balasubramanian et al. 2004). The precise physiological significance of this association, however, remains to be elucidated as no in situ data were provided by this study. Similarly, future in vivo studies should investigate whether this direct physical interaction occurs for both $sGABA_ARs$ and $eGABA_ARs$, though γ2S-containing $GABA_ARS$ are almost exclusively synaptic in location.

Following the original observation of an increase in tonic $GABA_A$ current by γ-hydroxybutyric acid (GHB), a weak $GABA_B$ agonist (Cope et al. 2009), two recent studies using GABA and the exogenous, selective $GABA_B$ agonists, baclofen, have now conclusively demonstrated a GPCR-mediated interaction between $GABA_BRs$ and $eGABA_ARs$. This interaction appears is present in TC neurons, cerebellar and dentate gyrus granule cells (Fig. 7.1), but not in hippocampal CA1 or cortical layer 2/3 pyramidal neurons (Tao et al. 2013). It does not result from changes in the release or uptake of GABA, since it is antagonized by direct intracellular infusion of

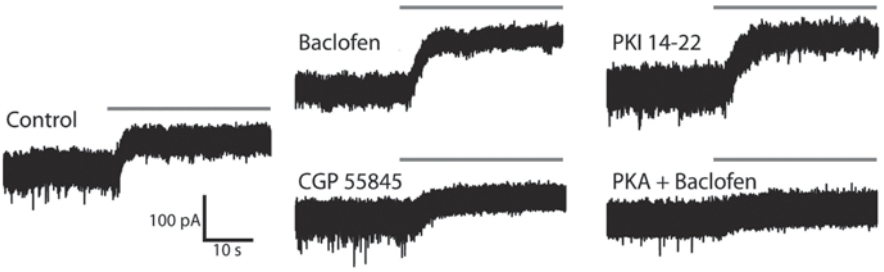

Fig. 7.1 $GABA_BRs$ regulation of $eGABA_A$ receptors. Whole cell voltage clamp recordings from VB TC neurons showing $GABA_BR$ modulation of the tonic $GABA_A$ current. The magnitude of the tonic current was revealed by application of gabazine (>100 μM) as shown by the grey bars. The $GABA_BR$ agonist R-baclofen (5 μM) increased the size of the tonic current relative to control, while the $GABA_BR$ antagonist CGP 55848 (1 μM) reduced it. The effect of baclofen could be mimicked by infusing the PKA inhibitor PKI 12–22 (50 ng/mL) or blocked by infusing the active catalytic subunit of PKA (10 IU/mL)

G-protein antagonists into the postsynaptic neuron (Fig. 7.1) (Connelly et al. 2013; Tao et al. 2013). The exact mechanism of this $GABA_BR$-mediated enhancement of $eGABA_AR$ activity is unclear, though it seems to be independent on the synthesis of new channels since a maximal tonic $GABA_A$ current augmentation can be achieved within 5–10 min from the application of baclofen. Indeed, by diffusing drugs intracellularly, it could be demonstrated that the signalling pathway is dependent on PKA (Connelly et al. 2013) (Fig. 7.1), indicating direct control of $eGABA_ARs$ via phosphorylation, as has been demonstrated in expression systems (Tang et al. 2010). Interestingly, both studies (Connelly et al. 2013; Tao et al. 2013) reported that a $GABA_B$ antagonist reduced the tonic $GABA_A$ current even in the absence of activation by a $GABA_B$ agonist (Fig. 7.1), indicating that at least in brain slices ambient GABA levels in thalamus, cerebellum and dentate gyrus are sufficient to activate this $GABA_BR$—$eGABA_A$ interaction.

The fact that $GABA_BRs$ appear to regulate the tonic $GABA_A$ current in a number of neuronal types gives us the possibility of speculating on the molecular make up of $eGABA_ARs$. Phosphorylation by PKA leads to an enhancement in $GABA_ARs$ containing β3 subunits, a decrease in function in those containing β1, and no effect in those with a β2 subunit (McDonald et al. 1998). Hence, the effect of $GABA_BR$ activation (direct PKA infusion in the postsynaptic neuron) on $eGABA_ARs$ could imply the presence of a significant proportion of β1-containing $GABA_A$ receptors in TC neurons, dentate gyrus and cerebellar granule cells. Anatomical studies have indicated an abundance of β1 subunits in cerebellar and dentate gyrus granule cells (though also large amounts of β3), whereas the thalamus has been reported to contain largely β2 subunits (Wisden et al. 1992; Pirker et al. 2000). Thus, we are left with the possibility that either PKA-mediated phosphorylation of β subunits can produce different responses depending on the neuronal type, or more likely that there are indeed significant complements of β1-containing receptors in the thalamus, dentate gyrus and cerebellum, which may be restricted to extrasynaptic sites.

Interpreting the circuit-level response to the $GABA_B$R-mediated increase of the tonic $GABA_A$ current requires careful considerations, as it is unlikely that endogenously released GABA could activate $GABA_B$Rs without activating $eGABA_A$Rs and vice versa. On the one hand, we have the situation where an increasing titre of GABA will activate more $eGABA_A$Rs and lead to an enhancement of their function via the concomitant recruitment of $GABA_B$Rs. That is, during a period of enhanced GABA release, the current mediated by $eGABA_A$Rs will be increased via activation of both $GABA_B$Rs and $eGABA_A$ receptors. On the other hand, $GABA_B$Rs are known to decrease the probability of vesicular GABA release. Thus, the sum effect of delivering a $GABA_B$ agonist in vivo is hard to predict, since it may decrease the release of GABA, and in turn the activity of $eGABA_A$Rs, to a greater extent than the $GABA_B$R-mediated enhancement of $eGABA_A$R activity, leading to a total reduction in the tonic $GABA_A$ current.

7.3 DARs—$eGABA_A$Rs Interaction

DARs are members of the GPCR super-gene family (Kebabian and Calne 1979), and dysfunction of central DA systems underlies the pathophysiology of many neurological and neuropsychiatric disorders, including depression, schizophrenia, attention deficit hyperactivity disorders (ADHD), drug abuse, Gilles de la Tourette's syndrome, Alzheimer's disease, Parkinson's disease (PD) and epilepsy (Di Giovanni 2008, 2010). DARs are divided into two subclasses, namely D1-like (D1 and D5) and D2-like (D2, D3 and D4) (Missale et al. 1998). In general, D1-like Rs are positively coupled to AC whereas D2-like Rs usually inhibit this enzyme. D1Rs and D2Rs regulate PKA phosphorylation via differing second-messenger cascade systems: D1Rs agonists activate and D2R agonists inactivate PKA (Chen et al. 2006) through Gs/Golf and Gi/o proteins, respectively (Stoof and Kebabian 1984).

Increasing evidence indicates that the interaction between the DA and GABA systems in the brain can be mostly attributed to the functional interactions between their Rs (Liu et al. 2000; Lee et al. 2005). These are not only mediated by classical second-messenger systems (which are reviewed below) but a direct protein–protein interaction between these two Rs has also been reported which is mediated by the carboxyl terminus (CT) of the D5R and the second intracellular loop of the $GABA_A$R γ2 subunit (Liu et al. 2000). This D5R–$GABA_A$R coupling mutually inhibits the activity of both Rs. Thus, $GABA_A$R stimulation inhibits the ability of D5Rs to activate AC, whereas D5R activation decreases synaptic $GABA_A$R-mediated inhibition (Liu et al. 2000; Lee et al. 2005). Whether similar or different protein–protein interactions exist between DARs and $eGABA_A$Rs remains to be investigated.

7.3.1 Striatum

The striatum is the main input of the basal ganglia circuitry, involved in controlling motor behaviours and important cognitive functions (Di Giovanni 2007). It

not only receives large dopaminergic innervations from the mesencephalic nuclei mainly from the substantia nigra pars compacta (SNc) but also from the ventral tegmental area (VTA). DA released in the striatum binds to D1-like Rs and D2-like Rs on striatal neurons, modulating their intrinsic excitability and synaptic plasticity. The medium spiny neurons (MSNs) are the GABAergic principal projecting striatal neurons and selectively express D1Rs on those of the direct pathway (to the spiny neuron (SN)) and D2Rs on those of the indirect pathway (to the external part of pallidum) (Gerfen et al. 1990; Di Giovanni et al. 2009).

The subunit composition of $GABA_A$Rs expressed in the striatum have been the subject of intense research, sometimes with contrasting results (Liste et al. 1997; Flores-Hernandez et al. 2000; Schwarzer et al. 2001; Ade et al. 2008; Santhakumar et al. 2010). The striatum stains positively for α1–α5 subunits (though weakly for α3 and α5), all β subunits, as well as γ2 and δ subunits (Pirker et al. 2000). On the other hand, it has been reported that MSNs do not express α3 and α6 subunits (Liste et al. 1997; Rodriguez-Pallares et al. 2000; Schwarzer et al. 2001), while they do express α2 and β2/β3 subunits (Liste et al. 1997). Moreover, single-cell PCR suggests that β1 and β3 subunits are expressed by MSNs (Flores-Hernandez et al. 2000), while β2 subunits appear to be solely expressed on striatal interneurons (Schwarzer et al. 2001).

Vicini's (Ade et al. 2008; Janssen et al. 2009; Janssen et al. 2011; Luo et al. 2013) and other groups (Kirmse et al. 2008; Janssen et al. 2009; Santhakumar et al. 2010) have provided solid evidence for the presence and the developmental profile of a tonic $GABA_A$ current in MSNs. (Note, however, that one study did not find any evidence for a tonic $GABA_A$ current in $D2^+$ or $D1^+$ MSNs; Gertler et al. 2008.) D2+ MSNs have greater tonic $GABA_A$ current than D1+ MSNs (Ade et al. 2008). Moreover, D2+ MSNs are more sensitive to low doses of GABA than D1+ MSNs. In young mice (P 16–25), application of the $GABA_A$R antagonist, bicuculline, in addition to blocking spontaneous inhibitory postsynaptic currents (IPSCs), consistently induces a reduction in holding current in D2+ MSNs, suggestive of an endogenous tonic $GABA_A$ current. This effect can also be observed in D1+ MSNs, although its magnitude is significantly smaller, or in some neurons can be absent altogether (Ade et al. 2008). The strong Tetrodotoxin (TTX)-sensitivity of tonic $GABA_A$ current in MSN reveals that synaptic spillover is the primary source for the ambient GABA that in striatum is responsible for eliciting the tonic current (Ade et al. 2008), as it is the case in other brain regions (Bright et al. 2007).

The larger tonic current in D2+ cells of young mice is likely to be mediated by α5β3γ2 receptors. This is based on the evidence of (i) a differential expression α5- and β3-containing receptors in D2+ neurons compared to D1+ neurons, (ii) the similar expression and effect of δ-subunit-containing $GABA_A$ between the two MSN populations, with a minimal contribution to tonic GABA currents and (iii) the lack of effect of α1-containing $GABA_A$R receptors activation on both D2+ and D1+ MSNs (Ade et al. 2008; Janssen et al. 2009; Santhakumar et al. 2010; Janssen et al. 2011).

$GABA_A$R subunits, however, are developmentally regulated, with a progressive decline in α5 subunits and an increase in α4 and δ subunits with age (Laurie et al. 1992). Therefore, it is not surprising that there is a developmental reversal in the

tonic $GABA_A$ current profile of adult striatal MSNs (>P30) mice (magnitude and $GABA_A$R subunit contribution) i.e., larger tonic current in D1+ MSNs, due to an increase of δ-containing $GABA_A$Rs, and a smaller current in D2+ MSNs, due to a decreased expression of the α5 subunit (Santhakumar et al. 2010). This developmental switch in the tonic inhibitory control of the striatal output neurons from those of the indirect pathway to those of the direct pathway is likely to alter the input–output curve of the striatal circuit.

Importantly, the tonic $GABA_A$ current in MSNs is modulated by DA1Rs and DA2Rs in both young and adult mice (Janssen et al. 2009). Although DA is present in such low concentrations that it does not activate D1Rs and D2Rs in striatal slices, D2R stimulation with quinpirole decreases the tonic currents in D2+ MSNs, whereas D1R activation with SKF-81297 induces a tonic $GABA_A$ current in D1+ MSNs (Fig. 7.2) (Janssen et al. 2009). This dopaminergic modulation of the tonic current is likely due to changes in the phosphorylation state of e$GABA_A$Rs in both young and adult mice, and β1- and β3-subunits are substrates for PKA-mediated phosphorylation (Moss et al. 1992; Poisbeau et al. 1999; Flores-Hernandez et al. 2000; Vithlani and Moss 2009; Kang et al. 2011). Despite this, it has been shown in other brain areas that s$GABA_A$ response to phosphorylation is subunit-specific, with β1-subunit phosphorylation reducing and β3-subunit phosphorylation enhancing synaptic GABAergic currents (McDonald et al. 1998; Nusser et al. 1999; Flores-Hernandez et al. 2000) DA agonists and intracellular PKA application fail to significantly alter s$GABA_A$ currents in the striatum (Janssen et al. 2009). In conclusion, DA modulates exclusively tonic $GABA_A$ currents and not IPSCs in this nucleus.

7.3.2 Thalamus

The rodent thalamus receives a sparse dopaminergic innervation from the mesencephalic nuclei (Groenewegen 1988; Papadopoulos and Parnavelas 1990; Garcia-Cabezas et al. 2007, 2009) and expresses moderate levels of DA receptors (Wamsley et al. 1989; Weiner et al. 1991; Khan et al. 1998). The exact cellular localization of DARs within the thalamus is largely unknown. However, electrophysiological and immunohistochemical findings have shown that the nucleus reticularis thalami (NRT) is rich in D4Rs (Khan et al. 1998) expressed presynaptically on globus pallidus (GP) terminals, and their activation by DA release reduces this inhibitory input to the NRT neurons (Floran et al. 2004; Gasca-Martinez et al. 2010). Compelling in vitro electrophysiological evidence shows that DA is capable of modifying the excitability of thalamic neurons, an effect to which both D1Rs and D2Rs are suggested to contribute with cellular and nucleus specificity. For example, D2Rs but not D1Rs are involved in DA-mediated excitation of mediodorsal (MD) thalamic neurons (Lavin and Grace 1998), while DA acting via D1Rs leads to a membrane depolarization in dorsal lateral geniculate nucleus (dLGN) TC neurons (Govindaiah and Cox 2005). On the other hand, DA may indirectly inhibit these neurons via D2R excitation of local GABAergic interneurons, producing an increase of phasic

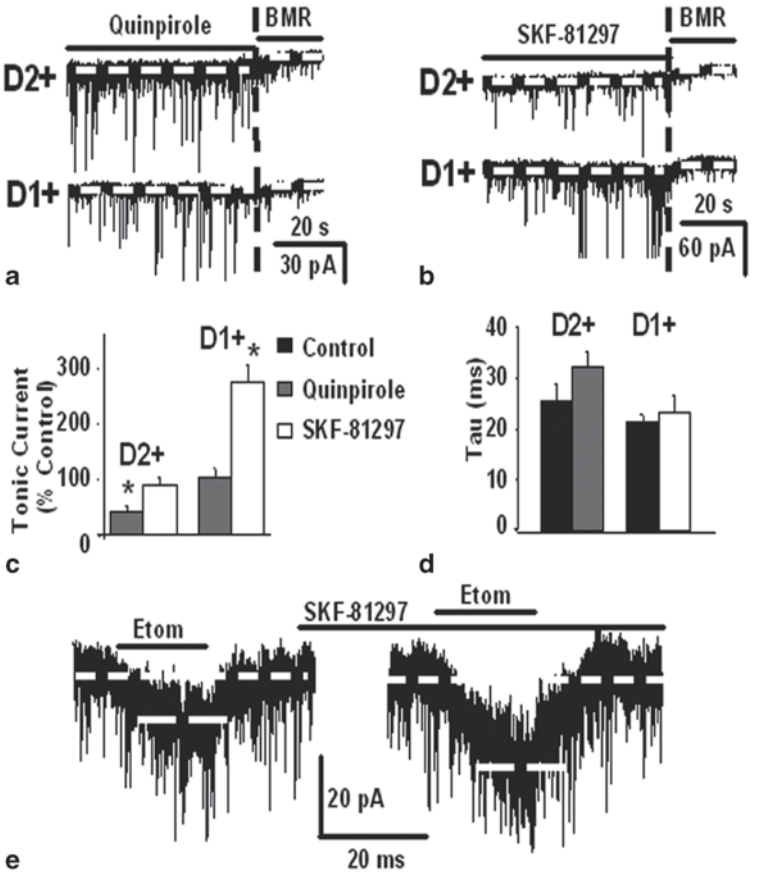

Fig. 7.2 Modulation of the $eGABA_A$ tonic current in the striatum. **a** Representative current traces from individual D2+ and D1+ MSN illustrating that the D2 agonist, quinpirole (10 μM), reduces tonic current in the D2+ MSN, while it does not affect tonic currents in the D1+ MSN. **b** Representative traces of a simultaneous dual recording between a D2+ and D1+ MSN illustrating that the D1 agonist, SKF-81297 (10 μM), induces a tonic current in the D1+ MSN, but also reduces it in the D2+ MSN. **c** Summary graph showing effects on tonic current with quinpirole and SKF-81297 application on D2+ ($n = 5$ and 3) and D1+ ($n = 6$ and 4). **d** Summary graph of phasic currents of both D2+ and D1+ in response to their respective agonists ($n = 6$ and 5 for D2+, $n = 8$ and 5 for D1+). **e** Representative current trace from a D1+ neuron where etomidate (3 μM) was given prior to and during SKF-81297 (10 μM) application. SKF-81297 was given for over 5 min before co-application with etomidate to allow full drug action. (Reproduced with permission from Janssen et al. 2009)

$GABA_A$ inhibition (Munsch et al. 2005). In agreement with this inhibitory role of DA in the dLGN, an increase of the tonic $GABA_A$ current during DA application has also been reported in rat TC neurons of this thalamic nucleus (Di Giovanni et al. 2008).

DA also modulates the activity of ventrobasal (VB) TC neurons via activation of both DAR subtypes (Govindaiah et al. 2010). In particular, DA, acting

postsynaptically at D2Rs, increases action-potential discharges, and via D1R activation, it induces membrane depolarization. As far as inhibition is concerned, DA has no effect on miniature IPSCs (mIPSCs) (Yague et al. 2013), but strongly depresses the amplitude of $eGABA_AR$-mediated tonic inhibition in VB TC neurons of Wistar rats (Yague et al. 2013). Quinpirole and PD-168,077 (D2R and D4R agonists respectively) also reduced the tonic current without altering phasic inhibition (Fig. 7.4). These effects are not due to a decreased vesicular GABA release, since $GABA_A$ sIPSC frequency, a measure of action-potential-dependent vesicular GABA release, is unaffected by DA and the two agonists. Based on the following evidence: (i) quinpirole binds with higher affinity at D3/4Rs than at D2Rs (Sokoloff et al. 1990) and mimics the DA effects, (ii) D3Rs are not considerably expressed in the thalamus (Gurevich and Joyce 1999) and (iii) PD-168,077 is a selective and potent D4 agonist (Glase et al. 1997), DA effects might be mediated by D4Rs.

It is difficult to speculate on the localization of the DARs that mediate the above effects on the tonic current using available electrophysiological data. DA might decrease eGABAR activity by reducing glial GABA release and its ambient concentration, since (i) evidence exists for astrocytic expression of DARs in some brain areas (Khan et al. 2001; Miyazaki et al. 2004), and (ii) astrocyte-neuron GABA signalling in the VB specifically targets $eGABA_ARs$ (Jimenez-Gonzalez et al. 2011). The only clear evidence regarding DAR localization in the thalamus is the high expression of D4Rs in the NRT (Ariano et al. 1997), which are located presynaptically on GP terminals and negatively control their inhibitory input to the NRT (Gasca-Martinez et al. 2010). D4R activation consequently decreases intra-NRT GABA release (Floran et al. 2004), leading, in turn, to an increased firing of GABAergic NRT neurons (Gasca-Martinez et al. 2010). However, selective D4R activation does not affect $sGABA_AR$ activity, indicating no change of NRT input to the VB. Thus, the effect of D4R activation on $eGABA_AR$ current (Yague et al. 2013) might be due to D4Rs express in VB TC neurons, in agreement with other recent electrophysiological evidence (Govindaiah et al. 2010).

Finally, in view of the increased $eGABA_AR$ function that is present in different experimental models of absence epilepsy (Cope et al. 2009), it is interesting to note that in the polygenic absence model called genetic absence epilepsy rats (GAERS) from Strasbourg, D4R activation decreases the tonic but not the phasic $GABA_A$ current (Fig. 7.3) (Yague et al. 2013). Since a selective reduction of the tonic current in the VB has been shown to drastically reduced absence seizures (Cope et al. 2009), it is possible that the known anti-absence effects of some dopaminergic drugs (Marescaux et al. 1992) may occur in part by their ability to decrease thalamic $eGABA_AR$ function.

---

and averaged IPSCs (*bottom*) from different TC neurons of the VB thalamus of Wistar rats under control conditions (*left*) and in the continuing presence of SKF39383 (50 μM), quinpirole (50 μM) and PD-168,077 (100 μM; *right*). (B_2, C_2, D_2) Summary of the effects of SKF39383, quinpirole and PD-168,077 on $GABA_A$ tonic current (*left*) and normalised $GABA_A$ tonic current (*right*). (E_1) Representative current traces (*right*) and averaged IPSCs (*left*) from different TC neurons in VB slices from GAERS under control conditions (*top*) and in the continuing presence of quinpirole (50 μM; *middle*) and PD-168,077 (100 μM; *bottom*). (E_2) Summary of the effects of quinpirole and PD-168,077 on $GABA_A$ tonic current (*top*) and normalised $GABA_A$ tonic current (*bottom*)

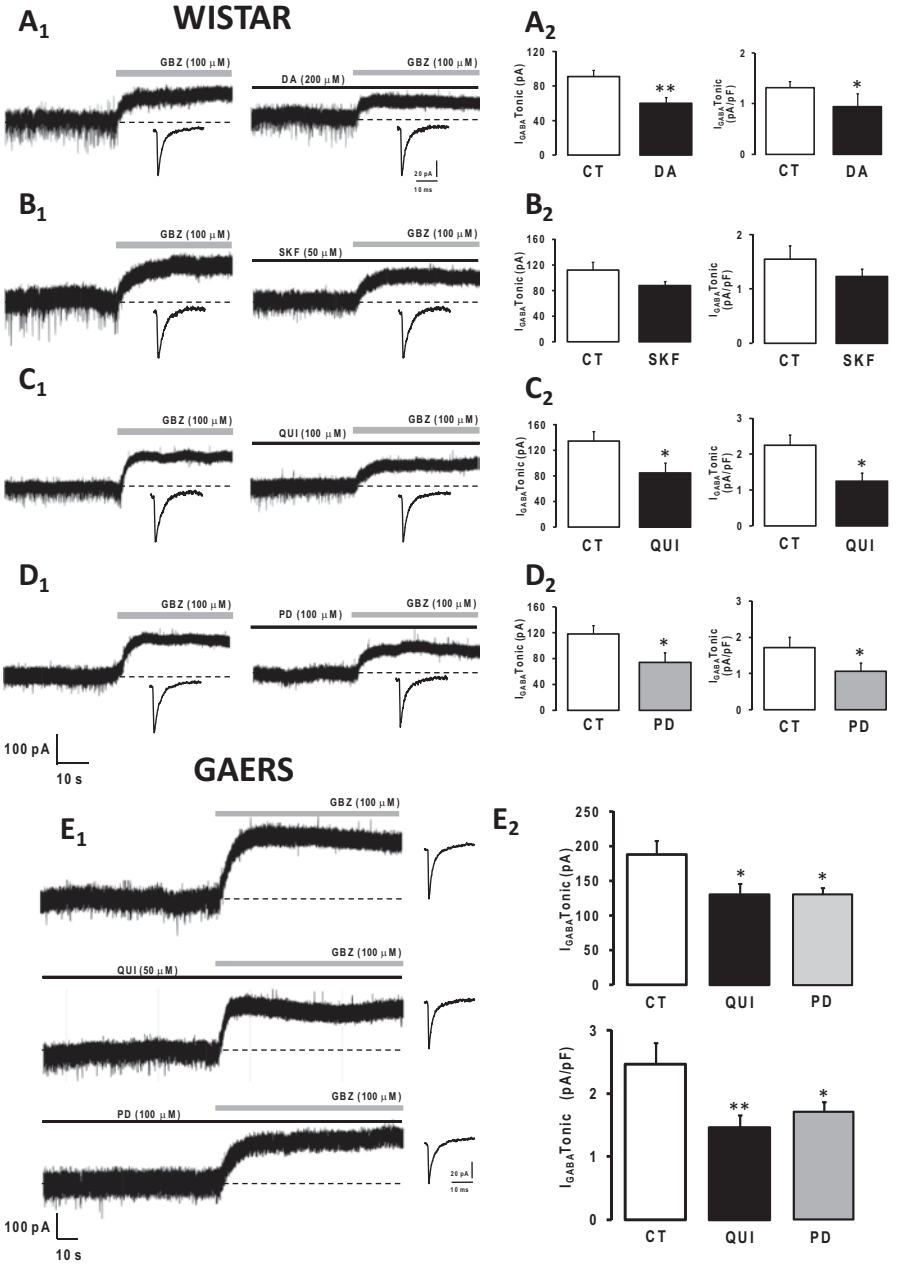

Fig. 7.3 Dopaminergic modulation of the tonic and phasic $GABA_A$ current of VB TC neurons of Wistar rats and Genetic Absence Epilepsy Rats from Strasbourg (GAERS). (A_1) Representative current traces (top) and averaged IPSCs (bottom) from different TC neurons in VB slices from Wistar rats, under control conditions (100 μM ascorbic added to aCSF) and in the continuing presence of DA (200 μM, right). Focal application of gabazine (GBZ, 100 μM, *grey bar*) reveals different magnitude of tonic $GABA_A$ tonic current. (A_2) Summary of the effects of DA on the tonic current (right) and normalised I_{GABA} tonic. (B_1, C_1, D_1) Representative current traces (*top*)

In summary, DA can excite VB neurons through different actions, one of which involves a reduction in the tonic $GABA_A$ current. This effect likely involves PKA-dependent phosphorylation similarly to DA modulation of the excitability of MSNs (Janssen et al. 2009).

7.4 5-HTRs—eGABA$_A$Rs Interaction

Virtually all brain regions receive innervation from serotonergic fibres arising from cell bodies of the two main subdivisions of the midbrain serotonergic nuclei, the dorsal (DR) and the median raphe (MR) (Dahlstrom and Fuxe 1964; Hillegaart 1991; Abrams et al. 2004; Di Giovanni et al. 2010; Hale and Lowry 2010). The diverse physiological effects of 5-HT in the brain are mediated by a variety of distinct receptors. These receptors are presently divided into seven classes (5-HT_1–5-HT_7), which are then subdivided into subclasses with a total of at least 14 different receptors (Barnes and Sharp 1999; Di Giovanni et al. 2011b), based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms (Hoyer et al. 2002). With the exception of the ionotropic 5-HT_3R, all other 5-HTRs are GPCRs which act through intracellular signalling pathways to have a myriad of effects on their host cells (Di Giovanni et al. 2011b).

5-HT-containing cell bodies of the raphe nuclei send projections to GABAergic cells in different brain areas and receive a reciprocal GABAergic innervation from raphe interneurons and projecting neurons from many other areas (Bagdy et al. 2000). Much attention has been devoted to the role of 5-HTRs in the control of GABA inhibition, because of their implication in the pathophysiology of many diseases that affect central GABAergic systems, including schizophrenia, depression, drug abuse, sleep disorders and epilepsy (Di Giovanni et al. 2001; Bankson and Yamamoto 2004; Invernizzi et al. 2007; Nikolaus et al. 2010). The precise nature of the interaction between 5-HT and GABA, however, has been difficult to elucidate, in that both inhibitory and excitatory roles for 5-HT have been demonstrated. These discrepancies may be attributable to the differential distribution, and/or the diverse functional roles of 5-HTR subtypes within different GABAergic systems in various brain regions (Jacobs and Azmitia 1992; Barnes and Sharp 1999; Hoyer et al. 2002).

5-HT increases the frequency of $GABA_A$ mIPSCs in a subpopulation of VTA and SNc dopaminergic neurons (Pessia et al. 1994; Theile et al. 2009), nucleus raphe magnus serotoninergic neurons (Inyushkin et al. 2010), spinal dorsal horn neurons (Inyushkin et al. 2010) and suprachiasmatic nucleus neurons (Bramley et al. 2005), but depresses evoked IPSCs in neurons of the rat dorsolateral septal nucleus (Matsuoka et al. 2004). Since the amplitude of mIPSCs is not affected by 5-HT or its ligands, it is highly likely that 5-HT augments GABAergic synaptic transmission via presynaptic mechanisms. Indeed, an increase in Ca^{2+} release from intracellular stores via 5-HT_{2C}R activation has been shown to be involved in the ethanol-induced enhancement of GABA release onto DA-containing VTA neurons (Theile et al. 2009).

The cross-communication between 5-HTRs and $GABA_A$Rs might also be postsynaptic. 5-HT_{2C}R activation produces long-lasting inhibition of $GABA_A$ current in Xenopus oocytes co-expressing both types of receptors, an effect that required elevation of intracellular Ca^{2+} levels (Huidobro-Toro et al. 1996). Interestingly, experiments in Xenopus oocytes using protein kinase and phosphatase inhibitors suggest that the 5-HT_{2C}R modulatory process does not involve changes in the phosphorylation state of $GABA_A$Rs with different subunit compositions (Huidobro-Toro et al. 1996). On the other hand, some evidence shows that PKA and PKC are involved in mediating the effects of 5-HT on $GABA_A$R function. Thus, activation of postsynaptic 5-HT_2Rs in prefrontal cortex pyramidal neurons has been shown to inhibit $GABA_A$ current via a PKC-induced phosphorylation of $GABA_A$ γ2 subunit, which is dependent on activation of the RACK1-anchored PKC (Feng et al. 2001). Moreover, 5-HT_4R activation modulates $GABA_A$R currents bi-directionally, depending on the basal PKA activation levels. Thus, elevated levels of PKA due to increased neuronal activity have been shown to reverse the enhancing effect of 5-HT_4R activation into depression of neuronal excitability (Cai et al. 2002).

7.4.1 Thalamus

The role of 5-HT in the thalamus is complex and not yet fully understood, most likely due to the plethora of effects that can be elicited by the activation of its 14 different receptor subtypes. Thalamic 5-HT projections mainly originate from the small-to-medium sized 5-HT neurons located in the DR and MR nuclei (Dahlstroem and Fuxe 1964; Vertes et al. 1999). Several studies have described a dense and heterogeneous distribution of 5-HT fibers within the thalamus; serotonergic fibers are heavily concentrated in midline, intralaminar and association thalamic nuclei, and with the exception of the dLGN, they are also weakly distributed in principal thalamic nuclei (Vertes et al. 2010; Rodriguez et al. 2011).

Among thalamic 5-HTRs (Mengod et al. 2010), 5-HT_{1A}Rs and 5-HT_{2A}Rs, which are coupled to Gαq/G11-proteins and activate phospholipase C (PLC) β (Di Giovanni et al. 2006), are relatively highly expressed in the GABAergic neurons of the NRT (Li et al. 2004; Bonnin et al. 2006; Rodriguez et al. 2011). 5-HT_{1A}Rs are mainly present on the soma and proximal dendrites of these neurons, whereas 5-HT_{2A}R are less abundant and moderately expressed on cell bodies and more abundant on fine and medium-sized dendrite (Rodriguez et al. 2011). 5-HT_{2A}Rs and 5-HT_{2C}Rs are also present in TC neurons of the rodent dLGN (Li et al. 2004; Coulon et al. 2010). A recent study has shown a preferential immunohistochemical staining for 5-HT_{2C}Rs versus 5-HT_{2A}Rs in mice dLGN TC neurons, although these receptors were not somatically expressed (Coulon et al. 2010). Indeed, 5-HT_2R mRNA has been detected in GABAergic interneurons of the dLGN, with similar pattern of expression for the 2A and 2C subtypes (Munsch et al. 2003).

Another receptor by which 5-HT can produce its function in the thalamus is the 5-HT_7R. Indeed, the highest expression of this 5-HTR in the rat brain does occur

in the intralaminar and midline thalamic nuclei, where it strongly modulates neuronal excitability by inhibiting the calcium-activated potassium conductance that is responsible for the slow afterhyperpolarization (Goaillard and Vincent 2002). In contrast, 5-HT_7Rs depolarize neurons of the anterodorsal thalamic nucleus primarily by increasing I_h through a cAMP-dependent and PKA-independent mechanism (Chapin and Andrade 2001).

7.4.2 Dorsal Lateral Geniculate Nucleus of the Thalamus

A common action of brainstem neurotransmitters is to depolarize the membrane potential of TC neurons, causing a shift from rhythmic bursting to tonic-firing activity (McCormick 1992b). Membrane depolarization by 5-HT is in large part caused by inhibition of a leak in K^+ conductance (Meuth et al. 2006) and by modulation of the hyperpolarization-activated, non-selective cation current I_h (Pape and McCormick 1989; Chapin and Andrade 2001). 5-HT and 5-HT_{2C}R activation produce comparable membrane depolarizations which depend on Gq-coupled intracellular signalling cascades (Coulon et al. 2010). The intracellular pathways that couple the 5-HT_2Rs to the Ca^{2+}-influx mechanism seems to depend on the PLC system: This does not involve Ca^{2+} release nor voltage-gated Ca^{2+} channels in the plasma membrane, but is critically dependent on the transient receptor potential (TRP) protein, transient receptor potential channel 4 (TRPC4) (Munsch et al. 2003).

Our experiments and observations show that 5-HTR modulation has complex effects on phasic and tonic $GABA_A$ currents. Interestingly, application of 5-HT itself does not result in a change in the tonic $GABA_A$ current in dLGN of TC neurons, but results in an increased frequency of sIPSCs (Fig. 7.4) (Di Giovanni et al. 2008). Similarly, application of the 5-$HT_{1A/7}$R agonist, 8-OH-DPAT, does not change the tonic current, but increases the weighted decay time constant and the frequency of mIPSCs. Application of the 5-$HT_{2A/2C}$R agonist, α-methyl-5-hydroxytryptamine (α-M-5-HT), leads to a massive increase in the tonic current and in the peak amplitude and frequency of mIPSCs. These effects are mediated by 5-HT_{2A}Rs since they were blocked by ketanserin, an antagonist with higher selectivity for 5-HT_{2A}Rs than 5-HT_{2C}Rs, but not by SB 242084, a selective 5-HT_{2C}R antagonist (Fig. 7.4). Moreover, concomitant application of 5-HT and ketanserin results in a decreased tonic $GABA_A$ current and an increase in the weighted decay time constant and charge transfer of mIPSCs. Finally, application of the unselective 5-HT_{2C}R agonist m-chlorophenylpiperazine (mCPP) markedly decreases the tonic current, whereas most mIPSC properties are unchanged with the exception of a decrease in peak amplitude. These effects of mCPP are likely mediated by 5-HT_{2C}Rs since they are blocked by co-application of SB 242084 (Fig. 7.4) (Di Giovanni et al. 2008).

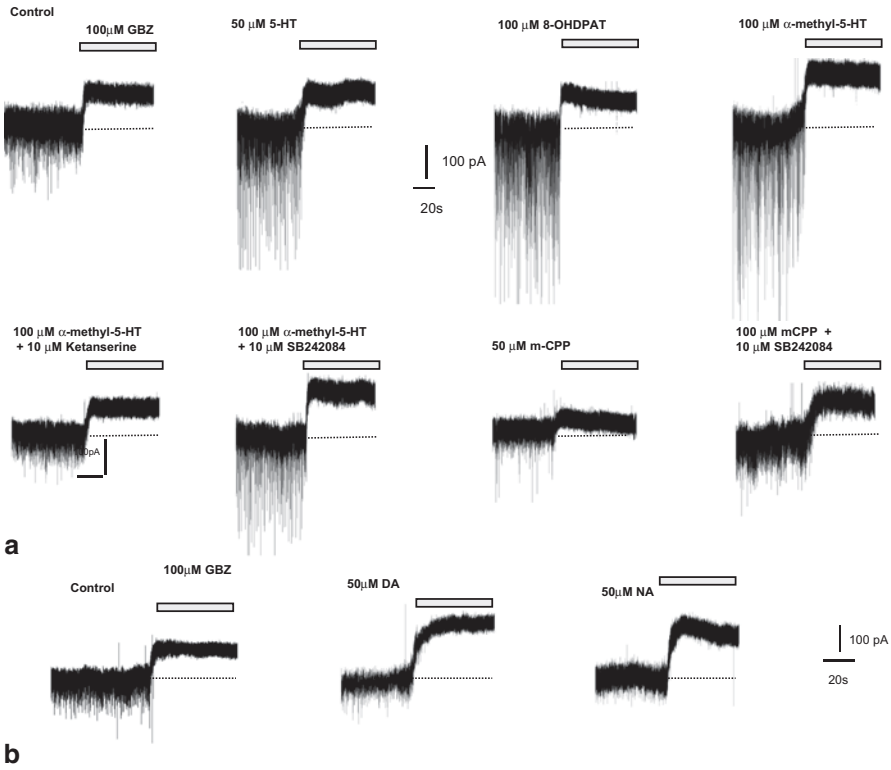

Fig. 7.4 Serotonergic modulation of tonic $GABA_A$ current in the dLGN of Wistar rats. **a** Focal application of gabazine (GBZ, 100 μM, *grey bar*) reveals different magnitude of tonic current. Representative current traces from different TC neurons of the dLGN thalamus of Wistar rats under control conditions (*top left*) and in the continuing presence of 5-HT (50 μM), 8-OHDPAT (100 μM), and α-methyl-5-HT (100 μM; *top traces*), and α-methyl-5-HT (100 μM) + Ketanserine (50 μM), α-methyl-5-HT (100 μM) + SB242084 (10 μM), and meta-chlorophenylpiperazine (mCPP) (50 μM) and mCPP (100 μM) + SB242084 (10 μM; *bottom traces*). **b** Representative current traces from different TC neurons in dLGN slices from Wistar rats under control conditions (*left*) and in the continuing presence of dopamine (DA) (50 μM; *middle*) and noradrenaline (NA) (100 μM; *right*)

7.4.3 Ventrobasal Nucleus of the Thalamus

5-HT is able to modulate the activity of VB TCs directly (McCormick 1992b), although early evidence indicated that it plays more of a modulatory role by facilitating the response of these neurons to excitatory amino acids (Eaton and Salt 1989) or by inhibiting acetylcholine-induced excitation (Andersen and Curtis 1964). Exogenous application of 5-HT in VB slices was reported to have no effect on sIPSC (Munsch et al. 2003). We recently confirmed this finding: 5-HT_{2A}R and 5-HT_{2C}R ligands lack any effect on phasic $GABA_A$ inhibition in VB TC neurons of Wistar rats (Cavaccini et al. 2012). Similarly to the dLGN, the 5-HT_{2A} selective agonist TCB-2

enhances the tonic $GABA_A$ current, an effect that is blocked by co-application of the 5-HT_{2A} antagonist MDL11,939. We further confirmed that TCB-2 was exerting its effects through e$GABA_A$ receptors by establishing that TCB-2 does not enhance the tonic current in TC cells of δ-knockout mice, though it does so in wild-type littermates. Conversely, RO 60–0175, a selective 5-HT_{2C} agonist, decreases the tonic current, and effect that is most likely mediated by 5-HT_{2C}Rs, since it is blocked by SB 242084. Moreover, MDL11,939 and SB242084 do not alter tonic inhibition when applied alone, indicating that in our experimental conditions endogenous 5-HT levels are insufficient to activate these receptors.

The opposite effects on the tonic $GABA_A$ current elicited by selective 5-HT_{2A}R and 5-HT_{2C}R agonists might depend on the activation of alternative signal transduction pathways. Alternatively, it may result from a different distribution of these receptors, i.e. a preferential postsynaptic expression of the 5-HT_{2C}Rs on VB TC neurons whilst 5-HT_{2A}Rs might instead be mostly located presynaptically on GABAergic NRT neurons and/or on their terminals. Since in our slices the NRT nucleus and its axons are preserved, both possibilities are realistic.

Finally, we investigated whether the 5-$HT_{2A/2C}$ ligands are capable of reducing the aberrant eGABA inhibition of TC neurons in the VB of the epileptic GAERS rats (Cope et al. 2009). Bath application of RO 60-0175 halved the tonic $GABA_A$ current in GAERS and non-epileptic control (NEC) rats. Moreover, MDL11,939 alone did not modify the tonic current in NECs, but it decreased it in GAERS (Cavaccini et al. 2012). This suggests that in absence epilepsy there might be an increased serotoninergic tone, and indeed 5-HT depletion has been shown in Long Evans rats, another strain which expresses spontaneous absence seizures (Bercovici et al. 2006). Alternatively, more 5-HTRs may be present in these epileptic animals or their efficacy may be increased compared to the NEC rats.

7.5 NARs—e$GABA_A$Rs Interaction

Locus coeruleus innervates almost all brain areas in a highly diffused manner. NA action is slow, as it acts through GPCRs, but its wide release in the brain makes NA a crucial regulator for various fundamental brain functions such as arousal, attention and memory processes (Sara 2009). The majority of studies on the NA effects on synaptic transmission has focused on excitatory glutamatergic transmission. However, NA also acts on GABAergic transmission. NA excites GABAergic interneurons (McCormick 1992a; Kawaguchi and Shindou 1998) and indirectly inhibits principal neuron activity in different CNS areas (Segal et al. 1991). On the other hand, NA can increase $GABA_A$ IPSCs of interneurons such as, cerebellar stellate cells (Kondo and Marty 1997), frontal cortex (Kawaguchi and Shindou 1998), cerebellum and have opposite effects in GABAergic neurons of different layers of sensory cortices depending on the type of NAR activated (Salgado et al. 2011).

As far as the thalamus is concerned, application of NA to dLGN relay neurons can result in a large slow depolarization through a decrease in a K^+ conductance

(McCormick and Prince 1988). Moreover, stimulation of beta-adrenergic receptors by NA elicits a marked enhancement of I_h (McCormick and Pape 1990). In this manner, NA may contribute to the switch from rhythmic burst firing to the transfer mode of action potential generation in the thalamus during increases in arousal and upon awakening from sleep. Surprisingly, the effect of NA on GABAergic transmission in the thalamus has not been investigated. Our preliminary data show that NA is capable of increasing both frequency of sIPSC and the tonic $GABA_A$ current in rat dLGN TC neurons (Fig. 7.4) (Di Giovanni et al. 2008).

7.6 Indirect mGluRs Modulation of $eGABA_ARs$

According to sequence homology and response to agonists, the eight subtypes of mGluRs that have so far been identified are subdivided into three groups: Group I (mGluR1 and 5), II (mGluR2 and 3) and III (mGluR4, 6, 7 and 8) (Conn and Pin 1997). Group I mGluRs stimulate inositol phosphate metabolism and mobilization of intracellular Ca^{2+}, whereas Group II and Group III couple to adenylyl cyclase (AC). Note, however, that a fourth (as yet unassigned) group of mGluRs which is coupled to phospholipase D has been reported recently (Ngomba et al. 2011).

Having reviewed the available evidence that support the presence of a direct modulation of $eGABA_ARs$ by GABA-, DA-, 5-HT- and NA-activated GPCRs, here we describe an interesting example of how another member of the GPCR family, the mGluR I, is capable of strongly regulating $eGABA_ARs$ in the thalamic dLGN by controlling dendritic GABA release from the local interneurons (Fig. 7.5).

mGluR1 are highly expressed in the dendrites, but not the soma of TC neurons in the dLGN (Coulon et al. 2010), where they are not located in the main body of asymmetric synapses, but are concentrated in the perisynaptic areas (Ngomba et al. 2011). In addition, mGluR1 mRNA is not detected in NRT neurons, which together with the local GABAergic interneurons, provide a strong inhibitory control to TC neurons in the dLGN (Shigemoto et al. 1992). These GABAergic interneurons innervation of dLGN TC neuron is peculiar, in that they form conventional axodendritic synapses, the so-called F1 terminals, and dendrodendritic synapses, or F2 terminals, that participate in specialized synaptic 'triads' where the interneuron dendrite is postsynaptic to retinogeniculate terminals and presynaptic to the TC neuron dendrite (Ohara et al. 1983; Hamos et al. 1985) (Fig. 7.5). GABA release from these two interneuron terminals differs in its action potential dependence, with F1 and F2 terminals being action potential-dependent and -independent, respectively (Cox and Sherman 2000; Govindaiah and Cox 2006b). Glutamate release from retinogeniculate terminals can therefore monosynaptically excite TC neuron dendrites and disynaptically inhibit them via glutamate receptor-dependent GABA release from F2 terminals (Cox and Sherman 2000; Govindaiah and Cox 2004; Blitz and Regehr 2005). Importantly, mGluRs are only present on F2 terminals (Godwin et al. 1996; Cox and Sherman 2000).

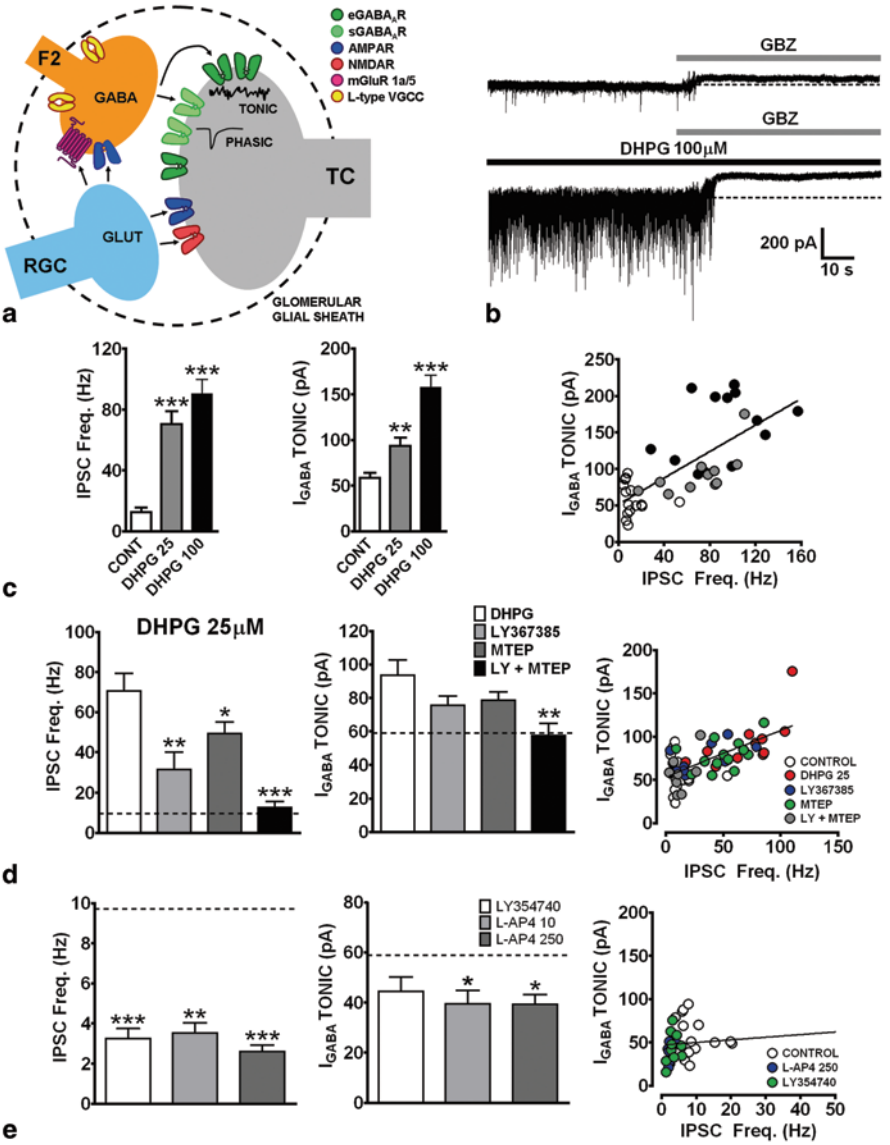

Fig. 7.5 mGluR-dependent modulation of tonic GABA signalling in visual thalamus. **a** Schematic representation of the specialised triadic circuitry involving retinal ganglion cell (*RGC*) afferents, interneuron dendritic GABA release sites (or F2 terminals) and thalamocortical (*TC*) neuron dendrites. Metabotropic glutamate receptors are located presynaptically on interneuron F2 terminals and their activation by RGC cell glutamate release modulates phasic and tonic GABAergic signalling in TC neurons. **b** Representative traces show the increase in phasic GABAergic IPSC frequency and tonic GABA current induced by application of the group I mGluR agonist DHPG. **c** IPSC frequency and magnitude of tonic GABA current in TC neurons is increased in a concentration-dependent manner by DHPG and the size of the tonic current correlates with the frequency of phasic events. **d** IPSC frequency and tonic GABA current are significantly increased above control levels (*white circles*) (dashed line on bar graphs) by DHPG (*red circles*). The increase relies

Application of the Group I-specific mGluR agonist (S)-3,5-Dihydroxyphenylglycine (DHPG) to thalamic slices of the dLGN causes a robust increase in synaptic GABA release from thalamic interneurons, leading to a marked increase in mIPSCs frequency and amplitude (Govindaiah and Cox 2006a) and a large increase in the tonic $GABA_A$ current of TC neurons (Fig. 7.5a) (Errington et al. 2011b). The latter effect does not simply result from the summation of IPSCs since DHPG fails to enhance the tonic current in δ-knockout mice (Errington et al. 2011b). Importantly, physiological recruitment of mGluRs via electrical stimulation of the retinogeniculate terminals is able to mimic DHPG action as it elicits a transient enhancement of the tonic $GABA_A$ current in dLGN TC neurons. Indeed, the evidence might suggest that activation of group I mGluRs (i.e. mGluR1a and mGluR5) on F2 terminals can initiate local dendro-dendritic GABA release, and this is sufficient to enhance the tonic $GABA_A$ current (Fig. 7.5).

Activation of Group II and III mGluRs has been shown to reduce phasic $GABA_A$ inhibition in TC neurons (Govindaiah and Cox 2006a), but a Group II agonist does not significantly reduce the tonic $GABA_A$ current (Errington et al. 2011b). Instead, activation of Group III mGluRs not only significantly reduces IPSC frequency but also decreases tonic current amplitude (Errington et al. 2011b). Unlike the activation of Group I mGluRs, when the amplitude of the tonic $eGABA_A$ current is plotted against IPSC frequency in the presence of Group II/III mGluR agonists, no significant correlation is observed. Thus, activation of mGluRs I and II can modulate vesicular GABA release and control tonic inhibition (Errington et al. 2011b).

In summary, we functionally demonstrated for the first time that $eGABA_A$Rs are located at putative postsynaptic locations on TC neuron dendrites that participate in the triadic arrangements characteristic of the dLGN, and that these receptors can be dynamically activated by robust dendro-dendritic GABA release (Fig. 7.5).

In contrast to the results in the dLGN, activation of Group I mGluRs does not increase tonic $GABA_A$ inhibition (or mIPSC frequency) in TC neurons of the VB complex of the thalamus. Unlike the dLGN, the rodent VB contains no interneurons (Barbaresi et al. 1986; Harris and Hendrickson 1987), and GABAergic innervation to VB TC neurons is provided solely by NRT neurons. The lack of effect of DHPG upon phasic and tonic $GABA_A$ inhibition in the VB, therefore, suggests that Group I mGluRs do not modulate output from NRT neurons. Note, also that Bright and Brickley (Bright and Brickley 2008) reported that postsynaptic depolarization of VB TC neurons induces a robust increase in the rate of spontaneous $GABA_A$ IPSCs, which, however, is not accompanied by an enhancement of the tonic $GABA_A$ current.

---

upon activation of both mGluR1 and mGluR5 subtypes being partially blocked by the mGluR1 antagonist LY367385 (*blue circles*) and mGluR5 antagonist MTEP (*green circles*) when applied individually, but completely reversed when both drugs are applied together (*grey circles*). **e** Unlike group I mGluRs, activation of group II and III mGluRs produces a marked reduction in interneuron GABA release and a corresponding decrease in both IPSC frequency and the amplitude of tonic $GABA_A$ currents in dLGN TC neurons. The reduction is likely to involve mGluR2 and 3 and mGluR 4 and 8 receptor subtypes. (Modified from Errington et al. 2011b with permission)

Conclusions

The amplitude of the $eGABA_AR$-mediated tonic current does not remain constant over time, but fluctuates in relation to the ambient GABA concentration (Pavlov et al. 2009). Moreover, $eGABA_ARs$ are dynamic entities showing plasticity in response to changes in $sGABA_ARs$ activity (Nani et al. 2013) and modulation by exogenous agents including neurosteroids, alcohol and anesthestetics (see Chap. 5; (Belelli et al. 2009)). As reviewed here, $eGABA_ARs$ are also subject to modulatory actions by a variety of GPCRs which can act presynaptically, modulating GABA release or postsynaptically, directly altering $eGABA_AR$ activity (Fig. 7.6). Since these GPCR-mediated modulations of $eGABA_AR$ are both nucleus- and neuronal type-selective, the functional interactions of GABA with other neurotransmitters are more complex than previously envisioned. A representative example is the striatum where DARs activate tonic and no phasic inhibition (Ade et al. 2008). Moreover, increasing evidence is showing that a similar scenario is present in other brain areas. For instance, it seems that the activation of $GABA_B$, D2 and $5\text{-}HT_{2A/2C}Rs$ preferentially modulates $eGABA_AR$ over $sGABA_AR$-mediated conductance in the VB complex of the thalamus (Di Giovanni et al. 2008; Cavaccini et al. 2012; Connelly et al. 2013; Yague et al. 2013), while mGlu, D2 and different 5-HTRs do affect both phasic and tonic inhibition in the dLGN (Munsch et al. 2005; Di Giovanni et al. 2008). This could be due to the different GPCRs synaptic localization and anatomical organization of dLGN and VB. From inference from our evidence on $GABA_B$ modulation in VB, cerebellum, hippocampus (Connelly et al. 2013) and other brain regions (Tao et al. 2013) and DA effects in the striatum (Janssen et al. 2009), it is possible that $eGABA_ARs$ might be under a reversible and dynamic regulation by different GPCRs and modulators through PKA/PKC-dependent phosphorylation (Fig. 7.6). Notwithstanding these data, a direct protein–protein interaction cannot be ruled out (Fig. 7.6).

These diverse modulations of $eGABA_ARs$ by GPCRs provide a vast array of mechanisms for the fine-tuning of single neuron and network excitability in response not only to a variety of physiological stimuli but also in neurological diseases. Since PD symptoms result from an imbalance in the two striatal pathways (Mallet et al. 2006; Esposito et al. 2007; Obeso et al. 2008) and Huntington's disease from a selective loss of D2+ -MSNs (Estrada Sanchez et al. 2008), the differential expression of the tonic $GABA_A$ currents in D1+ and D2+ MSNs may offer novel potential therapeutic targets for these diseases.

The ability of some monoamine sensing GPCRs to selectively modulate only one type of $GABA_AR$-mediated inhibition may also have important therapeutic relevance in pathologies such as absence epilepsy, where there is an aberrant increase in $eGABA_AR$ function but an unchanged phasic inhibition (Cope et al. 2009). Since this enhanced thalamic tonic $GABA_A$ current is a necessary and sufficient condition for the expression of typical absence epilepsy (Cope et al. 2005; Di Giovanni et al. 2011a; Errington et al. 2011c) (see Chap. 12), it is conceivable that the anti-absence

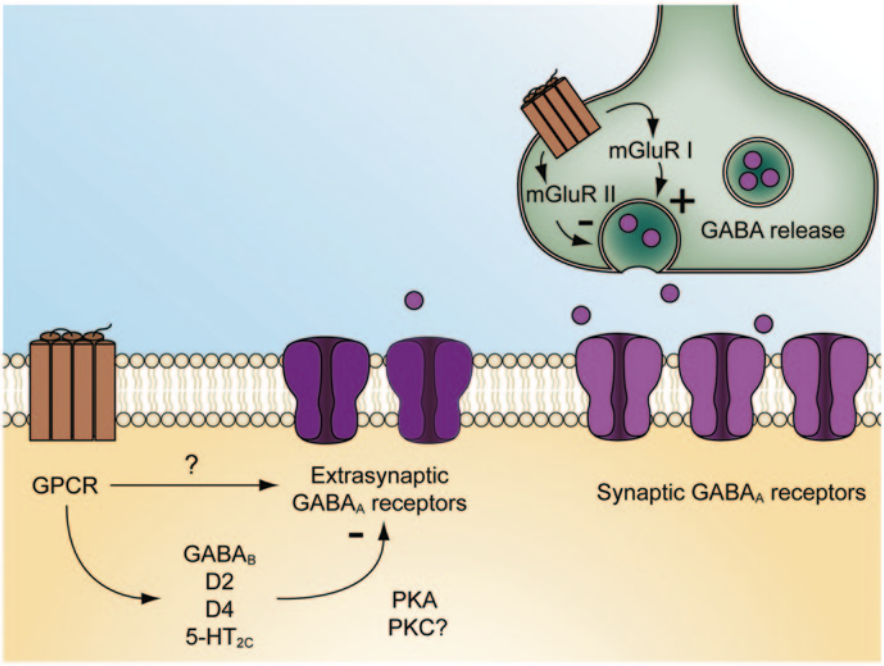

Fig. 7.6 Schematic of hypothetical GPCRs action on GABAergic synapses in the central nervous system. Pre-synaptic element: mGLURs might modulate the release of GABA (*purple*) from GABAergic neuron/interneuron, either increasing it via (1) mGLUR I or (2) decreasing it via mGLUR II (dLGN). Post-synaptic element: a postsynapse expressing synaptic and exstrasynaptic $GABA_A$Rs. Different GPCRs may modulate e$GABA_A$ current via either GPCR/second messenger/kinases (PKA/PKC) or G-protein-independent signalling. An increase of the e$GABA_A$ phosphorilation state of different subunits might lead to a decrease/increase of the $GABA_A$ inhibition. A direct GPCR–e$GABA_A$R might also occur

action of systemically injected 5-HT and DA ligands (Danober et al. 1998; Isaac 2005; Bagdy et al. 2007) occur in part via a modulation of the thalamic tonic $GABA_A$ inhibition. Strikingly, since no specific antagonist or inverse agonists exists for δ-subunit-containing e$GABA_A$Rs, the possibility of modulating the tonic $GABA_A$ current with GPCR ligands provides an interesting novel therapeutic target for this type of generalised epilepsy (Errington et al. 2011a) and other disorders for which an impairment of e$GABA_A$Rs has been reported. As a matter of fact, the cross-talk between GPCRs and e$GABA_A$Rs might be already targeted in the pharmacological actions of some of the drugs that act on GPCRs and are currently marketed for different neurological diseases.

Acknowledgments Our original research reviewed in this chapter was supported by Epilepsy Research UK (grant P1202), the Wellcome Trust (grant 91882) and EU COST CM1103.

References

Abrams JK, Johnson PL, Hollis JH, Lowry CA (2004) Anatomic and functional topography of the dorsal raphe nucleus. Ann N Y Acad Sci 1018:46–57

Ade KK, Janssen MJ, Ortinski PI, Vicini S (2008) Differential tonic GABA conductances in striatal medium spiny neurons. J Neurosci 28:1185–1197

Andersen P, Curtis DR (1964) The pharmacology of the synaptic and acetylcholine-induced excitation of ventrobasal thalamic neurones. Acta Physiol Scand 61:100–120

Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR (1997) Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. Brain Res 752:26–34

Bagdy E, Kiraly I, Harsing LG Jr (2000) Reciprocal innervation between serotonergic and GABAergic neurons in raphe nuclei of the rat. Neurochem Res 25:1465–1473

Bagdy G, Kecskemeti V, Riba P, Jakus R (2007) Serotonin and epilepsy. J Neurochem 100:857–873

Balasubramanian S, Teissere JA, Raju DV, Hall RA (2004) Hetero-oligomerization between $GABA_A$ and $GABA_B$ receptors regulates $GABA_B$ receptor trafficking. J Biol Chem 279:18840–18850

Bankson MG, Yamamoto BK (2004) Serotonin-GABA interactions modulate MDMA-induced mesolimbic dopamine release. J Neurochem 91:852–859

Barbaresi P, Spreafico R, Frassoni C, Rustioni A (1986) GABAergic neurons are present in the dorsal column nuclei but not in the ventroposterior complex of rats. Brain Res 382:305–326

Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38:1083–1152

Belley M, Sullivan R, Reeves A, Evans J, O'Neill G, Ng GY (1999) Synthesis of the nanomolar photoaffinity GABA(B) receptor ligand CGP 71872 reveals diversity in the tissue distribution of GABA(B) receptor forms. Bioorg Med Chem 7:2697–2704

Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ (2005) Extrasynaptic $GABA_A$ receptors of thalamocortical neurons: a molecular target for hypnotics. J Neurosci 25:11513–11520

Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW (2009) Extrasynaptic $GABA_A$ receptors: form, pharmacology, and function. J Neurosci 29:12757–12763

Bercovici E, Cortez MA, Wang X, Snead OC 3rd (2006) Serotonin depletion attenuates AY-9944-mediated atypical absence seizures. Epilepsia 47:240–246

Bettler B, Kaupmann K, Mosbacher J, Gassmann M (2004) Molecular structure and physiological functions of GABA(B) receptors. Physiol Rev 84:835–867

Blitz DM, Regehr WG (2005) Timing and specificity of feed-forward inhibition within the LGN. Neuron 45:917–928

Bonnin A, Peng W, Hewlett W, Levitt P (2006) Expression mapping of 5-HT1 serotonin receptor subtypes during fetal and early postnatal mouse forebrain development. Neuroscience 141:781–794

Bramley JR, Sollars PJ, Pickard GE, Dudek FE (2005) 5-HT1B receptor-mediated presynaptic inhibition of GABA release in the suprachiasmatic nucleus. J Neurophysiol 93:3157–3164

Brickley SG, Cull-Candy SG, Farrant M (1996) Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of $GABA_A$ receptors. J Physiol 497(Pt 3):753–759

Bright DP, Brickley SG (2008) Acting locally but sensing globally: impact of GABAergic synaptic plasticity on phasic and tonic inhibition in the thalamus. J Physiol 586:5091–5099

Bright DP, Aller MI, Brickley SG (2007) Synaptic release generates a tonic GABA(A) receptor-mediated conductance that modulates burst precision in thalamic relay neurons. J Neurosci 27:2560–2569

Cai X, Flores-Hernandez J, Feng J, Yan Z (2002) Activity-dependent bidirectional regulation of GABA(A) receptor channels by the 5-HT(4) receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. J Physiol 540:743–759

Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA (2004) Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. Proc Natl Acad Sci U S A 101:3662–3667

Cavaccini A, Yagüe JG, Errington AC, Crunelli V, Di Giovanni G (2012) Opposite effects of thalamic 5-HT2A and 5-HT2C receptor activation on tonic GABA-A inhibition: implications for absence epilepsy. In: Annual meeting of Neuroscience Society, New Orleans, USA, p 138.103/B157

Chapin EM, Andrade R (2001) A 5-HT(7) receptor-mediated depolarization in the anterodorsal thalamus. II. Involvement of the hyperpolarization-activated current I(h). J Pharmacol Exp Ther 297:403–409

Chen G, Kittler JT, Moss SJ, Yan Z (2006) Dopamine D3 receptors regulate $GABA_A$ receptor function through a phospho-dependent endocytosis mechanism in nucleus accumbens. J Neurosci 26:2513–2521

Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST (2011) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature 468:305–309

Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37:205–237

Connelly WM, Fyson SJ, Errington AC, McCafferty CP, Cope DW, Di Giovanni G, Crunelli V (2013) $GABA_B$ Receptors regulate extrasynaptic $GABA_A$ receptors. J Neurosci 33:3780–3785

Cope DW, Hughes SW, Crunelli V (2005) $GABA_A$ receptor-mediated tonic inhibition in thalamic neurons. J Neurosci 25:11553–11563

Cope DW, Di Giovanni G, Fyson SJ, Orban G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V (2009) Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. Nat Med 15:1392–1398

Coulon P, Kanyshkova T, Broicher T, Munsch T, Wettschureck N, Seidenbecher T, Meuth SG, Offermanns S, Pape HC, Budde T (2010) Activity modes in thalamocortical relay neurons are modulated by G(q)/G(11) family G-proteins - Serotonergic and glutamatergic signaling. Front Cell Neurosci 4:132

Cox CL, Sherman SM (2000) Control of dendritic outputs of inhibitory interneurons in the lateral geniculate nucleus. Neuron 27:597–610

Dahlstroem A, Fuxe K (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol Scand Suppl: S 232:231–255

Dahlstrom A, Fuxe K (1964) Localization of monoamines in the lower brain stem. Experientia 20:398–399

Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C (1998) Pathophysiological mechanisms of genetic absence epilepsy in the rat. Prog Neurobiol 55:27–57

Di Giovanni G (ed) (2007) The basal ganglia pathophysiology: recent advances. Transworld Research Network, Kerala

Di Giovanni G (2008) Will it ever become possible to prevent dopaminergic neuronal degeneration? CNS Neurol Disord Drug Targets 7:28–44

Di Giovanni G (2010) Dopamine interaction with other neurotransmitter systems: relevance in the pathophysiology and treatment of CNS disorders. CNS Neurosci Ther 16:125–126

Di Giovanni G, Di Matteo V, La Grutta V, Esposito E (2001) m-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. Neuroscience 103:111–116

Di Giovanni G, Di Matteo V, Pierucci M, Benigno A, Esposito E (2006) Central serotonin2C receptor: from physiology to pathology. Curr Top Med Chem 6:1909–1925

Di Giovanni G, Cope DW, Crunelli V (2008) Cholinergic and monoaminergic modulation of tonic $GABA_A$ inhibition in the rat dorsal lateral geniculate nucleus. Soc Neurosci 531.532/D533

Di Giovanni G, Di Matteo V, Esposito E (eds) (2009) Birth, life and death of dopaminergic neurons in the substantia nigra. J Neural Transm Supplementa, Supplement 73, Springer

Di Giovanni G, Esposito E, Di Matteo V (2010) Role of serotonin in central dopamine dysfunction. CNS Neurosci Ther 16:179–194
Di Giovanni G, Errington AC, Crunelli V (2011a) Pathophysiological role of extrasynaptic GABA A receptors in typical absence epilepsy. Malta Med J 3:4–9
Di Giovanni G, Esposito E, Di Matteo V (eds) (2011b) 5-HT2C Receptors in the Pathophysiology of CNS Disease. Springer/Humana Press, New York
Dohlman HG, Thorner J, Caron MG, Lefkowitz RJ (1991) Model systems for the study of seven-transmembrane-segment receptors. Annu Rev Biochem 60:653–688
Eaton SA, Salt TE (1989) Modulatory effects of serotonin on excitatory amino acid responses and sensory synaptic transmission in the ventrobasal thalamus. Neuroscience 33:285–292
Errington AC, Cope DW, Crunelli V (2011a) Augmentation of Tonic GABA(A) Inhibition in absence epilepsy: therapeutic value of inverse agonists at extrasynaptic GABA(A) receptors. Adv Pharmacol Sci 2011:790590
Errington AC, Di Giovanni G, Crunelli V, Cope DW (2011b) mGluR control of interneuron output regulates feedforward tonic $GABA_A$ inhibition in the visual thalamus. J Neurosci 31:8669–8680
Errington AC, Gibson KM, Crunelli V, Cope DW (2011c) Aberrant GABA(A) receptor-mediated inhibition in cortico-thalamic networks of succinic semialdehyde dehydrogenase deficient mice. PLoS One 6:e19021
Esposito E, Di Matteo V, Di Giovanni G (2007) Death in the substantia nigra: a motor tragedy. Expert Rev Neurother 7:677–697
Estrada Sanchez AM, Mejia-Toiber J, Massieu L (2008) Excitotoxic neuronal death and the pathogenesis of Huntington's disease. Arch Med Res 39:265–276
Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 6:215–229
Feng J, Cai X, Zhao J, Yan Z (2001) Serotonin receptors modulate GABA(A) receptor channels through activation of anchored protein kinase C in prefrontal cortical neurons. J Neurosci 21:6502–6511
Floran B, Floran L, Erlij D, Aceves J (2004) Activation of dopamine D4 receptors modulates [3H] GABA release in slices of the rat thalamic reticular nucleus. Neuropharmacology 46:497–503
Flores-Hernandez J, Hernandez S, Snyder GL, Yan Z, Fienberg AA, Moss SJ, Greengard P, Surmeier DJ (2000) D(1) dopamine receptor activation reduces GABA(A) receptor currents in neostriatal neurons through a PKA/DARPP-32/PP1 signaling cascade. J Neurophysiol 83:2996–3004
Garcia-Cabezas MA, Rico B, Sanchez-Gonzalez MA, Cavada C (2007) Distribution of the dopamine innervation in the macaque and human thalamus. Neuroimage 34:965–984
Garcia-Cabezas MA, Martinez-Sanchez P, Sanchez-Gonzalez MA, Garzon M, Cavada C (2009) Dopamine innervation in the thalamus: monkey versus rat. Cereb Cortex 19:424–434
Gasca-Martinez D, Hernandez A, Sierra A, Valdiosera R, Anaya-Martinez V, Floran B, Erlij D, Aceves J (2010) Dopamine inhibits GABA transmission from the globus pallidus to the thalamic reticular nucleus via presynaptic D4 receptors. Neuroscience 169:1672–1681
Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432
Gertler TS, Chan CS, Surmeier DJ (2008) Dichotomous anatomical properties of adult striatal medium spiny neurons. J Neurosci 28:10814–10824
Glase SA, Akunne HC, Georgic LM, Heffner TG, MacKenzie RG, Manley PJ, Pugsley TA, Wise LD (1997) Substituted [(4-phenylpiperazinyl)-methyl]benzamides: selective dopamine D4 agonists. J Med Chem 40:1771–1772
Goaillard JM, Vincent P (2002) Serotonin suppresses the slow afterhyperpolarization in rat intralaminar and midline thalamic neurones by activating 5-HT(7) receptors. J Physiol 541:453–465
Godwin DW, Van Horn SC, Eriir A, Sesma M, Romano C, Sherman SM (1996) Ultrastructural localization suggests that retinal and cortical inputs access different metabotropic glutamate receptors in the lateral geniculate nucleus. J Neurosci 16:8181–8192

Govindaiah G, Cox CL (2004) Synaptic activation of metabotropic glutamate receptors regulates dendritic outputs of thalamic interneurons. Neuron 41:611–623
Govindaiah G, Cox CL (2005) Excitatory actions of dopamine via D1-like receptors in the rat lateral geniculate nucleus. J Neurophysiol 94:3708–3718
Govindaiah G, Cox CL (2006a) Depression of retinogeniculate synaptic transmission by presynaptic D(2)-like dopamine receptors in rat lateral geniculate nucleus. Eur J Neurosci 23:423–434
Govindaiah G, Cox CL (2006b) Metabotropic glutamate receptors differentially regulate GABAergic inhibition in thalamus. J Neurosci 26:13443–13453
Govindaiah G, Wang Y, Cox CL (2010) Dopamine enhances the excitability of somatosensory thalamocortical neurons. Neuroscience 170:981–991
Groenewegen HJ (1988) Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. Neuroscience 24:379–431
Guidotti A, Auta J, Davis JM, Dong E, Grayson DR, Veldic M, Zhang X, Costa E (2005) GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. Psychopharmacology (Berl) 180:191–205
Gurevich EV, Joyce JN (1999) Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. Neuropsychopharmacology 20:60–80
Hale MW, Lowry CA (2010) Functional topography of midbrain and pontine serotonergic systems: implications for synaptic regulation of serotonergic circuits. Psychopharmacology (Berl) 213:243–264
Hamos JE, Van Horn SC, Raczkowski D, Uhlrich DJ, Sherman SM (1985) Synaptic connectivity of a local circuit neurone in lateral geniculate nucleus of the cat. Nature 317:618–621
Harris RM, Hendrickson AE (1987) Local circuit neurons in the rat ventrobasal thalamus-a GABA immunocytochemical study. Neuroscience 21:229–236
Hillegaart V (1991) Functional topography of brain serotonergic pathways in the rat. Acta Physiol Scand Suppl 598:1–54
Hines RM, Davies PA, Moss SJ, Maguire J (2012) Functional regulation of $GABA_A$ receptors in nervous system pathologies. Curr Opin Neurobiol 22:552–558
Hortnagl H, Tasan RO, Wieselthaler A, Kirchmair E, Sieghart W, Sperk G (2013) Patterns of mRNA and protein expression for 12 $GABA_A$ receptor subunits in the mouse brain. Neuroscience 236:345–372
Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71:533–554
Huidobro-Toro JP, Valenzuela CF, Harris RA (1996) Modulation of $GABA_A$ receptor function by G protein-coupled 5-HT2C receptors. Neuropharmacology 35:1355–1363
Invernizzi RW, Pierucci M, Calcagno E, Di Giovanni G, Di Matteo V, Benigno A, Esposito E (2007) Selective activation of 5-HT(2C) receptors stimulates GABA-ergic function in the rat substantia nigra pars reticulata: a combined in vivo electrophysiological and neurochemical study. Neuroscience 144:1523–1535
Inyushkin AN, Merkulova NA, Orlova AO, Inyushkina EM (2010) Local GABAergic modulation of the activity of serotoninergic neurons in the nucleus raphe magnus. Neurosci Behav Physiol 40:885–893
Isaac M (2005) Serotonergic 5-HT2C receptors as a potential therapeutic target for the design antiepileptic drugs. Curr Top Med Chem 5:59–67
Jacobs BL, Azmitia EC (1992) Structure and function of the brain serotonin system. Physiol Rev 72:165–229
Janssen MJ, Ade KK, Fu Z, Vicini S (2009) Dopamine modulation of GABA tonic conductance in striatal output neurons. J Neurosci 29:5116–5126
Janssen MJ, Yasuda RP, Vicini S (2011) GABA(A) Receptor beta3 subunit expression regulates tonic current in developing striatopallidal medium spiny neurons. Front Cell Neurosci 5:15
Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, Goldstein PA (2005) An extrasynaptic $GABA_A$ receptor mediates tonic inhibition in thalamic VB neurons. J Neurophysiol 94:4491–4501

Jimenez-Gonzalez C, Pirttimaki T, Cope DW, Parri HR (2011) Non-neuronal, slow GABA signalling in the ventrobasal thalamus targets delta-subunit-containing GABA(A) receptors. Eur J Neurosci 33:1471–1482

Kang SU, Heo S, Lubec G (2011) Mass spectrometric analysis of $GABA_A$ receptor subtypes and phosphorylations from mouse hippocampus. Proteomics 11:2171–2181

Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, Somogyi P (2010) Quantitative localisation of synaptic and extrasynaptic $GABA_A$ receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. Eur J Neurosci 32:1868–1888

Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A, Bettler B (1998) GABA(B)-receptor subtypes assemble into functional heteromeric complexes. Nature 396:683–687

Kawaguchi Y, Shindou T (1998) Noradrenergic excitation and inhibition of GABAergic cell types in rat frontal cortex. J Neurosci 18:6963–6976

Kebabian JW, Calne DB (1979) Multiple receptors for dopamine. Nature 277:93–96

Khan ZU, Gutierrez A, Martin R, Penafiel A, Rivera A, De La Calle A (1998) Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. J Comp Neurol 402:353–371

Khan ZU, Koulen P, Rubinstein M, Grandy DK, Goldman-Rakic PS (2001) An astroglia-linked dopamine D2-receptor action in prefrontal cortex. Proc Natl Acad Sci U S A 98:1964–1969

Kirmse K, Dvorzhak A, Kirischuk S, Grantyn R (2008) GABA transporter 1 tunes GABAergic synaptic transmission at output neurons of the mouse neostriatum. J Physiol 586:5665–5678

Kondo S, Marty A (1997) Protein kinase A-mediated enhancement of miniature IPSC frequency by noradrenaline in rat cerebellar stellate cells. J Physiol 498(Pt 1):165–176

Laurie DJ, Wisden W, Seeburg PH (1992) The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci 12:4151–4172

Lavin A, Grace AA (1998) Dopamine modulates the responsivity of mediodorsal thalamic cells recorded in vitro. J Neurosci 18:10566–10578

Lee FJ, Wang YT, Liu F (2005) Direct receptor cross-talk can mediate the modulation of excitatory and inhibitory neurotransmission by dopamine. J Mol Neurosci 26:245–252

Li QH, Nakadate K, Tanaka-Nakadate S, Nakatsuka D, Cui Y, Watanabe Y (2004) Unique expression patterns of 5-HT2A and 5-HT2C receptors in the rat brain during postnatal development: western blot and immunohistochemical analyses. J Comp Neurol 469:128–140

Liste I, Caruncho HJ, Guerra MJ, Labandeira-Garcia JL (1997) GABA(A) receptor subunit expression in intrastriatal striatal grafts comparison between normal developing striatum and developing striatal grafts. Brain Res Dev Brain Res 103:185–194

Liu F, Wan Q, Pristupa ZB, Yu XM, Wang YT, Niznik HB (2000) Direct protein-protein coupling enables cross-talk between dopamine D5 and gamma-aminobutyric acid A receptors. Nature 403:274–280

Luo R, Janssen MJ, Partridge JG, Vicini S (2013) Direct and GABA-mediated indirect effects of nicotinic ACh receptor agonists on striatal neurones. J Physiol 591:203–217

Luscher B, Shen Q, Sahir N (2011) The GABAergic deficit hypothesis of major depressive disorder. Mol Psychiatry 16:383–406

Luttrell DK, Luttrell LM (2004) Not so strange bedfellows: G-protein-coupled receptors and Src family kinases. Oncogene 23:7969–7978

Lydiard RB (2003) The role of GABA in anxiety disorders. J Clin Psychiatry 64 S 3:21–27

Maguire JL, Stell BM, Rafizadeh M, Mody I (2005) Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci 8:797–804

Mallet N, Ballion B, Le Moine C, Gonon F (2006) Cortical inputs and GABA interneurons imbalance projection neurons in the striatum of parkinsonian rats. J Neurosci 26:3875–3884

Marescaux C, Vergnes M, Depaulis A (1992) Genetic absence epilepsy in rats from Strasbourg-a review. J Neural Transm Suppl 35:37–69

Matsuoka T, Hasuo H, Akasu T (2004) 5-Hydroxytryptamine 1B receptors mediate presynaptic inhibition of monosynaptic IPSC in the rat dorsolateral septal nucleus. Neurosci Res 48:229–238
McCormick DA (1992a) Cellular mechanisms underlying cholinergic and noradrenergic modulation of neuronal firing mode in the cat and guinea pig dorsal lateral geniculate nucleus. J Neurosci 12:278–289
McCormick DA (1992b) Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. Prog Neurobiol 39:337–388
McCormick DA, Pape HC (1990) Noradrenergic and serotonergic modulation of a hyperpolarization-activated cation current in thalamic relay neurones. J Physiol 431:319–342
McCormick DA, Prince DA (1988) Noradrenergic modulation of firing pattern in guinea pig and cat thalamic neurons, in vitro. J Neurophysiol 59:978–996
McDonald BJ, Amato A, Connolly CN, Benke D, Moss SJ, Smart TG (1998) Adjacent phosphorylation sites on $GABA_A$ receptor beta subunits determine regulation by cAMP-dependent protein kinase. Nat Neurosci 1:23–28
Mengod G, Cortés R, Vilaró MT, Hoyer D (2010) Distribution of 5-HT Receptors in the Central Nervous System. In: Handbook of Behavioral Neuroscience, vol 21. Elsevier, New York pp 123–138
Meuth SG, Aller MI, Munsch T, Schuhmacher T, Seidenbecher T, Meuth P, Kleinschnitz C, Pape HC, Wiendl H, Wisden W, Budde T (2006) The contribution of TWIK-related acid-sensitive K+-containing channels to the function of dorsal lateral geniculate thalamocortical relay neurons. Mol Pharmacol 69:1468–1476
Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998) Dopamine receptors: from structure to function. Physiol Rev 78:189–225
Miyazaki I, Asanuma M, Diaz-Corrales FJ, Miyoshi K, Ogawa N (2004) Direct evidence for expression of dopamine receptors in astrocytes from basal ganglia. Brain Res 1029:120–123
Moss SJ, Smart TG, Blackstone CD, Huganir RL (1992) Functional modulation of $GABA_A$ receptors by cAMP-dependent protein phosphorylation. Science 257:661–665
Munsch T, Freichel M, Flockerzi V, Pape HC (2003) Contribution of transient receptor potential channels to the control of GABA release from dendrites. Proc Natl Acad Sci U S A 100:16065–16070
Munsch T, Yanagawa Y, Obata K, Pape HC (2005) Dopaminergic control of local interneuron activity in the thalamus. Eur J Neurosci 21:290–294
Nani F, Bright DP, Revilla-Sanchez R, Tretter V, Moss SJ, Smart TG (2013) Tyrosine phosphorylation of $GABA_A$ receptor gamma2-subunit regulates tonic and phasic inhibition in the thalamus. J Neurosci 33:12718–12727
Neer EJ (1995) Heterotrimeric G proteins: organizers of transmembrane signals. Cell 80:249–257
Ngomba RT, Santolini I, Salt TE, Ferraguti F, Battaglia G, Nicoletti F, van Luijtelaar G (2011) Metabotropic glutamate receptors in the thalamocortical network: strategic targets for the treatment of absence epilepsy. Epilepsia 52:1211–1222
Nikolaus S, Antke C, Beu M, Muller HW (2010) Cortical GABA, striatal dopamine and midbrain serotonin as the key players in compulsive and anxiety disorders-results from in vivo imaging studies. Rev Neurosci 21:119–139
Nusser Z, Mody I (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J Neurophysiol 87:2624–2628
Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693–1703
Nusser Z, Sieghart W, Mody I (1999) Differential regulation of synaptic $GABA_A$ receptors by cAMP-dependent protein kinase in mouse cerebellar and olfactory bulb neurones. J Physiol 521(Pt 2):421–435
Obeso JA, Rodriguez-Oroz MC, Benitez-Temino B, Blesa FJ, Guridi J, Marin C, Rodriguez M (2008) Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease. Mov Disord 23(Suppl 3):S548–559

Ohara PT, Lieberman AR, Hunt SP, Wu JY (1983) Neural elements containing glutamic acid decarboxylase (GAD) in the dorsal lateral geniculate nucleus of the rat; immunohistochemical studies by light and electron microscopy. Neuroscience 8:189–211

Padgett CL, Slesinger PA (2010) $GABA_B$ receptor coupling to G-proteins and ion channels. Adv Pharmacol 58:123–147

Papadopoulos GC, Parnavelas JG (1990) Distribution and synaptic organization of dopaminergic axons in the lateral geniculate nucleus of the rat. J Comp Neurol 294:356–361

Pape HC, McCormick DA (1989) Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current. Nature 340:715–718

Pavlov I, Savtchenko LP, Kullmann DM, Semyanov A, Walker MC (2009) Outwardly rectifying tonically active $GABA_A$ receptors in pyramidal cells modulate neuronal offset, not gain. J Neurosci 29:15341–15350

Pessia M, Jiang ZG, North RA, Johnson SW (1994) Actions of 5-hydroxytryptamine on ventral tegmental area neurons of the rat in vitro. Brain Res 654:324–330

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815–850

Pizzarelli R, Cherubini E (2011) Alterations of GABAergic signaling in autism spectrum disorders. Neural Plast 2011:297153

Poisbeau P, Cheney MC, Browning MD, Mody I (1999) Modulation of synaptic $GABA_A$ receptor function by PKA and PKC in adult hippocampal neurons. J Neurosci 19:674–683

Rodriguez-Pallares J, Labandeira-Garcia JL, Munoz A, Caruncho HJ (2000) Morphology and neurochemistry of two striatal neuronal subtypes expressing the GABA(A) receptor alpha3-subunit in the rat. Brain Res 876:124–130

Rodriguez JJ, Noristani HN, Hoover WB, Linley SB, Vertes RP (2011) Serotonergic projections and serotonin receptor expression in the reticular nucleus of the thalamus in the rat. Synapse 65:919–928

Salgado H, Garcia-Oscos F, Patel A, Martinolich L, Nichols JA, Dinh L, Roychowdhury S, Tseng KY, Atzori M (2011) Layer-specific noradrenergic modulation of inhibition in cortical layer II/III. Cereb Cortex 21:212–221

Santhakumar V, Jones RT, Mody I (2010) Developmental regulation and neuroprotective effects of striatal tonic $GABA_A$ currents. Neuroscience 167:644–655

Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci 10:211–223

Schwartz RD (1988) The $GABA_A$ receptor-gated ion channel: biochemical and pharmacological studies of structure and function. Biochem Pharmacol 37:3369–3375

Schwarzer C, Berresheim U, Pirker S, Wieselthaler A, Fuchs K, Sieghart W, Sperk G (2001) Distribution of the major gamma-aminobutyric acid(A) receptor subunits in the basal ganglia and associated limbic brain areas of the adult rat. J Comp Neurol 433:526–549

Segal M, Markram H, Richter-Levin G (1991) Actions of norepinephrine in the rat hippocampus. Prog Brain Res 88:323–330

Shigemoto R, Nakanishi S, Mizuno N (1992) Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an in situ hybridization study in adult and developing rat. J Comp Neurol 322:121–135

Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 347:146–151

Stell BM, Mody I (2002) Receptors with different affinities mediate phasic and tonic GABA(A) conductances in hippocampal neurons. J Neurosci 22:RC223

Stoof JC, Kebabian JW (1984) Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci 35:2281–2296

Tang X, Hernandez CC, Macdonald RL (2010) Modulation of spontaneous and GABA-evoked tonic alpha4beta3delta and alpha4beta3gamma2 L $GABA_A$ receptor currents by protein kinase A. J Neurophysiol 103:1007–1019

Tao W, Higgs MH, Spain WJ, Ransom CB (2013) Postsynaptic $GABA_B$ receptors enhance extrasynaptic $GABA_A$ receptor function in dentate gyrus granule cells. J Neurosci 33:3738–3743

Theile JW, Morikawa H, Gonzales RA, Morrisett RA (2009) Role of 5-hydroxytryptamine2C receptors in Ca2+-dependent ethanol potentiation of GABA release onto ventral tegmental area dopamine neurons. J Pharmacol Exp Ther 329:625–633

Ulrich D, Besseyrias V, Bettler B (2007) Functional mapping of GABA(B)-receptor subtypes in the thalamus. J Neurophysiol 98:3791–3795

Vertes RP, Fortin WJ, Crane AM (1999) Projections of the median raphe nucleus in the rat. J Comp Neurol 407:555–582

Vertes RP, Linley SB, Hoover WB (2010) Pattern of distribution of serotonergic fibers to the thalamus of the rat. Brain Struct Funct 215:1–28

Vithlani M, Moss SJ (2009) The role of $GABA_A$R phosphorylation in the construction of inhibitory synapses and the efficacy of neuronal inhibition. Biochem Soc Trans 37:1355–1358

Wamsley JK, Gehlert DR, Filloux FM, Dawson TM (1989) Comparison of the distribution of D-1 and D-2 dopamine receptors in the rat brain. J Chem Neuroanat 2:119–137

Weiner DM, Levey AI, Sunahara RK, Niznik HB, O'Dowd BF, Seeman P, Brann MR (1991) D1 and D2 dopamine receptor mRNA in rat brain. Proc Natl Acad Sci U S A 88:1859–1863

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 12:1040–1062

Yague JG, Cavaccini A, Errington AC, Crunelli V, Di Giovanni G (2013) Dopaminergic modulation of tonic but not phasic GABA-receptor-mediated current in the ventrobasal thalamus of Wistar and GAERS rats. Exp Neurol 247C:1–7

Chapter 8
Extrasynaptic $GABA_A$ Receptors and Tonic Inhibition in Spinal Cord

Emanuel Loeza-Alcocer, Carmen Andrés, Justo Aguilar, Ricardo Felix and Rodolfo Delgado-Lezama

Abstract γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the adult central nervous system (CNS), exerts its physiological effects by acting on ligand-gated chloride-permeable channels termed $GABA_A$ receptors ($GABA_AR$). The activation of these receptors produces two different types of inhibition: fast and tonic, mediated by synaptic and extrasynaptic $GABA_ARs$, respectively. The molecular conformation of the extrasynaptic $GABA_A$ receptors and the tonic inhibitory current they generate have been characterized in different brain structures, and their relevance in controlling neuronal excitability has been also demonstrated. Likewise, a role for these receptors has been suggested in a variety of neurological disorders such as schizophrenia, epilepsy, and Parkinson disease. In the spinal cord, the characterization of these receptors has initiated with the study of their relationship with motor control, chronic pain and anesthesia. This chapter highlights past and present developments in the field of extrasynaptic $GABA_A$ receptors and emphasizes their subunit composition, distribution, and physiological role in the spinal cord.

Keywords Spinal cord · Extrasynaptic $GABA_A$ receptors · GABA · Tonic inhibition · Neuron excitability

8.1 Introduction

The spinal cord is the region of the nervous system that receives information from the supraspinal nuclei, proprioceptors, mechanoreceptors, thermoreceptors, chemoreceptors and nociceptors. This information is processed by second order interneurons distributed on the dorsal horn in the susbstantia gelatinosa and laminae II and III along the spinal cord. These interneurons interact with complex circuits involved in motor control and generation of locomotor activity or other stereotyped motor

R. Delgado-Lezama (✉) · E. Loeza-Alcocer · C. Andrés · J. Aguilar
Department of Physiology, Biophysics and Neuroscience, Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), Avenida IPN 2508, Col. Zacatenco, Mexico City 07300, Mexico
e-mail: rdelgado@fisio.cinvestav.mx

R. Felix
Department of Cell Biology, Cinvestav-IPN, Avenida IPN 2508, Col. Zacatenco, Mexico City 07300, Mexico

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors*, The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_8

functions (e.g. micturition, ejaculation, scratching, respiration, pain behavior). Motoneurons located in laminae IX named by Sherrington as the final pathway of the nervous system, perform the crucial function of translating brain activity into different kinds of motor behavior. Therefore, the properties of motoneurons must be fine-tuned to produce precisely coordinated muscle activity in response to both external and internal stimuli. The stability and function of the spinal cord circuitries depend on the balance between the excitation and inhibition, the latter being mediated by glycinergic and GABAergic neurons. As we shall see, the GABAergic system plays fundamental roles in the development and function of the spinal cord.

8.1.1 GABA in the Spinal Cord

Investigation on the actions of γ-aminobutyric acid (GABA) in the spinal cord began in the decade of the fifties. Curtis et al. (1959) found that GABA depressed the excitatory (EPSP) and inhibitory postsynaptic potentials (IPSP) and reduced the excitability of motoneurons (Curtis et al. 1959). Working with the cat preparation, Eccles et al. (1963) characterized the presynaptic inhibition at primary afferents, and suggested that it was mediated by the activation of $GABA_A$Rs by GABA released from the interneuron GABAergic terminals synapsing primary afferent terminals (Eccles et al. 1963; Schmidt 1971; Rudomin and Schmidt 1999). These studies were the beginning of a series of investigations focused on the physiological role of GABA in the spinal cord (Curtis et al. 1959; Curtis 1969). Eccles and his colleagues also found that the presynaptic inhibition of the monosynaptic reflex was correlated with primary afferent depolarization (PAD), and reported that picrotoxin reduced both presynaptic inhibition and PAD (Eccles et al. 1963). Therefore, nowadays, it is well accepted that presynaptic inhibition of primary afferents is mediated by activation of synaptic $GABA_A$Rs.

8.1.2 Properties of Extrasynaptic $GABA_A$ Receptors

The biophysical and pharmacological properties of the extrasynaptic $GABA_A$Rs in general are reviewed and discussed in depth in another section of this book; therefore, our chapter will be focused on the distribution and function of extrasynaptic $GABA_A$Rs in the spinal cord.

The $GABA_A$ receptor is a hetero-oligomeric protein consisting of five proteins or subunits ($\alpha_{1–6}$, $\beta_{1–3,}$ $\gamma_{1–3}$, δ, ε, θ, π, $\rho_{1–3}$) (Olsen and Sieghart 2008; Olsen and Sieghart 2009). Theoretically, hundreds of $GABA_A$Rs can be produced; however, only a few dozens of $GABA_A$Rs has been found in the central nervous system (CNS). The stoichiometric composition of these receptors is 2α, 2β and γ subunits, where the latter can be replaced by one of the remaining subunits. Interestingly, with the aid of immunohistochemical and immunoprecipitation (Fritschy et al. 1998), immunoelectron microscopy (Somogyi et al. 1996), ligand binding, (Nusser et al.

1999) and quantitative immunogold techniques, (Nusser et al. 1995; Nusser et al. 1996; Nusser et al. 1997; Nusser et al. 1998) the expression of the $GABA_A$Rs subunits has been estimated in supraspinal neurons being $\alpha_1\beta_2\gamma_2$ and $\alpha_2\beta_3\gamma_2$ the most-favored combinations (Möhler et al. 2002). The majority of $GABA_A$Rs are located at the synapse, although a subpopulation is present in somatic, dendritic, and axonal membranes not in opposition to presynaptic terminals (Somogyi et al. 1989; Farrant and Nusser 2005; Kullmann et al. 2005). As mentioned earlier, these extrasynaptic receptors mediate an alternative (tonic) form of GABAergic inhibition.

Using functional assays, it has been shown that the application of bicuculline methiodide (BMI) and gabazine in addition to blocking the spontaneous phasic currents, also changes the holding current necessary to keep the cells at a predetermined holding membrane potential (Farrant and Nusser 2005; Kullmann et al. 2005; Brickley et al. 1996), unveiling the presence of a GABAergic tonic inhibition in different neuronal types from the hippocampus, thalamus, somatosensory cortex, and cerebellum (Farrant and Nusser 2005; Kullmann et al. 2005; Brickley et al. 1996). This is due to the activation of extrasynaptic $GABA_A$Rs by ambient GABA that produces a persistent increase in conductance or tonic shunt that modulates the excitability of the neurons (Farrant and Nusser 2005; Chadderton et al. 2004; Walker and Semyanov 2008).

Extrasynaptic $GABA_A$Rs predominantly contain α_4, α_5, and α_6 subunits in combination with δ and γ_2 (Farrant and Nusser 2005; Walker and Semyanov 2008; Semyanov et al. 2004). However, with the exception of receptors containing the δ subunit, the other $GABA_A$Rs have been found indistinctly in both synaptic and extrasynaptic locations (Nusser et al. 1995; Farrant and Nusser 2005; Walker and Semyanov 2008).

Based on its affinity for the endogenous GABA, it has been reported that $GABA_A$Rs containing $\alpha_6\beta_3\delta$ or $\alpha_4\beta_3\delta$ subunit combinations have the lowest EC_{50} values (~0.3–0.7 μM), whereas in $GABA_A$Rs of the $\alpha_1\beta_3\gamma_2$ or $\alpha_2\beta_3\gamma_2$ subtypes, the EC_{50} values are one order of magnitude higher (~6–14 μM). Interestingly, the first mentioned receptors are most commonly found in extrasynaptic locations while the latter are mainly contained in membranes at the synaptic connection (Farrant and Nusser 2005). In addition, GABA affinity is correlated with the sensitivity of $GABA_A$Rs to competitive and noncompetitive antagonists which are used mainly for investigating the presence of tonic inhibition. Hence, low concentrations (<10 μM) of gabazine (SR-95531) selectively block phasic GABAergic currents, while high concentrations of picrotoxin, SR-95531 or BMI are required to block tonic currents (Farrant and Nusser 2005; Walker and Semyanov 2008).

Interestingly, there are also $GABA_A$R antagonists and inverse agonists that display subunit selectivity. For instance, the diuretic furosemide, used in the treatment of congestive heart failure and edema, has ~100-fold selectivity for α_6 over α_1 subunit-containing receptors (Korpi et al. 1995), and therefore, it has been used to determine the presence and the functional role of extrasynaptic $GABA_A$Rs containing the α_6 subunit (Korpi et al. 1995; Sinkkonen et al. 2004). In addition, L-655708 an inverse agonist of α_5-containing $GABA_A$Rs has also been used to block tonic currents in hippocampal neurons (Zarnowska et al. 2009). Likewise, modulation

of tonic inhibition has been observed in the presence of endogenous neuroactive steroids, intravenous and volatile anesthetics, as well as alcohol. In particular, 3α, 21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone or THDOC) enhances tonic GABAergic currents mediated by δ containing receptors (Stell et al. 2003). Drugs targeting $GABA_AR$ subunits with much higher affinity and more clinical efficacy have now been developed (Rudolph and Knoflach 2011; Zeilhofer et al. 2012).

8.2 Expression of $GABA_AR$ Subunits in the Spinal Cord

The expression of $GABA_A$ receptor subunits in the spinal cord has been studied with different techniques including reverse transcriptase-polymerase chain reaction (RT-PCR), in situ hybridization and immunohistochemistry.

8.2.1 Expression of Transcripts for $GABA_ARs$ Subunits

The presence of mRNA encoding different subunits of $GABA_ARs$ has been evidenced in spinal cord using in situ hybridization and the RT-PCR technique.

Contrary to the high expression in the brain, the mRNA coding for the α_1 subunit is present only in very few scattered cells throughout the spinal cord mainly in layers VII and VIII (Ruano et al. 2000), and no expression is detectable over motoneuron areas (Wisden et al. 1991; Persohn et al. 1991). Likewise, the expression of the α_2 gene is confined to areas VIII and IX. The α_2 mRNA is abundantly expressed in motoneurons and smaller 'satellite' cells. These smaller cells are likely to be Renshaw cells (Wisden et al. 1991; Persohn et al. 1991; Petri et al. 2005; Ruano et al. 2000). In contrast, the α_3 mRNA is present in many neurons in all layers of the spinal cord. It is highly expressed in layers II–VII and X (Persohn et al. 1991; Ma et al. 1993; Petri et al. 2005; Ruano et al. 2000) but weakly expressed in some motoneurons (Wisden et al. 1991; Ruano et al. 2000). The α_5 probe in most cases present very weak labeling of motoneurons (Wisden et al. 1991), and layers VII and VIII are also moderately labeled. Medium-sized multipolar cells in layers IV–VI and cells in layers II and III contained a low but detectable signal for this subunit (Persohn et al. 1991; Ma et al. 1993). Interestingly, α_5 has been detected since E20 in developmental studies (Serafini et al. 1998). Last, in embryonic, postnatal, and adult rat spinal cord α_4 mRNA is barely expressed, while α_6 mRNA expression has not been detected (Ma et al. 1993; Petri et al. 2005).

The only β subunit transcript found in the adult lumbar spinal cord is β_3 which is highly expressed in the dorsal horn and is also present in ventral horn cells such as motoneurons (Wisden et al. 1991; Petri et al. 2005; Ma et al. 1993). Likewise, β_2 mRNA doubles over E17–20 during development (Serafini et al. 1998) and in the adult rat spinal cord its transcript can be detected in layers VII and VIII as well as in

layers IV–VI (Persohn et al. 1991). The γ_2 subunit mRNA is also found in many layers of spinal cord, with a similar pattern of distribution and intensity to α_3 mRNA. However, γ_2 mRNA is present at much higher levels than α_3 mRNA in motoneurons (Wisden et al. 1991; Ma et al. 1993; Petri et al. 2005).

During development, no signal has been detected with the δ subunit mRNA specific probe at any stage in the rat spinal cord (Persohn et al. 1991; Wisden et al. 1991; Ma et al. 1993). However, the presence of α_3, θ, and ε mRNAs expression has been reported during the E14–E19 period. As development progresses from E14-E10 to P0-P12, ε subunit expression expands into laminas X and IX and enters lamina VII (Pape et al. 2009).

Based on mRNA expression levels, it has been suggested that $GABA_A$Rs in motoneurons contain mainly the $\alpha_2\alpha_3\beta_3\gamma_2$ subunit composition, whereas complexes of the $\alpha_3\beta_3\gamma_2$ composition may predominate in the cells of the dorsal horn (Wisden et al. 1991). It is also possible to find $GABA_A$Rs with different molecular composition expressed in a single cell and that different cell types may express different $GABA_A$ receptors as it occurs in the brain (Mody and Pearce 2004; Ruano et al. 2000; Farrant and Nusser 2005).

8.2.2 Immunolocalization of $GABA_A$Rs in the Spinal Cord

Using radioligands Bowery et al. (1987) showed that $GABA_A$Rs are highly concentrated in laminae II and III, moderately expressed in laminae V, VI, VIII and IX, and presented at a low density in laminae I, IV, VII and X (Bowery et al. 1987). Immunolocalization using monoclonal antibodies against α and β subunits has evidenced the expression of $GABA_A$ receptors in the laminae II–IV, VI–VIII and X of rat (Richards et al. 1987) and human spinal cord (Waldvogel et al. 1990). At subcellular level, GABA immunoreactive axon terminals making axodendritic and axoaxonic synaptic contacts have been found in lamina II. Interestingly, Waldvogel et al. reported that $GABA_A$R immunoreactivity at these synaptic complexes was associated with presynaptic and postsynaptic membranes as well as with nonsynaptic membranes (Waldvogel et al. 1990). This is one of the first evidences for the presence of extrasynaptic $GABA_A$Rs in the spinal cord.

Likewise, immunocytochemical studies in the brain have documented the presence of $GABA_A$Rs containing $\alpha_{4/6}\beta\delta$, $\alpha_5\beta\gamma_2$, and $\alpha_1\beta\delta$ subunits at extrasynaptic locations (Farrant and Nusser 2005; Fritschy et al. 1992; Brickley and Mody 2012). Some of these subunits have been identified also in the spinal cord. For example, β_{2-3} has been found in synaptic and extrasynaptic membranes of dendrites and somas of neurons in laminae II–VII (Fig. 8.1a) (Alvarez et al. 1996). In addition, γ_2 has been immunolocalized in postsynaptic and presynaptic membranes opposed to presynaptic active zones and at extrasynaptic sites in membranes within boutons (Sur et al. 1995). More recently, the α_1 subunit has been found extrasynaptically in dendrites crossing the Renshaw cell area (Geiman et al. 2002). On the other hand, $GABA_A$Rs containing the β_3 subunit seems to be restricted primarily to a synaptic location (Todd et al. 1996).

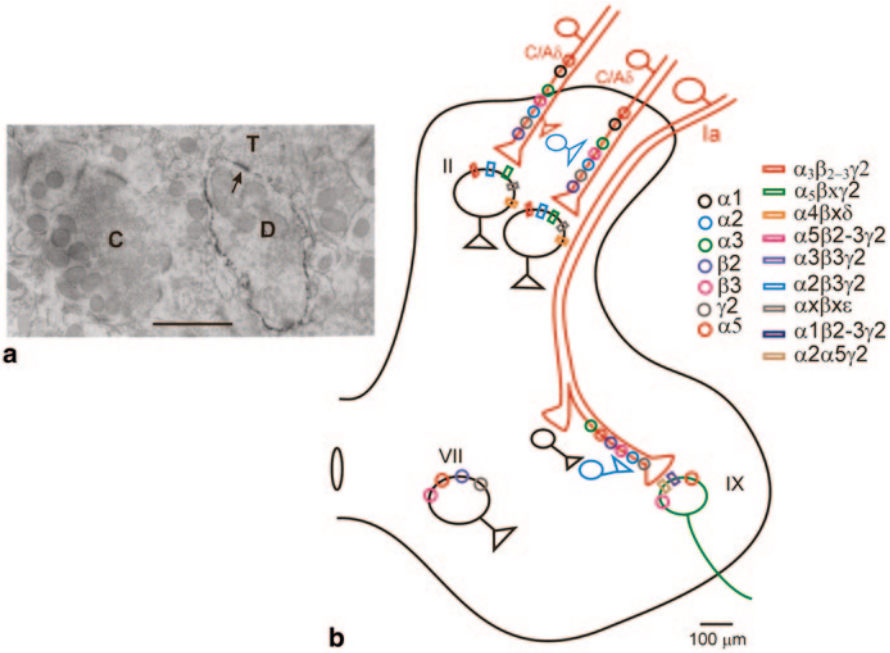

Fig. 8.1 Schematic representation of a transverse section of the spinal cord. **a** Subcellular localization of the $GABA_AR$ $\beta_{2/3}$ subunits in lamina II of the rat spinal cord. The image shows a dendrite (*D*) with synaptic and extrasynaptic plasma membrane $\beta_{2/3}$ subunits immunolabeling. The presynaptic terminal (*T*) contains flattened synaptic vesicles. Both, the pre- and postsynaptic membranes show $\beta_{2/3}$ immunoreactivity. An unlabeled central glomerular terminal (*C*) is also shown. Scale bar 1 μm. **b** Distribution of putative $GABA_ARs$ subtypes based on the localization of the distinct subunits. Panel A, modified with permission from Alvarez et al. (1996)

The expression of α_3, β_{2-3}, γ_2 subunits has been immunolocalized in cells from the dorsal and ventral horn in rats (Alvarez et al. 1996; Bohlhalter et al. 1996) and humans (Waldvogel et al. 2010). The α_2 subunit seems to be present also in all laminae although its expression is weak. In the dorsal horn, the expression pattern of the α_1 and α_5 subunit seems to be restricted to laminae III (Bohlhalter et al. 1996), while α_6 subunit expression has been found in laminae II (Gutiérrez et al. 1996).

Based on the co-localization of subunits within individual neurons, Bohlhalter et al. (1996) suggested distinct subunit combinations expected to be present in the spinal cord neurons, being $\alpha_3\beta_{2-3}\gamma_2$ the most likely to occur. In addition, $GABA_ARs$ with the $\alpha_1\beta_{2-3}\gamma_2$, $\alpha_5\beta_{2-3}\gamma_2$, and $\alpha_1\alpha_5\beta_{2-3}\gamma_2$ combinations are candidates to be present in laminae III–VIII and X (Fig. 8.1b). Motoneurons may express the atypical $\alpha_2\gamma_2$ and $\alpha_2\alpha_5\gamma_2$ combinations, and Renshaw cells may preferentially express α_3, α_5, β_{2-3}, and γ_2 $GABA_AR$ subunits (Geiman et al. 2002). The expression of the ε subunit in cells from laminae VII, X, and IX was reported recently. It has been also found in a specific population of cells of the thoracic spinal cord located in the intermediolateral column region (CML) in lamina VII (Pape et al. 2009).

8.3 GABAergic Tonic Current in the Spinal Cord

The presence of a GABAergic tonic current in supraspinal neurons was first observed when the application of an antagonist of the $GABA_A$Rs produced a substantial change in the holding current to clamp the membrane at a constant voltage (Farrant and Nusser 2005; Walker and Semyanov 2008; Mody and Pearce 2004). In the spinal cord, the first demonstration of a GABAergic tonic inhibitory current was obtained in chick embryo neurons, where it produced a slow depolarization. Based on an estimated single-channel conductance of 26 pS and an open probability of 0.33 (Yang and Zorumski 1989), it was estimated that approximately 30 $GABA_A$Rs were responsible for the tonic GABAergic current in these cells. In addition, the depolarization induced by this current was about 8 mV, which was very similar to the voltage change observed in the presence of BMI (Chub and O'Donovan 2001).

The GABAergic tonic currents have been recorded in neurons of the substatia gelatinosa in the spinal cord of mammals (Fig. 8.2a) (Ataka and Gu 2006; Takahashi et al. 2006; Mitchell et al. 2007; Maeda et al. 2010), and based on their pharmacological profile and RT-PCR data, it has been suggested that $GABA_A$Rs mediating these currents may contain $\alpha_5\beta_x\gamma_2$, $\alpha_x\beta_x\varepsilon$, and $\alpha_4\beta_x\delta$ subunits (Takahashi et al. 2006). A GABAergic tonic current can be activated by the anesthetic thiopental in mouse interneurons of the ventral horn which is abolished by 100 μM BMI (Fig. 8.2b). However, this tonic current apparently may not be activated by ambient GABA (Grasshoff et al. 2008).

Likewise, it has been reported that spontaneous synaptic activity in premotor interneurons of the adult turtle spinal cord can be blocked by strychnine but not by BMI (20 μM) or gabazine (100 μM). Interestingly, the holding current is not affected by these drugs, suggesting that spontaneous synaptic activity may not be mediating tonic inhibition. In contrast, application of BMI (100 μM) unmasks a GABAergic tonic current, which is not sensitive to furosemide but can be blocked by L-655708. Taken together, these data suggest the presence of α_5 subunit-containing $GABA_A$Rs tonically active by ambient GABA (Castro et al. 2011a). In line with this, a GABAergic tonic current mediated by $\alpha_5\gamma_2GABA_A$Rs has also been reported in preganglionic sympathetic neurons of the rat spinal cord (Wang et al. 2008).

The GABAergic tonic current may have a profound effect on neuronal excitability and may be involved in diverse physiological events at the level of both individual cells and local neuronal networks (Walker and Semyanov 2008; Semyanov et al. 2003; Walker 2008). For instance, in neurons of the *substantia gelatinosa* which are crucial elements for transmission and modification of noxious stimuli (review by Zeilhofer et al. 2012), the charge transfer carried by tonic inhibitory currents could be up to six times the total charge transferred by both GABAergic and glycinergic inhibitory postsynaptic currents (IPSCs) (Ataka and Gu 2006; Han and Youn 2008). This prominent action suggests a significant role of tonic inhibition in sensory processing as it is capable to modulate both cell and network behavior in supra spinal nuclei (Farrant and Nusser 2005; Nusser and Mody 2002). Additionally, evidence was obtained that the tonic current could be modulating the GABAergic spontaneous sIPSCs in these neurons of the *subtantia gelatinosa* (Ataka and Gu 2006; Gao and Smith 2010).

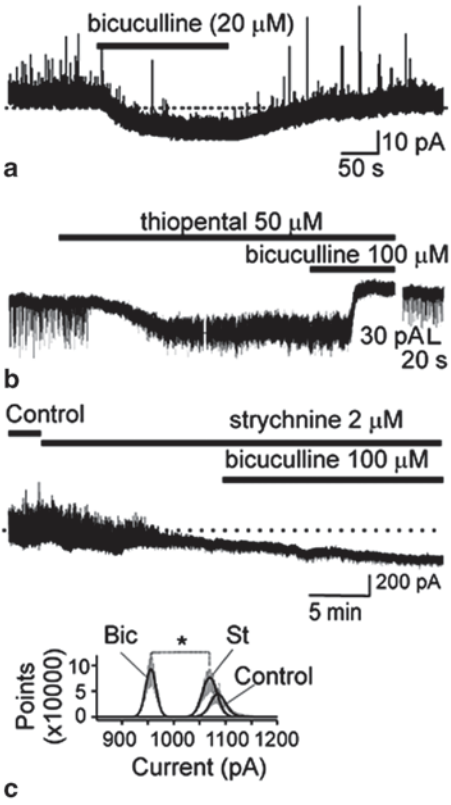

Fig. 8.2 Tonic current mediated by extrasynaptic $GABA_A$Rs in the spinal cord. **a** Shift of the baseline holding current following the application of 20 μM *bicuculline* from an adult mouse spinal cord neuron in laminae II recorded in whole cell patch clamp. **b** Time course of GABAergic tonic current recorded in whole cell mode in a mouse spinal interneuron activated by *thiopental* and its blockade by *bicuculline* (*BMI*). The inward current and the current fluctuation induced by *thiopental* were suppressed by BMI. The gap in the middle of the trace corresponds to a brief interruption of the recording for measurement of the electrode's access resistance. **c** GABAergic tonic current recorded in a turtle motoneuron held at 0 mV. *Strychnine* blocks the spontaneous IPSCs without affecting the holding current. In contrast, *bicuculline* (100 μM) application significantly modifies holding current. The lower panel shows Gaussian curves fitted to all-points histograms of values obtained from the holding current recorded in control Ringer and in the presence of *strychnine* and *bicucullline*. **a** Modified with permission from Ataka and Gu (2006). **b** Modified with permission from Grasshoff et al. (2008). **c** Modified with permission from Castro et al. (2011a)

8.4 Role of Extrasynaptic $GABA_A$ Receptors in Pain

Pain information originated in the periphery is transmitted to the CNS by specific neural pathways known as nociceptors. Nociceptive primary afferents project into the spinal cord through the dorsal root to synapse second order interneurons in the *substantia gelatinosa* which project to the supra spinal nuclei and activate

the spinal complex circuitries related with pain behaviors (Réthelyi and Capowski 1977; Yoshimura and Jessell 1990). At this level is where GABAergic inhibition plays an important role in suppressing or allowing the pain transmission to the brain (Melzack and Wall 1965).

The importance of the inhibition mediated by GABA in pain development has been evidenced by the observation that the blockade of $GABA_A$Rs induces hypersensitivity to light mechanical stimuli as well as allodynia and hyperalgesia (Roberts et al. 1986; Sivilotti and Woolf 1994; Torsney and MacDermott 2006; Baba et al. 2003). Although, in these experiments, the contribution of synaptic and extrasynaptic $GABA_A$Rs in the development of pain was not evaluated, subsequent investigations have shown that a benzodiazepine agonist, midazolam, can generate analgesia (Kohno et al. 2006), which has been attributed to the induction of a tonic GABAergic current in neurons from the *substantia gelatinosa* (Ataka and Gu 2006; Mitchell et al. 2007; Gao and Smith 2010; Maeda et al. 2010). Therefore, induction or restoration of this type of inhibition might be a useful tool for treatment of chronic pain.

8.4.1 Extrasynaptic $GABA_A$ Receptors in Nociceptive Primary Afferents

Nociceptive primary afferents are the pathway conveying pain information to the spinal cord (Yoshimura and Jessell 1990), and consequently they might be the first target to modulate pain. This modulation could be produced by activating $GABA_A$ receptors in primary afferents located in opposition to presynaptic terminals mediating PAD, and also in extrasynpatic sites such as the axons and somas in the dorsal root ganglion (DRG) cells (Deschenes et al. 1976; Morris et al. 1983; Désarmenien et al. 1984; Rudomin and Schmidt 1999; Carr et al. 2010). In addition, different subunits containing $GABA_A$ receptors (α_1, α_2, α_3, α_5, β_2, β_3, and γ_2) have been described in the central and peripheral terminals as well in the soma in the dorsal root ganglia by ligand binding assays (Singer and Placheta 1980), immunohistochemistry (Sur et al. 1995; Alvarez et al. 1996; Bohlhalter et al. 1996; Todd et al. 1996; Carlton et al. 1999; Witschi et al. 2011) and *in situ* hybridization studies (Persohn et al. 1991; Furuyama et al. 1992; Ma et al. 1993; Paul et al. 2012). In particular, activation of α_1GABA_A receptors placed in the peripheral nociceptive terminals by the intraplantar application of its agonist muscimol produced a dual effect in the cat formalin-pain model (Carlton et al. 1999). The pain behavior was attenuated at low concentration (2 μM); while at high concentration (1 mM), it was significantly enhanced. The authors suggested that the peripheral PAD induced by the low concentration of muscimol produces inhibition in the release of substance P (SP) and the calcitonin gene related peptide (CGRP) from nociceptive peripheral terminals as occurs with PAD at the central level. Nevertheless, when the PAD is facilitated by a high concentration of muscimol, peripheral terminal action potentials are generated and therefore, the nociceptive activity is potentiated (Carlton et al. 1999).

Another manner to decrease nociceptive information flow is inhibiting presynaptic excitatory transmitter release, which is associated with PAD, by activation of synaptic $GABA_A$ receptors (Eccles et al. 1963; Rudomin and Schmidt 1999; Willis 1999, Lin et al. 2000). In α_2 subunit null mice, diazepam is no longer capable to induce spinal antihyperalgesic action *in vivo* and its potentiating effect on PAD and presynaptic inhibition *in vitro* (Witschi et al. 2011). These results show that at least part of the antihyperalgesic action of spinally applied diazepam occurs through facilitated activation of α_2GABA_ARs on central terminal of primary nociceptors. Although PAD may inhibit neurotransmitter release, which is strong enough, it can reach the threshold for action potential firing (dorsal root reflex; DRR) which in turn may propagate retrogradely to the peripheral terminal evoking the release of inflammatory neuropeptides such as SP and CGRP (Rees et al. 1995; Willis 1999). Therefore, DRR recorded from Aδ and C nociceptors (Toennies 1938; Lisney 1979; Fitzgerald and Woolf 1981; Lin et al. 2000) may help to explain why denervation of the knee joint, capsaicin desensitization, and the administration of an antagonist of SP receptor can reduce carrageenan-induced arthritis (Lam and Ferrell 1989a, b). Cervero and Laird (1996) proposed a mechanism to explain hyperalgesia in which the DRR induced by inflammation might hypersensitize GABAergic interneurons evoking DRR in C fibers resulting in pain development (Cervero and Laird 1996). Hence, PAD might produce a dual action, (1) presynaptic inhibition and (2) hyperalgesia and allodynia due to the central and peripheral effects of DRR. Therefore, the alleviation of pain may implicate decreased excitability or shunting of primary afferents with subsequent increased threshold of DRR and lack of propagation, respectively. In line with this, it has been found that the activation of $GABA_A$ receptors in DRG neuronal cell bodies by muscimol or gaboxadol reduces pain behavior in a model of sciatic nerve crush injury (Naik et al. 2008). Additionally, glial cells transfected with glutamic acid decarboxylase (GAD) in the trigeminal ganglion diminish pain behavior by activating extrasynaptic $GABA_ARs$ in nociceptive primary afferents (Vit et al. 2009).

More recently, by using an inverse agonist of the α_5 (L-655708) it has been shown that the dorsal root potential (DRP) and the DRR are significantly depressed evidencing the contribution of $GABA_ARs$ containing this subunit to the tonic modulation of the excitability in primary afferents (Loeza-Alcocer et al. 2010). It is worth noting here, that L-655708 also blocks the tonic inhibitory current in hippocampal neurons (Glykys et al. 2008), and does not affect the phasic increase in excitability of primary afferents induced by the conditioning stimulation of an adjacent dorsal root, suggesting that the α_5GABA_ARs may be located at peri- or extrasynaptic axonal membranes. This has been confirmed by immunolocalization of theα_5 subunit in dorsal roots, DRG neuronal cell bodies, and peripheral processes (Loeza-Alcocer et al. 2010).

8.4.2 *Extrasynaptic $GABA_A$ Receptors as Novel Targets for Chronic Pain Treatment*

Depressed inhibition in the dorsal horn generates the most important symptoms of chronic pain. Thus, the pharmacological restoration of the spinal cord GABAergic inhibitory pathway through the activation of extrasynaptic $GABA_A$ receptors might be a viable therapeutic strategy for treating chronic pain (Sivilotti and Woolf 1994; Loomis et al. 2001; Malan et al. 2002; Moore et al. 2002; Baba et al. 2003; Torsney and MacDermott 2006) As mentioned earlier, the extrasynaptic $GABA_A$ receptor subunits identified in the spinal cord include $\alpha_{4/6}\,\beta_{2/3}\delta$, $\alpha_5\,\beta_{2/3}\gamma_2$, and $\alpha_1\,\beta\,\delta$, (Alvarez et al. 1996; Sur et al.1995; Geiman et al. 2002; Todd et al. 1996; Bohlhalter et al. 1996; Waldvogel et al. 2010; Gutiérrez et al. 1996; Pape et al. 2009) which also mediate the tonic GABAergic inhibition in the brain (Fritschy et al. 1992; Farrant and Nusser 2005; Brickley and Mody 2012). Support for this hypothesis is derived from the reports indicating that the activation of spinal $\delta GABA_ARs$ by the muscinol analogue 4,5,6,7-tetrahydroisoxazolo(5, 4-c)pyridin-3-ol (THIP) reduces the excitability of spinal mouse neurons, consequently inhibiting acute nociception, whereas the lack of $\delta GABA_ARs$ activity is associated with an increase in the second phase responses in the formalin test (Bonin et al. 2011).

Electrophysiological studies have evidenced the importance of the GABAergic tonic current in controlling neuronal excitability. For example, it has been reported that charge transfer is substantially higher through GABAergic tonic current activation than the phasic current in dorsal horn interneurons of laminae II (Ataka and Gu 2006; Takahashi et al. 2006). The large charge transfer carried by tonic GABAergic inhibitory current suggests a persistent increase in the input conductance in interneurons that decrease the size and duration of the excitatory postsynaptic potentials (EPSPs) affecting the ability of the cells to generate action potentials. This suggests that the activation of the GABAergic tonic inhibitory current may be an important mechanism to prevent central hyper-sensitization (Ataka and Gu 2006).

There are studies showing that the enhancement of GABAergic neuronal inhibition by $GABA_AR$ agonists or allosteric modulators of the benzodiazepine site in these receptors decrease certain types of pain (Grognet et al. 1983; Hammond and Drower 1984; Pelley and Vaught 1987; Kaneko and Hammond 1997; Eaton et al. 1999; Malan et al. 2002; Rode et al. 2005; Hugel et al. 2003; Kontinen and Dickenson 2000; Knabl et al. 2008; McKernan et al. 2000; Knabl et al. 2009; Griebel et al. 2001, 2003; Evans and Lowry 2007; Street et al. 2004; Munro et al. 2011). Though these drugs might affect tonic GABAergic inhibitory currents, it is not known to what extent the extrasynaptic receptors are participating in this process.

8.5 GABA and Motor Control

Motoneurons control the contraction or relaxation of muscles, which in most cases result in movement. By activating skeletal muscles, we are able to talk, walk, run, speak, breath, show our emotions, and execute motor activities with great accuracy. Consequently, motoneurons receive information from many neuronal centers located in supraspinal nuclei, muscle receptors, and propriospinal and spinal interneurons which relay information from skin, viscera, joints, and muscle receptors. This information is transmitted through synaptic boutons which are estimated to be ~50,000–140,000 in cat motoneurons (Ornung et al. 1998). GABA/glycine-immunoreactivity prevails in the stem dendrites between these boutons covering ~70% of the membrane, while glutamate-like immunoreactive terminals covers ~18% (4:1 ratio). However, the ratio of GABA/glycine-to glutamate falls to 1.5 at more distal dendrites (Ornung et al. 1998).

In order to respond to external demands with high precision, motoneurons should be able to discriminate the useless from relevant information. This means that motoneuron excitability should be under sophisticated control. As many neurons, motoneurons are provided with a vast arsenal of ionotropic receptors such as amino methyl isoxazole propionic acid (AMPA), N-methyl-D-aspartate (NMDA), glycine, $GABA_{A,}$ and $GABA_{C}$ as well as diverse metabotropic receptors in which endogenous ligands play important roles as neuromodulators (Rekling et al. 2000). In addition, the expression of K^+, Ca^{2+}, and Na^+ channels which determine the subthreshold membrane behavior, action potential shape, and firing properties contributes to control neuronal excitability. These proteins are also important targets for neuromodulators affecting excitability and present distinctive distribution patterns that in most cases are related to their main functions (Rekling et al. 2000).

The action of neurotransmitters and neuromodulators on these receptors and ionic channels should be integrated by the motoneuron to produce an action potential that will propagate along the efferent motor axon to activate muscle fibers. Among all receptors expressed by mature motoneurons, there are two that mediate inhibition: the ionotropic $GABA_A$ and the glycinergic receptors that play significant roles in controlling excitability. Immunohistochemical studies have demonstrated that GABA/glycine synaptic boutons are highly expressed in the soma and proximal dendrites in comparison to glutamatergic boutons, but show a uniform distribution in distal compartments (Ornung et al. 1998). As mentioned earlier, the presence of $GABA_A$ receptors with different subunit composition have been found in motoneurons, however, its significance in controlling excitability is not well understood.

One of the first studies concerning the action of GABA in the nervous system was performed by Curtis and coworkers (1959). These authors showed that GABA depressed the field potentials from dorsal horn interneurons and motoneurons evoked by stimulation of primary afferents (Curtis et al. 1959). They also reported that GABA reduced the magnitude of antidromic potentials, abolished orthodromic potentials, and depressed EPSPs and IPSPs by decreasing motoneuron's electrical excitability (Curtis et al. 1959). Despite similar GABA actions were recorded in γ

motoneurons and Renshaw cells (Curtis et al. 1959), it took many years to decipher that these findings were produced by activation of postsynaptic $GABA_A$Rs.

Subsequent work in the frog's (Peng and Frank 1989) and turtle (Delgado-Lezama et al. 2004) spinal cord showed that the EPSPs evoked by a primary afferent volley and dorso lateral funiculus (DLF) stimulation, respectively, were depressed by muscimol, and antagonized by BMI. The change in the quantum release (but not in the quantal size of the EPSPs), the reduction in the amplitude and the time constant of EPSP's falling phase as well as the increase of conductance in the motoneuron, led to the conclusion that muscimol was acting at the postsynaptic level (Peng and Frank 1989; Delgado-Lezama et al. 2004).

Interestingly, the depression of the EPSP amplitude and input resistance in turtle motoneurons is not reverted by 20 μM BMI and only occurs when the antagonist concentration is raised to 100 μM. Likewise, in supraspinal neurons, it is well known that the blockade of extrasynaptic $GABA_A$ receptors needs high concentration of antagonists (Farrant and Nusser 2005; Walker and Semyanov 2008). In line with this, it has been reported that the EPSPs evoked by DLF stimulation are facilitated and that the membrane resistance is increased in the presence of BMI (Delgado et al. 2004). Given that the $GABA_A$Rs are not expressed in DLF terminals (Rudomin et al. 1991; Curtis and Malik 1984; Curtis et al. 1984), these data suggested that BMI was acting on $GABA_A$Rs tonically active in motoneurons by ambient GABA. Similar actions have been observed in cerebellar and hippocampal granule cells, where activation of extrasynaptic $GABA_A$Rs produces a tonic inhibitory current which is evidenced when the holding current needed to clamp neurons at a predetermined voltage changes in the presence of $GABA_A$R antagonists (Farrant and Nusser 2005; Brickley et al. 1996; Walker and Semyanov 2008; Glykys et al. 2008).

A change in the holding current has also been recorded in motoneurons from the adult turtle in the presence of 100 μM BMI (Fig. 8.2c) (Castro et al. 2011a). It is worth mentioning that the participation of Ca^{2+} -dependent K^+ channels in this effect was ruled out since BMI did not affect the action potential afterhyperpolarization mediated by these channels. In addition, all IPSCs in adult turtle motoneurons were abolished by strychnine without affecting the holding current, confirming the role of glycine receptors as principal inhibitory mediators in these adult cells (Gao et al. 2001). These results also corroborated that spontaneous synaptic activity did not produce tonic inhibition.

On a molecular level, it is possible that extrasynaptic $GABA_A$Rs in turtle motoneurons are composed of $\alpha_{4/6}$ and α_5 subunits, since blockade of both type receptors with specific antagonists furosemide and L-655708, respectively, shifts the excitability curve to the left and increases the input resistance (unpublished data) as observed in cerebellar granule cells (Chadderton et al. 2004). Consistent with this, both receptors have been immunolocalized in turtle spinal cord motoneurons (unpublished data). Likewise, these findings confirm previous results showing the presence of α_5 subunit-containing $GABA_A$Rs in rat motoneurons (Ruano et al. 2000; Bohlhalter et al. 1996), and show that they are tonically active by ambient GABA. However, direct demonstration that $\alpha_{4/6}$ and α_5 subunit-containing $GABA_A$Rs mediating tonic inhibitory currents in motoneurons has not been documented.

From a functional point of view, $GABA_A$Rs in the spinal cord have shown to play an important role in stereotyped patterns of locomotor activity. For instance, in the adult lamprey spinal cord *in vitro*, the GABA system is active during normal locomotor activity and $GABA_A$ receptor activation affects burst frequency (Tegner et al. 1993). Indeed, fictive locomotor activity induced by NMDA shows decreased burst frequency when a blocker of GABA uptake is added. In addition, when a $GABA_A$R antagonist (BMI) is applied during fictive locomotion, an increase in burst rate is observed (Tegner et al. 1993). Similarly, the application of GABA or muscimol in the neonatal rat spinal cord suppresses fictive locomotor activity induced by NMDA in a dose-dependent manner, whereas the use of $GABA_A$R antagonists induces fictive locomotion or increases burst frequency during NMDA-evoked activity (Cazalets et al. 1994). Similar effects are observed in neonatal rat spinal cord displaying fictive locomotion induced by various compounds (serotonin, acetylcholine, NMDA) suggesting that these effects are specifically associated with the NMDA pathway (Cowley and Schmidt 1995). Kremer and Lev-Tov (1997) who reported that $GABA_A$R antagonist combined with low (subthreshold) doses of NMDA and serotonin induced rhythmic motor discharges in the ventral roots that were synchronous rather than alternating bilaterally. This suggested that left–right alternation is mediated mainly by strychnine-sensitive glycine receptors with a possible contribution of strychnine-resistant glycine receptors and/or $GABA_A$Rs. Other experiments using intracellular recording have shown postsynaptically GABA-mediated actions on motoneurons (Bertrand and Cazalets 1998). These results provide evidence that the GABAergic system plays a significant role in modulating the locomotor network (e.g., rhythm generator and left–right alternation), the synaptic motoneuronal inputs presynaptically via $GABA_B$ receptors (and perhaps $GABA_A$ receptors, see section below), and the motoneurons themselves via $GABA_A$ receptors in adult and neonatal species (Tegner et al. 1993; Cazalets et al. 1994; Cowley and Schmidt 1995; Kremer and Lev-tov 1997; Bertrand and Cazalets 1998; Schmitt et al. 2004). The question now arises whether the synaptic and extrasynaptic $GABA_A$ receptors are playing the same role in determining locomotor activity in the spinal cord as they do in supraspinal nuclei (Farrant and Nusser 2005; Semyanov et al. 2004).

8.5.1 $GABA_A$R-mediated Reflex Modulation

Monosynaptic reflex (MSR) activity mediated by glutamate release from Ia primary afferents synapsing motoneurons is a widely used paradigm to investigate motoneuron excitability modulation. Using a combined approach of electrophysiology and pharmacological tools, Eccles et al. initially reported that the long lasting EPSP depression produced by a conditioning antagonist afferent volley, reported by Frank and Fuortes (1957), resulted from inhibition of transmitter release from primary afferents which were depolarized (PAD). This response called PAD, was proposed to be mediated by GABA and is a key factor in producing presynaptic inhibition (Eccles et al. 1963; Eccles 1964).

Iontophoretic application of GABA in combination with immunohistochemical and morphology studies have demonstrated that $GABA_A$Rs activated by GABA release from terminals synapsing primary afferents are mediating presynaptic inhibition (Rudomin and Schmidt 1999; Willis 1999; Kullmann et al. 2005; Schmidt 1971). These findings constituted the first demonstration of the physiological role of GABA in the spinal cord. It is also worth noting that in the pharmacology approach used by Eccles and his group found that intravenous application of picrotoxin not only reversed the MSR depression induced by the conditioning stimulation but also significantly increased the reflex. In addition, picrotoxin also increased flexor reflexes evoked by cutaneous afferent volleys and produced convulsions. All these actions are attributed to the depression of presynaptic inhibition (Eccles et al. 1963).

Although Curtis et al. (1959) had shown that GABA depressed the EPSPs and IPSPs and changed the electrical membrane properties in motoneurons, Eccles and co-workers concluded that this action was due to a presynaptic inhibition of neurotransmitter release (Eccles et al. 1963). Morphological evidence (Rudomin and Schmidt 1999; Alvarez 1998) showing the presence of axoaxonic synapses between primary afferents and GABAergic terminals together with the depolarizing action of GABA on primary afferent terminals and DRG cells, which increase membrane conductance, led to the conclusion that $GABA_A$Rs are present in primary afferents fibers (Curtis et al. 1977; Curtis et al. 1995; Levy 1975; Rudomin et al. 1981; Willis 1999; Rudomin and Schmidt 1999).

By using high affinity $GABA_A$R antagonist such as gabazine, which at low concentration (1 μM) blocks only synaptic receptors without affecting the holding current (Farrant and Nusser 2005; Walker and Semyanov 2008), the effects of presynaptic inhibition on the MSR have been investigated in the turtle spinal cord (Bautista et al. 2010). Interestingly, the presynaptic inhibition of the MSR was abolished by gabazine (1 μM), picrotoxin (20 μM) or BMI (20 μM) without affecting the MSR (Fig. 8.3a, b). These antagonists also depressed the DRP, which is the electrotonic propagation of the PAD that mediates presynaptic inhibition (Fig. 8.3a, b) (Bautista et al. 2010). Furthermore, the increase in picrotoxin and BMI concentration to 100 μM significantly facilitates the MSR and depresses the DRP (Eccles et al. 1963; Bautista et al. 2010). Since BMI (100 μM) blocks the tonic current in motoneruons (Fig. 8.2c; Castro et al. 2011a) in a similar way as in neurons from supraspinal nuclei (Farrant and Nusser 2005; Walker and Semyanov 2008), it was concluded that the MSR facilitation may be induced by blockade of extrasynaptic $GABA_A$RS receptors in motoneurons. The additional depression observed in the DRP may be attributed to the removal of some tonic presynaptic inhibition that also contributes to the MSR facilitation.

Likewise, immunohistochemical studies have identified α_1 and α_2, but not $\alpha_{4/6}$ subunits in primary afferents. It is well known that $GABA_A$Rs containing $\alpha_{4/6}$ subunits are selectively blocked by furosemide at concentrations that did not affect the GABA transporters (Alvarez-Leefmans et al. 1988, 1998; Hamann et al. 2002). Interestingly, the actions of furosemide (200 μM) on the MSR have been investigated after blocking presynaptic inhibition with gabazine (1 μM). The result of this analysis shows that the reflex is facilitated without affecting the DRP (Fig. 8.3b).

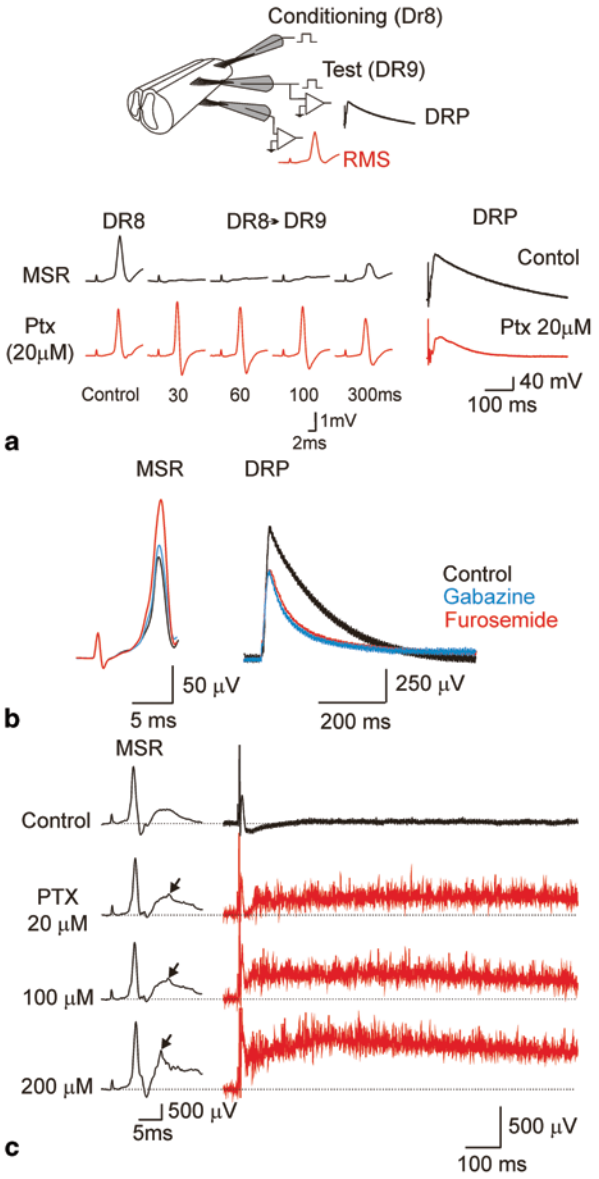

Fig. 8.3 Modulation of the monosynaptic reflex by synaptic and high affinity $GABA_A$ receptors. **a** Scheme showing two spinal cord segments and the experimental setup for evoking presynaptic inhibition of the monosynaptic reflex (*MSR*), and the recording of the dorsal root potential (*DRP*). Top traces in the lower panel represent the test *MSR* and conditioning *MSR* recorded at increasing interstimulus intervals as indicated. Bottom traces denote the test *MSR* and conditioning *MSR* at increasing interstimulus intervals recorded in the presence of picrotoxin. To the right, *DRP* recorded in Ringer control (*top trace*) and in the presence of picrotoxin *(bottom trace*). **b** *MSR* and *DRP* evoked by one volley of primary afferents recorded in Ringer control (*black*), and in the presence of gabazine (*blue*) alone or in combination with furosemide (*red*). **c** *MSR* and long lasting recordings evoked by one volley of primary afferents in Ringer control (*top trace*) and in the presence of picrotoxin at increasing concentrations. The *MSR* is shown at faster time scale. Arrows indicate the polysynaptic reflexes. **a**, **b** and **c** modified with permission from Bautista et al. (2010)

These data together with the action of BMI on the holding current observed in motoneurons suggested that MSR facilitation may not occur, as previously suggested, by depression of presynaptic inhibition mediated by presynaptic $GABA_A$Rs (Eccles et al. 1963), but may result from the blockade of extrasynaptic $GABA_A$Rs located in motoneurons.

Last, the convulsant action of picrotoxin may be explained by an increase in motoneuron excitability. In support of this interpretation, it has been found that in the presence of picrotoxin, BMI, or furosemide the MSR is not the only response to a primary afferents volley, but it is followed by an asynchronous long-lasting firing of motoneuron action potentials (Fig. 8.3c). During the post-discharge, it is not possible to activate the MSR. These initial studies reveal the presence of extrasynaptic $GABA_A$Rs in the turtle spinal cord motoneurons, and show that they might play important roles in controlling the overall excitability of the spinal network determining the motor output.

Concluding Remarks

Extrasynaptic $GABA_A$ receptors containing α_4, α_6, and α_5 subunits represent < 15 % of the total $GABA_A$R population in the CNS. However, they seem to play an important role in controlling neuronal excitability, as well as in motor control, and locomotor function.

In contrast to the extensive research conducted to characterize the expression and function of extrasynaptic $GABA_A$Rs in supraspinal neurons, much less research attention has been paid to the spinal cord. For example, though most of the extrasynaptic $GABA_A$R subunits have been identified in the spinal cord, little is known concerning their function in pre and postsynaptic membranes. To our knowledge, there is only one report demonstrating the presence of a GABAergic tonic current in spinal cord motoneurons (Castro et al. 2011a), and also, just a few characterizing the GABAergic tonic current in other cell types including ventral horn interneurons (Grasshoff et al. 2008; Castro et al. 2011b) dorsal horn interneurons (Takahashi et al. 2006; Mitchell et al. 2007; Han and Youn 2008; Bonin et al. 2011) and chick embryo spinal cord neurons (Yang and Zorumski 1989; Chub and O'Donovan 2001). Therefore, the physiological role of the GABAergic tonic current is far from being elucidated.

Recent studies have shown that spinal motoneuron excitability is modulated by high affinity $GABA_A$ receptors tonically active by ambient GABA. Although it is known that shunting its membrane allow neurons to filter out irrelevant inputs responding with an action potential only to pertinent information (Bautista et al. 2010), additional research is needed to understand which $GABA_A$Rs mediate the tonic inhibitory current in motoneurons and how they perform such an important task. Likewise, identifying the source of GABA is a relevant topic for future studies.

Interestingly, investigation into the field of pain has been performed to identify the $GABA_A$R isoforms responsible for spinal antihyperalgesia. Using a molecular approach, it has been possible to determine the benzodiazepine-sensitive α subunit involved in spinal in this process. Knabl et al. (2008) found strong spinal antihyperalgesia attenuation in mice harboring a point-mutation in the α_2 subunit and less attenuation in mice with a point-mutation in the α_3–α_5-subunits. In line with this, studies carried out in rodent pain models have shown that by modifying the activity

of pre- and postsynaptic $GABA_A$Rs with allosteric modulators, it is possible to induce antihyperalgesia. However, more research is necessary to confirm the possible participation of extrasynaptic $GABA_A$R activity in neurons and primary afferents involved in nociception (Zeilhofer et al. 2012). Of particular interest is the modulation of the DRR in primary afferents by extrasynaptic $GABA_A$ receptors because they can be blocked without producing the undesirable side effects of classical benzodiazepines such as sedation. Likewise, based on their modulatory effects on spinal reflexes and stereotyped locomotor activity in the spinal cord of different species (adults and neonates), it is reasonable to suggest that in addition to the potential therapeutic roles in anesthesia and nociception, novel $GABA_A$R ligands would provide novel therapeutic tools to improve motor and locomotor activity after spinal cord injury or related pathophysiological conditions (e.g., multiple sclerosis).

References

Alvarez FJ (1998) Anatomical basis for presynaptic inhibition of primary sensory fibers. In: Rudomin P, Romo R, Mendell LM (eds) Presynaptic inhibition and neural control. Oxford University Press, New York, pp 13–49

Alvarez FJ, Taylor-Blake B, Fyffe RE, De Blas AL, Light AR (1996) Distribution of immunoreactivity for the beta 2 and beta 3 subunits of the $GABA_A$ receptor in the mammalian spinal cord. J Comp Neurol 365:392–412

Alvarez-Leefmans FJ, Gamiño SM, Giraldez F, Noguerón I (1988) Intracellular chloride regulation in amphibian dorsal root ganglion neurones studied with ion-selective microelectrodes. J Physiol 406:225–246

Alvarez-Leefmans FJ, Nani A, Márquez S (1998) Chloride transport, osmotic balance, and presynaptic inhibition. In: Rudomin P, Romo R, Mendell LM (eds) Presynaptic inhibition and neural control. Oxford University Press, New York, pp 50–79

Ataka T, Gu JG (2006) Relationship between tonic inhibitory currents and phasic inhibitory activity in the spinal cord lamina II region of adult mice. Mol Pain 2:36

Baba H, Ji RR, Kohno T, Moore KA, Ataka T, Wakai A, Okamoto M, Woolf CJ (2003) Removal of GABAergic inhibition facilitates polysynaptic A fiber-mediated excitatory transmission to the superficial spinal dorsal horn. Mol Cell Neurosci 24:818–830

Bautista W, Aguilar J, Loeza-Alcocer JE, Delgado-Lezama R (2010) Pre- and postsynaptic modulation of monosynaptic reflex by $GABA_A$ receptors on turtle spinal cord. J Physiol 588:2621–2631

Bertrand S, Cazalets JR (1998) $GABA_A$ and $GABA_B$ modulation of synaptic transmission between L1-L2 locomotor network and the motoneurons in the newborn rat isolated spinal cord. Ann NY Acad Sci 16:470–471

Bohlhalter S, Weinmann O, Mohler H, Fritschy JM (1996) Laminar compartmentalization of $GABA_A$-receptor subtypes in the spinal cord: an immunohistochemical study. J Neurosci 16:283–297

Bonin RP, Labrakakis C, Eng DG, Whissell PD, De Koninck Y, Orser BA (2011) Pharmacological enhancement of δ-subunit-containing $GABA_A$ receptors that generate a tonic inhibitory conductance in spinal neurons attenuates acute nociception in mice. Pain 152:1317–1326

Bowery NG, Hudson AL, Price GW (1987) $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. Neuroscience 20:365–383

Brickley SG, Mody I (2012) Extrasynaptic $GABA_A$ receptors: their function in the CNS and implications for disease. Neuron 73:23–34

Brickley SG, Cull-Candy S, Farrant M (1996) Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of $GABA_A$ receptors. J Physiol 497:753–759

Carlton SM, Zhou S, Coggeshall RE (1999) Peripheral $GABA_A$ receptors: evidence for peripheral primary afferent depolarization. Neuroscience 93:713–722

Carr RW, Sittl R, Fleckenstein J, Grafe P (2010) GABA increases electrical excitability in a subset of human unmyelinated peripheral axons. PLoS ONE 5:e8780

Castro A, Aguilar J, Andrés C, Felix R, Delgado-Lezama R (2011a) $GABA_A$ receptors mediate motoneuron tonic inhibition in the turtle spinal cord. Neuroscience 192:74–80

Castro A, Aguilar J, González-Ramírez R, Loeza-Alcocer E, Canto-Bustos M, Felix R, Delgado-Lezama R (2011b) Tonic inhibition in spinal ventral horn interneurons mediated by α5 subunit-containing $GABA_A$ receptors. Biochem Biophys Res Commun 412:26–31

Cazalets JR, Sqalli-Houssaini Y, Clarac F (1994) GABAergic inactivation of the central pattern generators for locomotion in isolated neonatal rat spinal cord. J Physiol 474:173–181

Cervero F, Laird JM (1996) Mechanisms of touch-evoked pain (allodynia): a new model. Pain 68:13–23

Chadderton P, Margrie TW, Hausser M (2004) Integration of quanta in cerebellar granule cells during sensory processing. Nature 428:856–860

Chub N, O'Donovan MJ (2001) Post-episode depression of GABAergic tranmission in spinal neurons of the chick embryo. J Neurophysiol 85:2166–2176

Cowley KC, Schmidt BJ (1995) Effects of inhibitory amino acid antagonists on reciprocal inhibitory interactions Turing rhythmic motor activity in the in vitro neontal rat spinal cord. J Neurophysiol 74:1109–1117

Curtis DR (1969) The pharmacology of spinal postsynaptic inhibition. Prog Brain Res 31:171–189

Curtis DR, Malik R (1984) The effect of GABA on lumbar terminations of rubrospinal neurons in the cat spinal cord. Proc R Soc Lond B Biol Sci 223:25–33

Curtis DR, Phillis JW, Watkins JC (1959) The depression of spinal neurones by gamma-amino-n-butyric acid and beta-alanine. J Physiol 146:185–203

Curtis DR, Lodge D, Brand SJ (1977) GABA and spinal afferent terminal excitability in the cat. Brain Res 130:360–363

Curtis DR, Wilson VJ, Malik R (1984) The effect of GABA on the terminations of vestibulospinal neurons in the cat spinal cord. Brain Res 295:372–375

Curtis DR, Gynther BD, Beattie DT, Lacey G (1995) An in vivo electrophysiological investigation of group Ia afferent fibres and ventral horn terminations in the cat spinal cord. Exp Brain Res 106:403–417

Delgado-Lezama R, Aguilar J, Cueva-Rolón R (2004) Synaptic strength between motoneurons and terminals of the dorsolateral funiculus is regulated by GABA receptors in the turtle spinal cord. J Neurophysiol 91:40–47

Désarmenien M, Feltz P, Occhipinti G, Santangelo F, Schlichter R (1984) Coexistence of $GABA_A$ and $GABA_B$ receptors on A delta and C primary afferents. Br J Pharmacol 81:327–333

Deschenes M, Feltz P, Lamour Y (1976) A model for an estimate in vivo of the ionic basis of presynaptic inhibition: an intracellular analysis of the GABA-induced depolarization in rat dorsal root ganglia. Brain Res 118:486–493

Eaton MJ, Martinez MA, Karmally S (1999) A single intrathecal injection of GABA permanently reverses neuropathic pain after nerve injury. Brain Res 835(2):334–339

Eccles JC (1964) Presynaptic Inhibition in the spinal cord. Prog Brain Res 12:65–91

Eccles JC, Schmidt R, Willis WD (1963) Pharmacological studies on presynaptic inhibition. J Physiol 168:500–530

Evans AK, Lowry CA (2007) Pharmacology of the β-carboline FG-7142, a partial inverse agonist at the benzodiazepine allosteric site of the $GABA_A$ receptor: neurochemical, neurophysiological, and behavioral effects. CNS Drug Rev 13:475–501

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 6:215–229

Fitzgerald M, Woolf CJ (1981) Effects of cutaneous nerve and intraspinal conditioning of C-fiber afferent terminal excitability in decerebrate spinal rats. J Physiol 318:25–39

Frank K, Fourtes MGF (1957) Presynaptic and postsynaptic inhibition of monosynaptic reflexes. Fed Proc 16:39–40

Fritschy JM, Benke D, Mertens S, Oertel WH, Bachi T, Möhler H (1992) Five subtypes of type A gamma-aminobutyric acid receptors identified in neurons by double and triple immunofluorescence staining with subunit-specific antibodies. Proc Natl Acad Sci U S A 89:6726–6730

Fritschy JM, Weinmann O, Wenzel A, Benke D (1998) Synapse-specific localization of NMDA and $GABA_A$ receptor subunits revealed by antigen-retrieval immunohistochemistry. J Comp Neurol 390:194–210

Furuyama T, Sato M, Sato K, Araki T, Inagaki S, Takagi H, Tohyama M (1992) Co-expression of glycine receptor beta subunit and $GABA_A$ receptor gamma subunit mRNA in the rat dorsal root ganglion cells. Brain Res Mol Brain Res 12:335–338

Gao H, Smith BN (2010) Tonic $GABA_A$ receptor-mediated inhibition in the rat dorsal motor nucleus of the vagus. J Neurophysiol 103:904–914

Gao BX, Stricker C, Ziskind-Conhaim L (2001) Transition from GABAergic to glycinergic synaptic transmission in newly formed spinal networks. J Neurophysiol 86:492–502

Geiman EJ, Zheng W, Fritschy JM, Alvarez FJ (2002) Glycine and $GABA_A$ receptor subunits on Renshaw cells: relationship with presynaptic neurotransmitters and postsynaptic gephyrin clusters. J Comp Neurol 444:275–289

Glykys J, Mann EO, Mody I (2008) Which $GABA_A$ receptor subunits are necessary for tonic inhibition in the hippocampus? J Neurosci 28:1421–1426

Grasshoff C, Netzhammer N, Schweizer J, Antkowiak B, Hentschke H (2008) Depression of spinal network activity by thiopental: shift from phasic to tonic $GABA_A$ receptor-mediated inhibition. Neuropharmacology 55:793–802

Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Decobert M, Françon D, Avenet P, Depoortere H, Tan S, Oblin A, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B (2001) SL651498: an anxioselective compound with functional selectivity for a2- and a3-containing g-aminobutyric acid A ($GABA_A$) receptors. J Pharmacol Exp Ther 298:753–768

Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Depoortere H, Françon D, Avenet P, Schoemaker H, Evanno Y, Sevrin M, George P, Scatton B (2003) SL651498, a $GABA_A$ receptor agonist with subtype-seletive efficacy, as a potential treatment for generalized anxiety disorder and muscle spasm. CNS Drug Rev 9:3–20

Grognet A, Hertz F, DeFeudis FV (1983) Comparison of the analgesic actions of THIP and morphine. Gen. Pharmacol 14:585–589

Gutiérrez A, Khan ZU, De Blas AL (1996) Immunocytochemical localization of the alpha 6 subunit of the gamma-aminobutyric acidA receptor in the rat nervous system. J Comp Neurol 365:504–510

Hamann M, Rossi DJ, Attwell D (2002) Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. Neuron 33:625–633

Hammond DL, Drower EJ (1984) Effects of intrathecally administered THIP, baclofen and muscimol on nociceptive threshold. Eur J Pharmacol 103:121–125

Han SM, Youn DH (2008) $GABA_A$ receptor-mediated tonic currents in substantia gelatinosa neurons of rat spinal trigeminal nucleus pars caudalis. Neurosci Lett 441:296–301

Hugel H, Ellershaw JE, Dickman A (2003) Clonazepam as an adjuvant analgesic in patients with cancerrelated neuropathic pain. J Pain Symptom Manag 26:1073–1074

Kaneko M, Hammond DL (1997) Role of spinal gamma-aminobutyric acidA receptors in formalin-induced nociception in the rat. J Pharmacol Exp Ther 282:928–938

Knabl J, Witschi R, Hösl K, Reinold H, Zeilhofer UB, Ahmadi S, Brockhaus J, Sergejeva M, Hess A, Brune K, Fritschy JM, Rudolph U, Möhler H, Zeilhofer HU (2008) Reversal of pathological pain through specific spinal $GABA_A$ receptor subtypes. Nature 451:330–334

Knabl J, Zeilhofer UB, Crestani F, Rudolph U, Zeilhofer HU (2009) Genuine antihyperalgesia by systemic diazepam revealed by experiments in $GABA_A$ receptor point-mutated mice. Pain 141:233–238

Kohno T, Wakai A, Ataka T, Ikoma M, Yamakura T, Baba H (2006) Actions of midazolam on excitatory transmission in dorsal horn neurons of adult rat spinal cord. Anesthesiology 104:338–343

Kontinen VK, Dickenson AH (2000) Effects of midazolam in the spinal nerve ligation model of neuropathic pain in rats. Pain 85:425–431

Korpi ER, Kuner T, Seeburg PH, Lüddens H (1995) Selective antagonist for the cerebellar granule cell-specific gamma-aminobutyric acid type A receptor. Mol Pharmacol 47:283–289

Kremer E, Lev-Tov A (1997) Localization of the spinal network associated with generation of hindlimb locomotion in the neonatal rat and organization of its transverse coupling system. J Neurophysiol 77:1155–1170

Kullmann DM, Ruiz A, Rusakov DM, Scott R, Semyanov A, Walker MC (2005) Presynaptic, extrasynaptic and axonal $GABA_A$ receptors in the CNS: where and why? Prog Biophys Mol Biol 87:33–46

Lam FY, Ferrell WR (1989a) Capsaicin suppresses substance P-induced joint inflammation in the rat. Neurosci Lett 105:155–158

Lam FY, Ferrell WR (1989b) Inhibition of carrageenan induced inflammation in the rat knee joint by substance P antagonist. Ann Rheum Dis 48:928–932

Levy RA (1975) The effect of intravenously administered gamma-aminobutyric acid on afferent fiber polarization. Brain Res 92:21–34

Lin Q, Zou X, Willis WD (2000) Aδ and C primary afferents convey dorsal root reflexes after intradermal injection of capsaicin in rats. J Neurophysiol 84:2695–2698

Lisney SJ (1979) Evidence for primary afferent depolarization of single tooth-pulp afferents in the cat. J Physiol 288:437–447

Loeza-Alcocer J, Aguilar J, González-Ramírez R, Felix R, Delgado-Lezama R (2010) Modulation of the dorsal root and the monosynaptic reflex by high affinity α5 subunit-containing $GABA_A$ receptors in the turtle spinal cord. Annual Meeting of the Society for Neuroscience, San Diego, CA

Loomis CW, Khandwala H, Osmond G, Hefferan MP (2001) Coadministration of intrathecal strychnine and bicuculline effects synergistic allodynia in the rat: An isobolographic analysis. J Pharmacol Exp Ther 296:756–761

Ma W, Saunders PA, Somogyi R, Poulter MO, Barker JL (1993) Ontogeny of $GABA_A$ receptor subunit mRNAs in rat spinal cord and dorsal root ganglia. J Comp Neurol 338:337–359

Maeda A, Katafuchi T, Oba Y, Shiokawa H, Yoshimura M (2010) Enhancement of GABAergic tonic currents by midazolam and noradrenaline in rat *substantia gelatinosa* neurons *in vitro*. Anesthesiology 113:429–437

Malan TP, Heriberto HP, Poerreca F (2002) Spinal $GABA_A$ and $GABA_B$ receptor pharmacology in a rat model of neuropathic pain. Anesthesiology 96:1161–1167

McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ (2000) Sedative but not anxiolytic properties of benzodiazepines are mediated by the $GABA_A$ receptor α1 subtype. Nat Neurosci 3:587–592

Melzack R, Wall PD (1965) Pain mechanisms: a new theory. Science 150:971–979

Mitchell EA, Gentet LJ, Dempster J, Belelli D (2007) $GABA_A$ and glycine receptor-mediated transmission in rat lamina II neurons: relevance to the analgesic actions of neuroactive steroids. J Physiol 583:1021–1040

Mody I, Pearce RA (2004) Diversity of inhibitory neurotransmission through $GABA_A$ receptors. Trends Neurosci 27:569–575

Möhler H, Fritschy JM, Rudolph U (2002) A new benzodiazepine pharmacology. J Pharmacol Exp Ther 300:2–8

Moore KA, Kohno T, Karchewski LA, Scholz J, Baba H, Woolf CJ (2002) Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. J Neurosci 22:6724–6731

Morris M, Di Costanzo G, Fox S, Werman R (1983) Depolarizing action of GABA gamma-aminobutyric acid on myelinated fibers of peripheral nerve. Brain Res 278:117–126

Munro G, Erichsen HE, Rae MG, Mirza NR (2011) A question of balance positive versus negative allosteric modulation of $GABA_A$ receptor subtypes as a driver of analgesic efficacy in rat models of inflammatory and neuropathic pain. Neuropharmacol 61:121–132

Naik AK, Pathirathna S, Jevtovic-Todorovic V (2008) $GABA_A$ receptor modulation in dorsal root ganglia in vivo affects chronic pain after nerve injury. Neuroscience 154:1539–1553

Nusser Z, Mody I (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J Neurophysiol 87:2624–2628

Nusser Z, Roberts JD, Baude A, Richards JG, Somogyi P (1995) Relative densities of synaptic and extrasynaptic $GABA_A$ receptors on cerebellar granule cells as determined by a quantitative immunogold method. J Neurosci 15:2948–2960

Nusser Z, Sieghart W, Benke D, Fritschy JM, Somogyi P (1996) Differential synaptic localization of two major gamma-aminobutyric acid type A receptor alpha subunits on hippocampal pyramidal cells. Proc Natl Acad Sci U S A 93:11939–11944

Nusser Z, Cull-Candy S, Farrant M (1997) Differences in synaptic $GABA_A$ receptor number underlie variation in GABA mini amplitude. Neuron 19(3):697–709

Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693–1703

Nusser Z, Ahmad Z, Tretter V, Fuchs K, Wisden W, Sieghart W, Somogyi P (1999) Alterations in the expression of $GABA_A$ receptor subunits in cerebellar granule cells after the disruption of the alpha6 subunit gene. Eur J Neurosci 11:1685–1697

Olsen RW, Sieghart W (2008) International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Pharmacol Rev 60:243–260

Olsen RW, Sieghart W (2009) GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56:141–148

Ornung G, Ottersen OP, Cullheim S, Ulfhake B (1998) Distribution of glutamate, glycine- and GABA-immunoreactive nerve terminals on dendrites in the cat spinal motor nucleus. Exp Brain Res 118:517–532

Pape JR, Bertrand SS, Lafon P, Odessa MF, Chaigniau M, Stiles JK, Garret M (2009) Expression of $GABA_A$ receptor alpha3-, theta-, and epsilon-subunit mRNAs during rat CNS development and immunolocalization of the epsilon subunit in developing postnatal spinal cord. Neuroscience 160:85–96

Paul J, Zeilhofer HU, Fritschy JM (2012) Selective distribution of $GABA_A$ receptor subtypes in mouse spinal dorsal horn neurons and primary afferents. J Comp Neurol (in press)

Pelley KA, Vaught JL (1987) An antinociceptive profile of kojic amine: an analogue of gamma-aminobutyric acid (GABA). Neuropharmacology 26:301–307

Peng YY, Frank E (1989) Activation of $GABA_A$ receptors causes presynaptic and postsynaptic inhibition at synapses between muscle spindle afferents and motoneurons in the spinal cord of bullfrogs. J Neurosci 9:1516–1522

Persohn E, Malherbe P, Richards JG (1991) In situ hybridization histochemistry reveals a diversity of $GABA_A$ receptor subunit mRNAs in neurons of the rat spinal cord and dorsal root ganglia. Neuroscience 42:497–507

Petri S, Schmalbach S, Grosskreutz J, Krampfl K, Grothe C, Dengler R, Van Den Bosch L, Robberecht W, Bufler J (2005) The cellular mRNA expression of GABA and glutamate receptors in spinal motor neurons of SOD1 mice. J Neurol Sci 238:25–30

Rees H, Sluka KA, Westlund KN et al (1995) The role of glutamate and GABA receptors in the generation of dorsal root reflexes by acute arthritis in the anaesthetized rat. J Physiol 484:437–445

Rekling JC, Funk GD, Bayliss DA, Dong XW, Feldman JL (2000) Synaptic control of motoneuronal excitability. Physiol Rev 80:767–852

Réthelyi M, Capowski JJ (1977) The terminal arborization pattern of primary afferent fibers in the substantia gelatinosa of the spinal cord in the cat. J Physiol (Paris) 73:269–277

Richards JG, Schoch P, Häring P, Takacs B, Möhler H (1987) Resolving $GABA_A$/benzodiazepine receptors: cellular and subcellular localization in the CNS with monoclonal antibodies. J Neurosci 7:1866–1886

Roberts LA, Beyer C, Komisaruk BR (1986) Nociceptive responses to altered GABAergic activity at the spinal cord. Life Sci 39:1667–1674

Rode F, Jensen DG, Blackburn-Munro G, Bjerrum OJ (2005) Centrally-mediated antinociceptive actions of $GABA_A$ receptor agonists in the rat spared nerve injury model of neuropathic pain. Eur J Pharmacol 516:131–138

Ruano D, Létang V, Biton B, Avenet P, Benavides J, Scatton B, Vitorica J (2000) Subunit composition of rat ventral spinal cord $GABA_A$ receptors, assessed by single cell RT-multiplex PCR. Neuroreport 11:3169–3173

Rudolph U, Knoflach F (2011) Beyond classical benzodiazepines: novel therapeutic potential of $GABA_A$ receptor subtypes. Nat Rev Drug Discov 10:685–697

Rudomin P, Schmidt RF (1999) Presynaptic inhibition in the vertebrate spinal cord revisited. Exp Brain Res 129:1–37

Rudomin P, Engberg I, Jiménez I (1981) Mechanisms involved in presynaptic depolarization of group I and rubrospinal fibers in cat spinal cord. J Neurophysiol 46:532–548

Rudomin P, Jiménez I, Enriquez M (1991) Effects of stimulation of group I afferents from flexor muscles on heterosynaptic facilitation of monosynaptic reflexes produced by Ia and descending inputs: a test for presynaptic inhibition. Exp Brain Res 85:93–102

Schmidt RF (1971) Presynaptic inhibition in the vertebrate central nervous system. Ergeb Physiol 63:20–101

Schmitt DE, Hill RH, Grillner S (2004) The spinal GABAergic system is a strong modulator of burst frequency in the lamprey locomotor network. J Neurophysiol 92:2357–2367

Semyanov A, Walker MC, Kullmann DM (2003) GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nat Neurosci 6:484–490

Semyanov A, Walker MC, Kullmann DM, Silver RA (2004) Tonically active $GABA_A$ receptors: modulating gain and maintaining the tone. Trends Neurosci 27:262–269

Serafini R, Maric D, Maric I, Ma W, Fritschy JM, Zhang L, Barker JL (1998) Dominant GABA(A) receptor/Cl- channel kinetics correlate with the relative expressions of α_2, α_3, α5 and β_3 subunits in embryonic rat neurones. Eur J Neurosci 10:334–349

Singer E, Placheta P (1980) Reduction of [^{3}H]muscimol binding sites in rat dorsal spinal cord after neonatal capsaicin treatment. Brain Res 202:484–487

Sinkkonen ST, Vekovischeva OY, Möykkynen T (2004) Behavioural correlates of an altered balance between synaptic and extrasynaptic $GABA_A$ergic inhibition in a mouse model. Eur J Neurosci 20:2168–2178

Sivilotti L, Woolf CJ (1994) The contribution of $GABA_A$ and glycine receptors to central sensitization: disinhibition and touch-evoked allodynia in the spinal cord. J Neurophysiol 72:169–179

Somogyi P, Takagi H, Richards JG, Mohler H (1989) Subcellular localization of benzodiazepine/GABA, receptors in the cerebellum of rat, cat, and monkey using monoclonal antibodies. J Neurosci 9:2197–2209

Somogyi P, Fritschy JM, Benke D, Roberts JD, Sieghart W (1996) The γ_2 subunit of the $GABA_A$-receptor is concentrated in synaptic junctions containing the α_1 and $\beta_{2/3}$ subunits in hippocampus, cerebellum and globus pallidus. Neuropharmacology 35:1425–1444

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing $GABA_A$ receptors. Proc Natl Acad Sci U S A 100:14439–14444

Street LJ, Sternfeld F, Jelley RA, Reeve AJ, Carling RW, Moore KW, McKernan RM, Sohal B, Cook S, Pike A, Dawson GR, Bromidge FA, Wafford KA, Seabrook GR, Thompson SA, Marshall G, Pillai GV, Castro JL, Atack JR, MacLeod AM (2004) Synthesis and biological evaluation of 3-heterocyclyl-7,8,9,10-tetrahydro-(7,10-ethano)-1,2,4-triazolo[3,4-a]phthalazines and analogues as subtype-selective inverse agonists for theGABA(A)alpha5 benzodiazepine binding site. J Med Chem 47:3642–3657

Sur C, Mckernan R, Triller A (1995) Subcellular localization of the $GABA_A$ receptor γ_2 subunit in the rat spinal cord. Eur J Neurosci 7:1323–1332

Takahashi A, Mashimo T, Uchida I (2006) GABAergic tonic inhibition of substantia gelatinosa neurons in mouse spinal cord. Neuroreport 17:1331–1335

Tegner J, Matsushima T, El Manira A, Grillner S (1993) The spinal GABA system modulates burst frequency and intersegmental coordination in the lamprey: differential effects of $GABA_A$ and $GABA_B$ receptors. J Neurophysiol 69:647–657

Todd AJ, Watt C, Spike RC, Sieghart W (1996) Colocalization of GABA, glycine, and their receptors at synapses in the rat spinal cord. J Neurosci 16:974–982

Toennies G (1938) A sulfoxide of methionine. Science 88:545–546

Torsney C, MacDermott AB (2006) Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. J Neurosci 26:1833–1843

Vit JP, Ohara PT, Sundberg C, Rubi B, Maechler P, Liu C, Puntel M, Lowenstein P, Castro M, Jasmin L (2009) Adenovector GAD65 gene delivery into the rat trigeminal ganglion produces orofacial analgesia. Mol Pain 5:42

Waldvogel HJ, Faull RL, Jansen KL, Dragunow M, Richards JG, Mohler H, Streit P (1990) GABA, GABA receptors and benzodiazepine receptors in the human spinal cord: an autoradiographic and immunohistochemical study at the light and electron microscopic levels. Neuroscience 39:361–385

Waldvogel HJ, Baer K, Eady E, Allen KL, Gilbert RT, Mohler H, Rees MI, Nicholson LF, Faull RL (2010) Differential localization of gamma-aminobutyric acid type A and glycine receptor subunits and gephyrin in the human pons, medulla oblongata and uppermost cervical segment of the spinal cord: an immunohistochemical study. J Comp Neurol 518:305–328

Walker MC (2008) $GABA_A$ receptor subunit specificity: a tonic for the excited brain. J Physiol 586:921–922

Walker MC, Semyanov A (2008) Regulation of excitability by extrasynaptic $GABA_A$ receptors. Results Probl Cell Differ 44:29–48

Wang L, Spary E, Deuchars J, Deuchars SA (2008) Tonic GABAergic inhibition of sympathetic preganglionic neurons: a novel substrate for sympathetic control. J Neurosci 28:12445–12452

Willis WD (1999) Dorsal root potentials and dorsal root reflexes: a double-edged sword. Exp Brain Res 124:395–421

Wisden W, Gundlach AL, Barnard EA, Seeburg PH, Hunt SP (1991) Distribution of $GABA_A$ receptor subunit mRNAs in rat lumbar spinal cord. Brain Res Mol Brain Res 10:179–183

Witschi R, Punnakkal P, Paul J, Walczak JS, Cervero F, Fritschy JM, Kuner R, Keist R, Rudolph U, Zeilhofer HU (2011) Presynaptic α_2-$GABA_A$ receptors in primary afferent depolarization and spinal pain control. J Neurosci 31:8134–8142

Yang J, Zorumski CF (1989) Trifluoperazine blocks GABA-gated chloride currents in cultured chick spinal cord neurons. J Neurophysiol 61:363–373

Yoshimura M, Jessell T (1990) Amino acid-mediated EPSPs at primary afferent synapses with *substantia gelatinosa* neurones in the rat spinal cord. J Physiol 430:315–335

Zarnowska ED, Keist R, Rudolph U, Pearce RA (2009) $GABA_A$ receptor α_5 subunits contribute to $GABA_A$, slow synaptic inhibition in mouse hippocampus. J Neurophysiol 101:1179–1191

Zeilhofer HU, Benke D, Yevenes GE (2012) Chronic pain states: pharmacological strategies to restore diminished inhibitory spinal pain control. Annu Rev Pharmacol Toxicol 52:111–133

Chapter 9
The Role of Peri-synaptic GABA Receptors After Stroke

Andrew N. Clarkson and Mary Chebib

Abstract An attempt to find pharmacological therapies to treat stroke patients and minimize the extent of cell death has seen the failure of dozens of clinical trials. As a result, stroke/cerebral ischemia remains the second leading cause of death and is the leading cause of lasting adult disability worldwide. Stroke-induced cell death occurs due to an excess release of glutamate. As a consequence to this, a compensatory increased release of γ-aminobutyric acid (GABA) occurs that results in the subsequent internalization of synaptic $GABA_A$ receptors and spillover onto peri-synaptic/extrasynaptic $GABA_A$ receptors, resulting in an increase in tonic inhibition. Recent studies show that the brain can engage in a limited process of neural repair after stroke. Changes in cortical sensory and motor maps and alterations in axonal structure are dependent on patterned neuronal activity. The central cellular process in these events is alteration in neuronal response to incoming inputs—manipulations that increase neuronal firing to a given input are likely to induce changes in neuronal structure and alterations in cortical maps. It has been assumed that changes in neuronal excitability underlie processes of neural repair and remapping of cortical sensory and motor representations. Indeed, recent evidence suggests that local inhibitory and excitatory currents are altered after stroke and modulation of these networks to enhance excitability during the repair phase can facilitate functional recovery after stroke. More specifically, dampening tonic GABA inhibition from 3 days poststroke can afford an early and robust improvement in functional recovery after stroke. Further, recent data also suggest that boosting tonic GABA inhibition early after a stroke can afford significant protection and minimize the extent of neuronal cell loss.

Keywords Cortical excitability · Disinhibition · Neuroprotection · Neurorepair · Plasticity

A. N. Clarkson (✉)
Departments of Anatomy and Psychology, University of Otago,
PO Box 913, Dunedin 9013, New Zealand
e-mail: andrew.clarkson@otago.ac.nz

M. Chebib
Department of Pharmacy A15, University of Sydney, Sydney, NSW, Australia

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_9

Table 9.1 GABA signaling and the effects after stroke. (Source: Modified from Carmichael 2012)

Physiological process	Anatomical location	Neuronal function	Response to stroke during the acute phase	Response to stroke during the late phase	Role in functional recovery
Synaptic GABA inhibition	Synaptic	Fast inhibition between neuronal networks	Neuroprotection	Current impaired, role during late phase not yet known	Increasing synaptic current may promote mild recovery
Tonic GABA inhibition	Extrasynaptic	Slow inhibition, controls neuronal excitability	Neuroprotection	Increased current activation impairs recovery	Dampening tonic GABA currents facilitates improved functional recovery after stroke

9.1 γ-Aminobutyric Acid

γ-aminobutyric acid (GABA) is the principle inhibitory neurotransmitter within the mammalian central nervous system. Of all synapses, 20–50% within the CNS use GABA as a neurotransmitter, mediating both fast and slow inhibitory synaptic transmission (Sieghart 1995). GABA is an endogenous ligand for the $GABA_A$, $GABA_B$, and $GABA_C$ receptors (Chebib and Johnston 1999) and these receptor subtypes have been classified according to differences in both structure and pharmacology. $GABA_A$Rs are ligand-gated chloride channels (Chebib and Johnston 1999; D'Hulst et al. 2009) formed from five subunits arranged around a central ion pore. At least 19 mammalian genes encoding for the various $GABA_A$R subunits exist: α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, φ, π, and ρ_{1-3}, with slice variants also contributing to variations in receptor functions (Macdonald and Olsen 1994; Olsen and Sieghart 2008, 2009; Barrera et al. 2008; Whiting 2003a, b). The most common subunit combinations are believed to be composed of 2α, 2β, and γ, with the γ-subunit being able to be substituted for either an ε- or a δ-subunit (Barrera et al. 2008; Whiting 2003a, b).

GABA signaling within the brain occurs through two main systems (Table 9.1). Depolarization of inhibitory interneurons produces a phasic release of GABA and inhibition of postsynaptic neurons. Extrasynaptic $GABA_A$Rs respond to ambient levels of GABA present in the extracellular space to regulate baseline pyramidal neuron excitability and show reduced desensitization remaining active for long periods of time (Glykys and Mody 2007a). Tonic $GABA_A$Rs in the hippocampus and cortex contain either α5 or δ-subunits (Olsen and Sieghart 2009; Glykys and Mody 2007a). Reduced activity of α5 or δ-subunits enhances pyramidal neuron firing to afferent inputs (Glykys and Mody 2006, 2007a; Drasbek and Jensen 2006), enhances neuronal network excitability (Walker and Semyanov 2008), and facilitates long-term potentiation (LTP) and cognitive performance (Atack et al. 2006; Collinson et al. 2002; Dawson et al. 2006; Crestani et al. 2002). GABA transporters modulate

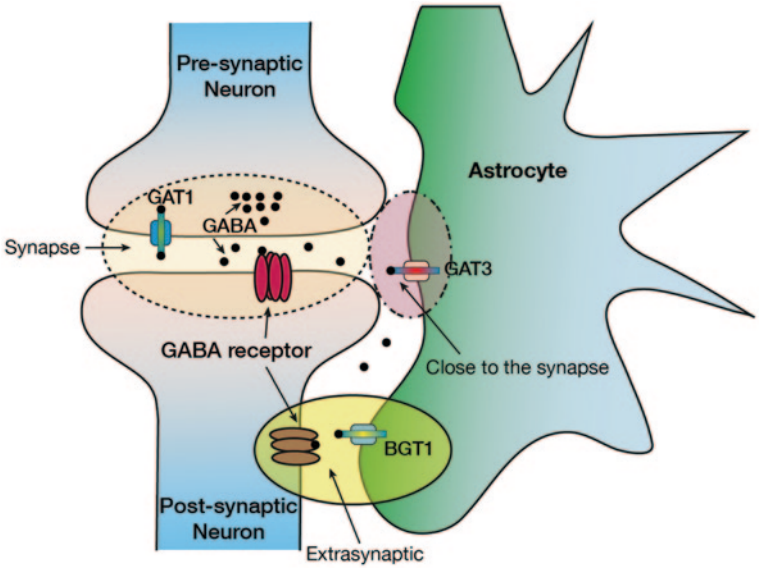

Fig. 9.1 The primary subcellular localization of *GATs* in relation to *$GABA_A$Rs*. *GAT-1* is primarily located *presynaptically*, *GAT-3* is primarily located on distal *astrocytic* processes in close proximity to the *synapse* (peri-synaptically) and *BGT-1* is located *extrasynaptically*. (Modified from Madsen et al. 2009)

the level of tonic $GABA_AR$ activity (Keros and Hablitz 2005) with the uptake of GABA into neurons and astrocytes for recycling (see Fig. 9.1). Low GABA concentrations activate extrasynaptic $GABA_ARs$, leading to persistent or tonic inhibition (Belelli et al. 2009; Mody 2001). Synaptic and extrasynaptic $GABA_ARs$ exhibit distinct pharmacological and biophysical properties that differentially influence brain physiology and behavior (Belelli et al. 2009).

Synaptic $GABA_ARs$ are composed of α_{1-3}, β_{1-3}, and γ_{1-3} subunits, and are the site of action for a variety of clinically important drugs, such as benzodiazepines, neurosteroids, and anesthetics, whereas extrasynaptic $GABA_ARs$ are composed of subunit combinations containing α_{4-6}, β_{1-3}, and γ_2- or δ-subunits. Of these receptors, the δ-containing $GABA_ARs$ co-assembled as $\alpha_4\beta\delta$—located in the cortex, hippocampus and thalamus—or $\alpha_6\beta\delta$—located in the cerebellum—that are emerging as unique and fundamental players in GABAergic neurotransmission (Belelli et al. 2009). In addition to δ-containing $GABA_ARs$ having a functional role in the cortex, the α5-containing $GABA_ARs$ co-assembled primarily as $\alpha_5\beta\gamma_2$ have also been implicated in poststroke repair (Clarkson et al. 2010). Even though the expression of the α5-subunit is low in the cortex compared to the δ-subunit, greater functional improvements in motor recovery are seen following modulation of the α5-subunit (Clarkson et al. 2010). The pharmacology of these extrasynaptic receptors is inconsistent between research groups (Borghese and Harris 2007) and has been hampered by the lack of selective agents to probe function in recombinant, native, and whole animal systems (Wafford et al. 2009). Conflicting data are also present with respect

to the ability of these receptors to desensitize (Belelli et al. 2009; Bright et al. 2011). Determining the composition and pharmacology of this receptor will enable the development of much needed therapies for use in stroke.

9.2 Disability in Stroke

Stroke is the leading cause of death and long-term disability in adults worldwide. Stroke-induced sensory and motor loss of limb function in particular prevents patients from returning to work and accounts for the statistic that almost one-third of stroke survivors become institutionalized after having a stroke (Ng et al. 2007; Lai et al. 2002; Dobkin 2004, 2008). Recent studies have shown that the brain has a limited capacity to repair after stroke. In both humans and animals, neural repair after stroke has been shown to involve remapping of cognitive functions and sprouting of new connections in tissue adjacent to the stroke site, the peri-infarct cortex (Carmichael 2006; Nudo 2006). However, mechanisms associated with poststroke neural repair and recovery have not been well characterized and it has been assumed that changes in cortical representational maps underlying the recovery involve changes in neuronal excitability. Consistent with this, animal studies suggest that therapies associated with rehabilitation can promote plasticity changes in tissue that survives the stroke (Maldonado et al. 2008).

Functional recovery within the peri-infarct cortex involves changes in neuronal excitability. Clinical studies using direct current stimulation of the peri-infarct cortex, with protocols that boost local neuronal excitability, have been shown to improve use of the affected limb in stroke patients (Alonso-Alonso et al. 2007; Hummel and Cohen 2006). Further, forced use or task-specific repetition of the affected limb has also been shown to activate the peri-infarct cortex and improve functional recovery (Wittenberg and Schaechter 2009). Studies suggest that decreases in GABA activity within the motor cortex could facilitate structural changes (Hess et al. 1996) and promote recovery of motor function (Cicinelli et al. 2003). Alterations in neuronal excitability underlie fundamental changes in information transfer in neuronal circuits (Citri and Malenka 2008) such as LTP and long-term depression (LTD) as well as the unmasking of quiescent synaptic connections and remodeling of cortical maps (Jacobs and Donoghue 1991). Further, changes in LTP and cortical map formation occur within the peri-infarct cortex adjacent to the stroke (Carmichael 2006). These data suggest a critical role for modulating cortical excitability as a means for promoting functional recovery after stroke.

9.2.1 Brain Excitability in Learning, Memory, and Repair

The processes of neurorehabilitation involve physical, occupational, and cognitive therapies (Dobkin 2004, 2008). Further, changes in poststroke cortical plasticity

play a critical role in mediating repair mechanisms. While these modalities clearly promote functional recovery, no drug treatments exist that promote poststroke brain repair and recovery. Recent evidence suggests that suppression of either cortical tonic GABA inhibition or stimulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor currents can promote poststroke function gain (Clarkson et al. 2010, 2011). This ability to regain function relies heavily on the ability to learn or relearn after stroke and likely follows classical activity-dependent processes associated with motor learning and memory (Conner et al. 2005; Krakauer 2006). In addition to these behavioral links, stroke recovery and classical learning and memory pathways share similar molecular and cellular links. For instance, genes that are important for learning and memory are also elevated during periods of poststroke repair and include membrane-associated phosphoproteins GAP43 and MARCKS, the transcription factor c-jun, and the cell adhesion molecule L1 (Carmichael et al. 2005).

Modulation of learning and memory pathways have previously been shown to promote functional recovery and poststroke axonal sprouting following administration of pharmacological agents such as amphetamines and phosphodiesterase type-4 inhibitors that boost cAMP/CREB signaling and learning and memory function (Stroemer et al. 1998; MacDonald et al. 2007). These data indicate that manipulating learning and memory pathways can offer a novel means for promoting recovery. As with stroke recovery, the processes of learning and memory can be enhanced by manipulations that increase neuronal excitability, which has also been shown to promote function recovery (Clarkson et al. 2010). Significant data are accumulating indicating an imbalance in inhibitory and excitatory pathways after stroke and modulation of these pathways by either enhancing glutamate-mediated transmission or dampening the tonic form of GABA can facilitate functional recovery (Clarkson et al. 2010, 2011; Clarkson and Carmichael 2009; Schmidt et al. 2010, 2011; Jaenisch et al. 2010). α5-$GABA_A$R-negative allosteric modulators are part of a broad class of drugs that boost learning and memory function by influencing key elements in neuronal memory storage, such as LTP (Atack et al. 2006; Dawson et al. 2006). α5-$GABA_A$R-negative allosteric modulators, and indeed any mechanism that dampens tonic GABA signaling, could significantly improve poststroke recovery (Clarkson et al. 2010). This suggests that the similarities between neuronal mechanisms of learning and memory, and those of functional recovery after stroke extend to common treatment strategies for both.

Most strategies that promote functional recovery after stroke, such as axonal sprouting, neurogenesis, or angiogenesis, focus or rely on inducing structural changes in the brain as a means to promote functional recovery after stroke (Carmichael 2008, 2010; Ohab et al. 2006; Zhang and Chopp 2009; Liauw et al. 2008). In order to promote structural change in the brain however, these treatments take time to develop a functional effect. Blocking tonic GABA inhibition induces a rapid improvement in behavioral recovery in the absence of any change in axonal sprouting within the peri-infarct cortex (Clarkson et al. 2010). These data suggest that treatments that focus on inducing molecular memory systems after stroke may have the advantage of promoting synaptic plasticity in peri-infarct cortex rapidly and without

altering the tissue reorganization that normally occurs after stroke. These therapies are highly translatable into the clinic due to their timing of drug administration, 3–7 days after stroke in rodents, and with the early effects seen with functional recovery, will aid in the huge social and economical burdens seen after stroke.

9.2.2 *Attenuating $GABA_A$ Receptor Function in Neural Repair After Stroke*

As with stroke recovery, the processes of learning and memory can be enhanced by manipulations that increase neuronal excitability. However, unlike the stroke recovery field, basic science studies in learning and memory have defined specific cellular pathways that lead to enhanced neuronal excitability and improved function.

Recent work has shown that enhanced neuronal excitability occurs following the dampening of the baseline level of inhibition in neurons. This baseline inhibition is in part set by a tonic, always present, degree of inhibitory signaling from the major inhibitory neurotransmitter, GABA. Unlike the phasic nature of synaptically released GABA, the action of GABA via extrasynaptic receptors is to tonically suppress neuronal excitability and to help regulate neuronal action potential firing. These extrasynaptic GABA receptors consist of α5 and δ-subunit-containing $GABA_A$Rs. Recent evidence using α5-$GABA_A$R "knockout" and point-mutated mice have clearly shown that the α5-subunitplays a key role in cognitive processing (Collinson et al. 2002; Crestani et al. 2002). In addition, in vitro and in vivo work has shown that α5-$GABA_A$R-negative allosteric modulators can enhance cognition within the Morris water maze, enhance hippocampal LTP, and do not have any pro-convulsant effects (Atack et al. 2006; Dawson et al. 2006). Using pharmacological and genetic manipulations of extrasynaptic $GABA_A$R's, we have shown marked improvements in functional recovery when starting treatments from 3 days after the stroke (Clarkson et al. 2010). These data are consistent for offering a potential role for extrasynaptic $GABA_A$Rs in processes involving synaptic plasticity and learning and memory and more recently post-stroke recovery.

Neuronal inhibition and network function is disturbed in peri-infarct tissue during periods of cortical plasticity, remapping, and recovery. The increase in tonic inhibition in cortical pyramidal neurons reported by Clarkson and colleagues (Clarkson et al. 2010) occurs at precisely the same time as cortical map plasticity and recovery (Brown et al. 2009). Behavioral recovery in stroke is closely correlated with functional plasticity in peri-infarct and connected cortical regions. In human stroke patients, an expansion in motor representation maps is seen in tissue adjacent to or connected to stroke (Carmichael 2006; Calautti and Baron 2003). In animal models, when stroke damages primary motor or somatosensory areas, motor and sensory representations remap in peri-infarct cortex (Brown et al. 2009; Dijkhuizen et al. 2003). These processes of recovery identify plasticity in the cortical circuits in peri-infarct cortex as key elements in functional recovery.

9.3 GABA and Cerebral Ischemia

A large body of work has been devoted to developing and exploring neuroprotectants that act to block glutamate-mediated neurotransmission in animal models of cerebral ischemia (Lipton 1999; Nicotera and Lipton 1999). Increased inhibitory neurotransmission associated with GABA has been shown to normalize the balance of glutamate-mediated excitation. Therefore, pharmacological enhancement of $GABA_AR$ neurotransmission provides an alternative means for neuroprotection. Indeed, over recent years, changes in GABA function following cerebral ischemia and possible protective benefits of GABAergic drugs have been extensively assessed (Schwartz et al. 1995; Green et al. 2000; Schwartz-Bloom and Sah 2001; Schwartz-Bloom et al. 2000; Alicke and Schwartz-Bloom 1995; Clarkson et al. 2005, 2007). Even though it has been proposed that enhancing GABA transmission may elicit protection against cerebral ischemia (Green et al. 2000; Schwartz-Bloom and Sah 2001; Schwartz-Bloom et al. 2000; Clarkson et al. 2005), the exact mechanisms that are associated with these neuroprotectants have, as yet, not been fully elucidated (see Fig. 9.2) and increasing GABA function may be protective during cerebral ischemia for different reasons (Schwartz et al. 1995; Green et al. 2000; Schwartz-Bloom and Sah 2001; Schwartz-Bloom et al. 2000; Alicke and Schwartz-Bloom 1995; Clarkson et al. 2005, 2007). Even though GABA agonists have shown great promise in animal models, these compounds have failed to translate into the clinic (Ginsberg 2008; Lyden et al. 2002). The failure of these compounds highlights the need to firstly establish better preclinical rodent models of stroke that better mimic what occurs in humans. Secondly, the use of subunit-specific GABA compounds is more likely to show an effect, due to them having less side effects, such as drug-induced hypothermia and sedation. However, even with recent developments in this area, studies are lacking. The need to assess subunit-specific GABA compounds to help understand what is happening after stroke in terms of GABA function is highlighted with clinical reports showing that zolpidem an α1 subunit $GABA_AR$ modulator can result in a transient improvement in aphasia in chronic stroke survivors (Cohen et al. 2004).

During situations of cerebral ischemia, it has been shown that the extracellular concentrations of GABA increase (approximately 50-fold compared to basal levels) to the micromolar range (Schwartz et al. 1995; Inglefield et al. 1995) and remain elevated for at least 30 min during periods of reperfusion. Prolonged exposure of the $GABA_ARs$ to high concentrations of GABA agonists in vitro has routinely shown desensitization and/or downregulation of the receptors (Cash and Subbarao 1987; Huguenard and Alger 1986; Tehrani and Barnes 1991). Similarly, the $GABA_AR$ is also downregulated in the gerbil hippocampus following transient cerebral ischemia (Alicke and Schwartz-Bloom 1995). In this model, receptor downregulation was shown to be via internalization, as there was a rapid decrease in binding of the hydrophilic ligand [3H]-SR-95531, but not the hydrophobic ligand [3H]-flunitrazepam (Alicke and Schwartz-Bloom 1995). This increase in extracellular GABA is likely to result in the spillover onto peri-synaptic $GABA_ARs$ resulting in an increase in tonic inhibition (see Fig. 9.3). Indeed, recent evidence showing an

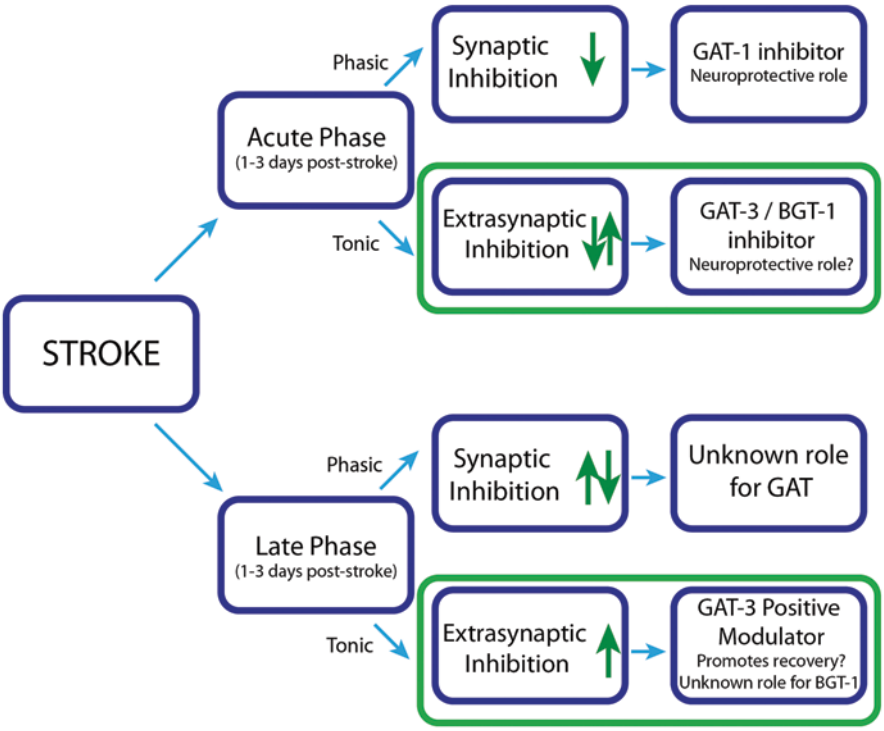

Fig. 9.2 GABA signaling and the role of *GAT* modulation after stroke. The *green boxes* highlight the *biphasic* nature of *extrasynaptic* GABA signaling. Early tonic GABA signaling limits the extent of neuronal cell death, whereas *tonic* GABA signaling during the *late phase* impairs stroke *recovery*. Here, we propose that early inhibition of *GAT* (*GAT-1*, *GAT-3*, and *BGT-1*) signaling can limit the extent of neuronal cell death, whereas, *positive modulation* of at least *GAT-3* and *BGT-1* during the *late phase* can aid in the *recovery* of lost functions after stroke. ↑—increased GABA currents, ↓—decreased GABA currents, ↑↓—no change in GABA currents

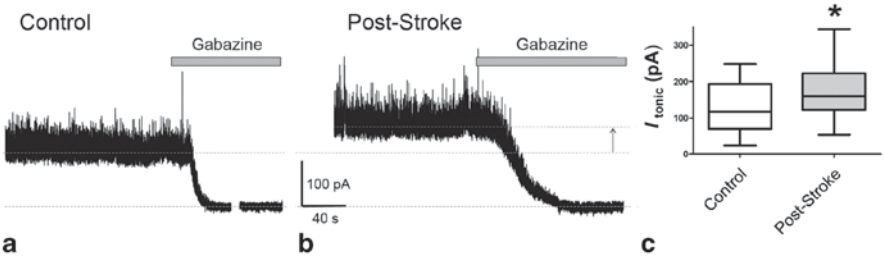

Fig. 9.3 Elevated tonic GABA inhibition within peri-infarct cortical regions. **a** and **b** Representative traces showing tonic inhibitory currents in *control* and *stroke* peri-infarct neurons, respectively. **c** Box plot (*boxes*: 25–75%, *whiskers*: 10–90%, *lines*: median) showing significantly elevated tonic inhibition in peri-infarct cortex ($^*P < 0.05$). Tonic currents were revealed by the shift in holding currents after blocking all $GABA_A$Rs with *gabazine* (>100 μM). Cells were voltage-clamped at +10 mV

increase in tonic inhibition after stroke supports this notion (Clarkson et al. 2010). This increase in tonic inhibition is most likely a safety mechanism imposed by the brain as a means to minimize neuronal damage. However, as this increase in tonic inhibition persists for at least 2 weeks after the stroke, this safety mechanism is likely to have either the wrong or no feedback mechanism formed to compensate for such a change in tonic GABA.

9.4 Poststroke Tonic Inhibition

Changes in neuronal excitability, loss of GABAergic inhibition, enhanced glutamatergic transmission, and synaptic plasticity all contribute to neuronal reorganization after stroke. Studies that promote an increase in local brain excitability result in improved function (Clarkson et al. 2010, 2011; Wittenberg and Schaechter 2009; Clarkson and Carmichael 2009) and suggest that decreasing GABA activity within the brain could facilitate structural changes that promote functional recovery (Fig. 9.2; Clarkson et al. 2010; Wittenberg and Schaechter 2009; Clarkson and Carmichael 2009). In particular, this enhancement of neuronal excitability involves dampening baseline levels of inhibition.

Tonic or continuous signaling from GABA sets baseline inhibition. GABA acts via extrasynaptic $GABA_A$Rs to tonically suppress neuronal excitability and regulate neuronal action potential firing. Therefore, in order to facilitate functional recovery, an increase in brain excitability is required to overcome this hypofunctionalism (Wittenberg and Schaechter 2009). Recently, Clarkson and colleagues have demonstrated marked improvements in poststroke functional recovery using pharmacological manipulations of extrasynaptic $GABA_A$Rs, implicating α5 or δ-containing $GABA_A$Rs as novel targets for developing agents to help stroke sufferers.

GABA has been shown to mediate both fast and slow inhibitory synaptic transmission (Sieghart 1995). During development however, the $GABA_A$Rs have been shown to mediate excitation as well as play an important role in neural migration and synaptogenesis (Ben-Ari et al. 1997; McCarthy et al. 2002). During situations of cerebral ischemia, extracellular concentrations of GABA are significantly elevated (Schwartz et al. 1995; Inglefield et al. 1995), resulting in $GABA_A$ receptor desensitization and/or downregulation (Alicke and Schwartz-Bloom 1995; Huguenard and Alger 1986). This is supported by immunohistochemical and autoradiographic data showing decreased expression of α1, α2, α3, α5, and γ2 subunits following photothrombotic stroke and freeze-lesion-induced cortical injury (Redecker et al. 2000, 2002; Que et al. 1999).

Recent work has shown that epileptogenesis results in the suppression of functionally active α5-$GABA_A$Rs and results in an increase/substitution of other $GABA_A$Rs with a subsequent increase in rather than suppression of tonic inhibitory currents (Scimemi et al. 2005). A similar compensatory increase in α4δ-mediated tonic currents has been seen in the α5 knockout mice within region CA1 of the hippocampus (Glykys and Mody 2006). Extracellular GABA concentrations and thus

tonic inhibition have been shown to be elevated as a consequence of the increase in excitatory drive, resulting in the modulation of neuronal excitability and prevention of neuronal saturation (Mitchell and Silver 2003). Consistent with these findings, Clarkson and colleagues reported an increase in GABA tonic inhibitory currents from 3 to 14 days poststroke in layer II cortical pyramidal neurons (Fig. 9.3; Clarkson et al. 2010). This poststroke increase in tonic inhibition may act as a compensatory mechanism to prevent further neuronal injury. However, this prolonged increase in tonic inhibition during the repair phase is acting as a hindrance by preventing cortical expansion and improvements in functional recovery. This is supported by findings by Clarkson and colleagues who show that both pharmacological and genetic modulation of tonic inhibition, dampening either α5 or δ-mediated increase in tonic GABA currents, results in early and marked improvements in functional recovery (Clarkson et al. 2010).

Understanding the profile for how cortical plasticity occurs and is altered after a stroke is critical for fully determining when to start treatments and with what therapeutic compound to use. Based on our findings, we have clearly shown that dampening of tonic GABA currently from 3 days results in robust functional improvements of motor recovery (Clarkson et al. 2010). These improvements however, may not be the same if treatments are started weeks after stroke onset as previously shown in humans using zolpidem, which was shown to transiently improve aphasia in chronic stroke survivors (Cohen et al. 2004). The α1 and β2 $GABA_AR$ subunits are densely localized within the cortex and co-assembly with the γ2-subunit accounts for about 40% of all $GABA_ARs$ within the cortex (McKernan and Whiting 1996). Assembly of $GABA_ARs$ containing α1β2γ2 has been shown to be enriched at synaptic sites throughout the cortex (Farrant and Nusser 2005) and involved in changes in synaptic plasticity. However, studies have also shown that the δ subunit can co-assemble with α1 subunits to form functional recombinant receptors (Saxena and Macdonald 1994; Bianchi and Macdonald 2003). Further, immunoprecipitation studies have shown that δ subunits can associate with α1 subunits (Mertens et al. 1993) and these α1-containing $GABA_ARs$ have been found extrasynaptically (Baude et al. 2007; Sun et al. 2004), consistent with the typical localization of δ-containing $GABA_ARs$ (Farrant and Nusser 2005). These data could suggest an alternative method for why zolpidem was having an effect in chronic stroke patients to alleviate the burden of aphasia. However, further studies are needed, as one previous study would suggest that the γ2-subunit is required in order for zolpidem to have an effect (Cope et al. 2004).

9.4.1 Dampening Cortical Inhibition Alters Cortical Responsiveness

Disinhibition of cortical connections within the peri-infarct or regions associated with the peri-infarct cortex have been argued as either occurring as a direct consequence of the stroke or as a potential compensatory mechanism related to the recov-

ery (Liepert 2006). This argument has come about based on a number of observations such as: local blockage of GABAergic inhibition unmasks preexisting horizontal connections within the rat motor cortex (Jacobs and Donoghue 1991); LTP of adult rat motor cortex horizontal connections is dependent on GABA disinhibition during theta burst stimulation, unlike other regions such as the hippocampus or somatosensory cortex (Hess et al. 1996); and finally, modulation of GABA has been shown to be involved in learning in healthy humans as shown using imaging studies showing a correlation between a decrease in GABA concentration in motor cortex and motor skill learning (Floyer-Lea et al. 2006). Consistent with the notion that cortical disinhibition is occurring as a compensatory mechanism, Clarkson and colleagues have shown a robust and persistent increase in tonic inhibition in the peri-infarct cortex after stroke and blockade of this tonic inhibition at the time of stroke with the extrasynaptic $GABA_A$R-negative allosteric modulator, L655–708, exacerbated the lesion (Clarkson et al. 2010). Further to this, Clarkson and colleagues showed for the first time that delayed treatment L655–708, which has previously been shown to induce LTP (Atack et al. 2006), provides an early and robust reversal in behavioral deficits (Clarkson et al. 2010). Given the early behavioral effects seen and the lack of effect on sprouting of new connections, cortical disinhibition following L655–708 treatment seems a logical argument. To support the notion that dampening GABA activity is having a beneficial effect, no improvement in motor function was observed after stroke following administration of the GABA agonist, muscimol (Clarkson et al. 2010). This is backed by clinical studies illustrating the re-immergence of stroke symptoms following administration of the GABA agonist midazolam in chronic stroke patients that have shown significant improvements in function (Lazar et al. 2002). The peri-infarct cortex exhibits neuronal metabolic dysfunction over a 1-month period (van der Zijden et al. 2008), which would indicate a therapeutic time window for blockade of tonic GABA signaling of at least 1 month after stroke. Consistent with this is the fact when L655–708 treatment is discontinued after a 2-week period of administration after stroke, a slight rebound effect/reversal in functional recovery is observed compared to animals that received treatment for the 6-week period (Clarkson et al. 2010).

9.4.2 Role of Tonic GABA in Poststroke Protection

Attempts to develop pharmacological therapies to treat stroke patients and minimize the extent of neuronal cell death have resulted in the failure of dozens of clinical trials. Over the past two decades, changes in GABA function following cerebral ischemia and the possible protective benefits of GABAergic compounds have been extensively assessed (Clarkson et al. 2005, 2010; Clarkson and Carmichael 2009; Schwartz-Bloom and Sah 2001; Clarkson 2012b). The enhancement of GABA transmission elicits protection against cerebral ischemia (Schwartz-Bloom and Sah 2001); in several animal models, however, the mechanisms by which GABAergic compounds achieve neuroprotection have not been fully elucidated (Clarkson et al.

2005, 2010; Clarkson and Carmichael 2009; Schwartz-Bloom and Sah 2001; Clarkson 2012b). Moreover, despite GABA agonists showing great promise in animal models of stroke, they have failed to translate into positive clinical outcomes (Ginsberg 2008; Lyden et al. 2002).

One strategy to understand the mechanisms of action of GABA in neuroprotection and to improve the performance of GABAergic compounds is to target GABA compounds to specific $GABA_AR$ subunits. Subunit-specific $GABA_AR$ compounds are more likely to show an effect and have fewer side effects, such as drug-induced hypothermia and sedation. The need to assess subunit-specific GABA compounds to help understand what is happening after stroke in terms of GABA function is highlighted with clinical reports showing that Zolpidem, an α1-subunit-specific $GABA_AR$ modulator, can result in a transient improvement in aphasia in stroke survivors (Cohen et al. 2004). In addition, the δ-$GABA_AR$ agonist, THIP, has been shown to protect against NMDA-induced cell death in vitro (Kristensen et al. 2003).

As discussed previously, the elevation in tonic inhibition after stroke could be an endogenous protective mechanism put in place to minimize the extent of cell death. However, the elevation in tonic inhibition likely comes on at such a delay to not be of any benefit. Therefore, by elevating tonic GABA inhibition early (hours after a stroke), significant neuroprotection may be achieved. Consistent with this, previous studies have shown that application of 100–1000 μM of the $GABA_A$ receptor agonist THIP to hippocampal slice cultures can protect against NMDA-induced cell death (Kristensen et al. 2003). Application of concentrations higher than 100 μM however, provided a tendency for less protection, most likely due to rapid desensitization of the GABA receptors at the higher concentrations. In vivo, intraperitoneal (i.p.) injections of THIP (5 × 15 mg/kg) offered no significant neuroprotection of CA1 in the rat hippocampus after global cerebral ischemia when given alone, but when administered together with diazepam, it significantly decreased the extent of CA1 pyramidal cell loss (Johansen and Diemer 1991).THIP is the prototypical δ-$GABA_AR$ agonist, which binds to the GABA binding site. Similar to GABA (Karim et al. 2012), THIP activates the α4β3δ $GABA_ARs$ with two distinct potencies, most likely the result of activating two different receptor stoichiometries (Meera et al. 2011). Pharmacokinetic studies have shown that a 2.5–10 mg/kg injection correlates to 0.5–3 μM THIP in the brain (Cremers and Ebert 2007). At these concentrations, THIP has about 5–10 % receptor occupancy at the low-affinity stoichiometric binding site of the α4β3δ $GABA_ARs$, but does not occupy α4β2δ $GABA_ARs$ (Meera et al. 2011; Sundstrom-Poromaa et al. 2002). In contrast, at these concentrations THIP has saturated the high-affinity stoichiometric binding site of the α4β3δ $GABA_ARs$ (Meera et al. 2011). Binding of THIP at these two different binding sites is likely to have differing functional properties; therefore, studies need to be carried out to test both low and high concentrations of THIP and the effects that this may have on protecting against neuronal cell death.

We have recently shown that low doses (1 mg/kg i.p.) of THIP given 1 h post photothrombotic stroke can afford significant protection (see Fig. 9.4; Clarkson et al., unpublished data). These data confirm the hypothesis that elevating tonic inhibition early after stroke can afford significant protection. However, further work is

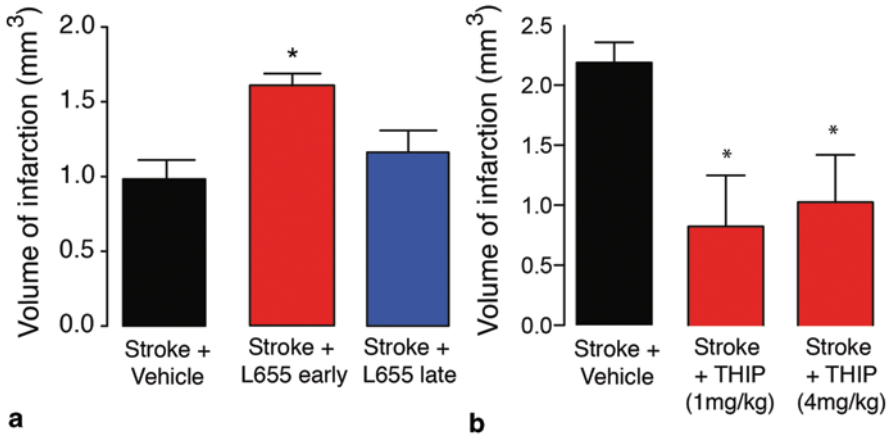

Fig. 9.4 Inflection point in tonic GABA modulation and effect on infarct size. **a** Quantification of the stroke volume for $n=4-5$ animals from *stroke + vehicle*-treatment, *stroke + L655,708*-treatment starting at the time of stroke, and *stroke + L655,708*-treatment starting from 3 days post insult. Dampening tonic GABA currents early results in an exacerbation in infarct size compared to vehicle-treated controls. Delaying starting treatment by 3 days had no effect on infarct volume. **b** Quantification of infarct volume for $n=5$ animals from *stroke + vehicle*-treatment and *stroke + THIP*-treatment starting 1 h post stroke. Increasing the level of tonic GABA early after stroke affords significant protection ($*P<0.05$)

still required to fully understand the dosing required to achieve these effects given the controversies that exist.

9.5 Role of Astrocytes in Regulating Tonic GABA

Glial cells and in particular astrocytes have for a long time only been considered to play a supporting role within the central nervous system. However, substantial evidence accumulated over the past two decades has started to shed light on the various physiological functions that they perform, which has led to the current view that they play an active role within the tripartite synapse (Perea et al. 2009), a site consisting of both presynaptic and postsynaptic neurons and an astrocyte. Several studies have demonstrated that astrocytes can sense, respond, and in turn regulate the function of neurons; and possess the necessary transporters and receptors to be able to detect both GABA and glutamate.

Astrocytes express both GABA (Schousboe et al. 2011) and glutamate (Danbolt 2001) transporters, as well as ionotropic and metabotropic GABA (Velez-Fort et al. 2012; Domingues et al. 2010; Madsen et al. 2011) and glutamate receptors (Biber et al. 1999). The activation of these transporters or receptors can trigger the initiation of various intracellular signaling pathways such as altered Ca^{2+} signaling (Doengi et al. 2009; Haydon and Carmignoto 2006), and ultimately leads to their

lease of GABA or glutamate in either a vesicular (Hamilton and Attwell 2010) or nonvesicular (Allen et al. 2004) manner.

Among the various mechanisms by which astrocytes control neuronal functions, regulation of tonic inhibition may play a major role. Modulation of tonic inhibition has recently been shown to play a vital role in the treatment of various pathophysiological conditions, such as epilepsy (Cope et al. 2009) and stroke (Clarkson et al. 2010). Despite the significant role that tonic GABA is playing in such pathologies of stroke and epilepsy, the source of the GABA that is contributing to the elevation in tonic GABA currents still remains a matter to be debated and remains largely unknown.Some groups claim that ambient GABA concentration is set almost exclusively by vesicular GABA being released from the synapse (Glykys and Mody 2007b), while others argue that release from astrocytes constitutes a significant source for extrasynaptic GABA (Wu et al. 2006; Lee et al. 2010) and increase in tonic inhibition. The release of GABA from astrocytes in the cortex has not yet been confirmed; however, in regions such as the hippocampus, the release of GABA from astrocytes occurs in response to astrocytic uptake of glutamate and significantly contributes to the increase in tonic inhibition of neurons during intense excitation (Heja et al. 2012). The physiological importance in the exchange/regulation of GABA and glutamate in astrocytes needs to be investigated further, especially with respect to stroke recovery given that the expression of the GABA transporter, (GAT)-3, is impaired after stroke (see Fig. 9.5; Clarkson et al. 2010).

9.5.1 GABA Transporters and Tonic GABA

GATs are membrane proteins critically involved in the regulation of mammalian inhibitory GABAergic neurotransmission. Four different GAT subtypes have been cloned (termed GAT-1, BGT-1, GAT-2, and GAT-3 in humans). The transporters belong to the 12 transmembrane family (SLC) of Na^+/Cl^--dependent transporters, which also comprises transporters for other neurotransmitters (Kristensen et al. 2011). The bacterial SLC6 transporter LeuT crystal structure (Yamashita et al. 2005) is now successfully being used to model the structure and function of the GATs (Forrest et al. 2008; Shi et al. 2008). The transport of GABA is electrogenic and primarily driven by co-transport of sodium ions across the cell plasma membrane (Kristensen et al. 2011). This property is utilized when measuring the functional pharmacology of GATs in vitro. The fact that the different GAT subtypes have unique subcellular distributions (see Fig. 9.1) argues for distinct functional roles (Madsen et al. 2009).

In a recent study by Clarkson and colleagues, it was reported that GAT-3 plays a direct role during this delayed phase, being impaired and downregulated, whereas protein levels and function of GAT-1 are preserved (Fig. 9.3). GAT-3 transporters are expressed mostly in astrocytes (Heja et al. 2009). Furthermore, ultrastructural (Minelli et al. 1996) and functional (Kinney and Spain 2002) data have shown that GAT-3 is expressed on glial processes facing the extrasynaptic space (Minelli et al. 1996; Kinney and Spain 2002) where the $GABA_A$Rs responsible for mediating

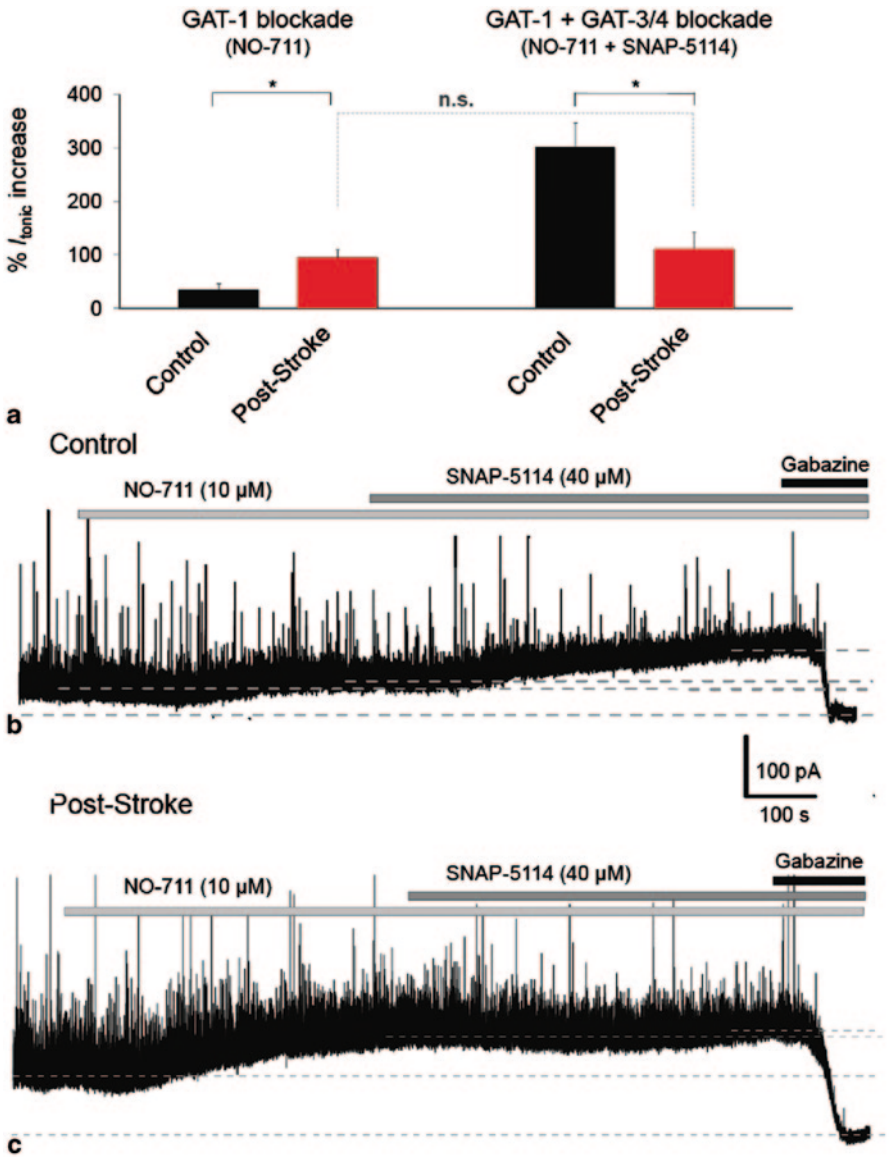

Fig. 9.5 *Poststroke* impairment in GABA transport. **a** Blocking *GAT-1* (*NO-711*) produced a higher % increase in I_{tonic} after stroke; combined blockade of *GAT-1* and *GAT-3/4* (*NO-711 + SNAP-5114*) produced a substantial I_{tonic} increase in *controls* but only an increase equivalent to blocking *GAT-1* alone after *stroke*. **b** and **c** I_{tonic} in sequential drug applications in *control* and stroke peri-infarct neurons, respectively. Note the lack of response to *SNAP-5114* application in the *poststroke slice*

tonic inhibition reside (Farrant and Nusser 2005). Thus, extrasynaptically expressed GATs may be interesting and clinically relevant targets for future anti-stroke pharmacological intervention. GAT-3 has already been proposed as a key player in stroke pathogenesis (Clarkson et al. 2010); however, the role of BGT-1 in stroke mechanism is unknown although it is proposed to be an extrasynaptic protecting mechanism following excitotoxic brain injury (Zhu and Ong 2004a, b).

9.6 The Role of $GABA_C$ Receptors and Poststroke Recovery

Like $GABA_A$ receptors, $GABA_C$ receptors are Cl^- channels that mediate fast synaptic inhibition. These transmitter-gated ion channels are believed to be structurally very similar, composed of five subunits, which arrange to form a homomeric ion channel composed of the rho (ρ) subunit. Five different subtypes of the ρ-subunit have been cloned from different species, which include human (ρ_{1-2}), mouse (ρ_{1-2}), rat (ρ_{1-3}), chicken (ρ_{1-2}), and white perch ($\rho_{1A\text{-}B}$, $\rho_{2A\text{-}B}$, ρ_3; Enz 2001) sharing >90% amino acid similarity between the different species (Zhang et al. 2001; Wang et al. 1994; Qian et al. 1998).

Receptors composed of ρ-subunits are biochemically, pharmacologically, and physiologically different from $GABA_A$ receptors (Chebib and Johnston 1999, 2000; Bormann 2000). GABA is an order of magnitude less potent at $GABA_A$ than at $GABA_C$ receptors. $GABA_A$ receptors are selectively blocked by the alkaloid, bicuculline, and modulated by benzodiazepines, steroids, and barbiturates (Chebib and Johnston 1999, 2000; Bormann 2000). $GABA_C$ receptors are not blocked by bicuculline, nor are they modulated by benzodiazepines, steroids, and barbiturates (Chebib and Johnston 1999, 2000). Instead, $GABA_C$ receptors are activated by *Z*-4-aminobut-2-enoic acid (*cis*-aminocrotonic acid, CACA; Chebib and Johnston 1999, 2000) and (1*S*,2*R*)-(+)-2-(aminomethyl)-cyclopropane-1-carboxylic acid ((1*S*,2*R*)-(+)-CAMP; Duke et al. 2000), and selectively blocked by (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA; Murata et al. 1996; Ragozzino et al. 1996). For a comprehensive review on the pharmacology of $GABA_C$ receptors, see Ng et al. (2011).

$GABA_A$ receptors are linked via the γ2 subunit to the cytoskeleton by $GABA_A$ receptor-associated proteins (GABARAP; Wang et al. 1994) while $GABA_C$ receptors are linked via the ρ1 subunit to the cytoskeleton by microtubule-associated protein 1B (MAP-1B; Hanley et al. 1999). The fact that two different proteins associate with the $GABA_A$ and $GABA_C$ receptors allows these receptors to exist and function separately. Although some studies have reported the possible co-assembly of the ρ subunits with other $GABA_A$ subunits, for example, the α_1 and γ_2 subunits, this remains controversial and it is still unknown if these heteromeric receptors only exist in vitro (Enz and Cutting 1998; Hackam et al. 1998; Qian and Ripps 1999; Ekema et al. 2002; Qian and Pan 2002; Milligan et al. 2004).

Immunohistochemical and electrophysiological studies have shown that, in most species, ρ_1 subunits are predominately expressed in bipolar cells (Fletcher et al.

2001; Lukasiewicz and Wong 1996; Eggers and Lukasiewicz 2006; Lukasiewicz 2005; Lukasiewicz et al. 2004; Lukasiewicz and Shields 1998) and horizontal cells in the retina; while the rat $\rho_2$subunits are found not only in the retina but also in the spinal cord (Rozzo et al. 2002) and in several brain regions, such as the hippocampus, cortex, pituitary, cerebellum, and thalamus (Enz 2001; Enz and Cutting 1999). Recently, the $\rho_2$subunit has been found on cerebellar astrocytes indicating that these receptor subtypes are not restricted to neurons (Martinez-Delgado et al. 2011).

A number of roles for the ρ_1 receptor have been identified. Both ρ_1 and ρ_2 $GABA_C$ receptors are found in the hippocampus where there is evidence for a functional role as extrasynaptic receptors activated via GABA spillover (Alakuijala et al. 2006) and in paired-pulse depression of inhibitory postsynaptic currents (Xu et al. 2009). In addition, $GABA_C$ antagonists including TPMPA have been shown to inhibit the development of myopia and improve learning and memory (Stone et al. 2003; Gibbs and Johnston 2005; Chebib et al. 2009b). Both TPMPA and P4MPA have been used to study the role of $GABA_C$ receptors in short-term memory formation in young chicks using a single trial passive and discriminated avoidance task. Further, SGS742, (3-aminopropyl)-*n*-butylphosphinic acid (also known as CGP36742) has been shown to have therapeutic potential for the treatment of cognitive deficits, petit mal epilepsy, and depression (Froestl et al. 2004), reaching Phase II trials for the treatment of cognitive impairment due to Alzheimer's disease.

These studies lead to the development of cyclopentane, such as (±)-*cis*-3-ACPBuPA, (±)-*cis*-(3-aminocyclopentane)butylphosphinic acid, which has been shown to be a selective $GABA_C$ antagonist that enhances learning and memory following i.p. injection in rats and inhibits the development of myopia following intravitreal injection in chicks (Chebib et al. 2009a). (±)-*cis*-3-ACPBuPA and related cyclopentane (Gavande et al. 2010) and cyclopentene analogs (Kumar et al. 2008) have been patented for use in enhancing cognitive activity (Chebib et al. 2003).

It has been suggested that homomeric $GABA_C$ receptors composed of $\rho 2$ rather than $\rho 1$ are located extrasynaptically (Schlicker et al. 2009). Given the importance of extrasynaptic GABA receptors consisting of α5- or δ-containing $GABA_A$Rs in motor recovery after stroke, assessment of motor functions following administration of the $GABA_C$ antagonists (S)- or (R)-ACPBPA have recently been evaluated, given also their importance in modulating learning and memory pathways. It was found that the $\rho 2$-preferring (R)-ACPBPA had a greater effect compared with the $\rho 1$-preferring (S)-ACPBPA in mice following delivery via osmotic minipumps implanted from day 3 poststroke (Clarkson 2012a). These functional improvements were almost as comparable to the levels seen following treatment with the α5-$GABA_A$R inverse agonist L-655,708, suggesting that these compounds may be of benefit in treating stroke recovery. We have further recently shown that this recovery maybe mediated in part by the modulation of the glial scar and subsequent increase in GAT3 expression (Clarkson et al., unpublished findings 2012); however, further work is required to confirm these findings.

Thus, given the localization of the various ρ $GABA_C$ receptors both on synaptic and extrasynaptic sites along with expression on astrocytes, knockout and pharma-

cological studies indicate critical roles for the $GABA_C$ receptor in a wide range of pathophysiological and neurological disorders. Thus, the development of selective $GABA_C$ receptor ligands will become important agents for a wide range of therapies and to help delineate the role these receptors play in the central and peripheral nervous systems.

Conclusions

Therapies that promote functional recovery after stroke are limited to physical rehabilitation measures. While specific measures, such as constraint-induced therapies, promote recovery of motor function, no pharmacological therapies are available that aid in recovery. Functional recovery after stroke follows psychological learning rules (Krakauer 2006) that indicate learning and memory principles may underlie behavioral recovery. The idea that certain aspects of learning and memory rules and changes in neuronal excitability can alter the profile of recovery after a stroke has led to ad hoc attempts to treat patients with any available drug known to stimulate learning and memory, such as amphetamine, dopamine agonists, and methylphenidate, to name just a few. As these compounds work through multiple receptor systems in the brain and were designed to treat neurological conditions other than being a neurorehabilitative aid, they have failed to translate into the clinic (Berends et al. 2009). At the cellular level, learning and memory principles are mediated by specific excitatory neuronal responses, such as LTP, and are potentiated by drugs that facilitate aspects of excitatory neuronal signaling (Walker and Semyanov 2008), such as tonic $GABA_AR$ antagonists (Glykys and Mody 2007a). Recent data show that stroke alters the balance of excitatory and inhibitory inputs to neurons in the peri-infarct cortex, by increasing inhibitory tone. This altered excitatory balance occurs through a decrease in the normal cellular uptake of GABA. Dampening GABA-mediated tonic inhibition restores the excitatory/inhibitory balance in peri-infarct motor cortex ex vivo, and promotes recovery of motor function in vivo (see Fig. 9.6). These effects occur through blockade of α5- or δ-containing $GABA_ARs$. These data indicate a novel role for tonic $GABA_AR$ function in promoting poststroke recovery most likely via cortical disinhibition (Jacobs and Donoghue 1991; Stinear et al. 2009; Stinear and Byblow 2002) and suggest a new avenue for pharmacological treatment of neurorehabilitation in stroke. This early effect on stroke recovery opens the possibility for treatments that block tonic GABA signaling and may be used in conjunction with later-acting stroke repair therapies in a combinatorial manner. More generally, tonic GABA signaling has a biphasic role in stroke. Early tonic GABA signaling limits stroke size, later tonic GABA signaling limits stroke recovery. These data identify a promising molecular system for future stroke recovery therapies and implicate molecular memory systems as likely key players in recovery from stroke.

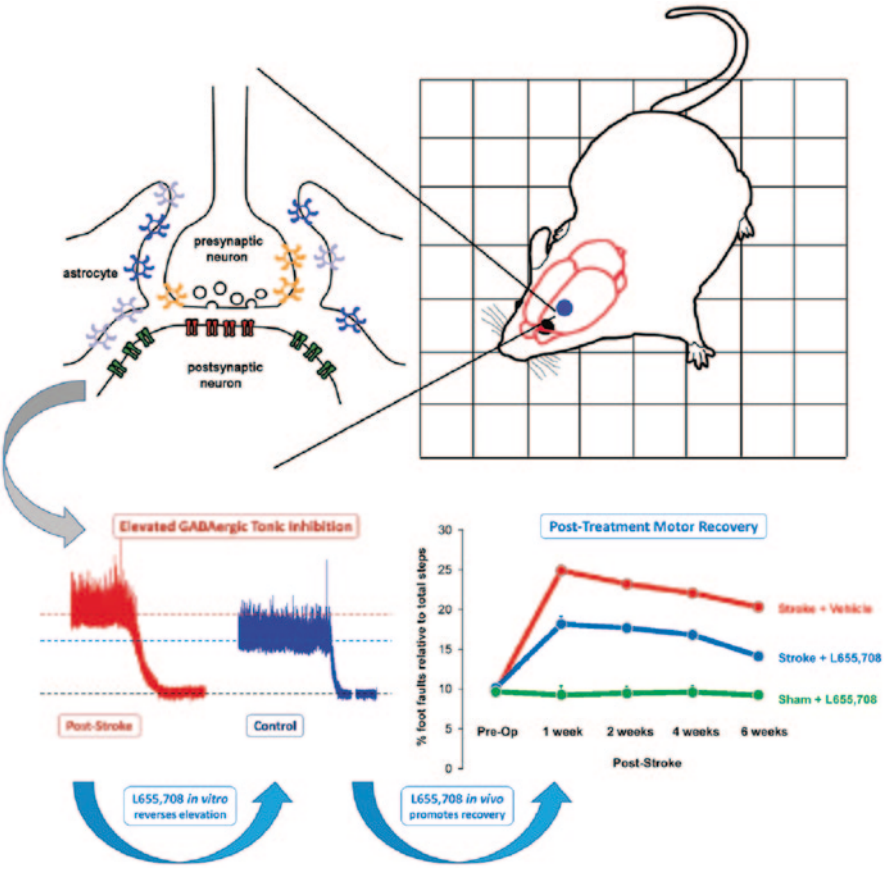

Fig. 9.6 Schematic summary of key changes in tonic GABA and stroke recovery. Stroke increases peri-infarct GABA by reducing the level of the GABA transporter, GAT-3/4, indicated by lighter shading in a subset of GAT-3/4 (*blue pinwheels*). The function and the level of GAT-1 are unaltered (*orange pinwheels*). Due to different subunit-associated properties, extrasynaptic $GABA_A$Rs (*green*) mediate a tonic form of inhibition that is distinct from the phasic form mediated by synaptic $GABA_A$Rs (*orange*).Tonic inhibitory currents in peri-infarct pyramidal neurons (*red trace*) are increased compared to control neurons (*blue trace*). Reducing tonic inhibition with a selective α5-$GABA_A$R inverse agonist (*L655,708*) reverses the increase in tonic inhibition and improves behavioral recovery in forelimb motor control after stroke

Acknowledgments This manuscript was completed during tenure of a Sir Charles Hercus Fellowship from the Health Research Council of New Zealand.

References

Alakuijala A, Alakuijala J, Pasternack M (2006) Evidence for a functional role of GABA receptors in the rat mature hippocampus. Eur J Neurosci 23(2):514–520

Alicke B, Schwartz-Bloom RD (1995) Rapid down-regulation of $GABA_A$ receptors in the gerbil hippocampus following transient cerebral ischemia. J Neurochem 65(6):2808–2811

Allen NJ, Rossi DJ, Attwell D (2004) Sequential release of GABA by exocytosis and reversed uptake leads to neuronal swelling in simulated ischemia of hippocampal slices. J Neurosci 24(15):3837–3849

Alonso-Alonso M, Fregni F, Pascual-Leone A (2007) Brain stimulation in poststroke rehabilitation. Cerebrovasc Dis 24(Suppl 1):157–166

Atack JR, Bayley PJ, Seabrook GR, Wafford KA, McKernan RM, Dawson GR (2006) L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing $GABA_A$ receptors. Neuropharmacology 51(6):1023–1029

Barrera NP, Betts J, You H, Henderson RM, Martin IL, Dunn SM et al (2008) Atomic force microscopy reveals the stoichiometry and subunit arrangement of the alpha4beta3delta GABA(A) receptor. Mol Pharmacol 73(3):960–967

Baude A, Bleasdale C, Dalezios Y, Somogyi P, Klausberger T (2007) Immunoreactivity for the $GABA_A$ receptor alpha1 subunit, somatostatin and Connexin36 distinguishes axoaxonic, basket, and bistratified interneurons of the rat hippocampus. Cereb Cortex 17(9):2094–2107

Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW (2009) Extrasynaptic $GABA_A$ receptors: form, pharmacology, and function. J Neurosci 29(41):12757–12763

Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa JL (1997) $GABA_A$, NMDA and AMPA receptors: a developmentally regulated 'menage a trois'. Trends Neurosci 20(11):523–529

Berends HI, Nijlant JM, Movig KL, Van Putten MJ, Jannink MJ, Ijzerman MJ (2009) The clinical use of drugs influencing neurotransmitters in the brain to promote motor recovery after stroke; a Cochrane systematic review. Eur J Phys Rehabil Med 45(4):621–630

Bianchi MT, Macdonald RL (2003) Neurosteroids shift partial agonist activation of GABA(A) receptor channels from low- to high-efficacy gating patterns. J Neurosci 23(34):10934–10943

Biber K, Laurie DJ, Berthele A, Sommer B, Tolle TR, Gebicke-Harter PJ et al (1999) Expression and signaling of group I metabotropic glutamate receptors in astrocytes and microglia. J Neurochem 72(4):1671–1680

Borghese CM, Harris RA (2007) Studies of ethanol actions on recombinant delta-containing gamma-aminobutyric acid type A receptors yield contradictory results. Alcohol 41(3):155–162

Bormann J (2000) The 'ABC' of GABA receptors. Trends Pharmacol Sci 21:16–19

Bright DP, Renzi M, Bartram J, McGee TP, MacKenzie G, Hosie AM et al (2011) Profound desensitization by ambient GABA limits activation of delta-containing $GABA_A$ receptors during spillover. J Neurosci 31(2):753–763

Brown CE, Aminoltejari K, Erb H, Winship IR, Murphy TH (2009) In vivo voltage-sensitive dye imaging in adult mice reveals that somatosensory maps lost to stroke are replaced over weeks by new structural and functional circuits with prolonged modes of activation within both the peri-infarct zone and distant sites. J Neurosci 29(6):1719–1734

Calautti C, Baron JC (2003) Functional neuroimaging studies of motor recovery after stroke in adults: a review. Stroke 34(6):1553–1566

Carmichael ST (2006) Cellular and molecular mechanisms of neural repair after stroke: making waves. Ann Neurol 59(5):735–742

Carmichael ST (2008) Themes and strategies for studying the biology of stroke recovery in the poststroke epoch. Stroke 39(4):1380–1388

Carmichael ST (2010) Targets for neural repair therapies after stroke. Stroke 41(10 Suppl):S124–S126

Carmichael ST (2012) Brain excitability in stroke: the yin and yang of stroke progression. Arch Neurol 69(2):161–167

Carmichael ST, Archibeque I, Luke L, Nolan T, Momiy J, Li S (2005) Growth-associated gene expression after stroke: evidence for a growth-promoting region in peri-infarct cortex. Exp Neurol 193(2):291–311

Cash DJ, Subbarao K (1987) Two desensitization processes of GABA receptor from rat brain. Rapid measurements of chloride ion flux using quench-flow techniques. FEBS Lett 217(1):129–133

Chebib M, Johnston GA (1999) The 'ABC' of GABA receptors: a brief review. Clin Exp Pharmacol Physiol 26(11):937–940

Chebib M, Johnston GAR (2000) GABA-activated ligand gated ion channels: medicinal chemistry and molecular biology. J Med Chem 43:1427–1447
Chebib M, Johnston GAR, Hanrahan JR (2003) patent PCT Int Appl WO 200345897
Chebib M, Gavande N, Wong KY, Park A, Premoli I, Mewett KN et al (2009a) Guanidino acids act as rho 1 GABA(C) receptor antagonists. Neurochem Res 34:1704–1711
Chebib M, Hinton T, Schmid KL, Brinkworth D, Qian H, Matos S et al (2009b) Novel, potent, and selective $GABA_C$ antagonists inhibit myopia development and facilitate learning and memory. J Pharmacol Exp Ther 328:448–457
Cicinelli P, Pasqualetti P, Zaccagnini M, Traversa R, Oliveri M, Rossini PM (2003) Interhemispheric asymmetries of motor cortex excitability in the postacute stroke stage: a paired-pulse transcranial magnetic stimulation study. Stroke 34(11):2653–2658
Citri A, Malenka RC (2008) Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology 33(1):18–41
Clarkson AN (2012a) Modulating post-stroke tonic inhibition offers an extended therapeutic windowfor facilitating functional improvements. 7th international symposium on neuroprotection and neurorepair, The Magdeburg Meeting Series, Potsdam, Germany
Clarkson AN (2012b) Perisynaptic GABA receptors the overzealous protector. Adv Pharmacol Sci 2012:708428
Clarkson AN, Carmichael ST (2009) Cortical excitability and post-stroke recovery. Biochem Soc Trans 37(Pt 6):1412–1414
Clarkson AN, Liu H, Rahman R, Jackson DM, Appleton I, Kerr DS (2005) Clomethiazole: mechanisms underlying lasting neuroprotection following hypoxia-ischemia. FASEB J 19(8):1036–1038
Clarkson AN, Clarkson J, Jackson DM, Sammut IA (2007) Mitochondrial involvement in transhemispheric diaschisis following hypoxia-ischemia: Clomethiazole-mediated amelioration. Neuroscience 144(2):547–561
Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature 468(7321):305–309
Clarkson AN, Overman JJ, Zhong S, Mueller R, Lynch G, Carmichael ST (2011) AMPA receptor-induced local brain-derived neurotrophic factor signaling mediates motor recovery after stroke. J Neurosci 31(10):3766–3775
Cohen L, Chaaban B, Habert MO (2004) Transient improvement of aphasia with zolpidem. N Engl J Med 350(9):949–950
Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C et al (2002) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. J Neurosci 22(13):5572–5580
Conner JM, Chiba AA, Tuszynski MH (2005) The basal forebrain cholinergic system is essential for cortical plasticity and functional recovery following brain injury. Neuron 46(2):173–179
Cope DW, Wulff P, Oberto A, Aller MI, Capogna M, Ferraguti F et al (2004) Abolition of zolpidem sensitivity in mice with a point mutation in the $GABA_A$ receptor gamma2 subunit. Neuropharmacology 47(1):17–34
Cope DW, Di Giovanni G, Fyson SJ, Orban G, Errington AC, Lorincz ML et al (2009) Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. Nat Med 15(12):1392–1398
Cremers T, Ebert B (2007) Plasma and CNS concentrations of Gaboxadol in rats following subcutaneous administration. Eur J Pharmacol 562(1–2):47–52
Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L et al (2002) Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. Proc Natl Acad Sci U S A 99(13):8980–8985
D'Hulst C, Atack JR, Kooy RF (2009) The complexity of the $GABA_A$ receptor shapes unique pharmacological profiles. Drug Discov Today 14(17–18):866–875
Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65(1):1–105
Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM et al (2006) An inverse agonist selective for alpha5 subunit-containing $GABA_A$ receptors enhances cognition. J Pharmacol Exp Ther 316(3):1335–1345

Dijkhuizen RM, Singhal AB, Mandeville JB, Wu O, Halpern EF, Finklestein SP et al (2003) Correlation between brain reorganization, ischemic damage, and neurologic status after transient focal cerebral ischemia in rats: a functional magnetic resonance imaging study. J Neurosci 23(2):510–517

Dobkin BH (2004) Strategies for stroke rehabilitation. Lancet Neurol 3(9):528–536

Dobkin BH (2008) Training and exercise to drive poststroke recovery. Nat Clin Pract Neurol 4(2):76–85

Doengi M, Hirnet D, Coulon P, Pape HC, Deitmer JW, Lohr C (2009) GABA uptake-dependent Ca(2+) signaling in developing olfactory bulb astrocytes. Proc Natl Acad Sci U S A 106(41):17570–17575

Domingues AM, Taylor M, Fern R (2010) Glia as transmitter sources and sensors in health and disease. Neurochem Int 57(4):359–366

Drasbek KR, Jensen K (2006) THIP, a hypnotic and antinociceptive drug, enhances an extrasynaptic $GABA_A$ receptor-mediated conductance in mouse neocortex. Cereb Cortex 16(8):1134–1141

Duke RK, Chebib M, Balcar VJ, Allan RD, Mewett KN, Johnston GAR (2000) (+)- and (−)-*cis*-2-Aminomethylcyclopropanecarboxylic acids show opposite pharmacology at recombinant ρ1 and ρ2 $GABA_C$ receptors. J Neurochem 75:2602–2610

Eggers ED, Lukasiewicz PD (2006) GABA(A), GABA(C) and glycine receptor-mediated inhibition differentially affects light-evoked signalling from mouse retinal rod bipolar cells. J Physiol London 572(1):215–225

Ekema GM, Zheng W, Lu L (2002) Interaction of GABA receptor/channel ρ_1 and γ_2 subunit. Invest Ophthalmol Vis Sci 43:2326–2333

Enz R (2001) $GABA_C$ receptors: a molecular view. Biol Chem 382:1111–1122

Enz R, Cutting GR (1998) Molecular composition of $GABA_C$ receptors. Vision Res 38:1431–1441

Enz R, Cutting GR (1999) $GABA_C$ receptor ρ subunits are heterogeneously expressed in the human CNS and form homo- and heterooligomers with distinct physical properties. Eur J Neurosci 11:41–50

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 6(3):215–229

Fletcher EL, Clark MJ, Senior P, Furness JB (2001) Gene expression and localisation of $GABA_C$ receptors in neurons of the rat gastrointestinal tract. Neuroscience 107:181–189

Floyer-Lea A, Wylezinska M, Kincses T, Matthews PM (2006) Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. J Neurophysiol 95(3):1639–1644

Forrest LR, Zhang YW, Jacobs MT, Gesmonde J, Xie L, Honig BH et al (2008) Mechanism for alternating access in neurotransmitter transporters. Proc Natl Acad Sci U S A 105(30):10338–10343

Froestl W, Gallagher M, Jenkins H, Madrid A, Melcher T, Teichman S et al (2004) SGS742: the first GABA(B) receptor antagonist in clinical trials. Biochem Pharmacol 68:1479–1487

Gavande N, Yamamoto I, Salam NK, Ai T-H, Burden PM, Johnston GAR et al (2010) Novel cyclic phosphinic acids as GABAC ρ receptor antagonists: design, synthesis, and pharmacology. Med Chem Lett. doi:10.1021/ml1001344

Gibbs ME, Johnston GAR (2005) Opposing Roles for $GABA_A$ and $GABA_C$ receptors in short-term memory formation in young chicks. Neuroscience 131:567–576

Ginsberg MD (2008) Neuroprotection for ischemic stroke: past, present and future. Neuropharmacology 55(3):363–389

Glykys J, Mody I (2006) Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABA A receptor alpha5 subunit-deficient mice. J Neurophysiol 95(5):2796–2807

Glykys J, Mody I (2007a) Activation of $GABA_A$ receptors: views from outside the synaptic cleft. Neuron 56(5):763–770

Glykys J, Mody I (2007b) The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. J Physiol 582(Pt 3):1163–1178

Green AR, Hainsworth AH, Jackson DM (2000) GABA potentiation: a logical pharmacological approach for the treatment of acute ischaemic stroke. Neuropharmacology 39(9):1483–1494

Hackam AS, Wang T-L, Guggino WB, Cutting GR (1998) Sequences in the amino termini of GABA ρ and $GABA_A$ subunits specify their selective interaction in vitro. J Neurochem 70:40–46

Hamilton NB, Attwell D (2010) Do astrocytes really exocytose neurotransmitters? Nat Rev Neurosci 11(4):227–238

Hanley JG, Koulen P, Bedford F, Gordon-Weeks PR, Moss SJ (1999) The protein MAP-1B links GABA(C) receptors to the cytoskeleton at retinal synapses. Nature 397(6714):66–69

Haydon PG, Carmignoto G (2006) Astrocyte control of synaptic transmission and neurovascular coupling. Physiol Rev 86(3):1009–1031

Heja L, Barabas P, Nyitrai G, Kekesi KA, Lasztoczi B, Toke O et al (2009) Glutamate uptake triggers transporter-mediated GABA release from astrocytes. PLoS ONE 4(9):e7153

Heja L, Nyitrai G, Kekesi O, Dobolyi A, Szabo P, Fiath R et al (2012) Astrocytes convert network excitation to tonic inhibition of neurons. BMC Biol 10:26

Hess G, Aizenman CD, Donoghue JP (1996) Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. J Neurophysiol 75(5):1765–1778

Huguenard JR, Alger BE (1986) Whole-cell voltage-clamp study of the fading of GABA-activated currents in acutely dissociated hippocampal neurons. J Neurophysiol 56(1):1–18

Hummel FC, Cohen LG (2006) Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? Lancet Neurol 5(8):708–712

Inglefield JR, Perry JM, Schwartz RD (1995) Postischemic inhibition of GABA reuptake by tiagabine slows neuronal death in the gerbil hippocampus. Hippocampus 5(5):460–468

Jacobs KM, Donoghue JP (1991). Reshaping the cortical motor map by unmasking latent intracortical connections. Science 251(4996):944–947

Jaenisch N, Witte OW, Frahm C (2010) Downregulation of potassium chloride cotransporter KCC2 after transient focal cerebral ischemia. Stroke 41(3):e151–e159

Johansen FF, Diemer NH (1991) Enhancement of GABA neurotransmission after cerebral ischemia in the rat reduces loss of hippocampal CA1 pyramidal cells. Acta Neurol Scand 84(1):1–6

Karim N, Wellendorph P, Absalom N, Bang LH, Jensen ML, Hansen MM et al (2012) Low nanomolar GABA effects at extrasynaptic alpha4beta1/beta3delta GABA(A) receptor subtypes indicate a different binding mode for GABA at these receptors. Biochem Pharmacol 84(4):549–557

Keros S, Hablitz JJ (2005) Subtype-specific GABA transporter antagonists synergistically modulate phasic and tonic $GABA_A$ conductances in rat neocortex. J Neurophysiol 94(3):2073–2085

Kinney GA, Spain WJ (2002) Synaptically evoked GABA transporter currents in neocortical glia. J Neurophysiol 88(6):2899–2908

Krakauer JW (2006) Motor learning: its relevance to stroke recovery and neurorehabilitation. Curr Opin Neurol 19(1):84–90

Kristensen BW, Noraberg J, Zimmer J (2003) The $GABA_A$ receptor agonist THIP is neuroprotective in organotypic hippocampal slice cultures. Brain Res 973(2):303–306

Kristensen AS, Andersen J, Jorgensen TN, Sorensen L, Eriksen J, Loland CJ et al (2011) SLC6 neurotransmitter transporters: structure, function, and regulation. Pharmacol Rev 63(3):585–640

Kumar RJ, Chebib M, Hibbs DE, Kim H-L, Johnston GAR, Salam NK et al (2008) Novel γ-aminobutyric acid ρ1 receptor antagonists; synthesis, pharmacological activity and structure-activity relationships. J Med Chem 51:3825–3840

Lai SM, Studenski S, Duncan PW, Perera S (2002) Persisting consequences of stroke measured by the stroke impact scale. Stroke 33(7):1840–1844

Lazar RM, Fitzsimmons BF, Marshall RS, Berman MF, Bustillo MA, Young WL et al (2002) Reemergence of stroke deficits with midazolam challenge. Stroke 33(1):283–285

Lee S, Yoon BE, Berglund K, Oh SJ, Park H, Shin HS et al (2010) Channel-mediated tonic GABA release from glia. Science 330(6005):790–796

Liauw J, Hoang S, Choi M, Eroglu C, Sun GH, Percy M et al (2008) Thrombospondins 1 and 2 are necessary for synaptic plasticity and functional recovery after stroke. J Cereb Blood Flow Metab 28(10):1722–1732

Liepert J (2006) Motor cortex excitability in stroke before and after constraint-induced movement therapy. Cogn Behav Neurol 19(1):41–47

Lipton P (1999) Ischemic cell death in brain neurons. Physiol Rev 79(4):1431–1568
Lukasiewicz PD (2005) Synaptic mechanisms that shape visual signaling at the inner retina. Prog Brain Res 147:205–218
Lukasiewicz PD, Shields CR (1998) Different combinations of GABA(A) and GABA(C) receptors confer distinct temporal properties to retinal synaptic responses. J Neurophysiol 79(6):3157–3167
Lukasiewicz PD, Wong ROL (1996) The properties of GABA(c) receptors on ferret retinal bipolar cells. Invest Ophthalmol Vis Sci 37(3):1940–1940
Lukasiewicz PD, Eggers ED, Sagdullaev BT, McCall MA (2004) GABA(C) receptor-mediated inhibition in the retina. Vision Res 44(28):3289–3296
Lyden P, Shuaib A, Ng K, Levin K, Atkinson RP, Rajput A et al (2002) Clomethiazole Acute Stroke Study in ischemic stroke (CLASS-I): final results. Stroke 33(1):122–128
MacDonald E, Van der Lee H, Pocock D, Cole C, Thomas N, VandenBerg PM et al (2007) A novel phosphodiesterase type 4 inhibitor, HT-0712, enhances rehabilitation-dependent motor recovery and cortical reorganization after focal cortical ischemia. Neurorehabil Neural Repair 21(6):486–496
Macdonald RL, Olsen RW (1994) $GABA_A$ receptor channels. Annu Rev Neurosci 17:569–602
Madsen KK, Clausen RP, Larsson OM, Krogsgaard-Larsen P, Schousboe A, White HS (2009) Synaptic and extrasynaptic GABA transporters as targets for anti-epileptic drugs. J Neurochem 109:S139–S144
Madsen KK, Ebert B, Clausen RP, Krogsgaard-Larsen P, Schousboe A, White HS (2011) Selective GABA transporter inhibitors tiagabine and EF1502 exhibit mechanistic differences in their ability to modulate the ataxia and anticonvulsant action of the extrasynaptic GABA(A) receptor agonist gaboxadol. J Pharmacol Exp Ther 338(1):214–219
Maldonado MA, Allred RP, Felthauser EL, Jones TA (2008) Motor skill training, but not voluntary exercise, improves skilled reaching after unilateral ischemic lesions of the sensorimotor cortex in rats. Neurorehabil Neural Repair 22(3):250–261
Martinez-Delgado G, Reyes-Haro D, Espino-Saldana AE, Rosas-Arellano A, Petriz A, Juarez-Mercado P et al (2011) Dynamics of GABArho2 receptors in retinal bipolar neurons and cerebellar astrocytes. Neuroreport 22(1):4–9
McCarthy MM, Auger AP, Perrot-Sinal TS (2002) Getting excited about GABA and sex differences in the brain. Trends Neurosci 25(6):307–312
McKernan RM, Whiting PJ (1996) Which $GABA_A$-receptor subtypes really occur in the brain? Trends Neurosci 19(4):139–143
Meera P, Wallner M, Otis TS (2011) Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors. J Neurophysiol 106(4):2057–2064
Mertens S, Benke D, Mohler H (1993) $GABA_A$ receptor populations with novel subunit combinations and drug binding profiles identified in brain by alpha 5- and delta-subunit-specific immunopurification. J Biol Chem 268(8):5965–5573
Milligan CJ, Buckley NJ, Garret M, Deuchars J, Deuchars SA (2004) Evidence for inhibition mediated by coassembly of $GABA_A$ and $GABA_C$ receptor subunits in native central neurons. J Neurosci 24:7241–7250
Minelli A, DeBiasi S, Brecha NC, Zuccarello LV, 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. J Neurosci 16(19):6255–6264
Mitchell SJ, Silver RA (2003) Shunting inhibition modulates neuronal gain during synaptic excitation. Neuron 38(3):433–445
Mody I (2001) Distinguishing between GABA(A) receptors responsible for tonic and phasic conductances. Neurochem Res 26(8–9):907–913
Murata Y, Woodward RM, Miledi R, Overman LE (1996) The first selective antagonists for a $GABA_C$ receptor. Bioorg Med Chem Lett 6:2073–2076
Ng YS, Stein J, Ning M, Black-Schaffer RM (2007) Comparison of clinical characteristics and functional outcomes of ischemic stroke in different vascular territories. Stroke 38(8):2309–2314

Ng CK, Kim HL, Gavande N, Yamamoto I, Kumar RJ, Mewett KN et al (2011) Medicinal chemistry of rho GABAC receptors. Future Med Chem 3(2):197–209
Nicotera P, Lipton SA (1999) Excitotoxins in neuronal apoptosis and necrosis. J Cereb Blood Flow Metab 19(6):583–591
Nudo RJ (2006) Mechanisms for recovery of motor function following cortical damage. Curr Opin Neurobiol 16(6):638–644
Ohab JJ, Fleming S, Blesch A, Carmichael ST (2006) A neurovascular niche for neurogenesis after stroke. J Neurosci 26(50):13007–13016
Olsen RW, Sieghart W (2008). International union of pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol Rev 60(3):243–260
Olsen RW, Sieghart W (2009) GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56(1):141–148
Perea G, Navarrete M, Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32(8):421–431
Qian H, Pan Y (2002) Co-assembly of GABA ρ subunits with the $GABA_A$ receptor γ_2 subunit cloned from white perch retina. Mol Brain Res 103:62–70
Qian H, Ripps H (1999) Response kinetics and pharmacological properties of heteromeric receptors formed by coassembly of GABA ρ- and γ_2-Subunits. Proc R Soc (Lond B) 266:2419–2425
Qian H, Dowling JE, Ripps H (1998) Molecular and pharmacological properties of GABA-r subunits from white perch retina. J Neurobiol 37:305–320
Que M, Schiene K, Witte OW, Zilles K (1999) Widespread up-regulation of N-methyl-D-aspartate receptors after focal photothrombotic lesion in rat brain. Neurosci Lett 273(2):77–80
Ragozzino D, Woodward RM, Murata Y, Eusebi F, Overman LE, Miledi R (1996) Design and *in vitro* pharmacology of a selective γ-aminobutyric $acid_C$ receptor antagonist. Mol Pharmacol 50:1024–1030
Redecker C, Luhmann HJ, Hagemann G, Fritschy JM, Witte OW (2000) Differential downregulation of $GABA_A$ receptor subunits in widespread brain regions in the freeze-lesion model of focal cortical malformations. J Neurosci 20(13):5045–5053
Redecker C, Wang W, Fritschy JM, Witte OW (2002) Widespread and long-lasting alterations in GABA(A)-receptor subtypes after focal cortical infarcts in rats: mediation by NMDA-dependent processes. J Cereb Blood Flow Metab 22(12):1463–1475
Rozzo A, Armellin M, Franzot J, Chiaruttini C, Nistri A, Tongiorgi E (2002) Expression and dendritic mRNA localization of $GABA_C$ receptor ρ1 and ρ2 subunits in developing rat brain and spinal cord. Eur J Neurosci 15:1747–1758
Saxena NC, Macdonald RL (1994) Assembly of $GABA_A$ receptor subunits: role of the delta subunit. J Neurosci 14(11 Pt 2):7077–7086
Schlicker K, McCall MA, Schmidt M (2009) $GABA_C$ receptor-mediated inhibition Is altered but not eliminated in the superior colliculus of $GABA_C$ρ1 knockout mice. J Neurophysiol 101:2947–2983
Schmidt S, Redecker C, Bruehl C, Witte OW (2010). Age-related decline of functional inhibition in rat cortex. Neurobiol Aging 31(3):504–511
Schmidt S, Bruehl C, Frahm C, Redecker C, Witte OW (2012) Age dependence of excitatory-inhibitory balance following stroke. Neurobiol Aging 33(7):1356–1363
Schousboe A, Madsen KK, White HS (2011) GABA transport inhibitors and seizure protection: the past and future. Future Med Chem 3(2):183–187
Schwartz RD, Yu X, Katzman MR, Hayden-Hixson DM, Perry JM (1995) Diazepam, given post-ischemia, protects selectively vulnerable neurons in the rat hippocampus and striatum. J Neurosci 15(1 Pt 2):529–539
Schwartz-Bloom RD, Sah R (2001) Gamma-aminobutyric acid(A) neurotransmission and cerebral ischemia. J Neurochem 77(2):353–371
Schwartz-Bloom RD, Miller KA, Evenson DA, Crain BJ, Nadler JV (2000) Benzodiazepines protect hippocampal neurons from degeneration after transient cerebral ischemia: an ultrastructural study. Neuroscience 98(3):471–484

Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker MC (2005) Multiple and plastic receptors mediate tonic $GABA_A$ receptor currents in the hippocampus. J Neurosci 25(43):10016–10024

Shi L, Quick M, Zhao Y, Weinstein H, Javitch JA (2008) The mechanism of a neurotransmitter:sodium symporter-inward release of Na^+ and substrate is triggered by substrate in a second binding site. Mol Cell 30(6):667–677

Sieghart W (1995) Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. Pharmacol Rev 47(2):181–234

Stinear JW, Byblow WD (2002) Disinhibition in the human motor cortex is enhanced by synchronous upper limb movements. J Physiol 543(Pt 1):307–316

Stinear CM, Coxon JP, Byblow WD (2009) Primary motor cortex and movement prevention: where Stop meets Go. Neurosci Biobehav Rev 33(5):662–673

Stone RA, Liu J, Sugimoto R, Capehart C, Zhu X, Pendrak K (2003) GABA, experimental myopia, and ocular growth in chick. Invest Ophthalmol Vis Sci 44:3933–3946

Stroemer RP, Kent TA, Hulsebosch CE (1998) Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with D-amphetamine therapy after neocortical infarction in rats. Stroke 29(11):2381–2393; discussion 93–95

Sun C, Sieghart W, Kapur J (2004) Distribution of alpha1, alpha4, gamma2, and delta subunits of $GABA_A$ receptors in hippocampal granule cells. Brain Res 1029(2):207–216

Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A et al (2002) Hormonally regulated alpha(4)beta(2)delta GABA(A) receptors are a target for alcohol. Nat Neurosci 5(8):721–722

Tehrani MH, Barnes EM Jr (1991) Agonist-dependent internalization of gamma-aminobutyric acidA/benzodiazepine receptors in chick cortical neurons. J Neurochem 57(4):1307–1312

van der Zijden JP, van Eijsden P, de Graaf RA, Dijkhuizen RM (2008) 1H/13C MR spectroscopic imaging of regionally specific metabolic alterations after experimental stroke. Brain 131(Pt 8):2209–2219

Velez-Fort M, Audinat E, Angulo MC (2012) Central role of GABA in neuron-glia interactions. Neuroscientist 18(3):237–250

Wafford KA, van Niel MB, Ma QP, Horridge E, Herd MB, Peden DR et al (2009) Novel compounds selectively enhance delta subunit containing GABA A receptors and increase tonic currents in thalamus. Neuropharmacology 56(1):182–189

Walker MC, Semyanov A (2008) Regulation of excitability by extrasynaptic GABA(A) receptors. Results Probl Cell Differ 44:29–48

Wang T-L, Guggino WB, Cutting GR (1994) A novel y-aminobutyric acid receptor subunit (r_2) cloned from human retina forms bicuculline-insensitive homooligomeric receptors in Xenopus oocytes. J Neurosci 14:6524–6531

Whiting PJ (2003a) The $GABA_A$ receptor gene family: new opportunities for drug development. Curr Opin Drug Discov Devel 6(5):648–657

Whiting PJ (2003b) GABA-A receptor subtypes in the brain: a paradigm for CNS drug discovery? Drug Discov Today 8(10):445–450

Wittenberg GF, Schaechter JD (2009) The neural basis of constraint-induced movement therapy. Curr Opin Neurol 22:582–588

Wu Y, Wang W, Richerson GB (2006) The transmembrane sodium gradient influences ambient GABA concentration by altering the equilibrium of GABA transporters. J Neurophysiol 96(5):2425–2436

Xu JY, Yang B, Sastry BR (2009) The involvement of GABA-C receptors in paired-pulse depression of inhibitory postsynaptic currents in rat hippocampal CA1 pyramidal neurons. Exp Neurol 216(1):243–246

Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E (2005) Crystal structure of a bacterial homologue of Na+/Cl-dependent neurotransmitter transporters. Nature 437(7056):215–223

Zhang ZG, Chopp M (2009) Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. Lancet Neurol 8(5):491–500

Zhang D, Pan Z-H, Awobuluyi M, Lipton SA (2001) Structure and function of $GABA_C$ receptors: a comparison of native versus recombinant receptors. Trends Pharmacol Sci 22:121–132

Zhu XM, Ong WY (2004a) A light and electron microscopic study of betaine/GABA transporter distribution in the monkey cerebral neocortex and hippocampus. J Neurocytol 33(2):233–240

Zhu XM, Ong WY (2004b) Changes in GABA transporters in the rat hippocampus after kainate-induced neuronal injury: decrease in GAT-1 and GAT-3 but upregulation of betaine/GABA transporter BGT-1. J Neurosci Res 77(3):402–409

Chapter 10
The Role of Extrasynaptic $GABA_A$ Receptors in Focal Epilepsy

Matthew C. Walker and Ivan Pavlov

Abstract Focal epilepsy can result from both genetic and environmental factors. Acquired focal epilepsy occurs following a specific brain insult such as stroke, head injury or prolonged seizures (status epilepticus). The time from the insult to the development of seizures is termed the epileptogenic period during which there are changes in connectivity, neurotransmission and neuronal excitability.

Epileptogenesis has conventionally been viewed as being associated with increased excitation and a loss of inhibition. This view has been reappraised in recent years due to a better understanding of the multifaceted roles of GABAergic signalling. There is growing evidence that loss of synaptic $GABA_A$ receptor-mediated inhibition observed in animal models of temporal lobe epilepsy is accompanied by compensatory upregulation of tonic currents mediated by extrasynaptic $GABA_A$ receptors.

Here, we provide evidence for such a change in $GABA_A$ receptor-mediated inhibition during epileptogenesis and speculate on the possible functional impact that such a shift in inhibition will have. In particular, we argue that shifts from phasic to tonic inhibition in the hippocampus will lead to a maintenance of "inhibition" of the network but will alter network gain, decreasing network stability. Furthermore, changes in the subunit composition of extrasynaptic $GABA_A$ receptors during epileptogenesis have implications for targeted pharmacotherapy of epilepsy.

Keywords Focal epilepsy · Tonic inhibition · Extrasynaptic $GABA_A$ receptors · Hippocampus · Hippocampal sclerosis

M. C. Walker (✉) · I. Pavlov (✉)
Department of Clinical and Experimental Epilepsy,
UCL Institute of Neurology, London, UK
e-mail: m.walker@ucl.ac.uk

I. Pavlov
e-mail: i.pavlov@ucl.ac.uk

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_10

10.1 Introduction

Focal epilepsy can arise as the result of: gene mutations leading to increased neuronal/network excitability (such as mutations in nicotinic acetylcholine receptor genes that result in autosomal dominant nocturnal frontal lobe epilepsy, and mutations in leucine-rich, glioma inactivated 1 resulting in autosomal dominant temporal lobe epilepsy with auditory hallucinations); malformations of cortical development (both genetically and environmentally determined); brain tumours; and acquired lesions following a specific brain insult such as prolonged seizures (status epilepticus), head injury and stroke. The role of extrasynaptic $GABA_A$ receptors has predominantly been determined in the acquired (rather than genetic) focal epilepsies and it is these epilepsies that we concentrate on in this chapter.

The observations that $GABA_A$ receptor antagonists induce seizures, and that drugs that directly potentiate $GABA_A$ receptors (such as benzodiazepines and barbiturates) or that decrease the uptake or breakdown of GABA (tiagabine and vigabatrin, respectively) prevent seizures, indicate the importance of the GABAergic system in seizure generation. Indeed, focal epilepsy has often been viewed as a disruption of the balance between glutamatergic excitation and GABAergic inhibition. The recognition that GABAergic inhibition can take varying forms over different timescales with different effects on neuronal excitability (Mody and Pearce 2004; Farrant and Nusser 2005; Farrant and Kaila 2007) has led to a reappraisal of this simplistic view. A critical observation is that even people or animals with severe epilepsy spend the majority of their time free of seizures. This suggests that the process leading to the development of epilepsy more likely creates networks of neurons that are intrinsically more unstable and in which significant changes in excitability are mostly compensated. However, this compensation either is insufficient or may itself lead to less stable network behaviour.

Most studies in animal models of focal epilepsy have determined changes in fast, synaptic inhibition mediated by the synaptic release of GABA onto postsynaptic $GABA_A$ receptors, and only more recently have changes in tonic currents, mediated by extrasynaptic $GABA_A$ receptors, been investigated. A role for tonic currents in human epilepsy has been suggested by the observation that polymorphisms and mutations in genes encoding extrasynaptic $GABA_A$ receptors, which reduce the magnitude of tonic currents, are associated with several human epilepsies (Dibbens et al. 2004; Feng et al. 2006; Eugène et al. 2007). These, however, are exclusively generalised epilepsies, but emphasise the importance of tonic currents in regulating network excitability. In this chapter, we will argue that, in focal epilepsies, tonic currents are usually increased, perhaps as a compensatory mechanism. This results in a shift from phasic to tonic inhibition, changing network behaviour. Increases in tonic inhibition compensate not just for loss of phasic $GABA_A$ receptor-mediated inhibition but also for loss of other membrane conductances such as the HCN-mediated conductance which is involved in the regulation of cell excitability (Chen et al. 2010), and the loss of which occurs in the development of focal epilepsy.

We also restrict ourselves to considering temporal lobe epilepsy, the most common focal epilepsy syndrome. This has been predominantly studied in an experimental paradigm in which animals are subjected a specific insult (e.g. prolonged seizure induced by a convulsant or traumatic brain injury), after which the animal develops epilepsy over a period of days or months (termed epileptogenesis). It is also worth noting that although we focus on extrasynaptic $GABA_A$ receptors and tonic inhibition, these must be viewed in the context of the panoply of changes in network connectivity, neuronal excitability and synaptic transmission that accompany the development of focal epilepsy.

10.2 Inhibition in Focal Epilepsy

10.2.1 Phasic Inhibition

Early evidence indicated that loss of $GABA_A$ receptor inhibition alone would permit the dominance of excitation and the generation of epileptiform activity (Schwartzkroin and Prince 1977; Gutnick et al. 1982; Connors 1984). Later studies of in vitro models of epileptic activity in acute rodent brain slices have highlighted the importance of a breakdown in feed-forward inhibition for the propagation of seizure-like activity (Trevelyan et al. 2006, 2007). There have been corresponding findings in brain slices from humans, who have previously undergone brain resection to cure their epilepsy, in which there is a loss of synaptic $GABA_A$ receptor-mediated inhibition prior to the occurrence of seizure-like activity (Huberfeld et al. 2011). It was, therefore, expected that epileptogenesis is associated with a loss of interneurons and/or a reduction in the number of or activity of inhibitory synapses on surviving principal neurons (Cossart et al. 2001; Wittner 2001; Buckmaster et al. 2002; Kobayashi and Buckmaster 2003; Shao and Dudek 2005; Hunt et al. 2009; Wyeth et al. 2010). These morphological and functional changes of inhibition occur quite rapidly following the epileptogenic insult, but may continue for a prolonged period of time extending to several months or beyond (Sloviter 1991; Friedman et al. 1994; Leroy et al. 2004; Pavlov et al. 2011). Some studies have suggested that the loss of dendritic-targeting interneurons may be partially compensated by surviving interneurons that project to the soma (Cossart et al. 2001), but the general consensus is that the net outcome of these changes is the loss of phasic (synaptic) inhibition.

10.2.2 Tonic Inhibition

A decrease in synaptic release would be expected to reduce the concentration of GABA present in the extracellular space and so be associated with a reduction in tonic currents mediated by extrasynaptic $GABA_A$ receptors. A correlation between synaptic release and tonic currents has been observed in CA1 pyramidal cells when

GABA uptake is reduced by inhibition of the GABA transporter GAT1 (Glykys and Mody 2007). Surprisingly, experimental evidence indicates that in post-status epilepticus animals, tonic $GABA_A$ receptor-mediated currents are maintained or even increased in many hippocampal neurons including CA1 pyramidal cells, *stratum radiatum* interneurons and dentate granule cells (Scimemi et al. 2005; Naylor et al. 2005; Zhang et al. 2007; Goodkin et al. 2008; Zhan and Nadler 2009; Rajasekaran et al. 2010). Studies in models of post-traumatic epilepsy indicate involvement of the hippocampus in the epileptogenic process and have found similar hippocampal pathology to that observed in post-status epilepticus animals (D'Ambrosio et al. 2005; Kharatishvili et al. 2006; Swartz et al. 2006). These hippocampal changes are also accompanied by maintenance of tonic currents, which have been reported to be preserved in dentate granule cells following lateral fluid-percussion brain injury (Pavlov et al. 2011), or to be enhanced after controlled cortical impact (Mtchedlishvili et al. 2010). Although there is no control comparator, significant tonic $GABA_A$ receptor-mediated currents are also present in neocortical pyramidal cells and dentate granule cells in resected tissue from humans with temporal lobe epilepsy (Scimemi et al. 2006). There are a number of explanations for the above. Recent data indicate that tonic currents in dentate granule cells are largely mediated by spontaneously opening $GABA_A$ receptors, and that extracellular GABA in the hippocampus under baseline conditions is kept at concentrations that are insufficient to activate extrasynaptic $GABA_A$ receptors (Wlodarczyk et al. 2013). Increases in tonic currents may be due to increases or changes in extrasynaptic $GABA_A$ receptors, changes of the sensitivity of extrasynaptic receptors for GABA or an increase in the GABA concentration surroundings neurons.

10.3 Extrasynaptic $GABA_A$ Receptor Plasticity in Focal Epilepsy

Epileptogenesis is associated with changes in the expression of $GABA_A$ receptor subunits in animal models (e.g. Brooks-Kayal et al. (1998) and in humans Loup et al. (2000)). The nature of these changes is partly dependent upon the model of focal epilepsy. Indeed, the heterogeneity of such changes indicates that the down- or upregulation of specific subunits is unlikely to be necessary for the development of epilepsy (Pavlov et al. 2011). In the hippocampus, α5β2/3γ2 and α4β2/3δ receptors generate most of the tonic current. While δ-containing $GABA_A$ receptors are exclusively extrasynaptic or perisynaptic (Wei et al. 2003), α5-containing receptors can also be found at postsynaptic sites (Serwanski et al. 2006). Double knockout mice lacking both δ and α5 subunits have almost no tonic $GABA_A$ receptor-mediated currents in pyramidal cells, dentate granule cells or molecular layer interneurons (Glykys et al. 2008). The relative contribution of different $GABA_A$ receptor subtypes to the generation of tonic currents in the hippocampus varies depending on the cell type. In pyramidal cells, α5-containing receptors contribute to ~50% of the tonic conductance, whilst in dentate granule cells, the majority of the tonic current

(~70%) is mediated by δ-containing receptors (Glykys et al. 2008). In addition, a small proportion (~10%) of extrasynaptic receptors on hippocampal pyramidal neurons lack both γ and δ subunits. These Zn^{2+}-sensitive αβ receptors may also contribute to tonic conductances in hippocampal neurons (Mortensen and Smart 2006).

10.3.1 Changes in α5 and δ Subunit Expression in the Hippocampus

Preservation of tonic $GABA_A$ receptor-mediated currents in epilepsy may be accompanied by their altered pharmacology. For example, despite tonic currents being preserved in CA1 pyramidal cells under baseline conditions and increased upon application of GABA in post-status epilepticus animals, there is a marked reduction in the sensitivity of these currents to an α5-specific inverse agonist L-655,708 (Scimemi et al. 2005). This is consistent with the decrease in α5 subunit expression in these animals (Fritschy et al. 1999; Houser and Esclapez 2003), thus suggesting that there is a substitution of the α5-containing receptors in epileptic tissue by extrasynaptic receptors that are either more numerous or have a greater sensitivity to GABA (Fig. 10.1). The subunit composition of these receptors has yet to be determined.

Changes in the δ subunit expression in dentate granule cells are less clear-cut. It has mostly been found to be reduced following status epilepticus in animal models (Schwarzer et al. 1997; Tsunashima et al. 1997; Peng et al. 2004; Rajasekaran et al. 2010), but see (Brooks-Kayal et al. 1998; Goodkin et al. 2008). Also, studies in post-traumatic epilepsy (Pavlov et al. 2011) and recordings from human epileptic tissue (Scimemi et al. 2006) do not find such a loss.

When the δ-containing $GABA_A$ receptors are lost in dentate granule cells, this is accompanied by an upregulation of α4 subunit expression, leading to increased perisynaptic expression of α4β2/3γ2 receptors (Zhang et al. 2007). However, α4γ2-containing receptors have a fivefold greater EC_{50} for GABA than α4δ-containing receptors (Brown et al. 2002). The observation that tonic currents are preserved can be explained by spontaneous $GABA_A$ receptor openings, greater number of receptors or higher concentrations of extracellular GABA. This latter explanation is supported by the observation of a larger SR95531-sensitive tonic current observed in dentate granule cells (Zhan and Nadler 2009). SR95531 acts as a pure antagonist and has minimal effect on the tonic current in dentate granule cells in control rats (Zhan and Nadler 2009; Wlodarczyk et al. 2013), which is mainly mediated by spontaneously opening receptors. Alternatively, although less likely, a large SR95531-sensitive tonic current in dentate granule cells from epileptic rats may be explained by the increased sensitivity of the receptors to GABA.

It has also been suggested that the contribution of α5-containing $GABA_A$ receptors to tonic currents in dentate granule cells is increased following status epilepticus (Zhan and Nadler 2009). Indeed, a slight increase of α5 mRNA level has been reported in these neurons (Fritschy et al. 1999; Houser and Esclapez 2003). Nonetheless, it is unlikely that such a change can fully compensate for the loss of

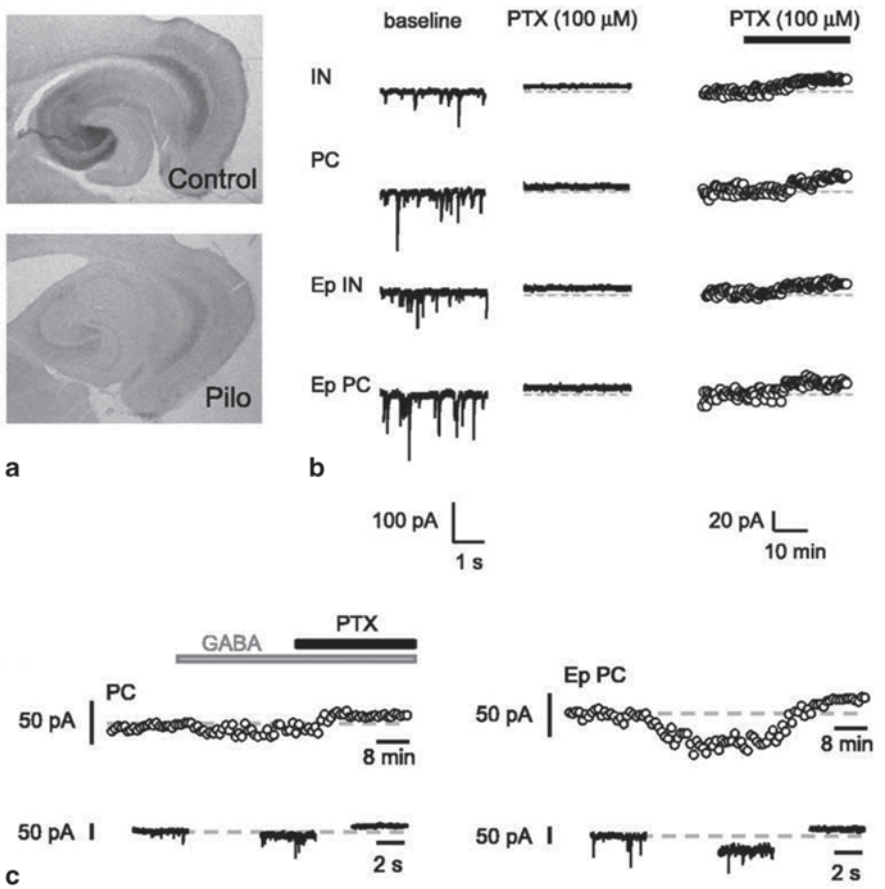

Fig. 10.1 Tonic currents are maintained/increased in CA1 pyramidal cells from epileptic animals. **a** Immunohistochemistry for the α5 subunit, showing decreased expression of this subunit in the hippocampus proper and entorhinal cortex of epileptic rats compared with that of control rats. Pilo, Pilocarpine. **b** Representative traces obtained from one interneuron and one pyramidal cell from a control and an epileptic rat. The tonic current revealed by application of $GABA_A$ receptor antagonist picrotoxin is no different between epilepstic and control neurons. **c** Application of GABA results in larger tonic current in epileptic than in control neurons. (After Scimemi et al. 2005)

the δ subunits and would be sufficient to augment tonic inhibition as reported in electrophysiological studies.

Changes in the subunit composition of extrasynaptic $GABA_A$ receptors during epilepsy have implications for the pharmacological treatment of epilepsy, as more specific drugs are being developed. It should also be noted that changes in the $GABA_A$ receptor subunits may be cell type specific. For example, one study has demonstrated that the reduction of δ subunit expression in dentate granule cells was accompanied by an increase in its expression in dentate interneurons (Peng et al. 2004). This further complicates the prediction of the effect of drugs targeting only specific $GABA_A$ receptors.

10.4 Extracellular GABA in Epilepsy

As detailed above, there is indirect evidence that epileptogenesis results in an increase in extracellular GABA. Such an increase could be due to increased release or decreased uptake. Since most studies show an overall decrease in synaptic inhibition, it is unlikely that increased vesicular release is contributing to increased tonic currents. GABA is also released non-synaptically but the contribution of this pool to extracellular GABA is unclear and the effects of epilepsy on non-synaptic GABA release have not been investigated. An alternative explanation is that there is a change in the number and/or function of GABA transporters (GATs). In addition, GATs are electrogenic such that during periods of depolarisation, GATs can reverse and pump GABA into the extracellular space (Wu et al. 2003, 2007). There are two main cortical GABA transporters, GAT1 and GAT3 (labelled GAT1 and GAT4, respectively, in mice): The former is predominantly neuronal and is located at presynaptic GABAergic terminals, while the latter is expressed in glia (Borden 1996; Ribak et al. 1996; Minelli et al. 1996; Conti et al. 2004). In the rat hippocampus, GAT1 predominantly determines the GABA concentration surrounding neurons, and GAT3 comes into play when extracellular GABA rises, in particular after inhibition of GAT1 (Kersanté et al. 2013).

There is evidence that epilepsy is associated with functional deficits in GABA uptake in human and rat dentate gyrus (Patrylo et al. 2001); other studies, however, have found unaltered GAT1 function in the CA1 region in epileptic rats (Stief et al. 2005). These studies indicate that there may be complex regional-specific changes in GABA transporter function. This has been confirmed in expression studies in resected hippocampi from people with epilepsy in which regional decreases in GAT1 but upregulation of GAT3 have been observed (Mathern et al. 1999; Lee et al. 2006). Under baseline conditions in epileptic tissue, GAT1-mediated transport takes up GABA (rather than acting in reverse), as GAT1 inhibitors significantly increase tonic currents in neurons from chronically epileptic rats (Frahm et al. 2003; Scimemi et al. 2005). However, epilepsy may facilitate reversal of GABA transporters. Interneurons in chronic epilepsy express more GAD and have elevated intracellular GABA concentrations (Esclapez and Houser 1999). Enhanced expression of GATs and an increase in GABA synthesis have also been demonstrated in dentate granule cells in the kainate model of chronic seizures (Sperk et al. 2003). Intracellular accumulation of GABA along with the depolarisation that occurs with seizure-like activity could result in reversal of GABA transporters and elevation of GABA in the extracellular space. However, in the hippocampus in vivo, significant depolarisation induced by raising the extracellular potassium was unable to reverse GAT1 (Kersanté et al. 2013). Nonetheless, such reversal could be facilitated by chronic changes in Cl^- homeostasis in epileptic tissue (Cohen et al. 2002; Palma et al. 2006) and/or gradual build-up of intracellular Cl^- during excessive network activity (Glykys et al. 2009). It is also plausible that increased glutamate uptake by astrocytes in response to massive release of the neurotransmitter during seizure activity may cause intracellular Na^+ accumulation (as a result of cotransport), and thus trigger the reversal of co-localised glial GABA transporters (Héja et al. 2009).

Indeed, there is growing evidence that glia transporters (GAT3) may be reversed during epileptic activity, so that blocking GAT3 increases epileptiform activity (Heja et al. 2012); we have not observed that this is a ubiquitous finding and it may depend upon the in vitro model used (unpublished data).

10.5 Functional Consequences of Enhanced Tonic Inhibition in Epilepsy

There has been scant work on the effects of tonic inhibition on epileptiform activity. Increasing extracellular GABA by inhibiting GABA transporters inhibits (although not universally) seizure-like activity in hippocampal slices and seizures in models of focal epilepsy (Pfeiffer et al. 1996; Sabau et al. 1999; Dalby 2003). It is important to note that this strategy will however increase the activity of both $GABA_A$ and $GABA_B$ receptors and as yet there has been no satisfactory dissection of these separate effects. Moreover, activation of $GABA_B$ receptors may further potentiate tonic $GABA_A$ receptor-mediated conductances (Connelly et al. 2013; Tao et al. 2013). Increasing extracellular GABA may also have cell type-specific actions. Some interneurons, including certain hippocampal subtypes, have $E_{GABA(A)}$ more positive than their resting membrane potential, and therefore GABA depolarises such cells (Martina et al. 2001; Chavas and Marty 2003; Vida et al. 2006; Song et al. 2011); this effect could become even more prominent following increased synaptic activity (Lamsa and Taira 2003). A small rise in extracellular GABA concentrations has an excitatory action on these interneurons through depolarisation, while higher concentrations produces inhibition through mainly a shunting effect (Song et al. 2011). In contrast, the firing of interneurons, in which activation of $GABA_A$ receptors does not produce depolarisation (Verheugen et al. 1999; Martina et al. 2001), will not show this biphasic action of increasing GABA but will solely be suppressed by elevated GABA. This will disinhibit excitatory neurons counteracting reduction of their excitability.

This voltage effect of tonic GABA is likely to be even more complex in principal cells. Generally, the reversal of $GABA_A$ receptors is close to the resting membrane potential such that the voltage effect of tonic inhibition will be minimal. However, in some pyramidal cells during epileptogenesis, the $GABA_A$ receptor reversal potential may become positive with respect to resting membrane potential due to loss of the chloride transporter KCC2 and upregulation of NKCC1 (Rivera et al. 2002; Jin et al. 2005; Muñoz et al. 2007; Huberfeld et al. 2007). It is feasible that tonic current should depolarise such neurons and such a depolarisation would bring the neuron closer to action potential threshold. However, such a depolarisation would also (in)activate other conductances including inactivation of sodium channels, activation of Kv7s and inactivation of HCN, all of which may act to reduce neuronal excitability. It is also worth noting that altered Cl^- homeostasis in epilepsy can itself act as an intracellular signal that controls GABAergic inhibition by modulating the expression of α and δ $GABA_A$ receptor subunits (Succol et al. 2012).

10.5.1 Effects of Tonic $GABA_A$ Receptor Conductance on Neuronal Computation

Perhaps a greater effect of tonic currents on pyramidal cells is the contribution to the input resistance. Persistent activation of $GABA_A$ receptors decreases the membrane input resistance of a neuron and therefore reduces voltage response to incoming excitation (i.e. decreases the amplitude of the excitatory postsynaptic potentials), so decreasing the probability of action potential generation. The relationship between the probability of action potential generation (or the frequency of action potentials in certain cell types) and the magnitude of the input conductance is termed the input–output (I–O) relationship of a neuron and reflects the computation performed by the cell (Silver 2010). The output of hippocampal pyramidal cells is best described by the probability of firing (rather than frequency of firing) in response to temporally correlated synaptic inputs (Azouz 2005; Carvalho and Buonomano 2009). The I–O relationship can be modified through a change in gain (slope) or in offset (threshold), which are equivalent to multiplicative/divisive and additive/subtractive operations, respectively. Since background synaptic noise can summate with the input, then previously subthreshold inputs can on occasions reach threshold, and conversely random inhibitory inputs can decrease the probability of previously suprathreshold inputs from reaching threshold. Therefore, the effect of background synaptic activity is to create voltage variations which reduce the slope of the I–O function, but increase its dynamic range (Wolfart et al. 2005). Decreasing phasic inhibition will decrease voltage fluctuations and so will increase neuronal gain; in addition, decreasing phasic inhibition will increase synaptic summation and so will increase the chance of a specific input reaching threshold, i.e. will offset the I–O function. A fundamental observation is that, partly due to outward rectification, tonic $GABA_A$ receptor-mediated conductances in hippocampal CA1 pyramidal neurons have little influence on subthreshold noise and only affect neurons at spiking threshold. This results in tonic inhibition primarily affecting the offset of the I–O relationship with minimal effect on the slope (Fig. 10.2; Pavlov et al. 2009). Thus, the effect of a shift from phasic to tonic inhibition will be maintenance of neuronal and network excitability when the input is of low magnitude but a change in the gain of the system such that as the input increases there will be a larger increase in output, leading to a potentially more unstable network (Fig. 10.2).

10.6 Pharmacological Implications of Changes in Extrasynaptic Receptors in Focal Epilepsy

As detailed above, decreasing GABA uptake or increasing extracellular GABA by decreasing GABA metabolism will have an action partly through extrasynaptic $GABA_A$ receptors. The enhanced sensitivity of neurons to increases in extracellular GABA that occurs with the development of epilepsy (Scimemi et al. 2005) indicates

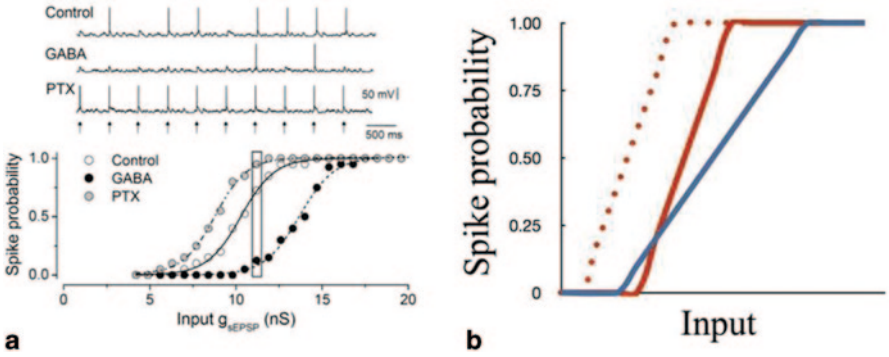

Fig. 10.2 Tonic currents in the hippocampus affect neuronal offset not gain. **a** Application of GABA in the presence of synaptic activity shifts the I–O curve of a neuron to the right without affecting the slope. Subsequent application of the $GABA_A$ receptor antagonist picrotoxin shifts the curve in the opposite direction beyond the control values without a change in slope. **b** In non-epileptic neurons, the I–O relationship as the *blue line*. Loss of synaptic inhibition results in a leftward shift and an increase in slope (*red dotted line*). Increased tonic inhibition compensates for the offset but the slope of the I–O relationship remains increased (*red solid line*). (After Pavlov et al. 2009)

that approaches which increase extracellular GABA are likely to be effective in focal epilepsy (as has been observed clinically), but may have detrimental effects on absence seizures (see Chap. XX). Even within focal epilepsies, such strategies could have complex and paradoxical effects as large increases in tonic currents in interneurons may decrease synaptic inhibition and so paradoxically have a pro-epileptic effect. An alternative strategy would be to target the extrasynaptic receptors directly. However, alterations in subunit composition that occur not only with epileptogenesis but also physiologically, such as during the menstrual cycle (Maguire et al. 2005) or at puberty (Shen et al. 2007, 2010), will alter the sensitivity to such drugs, complicating receptor subtype-targeted pharmacotherapy. Furthermore, the efficacy of different drugs targeting extrasynaptic $GABA_A$ receptors is dependent on the availability and concentration of ambient GABA (Houston et al. 2012).

An important observation has been the loss of benzodiazepine-sensitive synaptic $GABA_A$ receptors during prolonged seizures that contributes to drug resistance (Kapur and Macdonald 1997; Leroy et al. 2004; Feng et al. 2008), but the preservation of extrasynaptic receptors, indicating that therapies aimed at these receptors (either in a non-specific fashion, e.g. barbiturates, or specifically) may be more effective in the late treatment of prolonged seizures.

Conclusion

The development of focal epilepsy seems to be accompanied by a shift from inhibition mediated by synaptic $GABA_A$ receptors to inhibition mediated by extrasynaptic $GABA_A$ receptors. This may result in an increase in the gain of the network. This

would result in only small increases in input leading to a large increase in the probability of neuronal firing, resulting in potentially more unstable networks that would have the propensity to generate seizure activity.

Alterations in the subunit composition of extrasynaptic $GABA_A$ receptors during the development of epilepsy also have significant implications for targeted pharmacotherapy. It is likely that different insults may result in differing degrees and types of subunit alterations, suggesting that more specific therapies may be most useful in epilepsies with distinct and particular aetiologies.

Acknowledgments This work was supported by Pewterer's Fellowship to I.P., funding from Wellcome Trust, Medical Research Council (UK), Epilepsy Research UK and European Integrated Project "EPICURE" (EFP6-037315).

References

Azouz R (2005) Dynamic spatiotemporal synaptic integration in cortical neurons: neuronal gain, revisited. J Neurophysiol 94:2785–2796

Borden LA (1996) GABA transporter heterogeneity: pharmacology and cellular localization. Neurochem Int 29:335–356

Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA (1998) Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. Nat Med 4:1166–1172

Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA (2002) Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. Br J Pharmacol 136:965–974

Buckmaster PS, Yamawaki R, Zhang GUOF (2002) Axon arbors and synaptic connections of a vulnerable population of interneurons in the dentate gyrus in vivo. J Comp Neurol 445:360–373

Carvalho TP, Buonomano DV (2009) Differential effects of excitatory and inhibitory plasticity on synaptically driven neuronal input-output functions. Neuron 61:774–785

Chavas J, Marty A (2003) Coexistence of excitatory and inhibitory GABA synapses in the cerebellar interneuron network. J Neurosci 23:2019–2031

Chen X, Shu S, Schwartz LC, Sun C, Kapur J, Bayliss DA (2010) Homeostatic regulation of synaptic excitability: tonic GABA(A) receptor currents replace I(h) in cortical pyramidal neurons of HCN1 knock-out mice. J Neurosci 30:2611–2622

Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R (2002) On the origin of interictal activity in human temporal lobe epilepsy *in vitro*. Science 298:1418–1421

Connelly WM, Fyson SJ, Errington AC, McCafferty CP, Cope DW, Di Giovanni G, Crunelli V (2013) GABAB receptors regulate extrasynaptic GABAA receptors. J Neurosci 33:3780–3785

Connors BW (1984) Initiation of synchronized neuronal bursting in neocortex. Nature 310:685–687

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

Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben-Ari Y, Esclapez M, Bernard C (2001) Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nat Neurosci 4:52–62

D'Ambrosio R, Fender JS, Fairbanks JP, Simon EA, Born DE, Doyle DL, Miller JW (2005) Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat. Brain 128:174–188

Dalby NO (2003) Inhibition of γ-aminobutyric acid uptake: anatomy, physiology and effects against epileptic seizures. Eur J Pharmacol 479:127–137

Dibbens LM, Feng H-J, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC (2004) GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. Hum Mol Genet 13:1315–1319

Esclapez M, Houser CR (1999) Up-regulation of GAD65 and GAD67 in remaining hippocampal GABA neurons. J Comp Neurol 412:488–505

Eugène E, Depienne C, Baulac S, Baulac M, Fritschy JM, Le Guern E, Miles R, Poncer JC (2007) GABA(A) receptor gamma 2 subunit mutations linked to human epileptic syndromes differentially affect phasic and tonic inhibition. J Neurosci 27:14108–14116

Farrant M, Kaila K (2007) The cellular, molecular and ionic basis of GABA(A) receptor signalling. Prog Brain Res 160:59–87

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci 6:215–229

Feng H, Mathews GC, Kao C, Macdonald RL (2008) Alterations of GABA A -receptor function and allosteric modulation during development of status epilepticus. J Neurophysiol 99:1285–1293

Feng H-J, Kang J-Q, Song L, Dibbens L, Mulley J, Macdonald RL (2006) Delta subunit susceptibility variants E177A and R220H associated with complex epilepsy alter channel gating and surface expression of alpha4beta2delta GABAA receptors. J Neurosci 26:1499–1506

Frahm C, Stief F, Zuschratter W, Draguhn A (2003) Unaltered control of extracellular GABA-concentration through GAT-1 in the hippocampus of rats after pilocarpine-induced status epilepticus. Epilepsy Res 52:243–252

Friedman LK, Pellegrini-Giampietro DE, Sperber EF, Bennett VL, Moshe SL, Zukin RS (1994) Kainate-induced status epilepticus alters glutamate and GABAA receptor gene expression in adult rat hippocampus: an in situ hybridization study. J Neurosci 14:2697–2707

Fritschy JM, Kiener T, Bouilleret V, Loup F (1999) GABAergic neurons and GABA(A)-receptors in temporal lobe epilepsy. Neurochem Int 34:435–445

Glykys J, Mody I (2007) The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. J Physiol 582:1163–1178

Glykys J, Mann EO, Mody I (2008) Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? J Neurosci 28:1421–1426

Glykys J, Dzhala VI, Kuchibhotla KV, Feng G, Kuner T, Augustine G, Bacskai BJ, Staley KJ (2009) Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. Neuron 63:657–672

Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J (2008) Subunit-specific trafficking of GABA(A) receptors during status epilepticus. J Neurosci 28:2527–2538

Gutnick MJ, Connors BW, Prince DA (1982) Mechanisms of neocortical epileptogenesis *in vitro*. J Neurophysiol 48:1321–1335

Héja L, Barabás P, Nyitrai G, Kékesi KA, Lasztóczi B, Toke O, Tárkányi G, Madsen K, Schousboe A, Dobolyi A, Palkovits M, Kardos J (2009) Glutamate uptake triggers transporter-mediated GABA release from astrocytes. PLoS ONE 4:e7153

Heja L, Nyitrai G, Kekesi O, Dobolyi A, Szabo P, Fiath R, Ulbert I, Pal-Szenthe B, Palkovits M, Kardos J (2012) Astrocytes convert network excitation to tonic inhibition of neurons. BMC Biol 10:26

Houser CR, Esclapez M (2003) Downregulation of the alpha5 subunit of the GABA(A) receptor in the pilocarpine model of temporal lobe epilepsy. Hippocampus 13:633–645

Houston CM, McGee TP, MacKenzie G, Troyano-Cuturi K, Rodriguez PM, Kutsarova E, Diamanti E, Hosie AM, Franks NP, Brickley SG (2012) Are extrasynaptic GABAA receptors important targets for sedative/hypnotic drugs? J Neurosci 32:3887–3897

Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C (2007) Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. J Neurosci 27:9866–9873

Huberfeld G, Menendez de la Prida L, Pallud J, Cohen I, Le Van Quyen M, Adam C, Clemenceau S, Baulac M, Miles R (2011) Glutamatergic pre-ictal discharges emerge at the transition to seizure in human epilepsy. Nat Neurosci 14:627–634

Hunt RF, Scheff SW, Smith BN (2009) Posttraumatic epilepsy after controlled cortical impact injury in mice. Exp Neurol 215:243–252

Jin X, Huguenard JR, Prince DA (2005) Impaired Cl^- extrusion in layer V pyramidal neurons of chronically injured epileptogenic neocortex. J Neurophysiol 93:2117–2126

Kapur J, Macdonald RL (1997) Rapid seizure-induced reduction of benzodiazepine and Zn2+ sensitivity of hippocampal dentate granule cell GABA A receptors. J Neurosci 17:7532–7540

Kersanté F, Rowley S, Pavlov I, Gutièrrez-Mecinas M, Semyanov A, Reul J, Walker MC, Linthorst ACE (2013) A functional role for both GABA transporter-1 and GABA transporter-3 in the modulation of extracellular GABA and GABAergic tonic conductances in the rat hippocampus. J Physiol 591(Pt 10):2429–2441

Kharatishvili I, Nissinen JP, McIntosh TK, Pitkänen A (2006) A model of posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats. Neuroscience 140:685–697

Kobayashi M, Buckmaster PS (2003) Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. J Neurosci 23:2440–2452

Lamsa K, Taira T (2003) Use-dependent shift from inhibitory to excitatory GABAA receptor action in SP-O interneurons in the rat hippocampal CA3 area. J Neurophysiol 90:1983–1995

Lee T-S, Bjørnsen LP, Paz C, Kim JH, Spencer SS, Spencer DD, Eid T, De Lanerolle NC (2006) GAT1 and GAT3 expression are differently localized in the human epileptogenic hippocampus. Acta Neuropathol 111:351–363

Leroy C, Poisbeau P, Keller AF, Nehlig A (2004) Pharmacological plasticity of GABA(A) receptors at dentate gyrus synapses in a rat model of temporal lobe epilepsy. J Physiol 557:473–487

Loup F, Wieser H-G, Yonekawa Y, Aguzzi A, Fritschy J-M (2000) Selective alterations in GABAA receptor subtypes in human temporal lobe epilepsy. Neuron 20:5401–5419

Maguire JL, Stell BM, Rafizadeh M, Mody I (2005) Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci 8:797–804

Martina M, Royer S, Paré D (2001) Cell-type-specific GABA responses and chloride homeostasis in the cortex and amygdala. J Neurophysiol 86:2887–2895

Mathern GW, Mendoza D, Lozada A, Pretorius JK, Dehnes Y, Danbolt NC, Nelson N, Leite JP, Chimelli L, Born DE, Sakamoto AC, Assirati JA, Fried I, Peacock WJ, Ojemann GA, Adelson PD (1999) Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. Neurology 52:453–472

Minelli A, DeBiasi S, Brecha NC, Zuccarello LV, 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. J Neurosci 16:6255–6264

Mody I, Pearce RA (2004) Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci 27:569–575

Mortensen M, Smart TG (2006) Extrasynaptic alphabeta subunit GABAA receptors on rat hippocampal pyramidal neurons. J Physiol 577:841–856

Mtchedlishvili Z, Lepsveridze E, Xu H, Kharlamov EA, Lu B, Kelly KM (2010) Increase of GABAA receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury. Neurobiol Dis 38:464–475

Muñoz A, Méndez P, DeFelipe J, Alvarez-Leefmans FJ (2007) Cation-chloride cotransporters and GABA-ergic innervation in the human epileptic hippocampus. Epilepsia 48:663–673

Naylor DE, Liu H, Wasterlain CG (2005) Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. J Neurosci 25:7724–7733

Palma E, Amici M, Sobrero F, Spinelli G, Di Angelantonio S, Ragozzino D, Mascia A, Scoppetta C, Esposito V, Miledi R, Eusebi F (2006) Anomalous levels of Cl– transporters in the hippocampal subiculum from temporal lobe epilepsy patients make GABA excitatory. Proc Natl Acad Sci U S A 103:8465–8468

Patrylo PR, Spencer DD, Williamson A, Ortinski PI, Turner JR, Barberis A, Motamedi G, Yasuda RP, Wolfe B, Kellar KJ, Vicini S, Mcnay EC, Mccrimmon RJ, Sherwin RS (2001) GABA uptake and heterotransport are impaired in the dentate gyrus of epileptic rats and humans with temporal lobe sclerosis. J Neurophysiol 85:1533–1542

Pavlov I, Savtchenko LP, Kullmann DM, Semyanov A, Walker MC (2009) Outwardly rectifying tonically active GABAA receptors in pyramidal cells modulate neuronal offset, not gain. J Neurosci 29:15341–15350

Pavlov I, Huusko N, Drexel M, Kirchmair E, Sperk G, Pitkänen A, Walker MC (2011) Progressive loss of phasic, but not tonic, GABA(A) receptor-mediated inhibition in dentate granule cells in a model of post-traumatic epilepsy in rats. Neuroscience 194:208–219

Peng Z, Huang CS, Stell BM, Mody I, Houser CR (2004) Altered expression of the delta subunit of the GABAA receptor in a mouse model of temporal lobe epilepsy. J Neurosci 24:8629–8639

Pfeiffer M, Draguhn A, Meierkord H, Heinemann U (1996) Effects of gamma-aminobutyric acid (GABA) agonists and GABA uptake inhibitors on pharmacosensitive and pharmacoresistant epileptiform activity *in vitro*. Br J Pharmacol 119:569–577

Rajasekaran K, Joshi S, Sun C, Mtchedlishvilli Z, Kapur J (2010) Receptors with low affinity for neurosteroids and GABA contribute to tonic inhibition of granule cells in epileptic animals. Neurobiol Dis 40:490–501

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

Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M (2002) BDNF-induced TrkB activation down-regulates the K^+ –Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion. J Cell Biol 159:747–752

Sabau A, Frahm C, Pfeiffer M, Breustedt J, Piechotta A, Numberger M, Engel D, Heinemann U, Draguhn A (1999) Age-dependence of the anticonvulsant effects of the GABA uptake inhibitor tiagabine *in vitro*. Eur J Pharmacol 383:259–266

Schwartzkroin PA, Prince DA (1977) Penicillin-induced epileptilorm activity in the hippocampal *in vitro* preparation. Ann Neurol 1:463–469

Schwarzer C, Tsunashima K, Wanzenböck C, Fuchs K, Sieghart W, Sperk G (1997) GABA(A) receptor subunits in the rat hippocampus II: altered distribution in kainic acid-induced temporal lobe epilepsy. Neuroscience 80:1001–1017

Scimemi A, Semyanov A, Sperk G, Kullmann DM, Walker MC (2005) Multiple and plastic receptors mediate tonic GABAA receptor currents in the hippocampus. J Neurosci 25:10016–10024

Scimemi A, Andersson A, Heeroma JH, Strandberg J, Rydenhag B, McEvoy AW, Thom M, Asztely F, Walker MC (2006) Tonic GABA(A) receptor-mediated currents in human brain. Eur J Neurosci 24:1157–1160

Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, De Blas AL (2006) Synaptic and nonsynaptic localization of GABAA receptors containing the alpha5 subunit in the rat brain. J Comp Neurol 499:458–470

Shao L-R, Dudek FE (2005) Changes in mIPSCs and sIPSCs after kainate treatment: evidence for loss of inhibitory input to dentate granule cells and possible compensatory responses. J Neurophysiol 94:952–960

Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, Smith SS (2007) Reversal of neurosteroid effects at alpha4beta2delta GABAA receptors triggers anxiety at puberty. Nat Neurosci 10:469–477

Shen H, Sabaliauskas N, Sherpa A, Fenton AA, Stelzer A, Aoki C, Smith SS (2010) A critical role for alpha4betadelta GABAA receptors in shaping learning deficits at puberty in mice. Science 327:1515–1518

Silver RA (2010) Neuronal arithmetic. Nat Rev Neurosci 11:474–489

Sloviter RS (1991) Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. Hippocampus 1:41–66

Song I, Savtchenko L, Semyanov A (2011) Tonic excitation or inhibition is set by GABA(A) conductance in hippocampal interneurons. Nat Commun 2:376

Sperk G, Schwarzer C, Heilman J, Furtinger S, Reimer RJ, Edwards RH, Nelson N (2003) Expression of plasma membrane GABA transporters but not of the vesicular GABA transporter in dentate granule cells after kainic acid seizures. Hippocampus 13:806–815

Stief F, Piechotta A, Gabriel S, Schmitz D, Draguhn A (2005) Functional GABA uptake at inhibitory synapses in CA1 of chronically epileptic rats. Epilepsy Res 66:199–202

Succol F, Fiumelli H, Benfenati F, Cancedda L, Barberis A (2012) Intracellular chloride concentration influences the GABAA receptor subunit composition. Nat Commun 3:738

Swartz BE, Houser CR, Tomiyasu U, Walsh GO, DeSalles A, Rich JR, Delgado-Escueta A (2006) Hippocampal cell loss in posttraumatic human epilepsy. Epilepsia 47:1373–1382

Tao W, Higgs MH, Spain WJ, Ransom CB (2013) Postsynaptic GABAB receptors enhance extrasynaptic GABAA receptor function in dentate gyrus granule cells. J Neurosci 33:3738–3743

Trevelyan AJ, Sussillo D, Watson BO, Yuste R (2006) Modular propagation of epileptiform activity: evidence for an inhibitory veto in neocortex. J Neurosci 26:12447–12455

Trevelyan AJ, Sussillo D, Yuste R (2007) Feedforward inhibition contributes to the control of epileptiform propagation speed. J Neurosci 27:3383–3387

Tsunashima K, Schwarzer C, Kirchmair E, Sieghart W, Sperk G (1997) GABA(A) receptor subunits in the rat hippocampus III: altered messenger RNA expression in kainic acid-induced epilepsy. Neuroscience 80:1019–1032

Verheugen JA, Fricker D, Miles R (1999) Noninvasive measurements of the membrane potential and GABAergic action in hippocampal interneurons. J Neurosci 19:2546–2555

Vida I, Bartos M, Jonas P (2006) Shunting inhibition improves robustness of gamma oscillations in hippocampal interneuron networks by homogenizing firing rates. Neuron 49:107–117

Wei W, Zhang N, Peng Z, Houser CR, Mody I (2003) Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. J Neurosci 23:10650–10661

Wittner L (2001) Preservation of perisomatic inhibitory input of granule cells in the epileptic human dentate gyrus. Neuroscience 108:587–600

Wlodarczyk A, Sylantyev S, Herd MB, Kersanté F, Lambert JJ, Rusakov DA, Linthorst ACE, Semyanov A, Belelli D, Pavlov I, Walker MC (2013) GABA-independent GABAA receptor openings maintain tonic currents. J Neurosci 33(9):3905–3914

Wolfart J, Debay D, Le Masson G, Destexhe A, Bal T (2005) Synaptic background activity controls spike transfer from thalamus to cortex. Nat Neurosci 8:1760–1767

Wu Y, Wang W, Richerson GB (2003) Vigabatrin induces tonic inhibition via GABA transporter reversal without increasing vesicular GABA release. J Neurophysiol 89:2021–2034

Wu Y, Wang W, Díez-Sampedro A, Richerson GB (2007) Nonvesicular inhibitory neurotransmission via reversal of the GABA transporter GAT-1. Neuron 56:851–865

Wyeth MS, Zhang N, Mody I, Houser CR (2010) Selective reduction of cholecystokinin-positive basket cell innervation in a model of temporal lobe epilepsy. J Neurosci 30:8993–9006

Zhan R-Z, Nadler JV (2009) Enhanced tonic GABA current in normotopic and hilar ectopic dentate granule cells after pilocarpine-induced status epilepticus. J Neurophysiol 102:670–681

Zhang N, Wei W, Mody I, Houser CR (2007) Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. J Neurosci 27:7520–7531

Chapter 11
Gain-of-Function of Thalamic Extrasynaptic GABA-A Receptors in Typical Absence Seizures

Vincenzo Crunelli, Giuseppe Di Giovanni, H. Rheinallt Parri and Adam C. Errington

Abstract Epilepsy is generally viewed as resulting from an unbalanced excitatory/inhibitory drive, where either excitatory transmission is enhanced and/or inhibitory transmission is decreased. However, studies in genetic and pharmacological models of non-convulsive typical absence seizures have revealed that an increased activation of extrasynaptic γ-aminobutyric acid$_A$ (GABA$_A$) receptors (eGABA$_A$Rs), and the resulting enhanced tonic GABA$_A$ inhibition in thalamocortical (TC) neurons, is a necessary and sufficient condition for the expression of these seizures. Importantly, in genetic absence models, the mechanism underlying eGABA$_A$R gain of function is non-neuronal in nature as it results from a malfunction in the thalamic astrocytic GABA transporter, GAT-1. These results challenge the existing view that typical absence seizures are underpinned by a widespread loss of GABAergic function in TC circuits, and are supported by the evidence that drugs that increase GABAergic signalling elicit or aggravate absence seizures in animal model and humans. Furthermore, by highlighting a vital role for astrocytes and eGABA$_A$Rs in the pathophysiology of typical absence epilepsy, these new findings offer novel targets for the development of more effective anti-absence drugs.

Keywords Thalamus · Cortex · GABA$_B$ receptors · GABA transporters · THIP · Dopamine · Serotonin

V. Crunelli (✉) · G. Di Giovanni
Neuroscience Division, School of Biosciences, Cardiff University, Cardiff, UK
e-mail: crunelli@cardiff.ac.uk

G. Di. Giovanni
Department of Physiology and Biochemistry, Faculty of Medicine,
Malta University, Msida, Malta

H. R. Parri
School of Life and Health Sciences, Aston University, Birmingham, UK

A. C. Errington
Neuroscience and Mental Health Research Institute,
Institute of Psychological Medicine and Clinical Neuroscience,
School of Medicine, Cardiff University, Cardiff, UK

A. C. Errington et al. (eds.), *Extrasynaptic GABA$_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_11

11.1 Absence Epilepsy

A typical absence is a non-convulsive epileptic seizure that consists of a sudden, relatively brief impairment of consciousness accompanied by a generalized, synchronous, bilateral 2.5–4 Hz spike and slow wave discharges (SWDs) in the electroencephalogram (EEG) (Panyiotopoulos 1997; Avoli et al. 2001; Crunelli and Leresche 2002; Blumenfeld 2005). Typical absence seizures are an integral part of the EEG and clinical presentation of a number of idiopathic generalized epilepsies, but they are the only seizure phenotype in childhood absence epilepsy. This is a characteristic developmental neurological disease, since absence seizures start between 3 and 8 years of age and spontaneous remission occurs in about 70 % of patients during late adolescence.

Absence seizures are genetically determined, with a 16–45 % positive family history. Penetrance is incomplete, though a concordance of 70–85 % and 33 % has been reported in monozygotic twins and first-degree relatives, respectively (Crunelli and Leresche 2002). Indeed, the classical 2.5–4-Hz SWDs trait of different immunoglobulin E (IGEs) has been shown to be inherited as an autosomal dominant gene, and the current consensus is that the absence epilepsy is a familial disease with a complex genotype. Among the different genetic abnormalities that have been detected in childhood absence epilepsy sufferers, those leading to a putative T-type calcium channel gain-of-function or a $GABA_AR$ loss-of-function have received the greatest attention (Crunelli et al. 2012; Leresche et al. 2012). As discussed below, however, a critical analysis of the relevant literature indicates that a widespread decrease in GABAergic signalling in human and experimental absence seizures is not supported by solid and conclusive evidence.

11.2 Absence Seizures are Generated within the Thalamocortical Circuit

The expression of human typical absence seizures requires coordinated activity in reciprocally connected glutamatergic thalamocortical (TC) neurons and GABAergic thalamic reticular neurons as well as neocortical neurons (i.e., within TC circuits), as revealed by some old invasive studies and modern non-invasive imaging investigations (Williams 1953; Gotman et al. 2005; Hamandi et al. 2006; Tyvaert et al. 2009; Vaudano et al. 2009; Westmijse et al. 2009; Bai et al. 2010; Szaflarski et al. 2010). These studies have also confirmed the lack of involvement of other brain areas, including hippocampus, cerebellum and limbic regions. Importantly, the almost exclusive involvement of cortico-thalamo-cortical networks in the generation of typical absence seizures has also been widely confirmed in experimental absence models (Coenen et al. 1992; Danober et al. 1998; Crunelli and Leresche 2002; Budde et al. 2006).

Against the common belief that the EEG activity characteristic of absence seizures is generalized from their very start, one of the most important recent discoveries has been the identification of a cortical ‘initiation site’ of SWDs. Thus, standard

and high-density EEG studies together with magnetoencephalogram (MEG) and functional magnetic resonance imaging (fMRI) in absence epilepsy patients have shown the presence of SWDs (and associated changes in blood oxygenation level dependent (BOLD) signals) in discrete, mainly frontal cortical regions before they appear over other neocortical regions and the thalamus (Holmes et al. 2004; Gotman et al. 2005; Hamandi et al. 2006; Vaudano et al. 2009; Bai et al. 2010; Szaflarski 2010). Importantly, the presence of a cortical 'initiation site' for typical absence seizures has also been shown in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Wistar Albino Glaxo/Rijswijk rats (WAG), two well-established absence models (Coenen et al. 1992; Danober et al. 1998), where in contrast to human absences, it is located in the perioral region of the primary somatosensory cortex (Meeren et al. 2002; Manning et al. 2004; Polack et al. 2007).

11.3 Thalamic GABAergic Signalling and Absence Seizures

The current view that absence seizures require a widespread $GABA_A$Rs loss-of-function in the TC circuits (McCormick and Contreras 2001; Beenhakker and Huguenard 2009) has mainly evolved from three sets of data. Firstly, the original in vivo results showing that the intramuscular injection of the (weak) $GABA_A$R antagonist penicillin in cats elicits SWDs (Prince and Farrell 1969; Fisher and Prince 1977; Gloor 1978; Avoli and Gloor 1981; Kostopoulos et al. 1981a, b); secondly, the findings of electrophysiological in vitro studies performed in thalamic slices, where the block of thalamic $GABA_A$Rs was shown to elicit a rhythmic firing pattern at a frequency (3 Hz) similar to that of SWDs in humans (von Krosigk et al. 1993; Bal et al. 1995); and thirdly, the loss-of-function of phasic $GABA_A$R-mediated inhibition in different recombinant $GABA_A$Rs containing one of the diverse mutations of these receptors (Tanaka et al. 2008; MacDonald et al. 2010) that were previously identified in human absence patients (Wallace et al. 2001; Kananura et al. 2002; Maljevic et al. 2006; Lachance-Touchette et al. 2011). In view of the available evidence, however, the idea of a decrease in GABAergic transmission in all component neurons of the TC circuit is at present untenable, as discussed in detail below.

As far as the neocortex is concerned, decreased cortical GABAergic inhibition has been suggested to occur in layer 2/3 regular spiking neurons (Luhmann et al. 1995) and in layer 5 pyramidal neurons (D'Antuono et al. 2006) of adult WAG rats (Coenen et al. 1992). Moreover, a slight reduction in $GABA_A$ inhibitory postsynaptic potentials (IPSPs)/inhibitory postsynaptic potentials and currents (IPSPCs) in layer 2/3 has been reported in mice that carry the human $GABA_A$-γ2(R43Q) mutation and show absence seizures (Tan et al. 2007). However, no change is observed in mIPSCs in pyramidal cells and interneurons of cortical layers 2–3 of young, pre-seizure GAERS (Bessaïh et al. 2006), and cortical GABAergic inhibition is apparently intact in the feline generalized penicillin epilepsy model (Giaretta et al. 1985). Thus, a reduction in phasic $GABA_A$ inhibition is present in cortical neurons of some absence models, but the field will definitely gain from some systematic analysis of this type of GABAergic signalling in the neocortex.

As far as phasic $GABA_AR$ inhibition in nucleus reticularis thalami (NRT) neurons is concerned, there is either no change or an increase in intra-NRT phasic $GABA_A$ inhibition in rat and mouse models of typical absence epilepsy (e.g. GAERS rats, DBA/2J and succinic semiadlehyde dehydrogenase-deficient ($SSADH^{-/-}$) mice as well as mice carrying the $GABA_A$-γ2(R43Q) mutation found in absence epilepsy patients) (Bessaïh et al. 2006; Tan et al. 2007, 2008; Errington et al. 2011a). In contrast, in $GABA_AR$-β3 subunit knock-out (KO) mice, which show absence seizures as part of a much complex phenotype, $GABA_A$ IPSCs in NRT neurons are abolished (Huntsman et al. 1999), and in γ3 KO mice, which show an unexpected decrease in gamma-hydroxybutyric acid (GHB)-induced absence seizures, $GABA_A$ IPSCs in the NRT are increased (Schofield et al. 2009). Thus, it remains unclear which type of changes (if any) in intra-NRT phasic $GABA_A$ inhibition are a necessary condition for the expression of typical absence seizures.

As far as TC neurons are concerned, all findings from in vitro data and from the in vivo results in well-established absence models indicate that phasic $GABA_AR$ function is neither abolished nor decreased. Thus, the intramuscular injection of penicillin has no apparent effect on GABAergic function in TC neurons (Gloor 1968; Giaretta et al. 1985) and direct intrathalamic injection of either penicillin (Gloor 1969) or the more potent $GABA_AR$ antagonist bicuculline (Steriade and Contreras 1995) fails to elicit SWDs in the cat. Moreover, microiontophoretic application of bicuculline during SWDs drastically increases the firing of TC neurons in WAG rats in vivo (Staak and Pape 2001), indicating that $GABA_ARs$ are functional during this epileptic activity in an inbred model, and mice with the $GABA_A$-γ2(R43Q) mutation, express spontaneous absence seizures but no change in miniature IPSCs in TC neurons (Tan et al. 2007) as it is the case in $GABA_AR$-β3 subunit KO mice. Finally, unitary conductance of $GABA_A$ IPSCs in TC neurons of an intralaminar thalamic nucleus in WAG rats is increased compared to non-epileptic rats (Brockhaus and Pape 2011); the frequency and amplitude of spontaneous IPSCs is increased in TC neurons of $SSADH^{-/-}$ mice (Errington et al. 2011a), and evoked IPSCs are similar in lethargic and tottering mice compared to their wildtype littermates (Caddick et al. 1999) (for a comprehensive analysis of other evidence on phasic $GABA_AR$ function in TC neurons of absence models, see Crunelli et al. 2011, 2012; Leresche et al. 2012).

11.4 Enhanced Tonic $GABA_A$ Inhibition in Genetic Absence Epilepsy Models Results from a Malfunction of GAT-1

In contrast to phasic $GABA_A$inhibition, it has been demonstrated in vitro that the tonic $GABA_A$ current in TC neurons of the ventrobasal (VB) thalamus is enhanced in several genetic and pharmacological models of absence epilepsy compared to their respective non-epileptic control animals (Cope et al. 2009). This was first shown in the polygenic GAERS model, where up to postnatal day 16, the tonic current in VB of GAERS is similar to that of the non-epileptic control (NEC) strain. However, beyond P16 and prior to seizure onset, a significant increase in tonic current amplitude occurs in GAERS VB neurons that remains elevated in comparison

to NEC animals until well past the time of seizure onset (around postnatal day 30; Fig. 11.1a). As such, rather than being a consequence of the seizures, this indicates that the pathological enhancement of tonic GABA inhibition during development in GAERS may be pro-epileptogenic (Cope et al. 2009). An enhanced tonic $GABA_A$ current is also observed in TC neurons of monogenic absence models (i.e., stargazer and lethargic mice; Cope et al. 2009) as well as in $SSADH^{-/-}$ mice (Fig. 11.1b; Errington et al. 2011a) which show an absence phenotype together with other neurological deficits (Hosford and Wang 1997; Fletcher and Frankel 1999; Hogema et al. 2001). In contrast, no tonic $GABA_A$ current is present in the GABAergic NRT neurons of GAERS or NEC rats (Cope et al. 2009) as is indeed the case in normal Wistar rats (Cope et al. 2005).

The pathological augmentation of tonic $GABA_A$ currents in TC neurons of genetic absence models results from a dysfunction of GABA re-uptake by the transporter GAT-1 (Cope et al. 2009). In fact, in thalamic slices prepared from both GAERS rats and stargazer mice, the block of GAT-1 using the specific antagonist NO711 has no effect on the tonic $GABA_A$ current in VB TC neurons, whereas in the respective non-epileptic rats and mice it elicits a significant enhancement of tonic current that reached levels similar to those found in neurons from epileptic animals (Fig. 11.1c–e). Furthermore, in non-epileptic animals, blockade of GAT-3 using SNAP5114 results in an increase of the tonic $GABA_A$ current that was significantly less than that observed in GAERS or stargazer, suggesting that GAT-1's ability to compensate for the loss of GAT-3 is lost in the epileptic strains (Fig. 11.1c–e). A malfunction in GAT-1 also underlies the increased tonic $GABA_A$ current in TC neurons of lethargic mice (Cope et al. 2009), though in contrast to GAERS and stargazer mice, the action of this transporter is not inhibited in lethargic mice but appears to be reversed.

Interestingly, GAT-1 in the thalamus is expressed exclusively in astrocytes (De Biasi et al. 1998; Pow et al. 2005), indicating that one of the potential thalamic abnormalities underlying absence seizures is located in a non-neuronal cell type. Confirmation of these indirect findings has come from direct functional measurements of the GAT-1 transporter current in thalamic astrocytes of GAERS and NEC rats (Pirttimaki et al. 2013). Thus, in the presence of NO711, the amplitude of the GABA transporter current is unchanged in GAERS but significantly reduced in NEC rats (Fig. 11.2). Application of the GAT-3 blocker SNAP5114 markedly decreases the transporter current in NEC astrocytes, whereas co-application of NO711 and SNAP5114 nearly abolishes it. In contrast, SNAP5114 inhibits the astrocytic GABA transporter current in GAERS to a similar extent as the co-application of NO711 and SNAP5114 (Fig. 11.2). Together, these data provide direct confirmation of a dysfunctional GAT-1 activity in the epileptic GAERS rats (Pirttimaki et al. 2013). Finally, it is important to note that the function of GAT-1 is not compromised in brain areas that do not participate in the generation of typical absence seizures and where the distribution of this transporter is primarily neuronal (Cope et al. 2009). Thus, the tonic $GABA_A$ current of dentate gyrus granule cells is not different between GAERS and NEC, and in stargazer mice, tonic current in both dentate and cerebellar granule cells is actually reduced compared to wild-type littermates (Cope et al. 2009).

In summary, therefore, genetic models of typical absence seizures (i.e., GAERS, stargazer, and lethargic mice) show a brain region-specific gain-of-function of

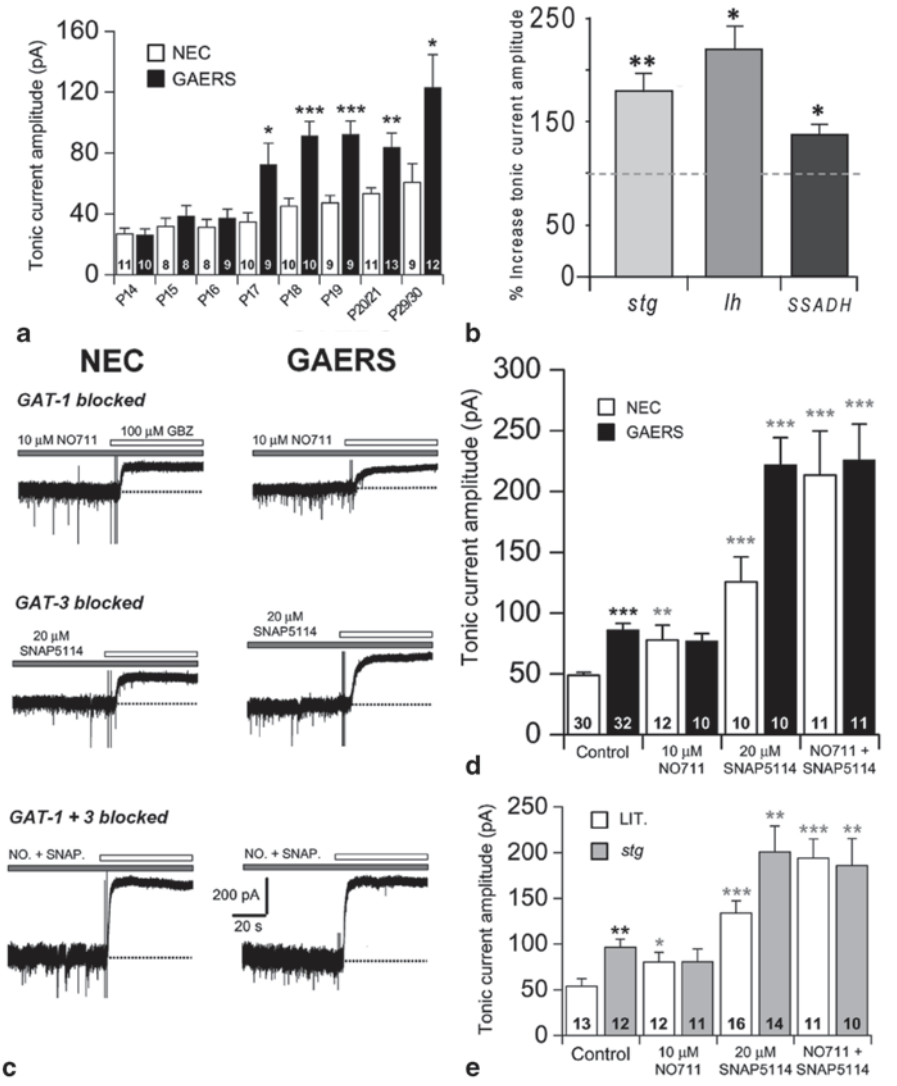

Fig. 11.1 The enhanced tonic $GABA_A$ current in TC neurons of experimental absence models results from a dysfunction in the GABA transporter GAT-1. **a** The developmental profile of enhanced thalamic tonic current observed in GAERS animals compared to NEC. At postnatal day 17 (P17) (prior to seizure onset), a significant increase in current amplitude is observed in the epileptic animal that remains elevated up to seizure onset (P30). **b** Tonic $GABA_A$ currents in VB TC neurons of stargazer (*stg*), lethargic and $SSADH^{-/-}$ mice are significantly greater than their respective non-epileptic littermates after seizure onset (values expressed as percentage of the respective wild-type littermates). **c** Block of GAT-1 using NO711 in NEC animals elevates tonic current amplitude to levels similar to those observed in GAERS animals. No further enhancement of tonic current in GAERS is observed when GAT-1 is blocked. Block of GAT-3 produces significant increases in tonic current in both NEC and GAERS animals although the increase is smaller in NEC where GAT-1 remains functional. Simultaneous block of GAT-1 and GAT-3 results in very large tonic currents in both GAERS and NEC animals, which are not significantly different

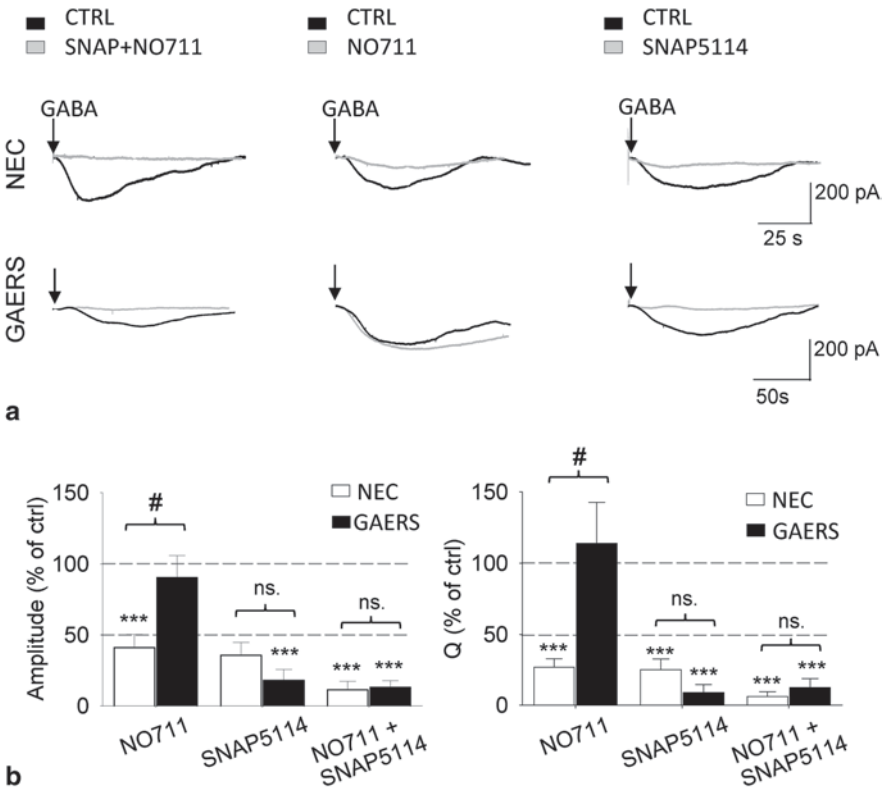

Fig. 11.2. Malfunction of GAT-1 but not GAT-3 transporter current in an experimental model of absence epilepsy. **a** GABA transporter currents in control condition (CTRL) and during application of GAT-1 (NO711, 30 μM) and GAT-3 (SNAP5114, 60 μM) blockers. Top and bottom panels show representative currents from NECs and GAERS, respectively. **b** Amplitude and charge transfer (Q) of the GABA transporter currents in NEC ($n=6$) and GAERS ($n=7$) expressed as % of those under control conditions. * indicates significance for each group against control conditions (*$p<0.05$, **$p<0.01$ or ***$p<0.005$); # ($p<0.05$) indicates significant difference between NEC and GAERS. **a**, **b** reproduced with permission from Pirttimaki et al. (2013)

eGABA$_A$Rs, which in TC neurons is due to increased extracellular GABA levels that result from a malfunction in GABA uptake by astrocytic GAT-1. Indeed, these data expand upon previous findings that demonstrated a reduction of GABA uptake by GAT-1 (Sutch et al. 1999) and increased extracellular GABA levels (Richards et al. 1995) in the VB thalamus of GAERS compared to NEC. Since no genetic abnormalities are present in the GAT-1 gene of GAERS and stargazer, and the protein expression of this GABA transporter are similar in the VB of these absence epilepsy models compared to non-epileptic animals (Cope et al. 2009), the nature of the GAT-1 malfunction remains to be determined.

from each other. **d** Graph summarising the experiments depicted in **c**. **e** Graph depicting the same series of experiments performed in stargazer mice illustrating the similar effects in both models. *$p<0.05$, **$p<0.01$, ***$p<0.001$. Number of recorded neurons for each condition is indicated in bars. **a**, **c**, **d** and **e**, reproduced with permission from Cope et al. (2009)

11.5 Enhanced Tonic $GABA_A$ Inhibition by Absence Seizures-Inducing Drugs and the Role of $GABA_B$Rs

Absence seizures can be generated in genetically "normal" animals via administration of various pharmacological agents. The best-established pharmacological model of typical absence seizures is achieved by the systemic administration of γ-hydroxybutyric acid (GHB) (Snead 1991, 1996; Banerjee et al. 1993; Crunelli et al. 2006). However, systemic administration of 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP), a selective agonist at the δ subunit-containing $eGABA_A$Rs in thalamus (Brown et al. 2006), also elicits SWDs in normal animals (Fariello and Golden 1987). In the context of the involvement of enhanced thalamic tonic $GABA_A$ inhibition in several genetic models of absence epilepsy, the pharmacological induction of seizures by THIP becomes readily explainable. This is because, as expected, THIP can potently enhance tonic $GABA_A$ currents of TC neurons in non-epileptic rats and mice (Cope et al. 2009), thus mimicking the enhanced thalamic tonic inhibition observed in genetic models.

On the other hand, the absence-inducing action of GHB, which does not bind to $GABA_A$Rs and elicits absence seizures by activation of $GABA_B$Rs (Crunelli et al. 2006), are difficult to interpret in light of the apparent necessity for enhanced $eGABA_A$R signalling during absence seizures. However, GHB does enhance the tonic $GABA_A$ current in TC neurons of normal Wistar in vitro, by an action that is mediated by $GABA_B$Rs (Fig. 11.3a and b; Cope et al. 2009). Moreover, the selective $GABA_B$ antagonist CGP55845 significantly reduces the tonic $GABA_A$ current amplitude in TC neurons of normal Wistar rats to 74% of the control values, indicating that facilitation of $eGABA_A$Rs by $GABA_B$Rs contributes approximately one quarter of the tonic $GABA_A$ current in normal rats (Cope et al. 2009). Importantly, CGP55845 also reduces the tonic current in GAERS, stargazer, and lethargic mice to about 55, 65, and 57% of control, respectively (Fig. 11.3c; Cope et al. 2009), suggesting that facilitation of $eGABA_A$R function by $GABA_B$R activation contributes up to half of the pathologically enhanced tonic current in these genetic models.

In summary, therefore, a GAT-1 malfunction in thalamic astrocytes of mouse and rat genetic models leads to an increase in ambient GABA in the sensory thalamus, which in turn elicits an enhancement in tonic $GABA_A$ inhibition through direct activation of $eGABA_A$Rs and indirect facilitation of $eGABA_A$Rs via activation of $GABA_B$Rs. Indeed, recent studies have shown that the selective $GABA_B$ agonist baclofen does increase the tonic $GABA_A$ current in TC neurons, an effect that is also present in cerebellar and dentate gyrus granule cells, but not in hippocampal CA1 neurons and layer 2/3 neocortical cells (Connelly et al. 2013). This modulation of the tonic $GABA_A$ current by $GABA_B$Rs involves a direct intracellular cross-talk between postsynaptic $eGABA_A$Rs and $GABA_B$Rs and occurs via a pathway involving $G_{i/o}$ G-proteins, adenylate cyclase and protein kinase A (for more details, see Chap. 7—Connelly et al.). Thus, the well-known ability of $GABA_B$R agonists and antagonists to aggravate/elicit and block experimental absence seizures (Lui et al. 1992; Hosford et al. 1995), respectively, does not result exclusively from the direct activation of these receptors but also from their protein kinase A-dependent modulation of $eGABA_A$Rs (Connelly et al. 2013). In this respect, it is worth men-

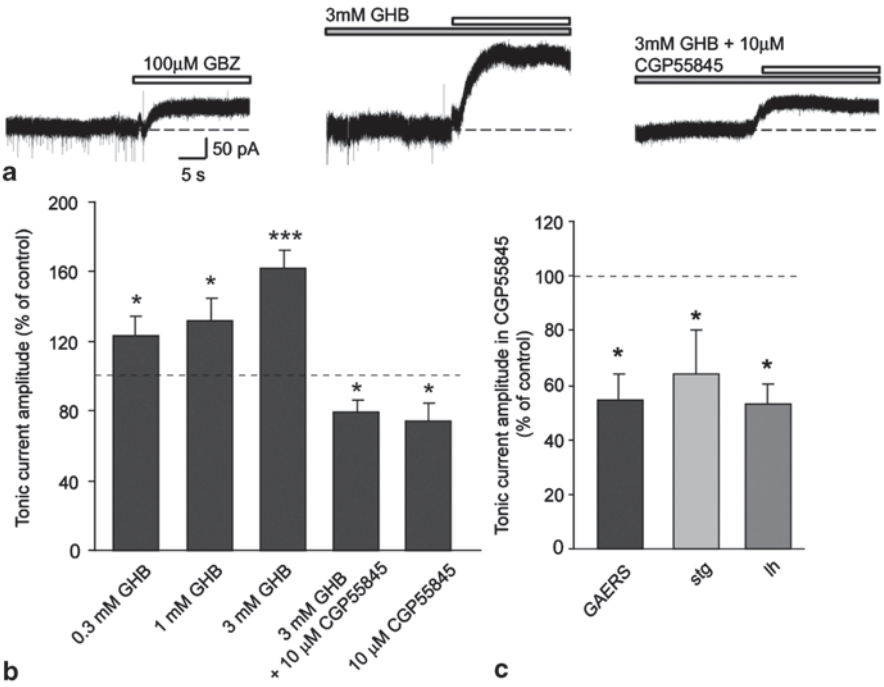

Fig. 11.3. $GABA_BR$ involvement in the tonic $GABA_A$ current of TC neurons in genetic and pharmacological models of typical absence seizures. **a** Current traces from TC neurons of normal Wistar rats showing the GHB-elicited increase in tonic $GABA_A$ current and its block by the $GABA_B$ antagonist CGP55845 (10 μM). **b** Comparison of the effects of different concentrations of GHB on tonic $GABA_A$ current amplitude in normal Wistar rats and its block by CGP55845 (10 μM). Note how CGP55845 alone decreases tonic current amplitude compared to control conditions. Values are normalized to the average tonic current in the absence of GHB or CGP55845. **c** Effect of bath application of 10 μM CGP55845 on tonic $GABA_A$ current in GAERS, stargazer (*stg*) and lethargic (*lh*) mice. Values are normalized to the average tonic current amplitude in the absence of CGP55845. B and C: $*p<0.05$, $**p<0.01$, and $***p<0.001$. **a**–**c**, reproduced with permission from Cope et al. (2009)

tioning here that the modulation of $eGABA_ARs$ by $GABA_BRs$ is not unique, since drugs that act on both 5-hydroxytryptamine (5-HT) and dopamine receptors, and are known to influence experimental absence seizures (Danober et al. 1998), are able to affect the tonic $GABA_ARs$ current of VB TC neurons (Cavaccini et al. 2012; Yagüe et al. 2013; see Chap. 7—Connelly et al.).

11.6 Enhanced Tonic $GABA_A$ Inhibition and the Generation of Typical Absence Seizures

The enhanced tonic $GABA_A$current measured in vitro in TC neurons of different pharmacological and genetic models has a significant impact on experimental absence seizures in freely moving animals. In particular, the following evidence in-

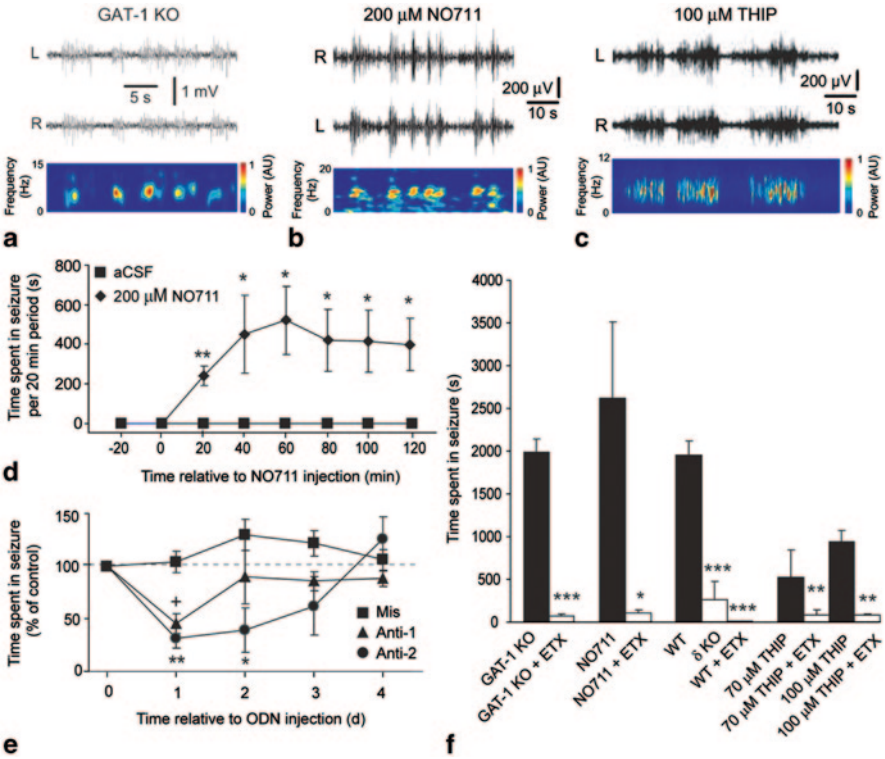

Fig. 11.4 The tonic $GABA_A$ inhibition in TC neurons is both necessary and sufficient for typical absence seizure generation. **a** Bilateral (*L* = left, *R* = right hemispheres) EEG traces from a freely moving GAT-1 knockout (*KO*) mouse showing spontaneous SWDs (spectrogram of the R trace is illustrated *below*). **b** Bilateral EEG traces from a normal Wistar rat following intrathalamic administration by reverse microdialysis of 200 µM of the selective GAT-1 blocker NO711 (spectrogram of the L trace is illustrated *below*). **c** Bilateral EEG traces from a normal Wistar rat following intrathalamic administration by reverse microdialysis of 100 µM THIP (spectrogram of the R trace is illustrated *below*). **d** Time course of the induction of SWDs by intrathalamic administration of NO711. **e** Spike-and-wave discharges are substantially reduced in GAERS 1 and 2 days following a single intrathalamic injection of a small and large dose of a δ-subunit antisense oligodeoxynucleotide (Anti-1 and Anti-2, respectively) but not of a missense oligo (*Mis*). **f** Summary histograms showing the cumulative (over 1–2 h) time spent in seizures for different transgenic and pharmacological mice and rat models and the abolition of their SWDs by systemic administration of ethosuximide (100 or 200 mg/kg/ip). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. **a**–**f**, reproduced with permission from Cope et al. (2009)

dicates that it is required for the expression of these non-convulsive seizures: (1) GAT-1 KO mice, whose TC neurons display enhanced tonic $GABA_A$ currents in vitro express ethosuximide-sensitive typical absence seizures (Fig. 11.4a and f; Cope et al. 2009); (2) the injection of the selective GAT-1 blocker NO-711 into the VB by reverse microdialysis elicits ethosuximide-sensitive typical absence seizures in non-epileptic Wistar rats (Fig. 11.4b, d, and f; Cope et al. 2009); (3) in $GABA_AR\delta$ KO mice, which have no tonic $GABA_A$ current in TC neurons, systemic admin-

istration of GHB fails to induce absence seizures (Cope et al. 2009); (4) intrathalamic injection of a δ-subunit-specific antisense oligodeoxynucleotide in GAERS strongly decreases both the tonic $GABA_A$ current and spontaneous seizures 1–2 days after injection, whereas a missense oligodeoxynucleotide does not (Fig. 11.4e; Cope et al. 2009); and (5) intrathalamic administration of THIP in non-epileptic Wistar rats elicits ethosuximide-sensitive absence seizures (Fig. 11.4c and f; Cope et al. 2009). Taken together, these data show that enhanced tonic $GABA_A$ inhibition in TC neurons is both necessary and sufficient for the generation of typical absence seizures.

Conclusions

As mentioned earlier, a decreased inhibitory drive and/or an increased excitatory drive are generally viewed as underlying epilepsy and epileptogenesis (see Chap. 10—Walker and Pavlov). Instead, the data reviewed above on the gain-of-function of $eGABA_ARs$ in both genetic and pharmacological models of absence seizures highlight a new perspective on the intricate mechanisms leading to the generation of absence seizures and provide powerful proof of concept for the development of novel anti-absence drugs with new neuronal and astrocytic targets (Errington et al. 2011b; Crunelli and Carmignoto 2013). Additional support for this view is provided by the too often neglected evidence that drugs that increase GABAergic function (such the GABA transaminase blocker vigabatrin and the GABA uptake blocker tiagabine) are known to elicit absence seizures in healthy humans and to aggravate them in absence epilepsy patients (Perucca et al. 1998; Ettinger et al. 1999). The development of new anti-absence drugs, and more importantly of anti-absence drugs that act on novel targets has been stressed by expert groups (Loscher and Schmidt 2011; Galanopoulou et al. 2012; Simonato et al. 2012), and it is strongly supported by recent meta-analyses which show that both the gold-standard anti-absence drug (i.e., ethosuximde) and the new generation of these medicines (i.e., valproate) leave about 60% of absence epilepsy patients unprotected (Glauser et al. 2010, 2013).

In particular, the work reviewed in this chapter suggests that a selective reduction of tonic $GABA_A$ inhibition in thalamic neurons by yet-unknown inverse agonists selective for $\alpha 4$-δ subunit containing $eGABA_ARs$ presents one solid route of therapeutic intervention in absence epilepsy. Another approach, of course, would be to target other neurotransmitter receptors, the activation of which indirectly reduces $eGAB_ARs$ function (see Chap. 7—Connelly et al., this book) or to develop drugs that *increase* the function of astrocytic GABA transporters, in particular GAT-1. Whether the pharma industry will be sensitive to this evidence and to any of these suggestions remain to be seen. Nevertheless, the findings summarized in this chapter clearly indicate the powerful control that $eGABA_ARs$ exerts over normal brain function and the drastic neurological consequences brought about by their gain-of-function.

Acknowledgments We would like to thank the Wellcome Trust, the MRC and the European Union for financial support. This study was also supported by Epilepsy Research UK (Grant No. P1202).

References

Avoli M, Gloor P (1981) The effects of transient functional depression of the thalamus on spindles and on bilateral synchronous epileptic discharges of feline generalized penicillin epilepsy. Epilepsia 22:443–452

Avoli M, Rogawski MA, Avanzini G (2001) Generalized epileptic disorders: an update. Epilepsia 42:445–457

Bai X, Vestal M, Berman R, Negishi M, Spann M, Vega C, Desalvo M, Novotny EJ, Constable RT, Blumenfeld H (2010) Dynamic time course of typical childhood absence seizures: EEG behaviour, and functional magnetic resonance imaging. J Neurosci 30:5884–5893

Bal T, von Krosigk M, McCormick DA (1995) Synaptic and membrane mechanisms underlying synchronized oscillations in the ferret lateral geniculate nucleus in vitro. J Physiol 483:641–663

Banerjee PK, Hirsch E, Snead III OC (1993) γ-hydroxybutyric acid induced spike and wave discharges in rats: relation to high-affinity [^{3}H]γ-hydroxybutyric acid binding sites in the thalamus and cortex. Neuroscience 56:11–21

Beenhakker MP, Huguenard JR (2009) Neurons that fire together also conspire together: is normal sleep circuitry hijacked to generate epilepsy? Neuron 62:612–632

Bessaïh T, Bourgeais L, Badiu CI, Carter DA, Toth TI, Ruano D, Lambolez B, Crunelli V, Leresche N (2006) Nucleus-specific abnormalities of GABAergic synaptic transmission in a genetic model of absence seizures. J Neurophysiol 96:3074–3081

Blumenfeld H (2005) Cellular and network mechanisms of spike-wave seizures. Epilepsia 46:21–33

Brockhaus J, Pape HC (2011) Abnormalities in GABAergic synaptic transmission of intralaminar thalamic neurons in a genetic rat model of absence epilepsy. Mol Cell Neurosci 46:444–451

Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA (2006) Pharmacological characterization of a novel cell line expressing human α4β3δ $GABA_A$ receptors. Br J Pharmacol 136:965–974

Budde T, Pape C-H, Kumar SS, Huguenard JR (2006) Thalamic, thalamocortical and corticocortical models of epilepsy with an emphasis on absence seizures. In: Pitkanen A, Schwartzkroin PA, Moshe SL (eds) Models of seizures and epilepsy. Elsevier, Amsterdam, pp 73–88

Caddick SJ, Wang C, Fletcher CF, Jenkins NA, Copeland NG, Hosford DA (1999) Excitatory but not inhibitory synaptic transmission is reduced in lethargic (*Cacnb4*^{lh}) and tottering (*Cacna1a*^{tg}) mouse thalami. J Neurophysiol 81:2066–2074

Cavaccini A, Yagüe JG, Errington AC, Crunelli V, Di Giovanni G (2012) Opposite effects of thalamic 5-HT2A and 5-HT2C receptor activation on tonic GABA-A inhibition: implications for absence epilepsy. Soc Neurosci Abstr 138:03

Coenen AM, Drinkenburg WH, Inoue M, van Luijtelaar EL (1992) Genetics models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. Epilepsy Res 12:75–86

Connelly WM, Fyson SJ, Errington AC, McCafferty CP, Cope DW, Di Giovanni G, Crunelli V (2013) $GABA_B$ receptors regulate extrasynaptic $GABA_A$ receptors. J Neurosci 33:3780–3785

Cope DW, Hughes SW, Crunelli V (2005) $GABA_A$ receptor-mediated tonic inhibition in thalamic neurons. J Neurosci 25:11553–11563

Cope DW, Di Giovanni G, Fyson SJ, Orbán G, Errington AC, Lörincz ML, Gould TM, Carter DA, Crunelli V (2009) Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy. Nat Med 15:1392–1398

Crunelli V, Carmignoto G (2013) New vistas on astroglia in convulsive and non-convulsive epilepsy highlight novel astrocytic targets for treatment. J Physiol 591:775–785

Crunelli V, Leresche N (2002) Childhood absence epilepsy: genes, channels, neurons and networks. Nat Rev Neurosci 3:371–382

Crunelli V, Emri Z, Leresche N (2006) Unravelling the brain targets of gamma-hydroxybutyric acid. Curr Opin Pharmacol 6:44–52

Crunelli V, Cope DW, Terry JR (2011) Transition to absence seizures and the role of GABA(A) receptors. Epilepsy Res 97:283–289

Crunelli V, Leresche N, Cope DW (2012) GABA-A receptor function in typical absence seizures. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV (eds), Jasper's basic mechanisms of the epilepsies. Oxford University Press, Oxford

D'Antuono M, Inaba Y, Biagini G, D'Arcangelo G, Trancredi V, Avoli M (2006) Synaptic hyperexcitability of deep layer neocortical cells in a genetic model of absence seizures. Genes Brain Behav 5:73–84

Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C (1998) Pathophysiological mechanisms of genetic absence epilepsy in the rat. Prog Neurobiol 55:27–57

De Biasi S, Vitellaro-Zuccarello L, Brecha NC (1998) Immunoreactivity for the GABA transporter-1 and GABA transporter-3 is restricted to astrocytes in the rat thalamus. A light and electron-microscopic immunolocalization. Neuroscience 83:815–828

Errington AC, Gibson KM, Crunelli V, Cope DW (2011a) Aberrant GABA(A) receptor-mediated inhibition in cortico-thalamic networks of succinic semialdehyde dehydrogenase deficient mice. PLoS ONE 6:e19021

Errington AC, Cope DW, Crunelli V (2011b) Augmentation of tonic GABA(A) inhibition in absence epilepsy: therapeutic value of inverse agonists at extrasynaptic GABA(A) receptors. Adv Pharmacol Sci 2011:790590

Ettinger AB, Bernal OG, Andriola MR, Bagchi S, Flores P, Just C, Pitocco C, Rooney T, Tuominen J, Devinsky O (1999) Two cases of nonconvulsive status epilepticus in association with tiagabine therapy. Epilepsia 40:1159–1162

Fariello RG, Golden GT (1987) The THIP-induced model of bilateral synchronous spike and wave in rodents. Neuropharmacology 26:161–165

Fisher RS, Prince DA (1977) Spike-wave rhythms in cat cortex induced by parenteral penicillin. I. Electroencephalographic features. Electroenceph Clin Neurophysiol 42:608–624

Fletcher CF, Frankel WN (1999) Ataxic mouse mutants and molecular mechanisms of absence epilepsy. Hum Mol Genet 8:1907–1912

Galanopoulou AS, Buckmaster PS, Staley KJ, Moshe SL, Perucca E, Engel J Jr, Loscher W, Noebels JL, Pitkaanen A, Stables J, White HS, O'Brien TJ, Simonato M (2012) Identification of new epilepsy treatments: issues in preclinical methodology. Epilepsia 53:571–582

Giaretta D, Kostopoulos G, Gloor P, Avoli M (1985) Intracortical inhibitory mechanisms are preserved in feline generalized penicillin epilepsy. Neurosci Lett 59:203–208

Glauser TA, Cnaan A, Shinnar S, Hirtz DG, Dlugos D, Masur D, Clark PO, Capparelli EV, Adamson PC, Childhood Absence Epilepsy Study Group (2010) Ethosuximide, valproic acid, and lamotrigine in childhood absence epilepsy. N Engl J Med 362:790–799

Glauser TA, Cnaan A, Shinnar S, Hirtz DG, Dlugos D, Masur D, Clark PO, Adamson PC, Childhood Absence Epilepsy Study Team (2013) Ethosuximide, valproic acid, and lamotrigine in childhood absence epilepsy: initial monotherapy outcomes at 12 months. Epilepsia 54:141–155

Gloor P (1968) Generalized cortico-reticular epilepsies. Some considerations on the pathophysiology of generalized bilaterally synchronous spike and wave discharges. Epilepsia 9:249–263

Gloor P (1969) Epileptogenic action of penicillin. Ann N Y Acad Sci 166:350–360

Gloor P (1978) Generalized epilepsy with bilateral synchronous spike and wave discharge. New findings concerning its physiological mechanisms. Electroen Clin Neuro Suppl 1978:245–249

Gotman J, Grova C, Bagshaw A, Kobayashi E, Aghakhani Y, Dubeau F (2005) Generalized epileptic discharges show thalamocortical activation and suspension of the default state of the brain. Proc Natl Acad Sci U S A 102:15236–15240

Hamandi K, Salek-Haddadi A, Laufs H, Liston A, Friston K, Fish DR, Duncan JS, Lemieux L (2006) EEG-fMRI of idiopathic an secondarily generalized epilepsies. Neuroimage 31:1700–1710

Hogema BM, Gupta M, Senephansiri H, Burlingame TG, Taylor M (2001) Pharmacologic rescue of lethal seizures in mice deficient in succinate semialdehyde dehydrogenase. Nat Genet 29:212–216

Holmes MD, Brown M, Tucker DM (2004) Are "generalized" seizures truly generalized? Evidence of localized mesial frontal and frontopolar discharges in absence. Epilepsia 45:1568–1579

Hosford DA, Wang Y (1997) Utility of the lethargic (*lh*/*lh*) mouse model of absence seizures in predicting the effects of lamotrigine, vigabatrin, tiagabine, gabapentin, and topiramate against human absence seizures. Epilepsia 38:408–414

Hosford DA, Lin FH, Kraemer DL, Cao Z, Wang Y, Wilson JT Jr (1995) Neural network of structures in which $GABA_B$ receptors regulate absence seizures in the lethargic (lh/lh) mouse model. J Neurosci 15:7367–7376

Huntsman MM, Porcello DM, Homanics GE, DeLorey TM, Huguenard JR (1999) Reciprocal inhibitory connections and network synchrony in the mammalian thalamus. Science 283:541–543

Kananura C, Haug K, Sander T, Runge U, Gu W, Hallmann K, Rebstock J, Heils A, Steinlein OK (2002) A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. Arch Neurol 59:1137–1141

Kostopoulos G, Gloor P, Pellegrini A, Gotman J (1981a) A study of the transition from spindles to spike and wave discharge in feline generalized penicillin epilepsy: microphysiological features. Exp Neurol 73:55–77

Kostopoulos G, Gloor P, Pellegrini A, Siatitsas I (1981b) A study of the transition from spindles to spike and wave discharge in feline generalized penicillin epilepsy: EEG features. Exp Neurol 73:43–54

Lachance-Touchette P, Brown P, Meloche C, Kinirons P, Lapointe L, Lacasse H, Lortie A, Carmant L, Bedford F, Bowie D, Cossette P (2011) Novel alpha1 and gamma2 GABA(A) receptor subunit mutations in families with idiopathic generalized epilepsy. Eur J Neurosci 34:237–249

Leresche N, Lambert RC, Errington AC, Crunelli V (2012) From sleep spindles of natural sleep to spike and wave discharges of typical absence seizures: is the hypothesis still valid? Pflugers Arch 463:201–212

Loscher W, Schmidt D (2011) Modern antiepileptic drug development has failed to deliver: ways out of the current dilema. Epilepsia 52:657–678

Luhmann HJ, Mittmann T, van Luijtelaar G, Heinemann U (1995) Impairment of introcortical GABAergic inhibition in a rat model of absence epilepsy. Epilepsy Res 22:43–51

Lui Z, Vergnes M, Depaulis A, Marescaux C (1992) Involvement of intrathalamic $GABA_B$ neurotransmission in the control of absence seizures in the rat. Neuroscience 48:87–93

MacDonald RL, Kang J-Q, Gallagher MJ (2010) Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. J Physiol 588:1861–1869

McCormick DA, Contreras D (2001) On the cellular and network bases of epileptic seizures. Annu Rev Physiol 63:815–846

Maljevic KK, Cobilanschi J, Tilgen N, Beyer S, Weber YG, Schlesinger F, Ursu D, Melzer W, Cossette P, Bufler J, Lerche H, Heils A (2006) A mutation in the GABA(A) receptor alpha(1)-subunit is associated with absence epilepsy. Ann Neurol 59:983–987

Manning JP, Richards DA, Leresche N, Crunelli V, Bowery NG (2004) Cortical-area specific block of genetically determined absence seizures by ethosuximide. Neuroscience 123:5–9

Meeren HKM, Pijn JPM, Van Liujtelaar ELJM, Coenen AML, da Silva FHL (2002) Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J Neurosci 22:1480–1495

Panyiotopoulos CP (1997) Absences epilepsies. In: Engel JJ, Pedley TA (eds) Epilepy: a comprehensive textbook. Lippicott-Raven, Philadelphia, pp 2327–2346

Perucca E, Gram L, Avanzini G, Dulac O (1998) Antiepileptic drugs as a cause of worsening seizures. Epilepsia 39:5–17

Pirttimaki T, Parri HR, Crunelli V (2013) Astrocytic GABA transporter GAT-1 dysfunction in experimental absence seizures. J Physiol 591:823–833

Polack PO, Guillemain I, Hu E, Deransart C, Depaulis A, Charpier S (2007) Deep layer somotosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. J Neurosci 27:6590–6599

Pow DV, Sullivan RK, Williams SM, Scott HL, Dodd PR, Finkelstein D (2005) Differential expression of the GABA transporters GAT-1 and GAT-3 in brains of rats, cats, monkeys and humans. Cell Tissue Res 320:379–392

Prince DA, Farrell D (1969) "Centrencephalic" spike-wave discharges following parental penicillin injection in the cat. Neurology 19:309–310

Richards DA, Lemos T, Whitton PS, Bowery NG (1995) Extracellular GABA in the ventrolateral thalamus of rats exhibiting spontaneous absence epilepsy: a microdialysis study. J Neurochem 65:1674–1680

Schofield CM, Kleiman-einer M, Rudolph U, Huguenard JR (2009) A gain in $GABA_A$ receptor synaptic strength in thalamus reduces oscillatory activity and absence seizures. Proc Natl Acad Sci U S A 106:7630–7635

Simonato M, Loscher W, Cole AJ, Dudek E, Engel J Jr, Kaminski RM, Loeb JA, Scharfman H, Staley KJ, Velíšek L, Klitgaard H (2012) Finding a better drug for epilepsy: preclinical screening strategies and experimental trial design. Epilepsia 53:1860–1867

Snead OC (1991) The gamma-hydroxybutyrate model of absence seizures: correlation of regional brain levels of gamma-hydroxybutyric acid and gamma-butyrolactone with spike wave discharges. Neuropharmacology 30:161–167

Snead OC (1996) Antiabsence seizure activity of specific $GABA_B$ and gamma-Hydroxybutyric acid receptor antagonist. Pharmacol Biochem Behav 53:73–79

Staak R, Pape HC (2001) Contribution of GABA(A) and GABA(B) receptors to thalamic neuronal activity during spontaneous absence seizures in rats. J Neurosci 21:1378–1384

Steriade M, Contreras D (1995) Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. J Neurosci 15:623–642

Sutch RJ, Davies CC, Bowery NG (1999) GABA release and uptake measured in crude synaptosomes from Genetic Absence Epilepsy Rats from Strasbourg (GAERS). Neurochem Int 34:415–425

Szaflarski JP, Difrancesco M, Hirschauer T, Banks C, Privitera MD, Gotman J, Holland SK (2010) Cortical and subcortical contributions to absence seizure onset examined with EEG/fMRI. Epilepsy Behav 18:404–413 PMID:20580319

Tan HO, Reid CA, Single FN, Davies PJ, Chiu C, Murphy S, Clarke AL, Dibbens L, Krestel H, Mulley JC, Jones MV, Seeburg PH, Sakmann B, Berkovic SF, Sprengel R, Petrou S (2007) Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy. Proc Natl Acad Sci U S A 104:17536–17541

Tan HO, Reid CA, Chiu C, Jones MV, Petrou S (2008) Increased thalamic inhibition in the absence seizure prone DBA/2J mouse. Epilepsia 49:921–925

Tanaka M, Olsen RW, Medina MT, Schwartz E, Alonso ME, Duron RM, Castro-Ortega R, Martinez-Juarez IE, Pascual-Castroviejo I, Machado-Salas J, Silva R, Bailey JN, Bai D, Ochoa A, Jara-Prado A, Pineda G, MacDonald RL, Delgado-Escueta AV (2008) Hyperglycosylation and reduced GABA currents of mutated GABRB3 polypeptide in remitting childhood absence epilepsy. Am J Hum Genet 82:1249–1261

Tyvaert L, Chassagnon S, Sadikot A, LeVan P, Dubeau F, Gotman J (2009) Thalamic nuclei activity in idiopathic generalized epilepsy: an EEG-fMRI study. Neurology 73:2018–2022

Vaudano AE, Laufs H, Kiebel SJ, Carmichael DW, Hamandi K, Guye M, Thornton R, Rodionov R, Friston KJ, Duncan JS, Lemieux L (2009) Causal hierarchy within the thalamo-cortical network in spike and wave discharges. PloS ONE 4:e6475

von Krosigk M, Bal T, McCormick DA (1993) Cellular mechanisms of a synchronized oscillation in the thalamus. Science 261:361–364

Yagüe JG, Cavaccini A, Errington AC, Crunelli V, Di Giovanni G (2013) Dopaminergic modulation of tonic but not phasic $GABA_A$-receptor-mediated current in the ventrobasal thalamus of Wistar and GAERS rats. Exp Neurol 247:1–7

Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF (2001) Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 28:49–52

Westmijse I, Ossenblok P, Gunning B, van Luijtelaar G (2009) Onset and propagation of spike and slow wave discharges in human absence epilepsy: a MEG study. Epilepsia 50:2538–2548

Williams D (1953) A study of thalamic and cortical rhythms in petit mal. Brain J Neurol 76:50–69

Chapter 12
GABAergic Control of the Hypothalamic–Pituitary–Adrenal (HPA) Axis: Role of Extrasynaptic $GABA_A$ Receptors

Jamie Maguire

Abstract The body's physiological response to stress is mediated by the hypothalamic–pituitary–adrenal (HPA) axis, the activity of which is tightly regulated by GABAergic inhibition. Parvocellular neurosecretory neurons located in the paraventricular nucleus (PVN) of the hypothalamus which release corticotropin-releasing hormone (CRH) govern the output of the HPA axis. CRH neurons are innervated by a high density of GABAergic terminals and are regulated by robust GABAergic inhibition. Furthermore, numerous other brain regions, including the hippocampus, prefrontal cortex, bed nucleus of the stria terminalis (BNST), and amygdala, exert control over the HPA axis via indirect GABAergic connections onto CRH neurons. CRH neurons express numerous γ-aminobutyric-acid type-A receptor ($GABA_AR$) subunits and are regulated by both phasic and tonic GABAergic inhibition, mediated by synaptic and extrasynaptic $GABA_ARs$, respectively. The GABAergic control of the HPA axis is highly plastic and is altered by both acute and chronic stress. Here, I review the role of extrasynaptic $GABA_ARs$ in the regulation of the HPA axis and the physiological response to stress.

Keywords GABA · Tonic inhibition · Extrasynaptic receptors · Stress · HPA axis · Neurosteroids · Corticotropin-releasing hormone (CRH)

This chapter will review the evidence to date on the role of extrasynaptic $GABA_ARs$ in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis. There is a paucity of data on the role of these receptors in regulating corticotropin-releasing hormone (CRH) neurons which in turn govern the HPA axis. Therefore, this chapter will also consider the role of extrasynaptic $GABA_ARs$ in other brain regions which exert control HPA axis function, with a focus on the hippocampus, prefrontal cortex, bed nucleus of the stria terminalis (BNST), and amygdala.

J. Maguire (✉)
Department of Neuroscience, Tufts University School of Medicine,
136 Harrison Ave., SC205, Boston, MA 02111, USA
e-mail: Jamie.Maguire@tufts.edu

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_12

12.1 HPA Axis

Stress activates parvocellular neurosecretory neurons in the paraventricular nucleus (PVN), which govern the activity of the HPA axis. Stress increases the activity of these neurons resulting in the release of corticotropin-releasing hormone (CRH) into the pituitary portal system. CRH then acts in the pituitary to signal the release of adrenocorticotropic hormone (ACTH), which then triggers the production and release of glucocorticoids from the adrenal cortex: cortisol in humans and corticosterone in rodents. Peripherally secreted glucocorticoids exert actions on target tissues in the periphery as well as in the brain, which largely involve rapid actions of glucocorticoids on mineralocorticoid receptors (MRs) and longer-lasting effects mediated by glucocorticoid receptors (GRs; for review, see Herman et al. 2003; Mody and Maguire 2011; Larsen et al. 2003; Ulrich-Lai and Herman 2009). In fact, one of the major effects of stress-induced glucocorticoid production is negative feedback onto the HPA axis itself (for review, see Buckingham 1979; de Kloet et al. 1998; Dallman et al. 1987).

The negative feedback of glucocorticoids on HPA axis function occurs on both rapid and delayed time scales. The negative feedback action of glucocorticoids has been a tenet of endocrine physiology for decades signaling (for review, see Watts 2005). It is widely accepted that the delayed inhibition of HPA axis function is mediated by the effects of glucocorticoids on gene transcription, resulting in the decreased expression, production, and secretion of the neuropeptides involved in stress signaling (for review, see Watts 2005). However, the rapid regulatory actions of glucocorticoids on the regulation of the HPA axis are not as well understood. The rapid actions of glucocorticoids are thought to be independent of gene transcription and have been proposed to be mediated by membrane glucocorticoid receptors (Tasker et al. 2005). Interestingly, the rapid negative feedback onto the HPA axis involves modulation of the synaptic inputs onto CRH neurons in the PVN (for review, see Hill and Tasker 2012) and, recently, there has been a great deal of interest in the synaptic control of the HPA axis (Levy and Tasker 2012; Wamsteeker and Bains 2010). In fact, although numerous brain regions play a role in the regulation of the HPA axis by impinging on CRH neurons in the hypothalamus, the activity of CRH neurons is largely controlled by GABAergic inhibition (for review, see Herman et al. 2004; Decavel and van den Pol 1990).

12.2 GABAergic Control of HPA Axis

The essential role of GABAergic inhibition in the regulation of the HPA axis is evident from the fact that 50 % of the synapses in the hypothalamus are GABAergic (Decavel and van den Pol 1990, 1992; Miklos and Kovacs 2002), and it has been estimated that there are 20×10^6 GABAergic synaptic contacts per cubic millimeter in the parvocellular division of the PVN (Miklos and Kovacs 2002). Furthermore, 79 % of all GABAergic boutons in the parvocellular subdivision of the

Table 12.1 Indirect GABAergic Projections to the PVN

Indirect connections to PVN: Role of GABAergic interneurons			
Brain Region	Primary Effect	Interneuron Relay	Primary Neurotransmitter
Prefrontal Cortex	Inhibitory	peri-PVN, BNST, hypothalamic regions	GABA
Hippocampus	Inhibitory	peri-PVN, BNST, hypothalamic regions	GABA
Amygdala (CeA)	Excitatory	BNST	GABA
Amygdala (MeA)	Excitatory	peri-PVN, BNST, hypothalamic regions	GABA
Thalamus	Excitatory/Inhibitory	prefrontal cortex, amygdala, subiculum	GABA
Hypothalamus	Inhibitory	peri-PVN, hypothalamic regions	GABA
lateral septum	Inhibitory	peri-PVN, hypothalamic regions	GABA

The prefrontal cortex, hippocampus, amygdale, thalamus, lateral septum, and other hypothalamic regions exert control over HPA axis function via GABAergic interneurons residing in the peri-PVN region, BNST, and other hypothalamic regions.

hypothalamus terminate on CRH neurons (Miklos and Kovacs 2002). GABAergic terminals have been identified on the dendritic shafts, spines, and soma of CRH neurons in the dorsal medial parvocellular division of the hypothalamus (Miklos and Kovacs 2002). The high density of GABAergic innervation onto CRH neurons suggest, at least at a morphological level, an important role for GABA in the regulation of CRH neurons and, thus, the HPA axis.

GABAergic inputs onto CRH neurons largely originate from the anterior hypothalamic area, dorsomedial hypothalamic nucleus, the medial preoptic area, lateral hypothalamic area, from multiple nuclei within the BNST, and a local interneuron population surrounding the PVN (peri-PVN; Boudaba et al. 1996; Roland and Sawchenko 1993; Tasker and Dudek 1993; for review, see Cullinan et al. 2008; Herman et al. 2004). In addition to the direct GABAergic connections from these interneuronal populations, CRH neurons are also indirectly regulated by numerous other brain regions including the hippocampus, prefrontal cortex, amygdala, thalamus, and other hypothalamic regions which are summarized in Table 12.1 (for review, see Cullinan et al. 2008; Herman et al. 2004). However, there are minimal direct connections from these brain regions onto CRH neurons. These brain regions primarily exert their control over HPA axis activity via GABAergic interneuron connections (for review, see Cullinan et al. 2008; Table 12.1).

12.3 $GABA_AR$ Subunit Expression and HPA Axis Regulation

As outlined in the section above, $GABA_ARs$ play an important role in the regulation of the HPA axis. $GABA_ARs$ regulate neurons directly involved in HPA axis function as well as in brain regions which exert indirect control over the HPA axis. Given the importance of $GABA_AR$ subunit expression in the kinetics, pharmacology, and sub-

cellular localization of these receptors, it is informative to identify which $GABA_AR$ subtypes play a role in the regulation of the HPA axis.

12.3.1 $GABA_AR$ Subunit Expression in CRH Neurons

Despite the clear evidence of the role for GABA in the regulation of parvocellular neurosecretory neurons controlling the HPA axis, very few studies have investigated which $GABA_AR$ subunits are expressed in this region. This lack of knowledge is largely due to the fact that the PVN is a heterogeneous nucleus and it is difficult to determine which $GABA_AR$ subunits are expressed in the different cell types in this region. However, a few careful studies have elucidated some of the $GABA_AR$ subunits participating in the regulation of the HPA axis. Diffuse labeling of the majority of $GABA_AR$ receptor subunits was found in the hypothalamus, except for the α6 subunit (Pirker et al. 2000; Table 12.2). Densely labeled processes expressing the $GABA_AR$ α1, α2, β1–3, and γ2 subunits were observed in the hypothalamus (Pirker et al. 2000; Table 12.2). Interestingly, cell bodies expressing the extrasynaptic $GABA_AR$ α5 and δ subunits were also observed in the majority of hypothalamic regions (Pirker et al. 2000; Table 12.2). The significance of the diffuse labeling of $GABA_AR$ subunits in the hypothalamus is difficult to interpret based solely on immunostaining. However, the expression of the extrasynaptic $GABA_ARs$ in the cell bodies of hypothalamic neurons suggests that these receptors may play a significant role in the regulation of these neurons.

Within the PVN of the hypothalamus, transcript expression of the $GABA_AR$ α1, β1, β3, and γ2 subunits have been identified (Fenelon and Herbison 1995; Table 12.2). At the protein level, the expression of the majority of $GABA_AR$ subtypes were observed, except α4, α6, and γ1 (Table 12.2). Table 12.2 summarizes $GABA_AR$ subunit expression in the PVN. Diffuse labeling of the α1 and γ2 subunits was observed in the processes of neurons in the PVN (Pirker et al. 2000; Table 12.2). Cell bodies in this region express the α5, β1, β3, γ3, and δ subunits (Cullinan 2000; Pirker et al. 2000; Sarkar et al. 2011; Table 12.2), suggesting a role for extrasynaptic $GABA_ARs$ in this region. The expression of the $GABA_AR$ δ subunit in the PVN was further confirmed in this region by comparing expression to $GABA_AR$ δ subunit knockout mice (*Gabrd*$^{-/-}$ mice; Sarkar et al. 2011; Table 12.2). It is interesting to note that the α4 subunit co-assembles with the δ subunit in other brain regions (Sur et al. 1999; Jones et al. 1997), but given the lack of α4 expression in the PVN suggests that δ may partner with another α subunit in this region.

12.3.2 $GABA_AR$ Subunit Expression in Brain Regions Controlling the HPA Axis

In addition to the direct role of extrasynaptic $GABA_ARs$ in the regulation of the HPA axis at the level of CRH neurons, extrasynaptic $GABA_ARs$ also play an important role in the regulation of neurons in brain regions known to control HPA

Table 12.2 $GABA_A$ receptor subunit expression in the PVN

$GABA_AR$ subunit	Expression in PVN	References
α1	**, mRNA	Pirker, 2000; Cullinan, 2000; Fenelon, 1996
α2	**, mRNA	Pirker, 2000; Cullinan, 2000; Fenelon, 1996
α3	-	Pirker, 2000
α4	-	Pirker, 2000
α5	*	Pirker, 2000
α6		
β1	**, mRNA	Pirker, 2000; Cullinan, 2000
β2	*, mRNA	Pirker, 2000; Cullinan, 2000; Fenelon, 1996
β3	*, mRNA	Pirker, 2000; Cullinan, 2000
γ1	-	Pirker, 2000
γ2	**	Pirker, 2000; Fenelon, 1996
γ3	**	Pirker, 2000
δ	**	Pirker, 2000

Despite the limited number of studies exploring $GABA_AR$ expression in the hypothalamus, numerous $GABA_AR$ subunits have been identified in the PVN either at the protein level and/or at the transcript level. Extrasynaptic $GABA_AR$ α5 and δ subunits have been identified in parvocellular neurosecretory neurons in the PVN (red box).
*=low/diffuse expression, **=high expression, –=no expression

axis activity, including the hippocampus, prefrontal cortex, and amygdala (for review, see Herman et al. 2005). Extrasynaptic $GABA_A$ δ subunit-containing receptors are highly expressed in these brain regions (Fig. 12.1) and likely contribute to the regulation of the HPA axis via indirect actions on parvocellular neurosecretory neurons in the PVN.

12.3.2.1 Hippocampus

The hippocampus plays a role in the regulation of the HPA axis largely via outflow from the ventral subiculum. Indirect connections from the ventral subiculum by way of the BNST, the medial preoptic area, the dorsomedial hypothalamus, and other hypothalamic nuclei influence HPA axis function (for review, see Jankord and Herman 2008). The hippocampus primarily exerts inhibitory effects on the activity of the HPA axis (Jacobson and Sapolsky 1991; Herman and Cullinan 1997; for review, see Herman et al. 2005). Indeed, the hippocampus expresses a high density of both MRs and GRs (Reul and de Kloet 1985, 1986; Herman et al. 1989), demonstrating the importance of this region in regulating stress reactivity (for re-

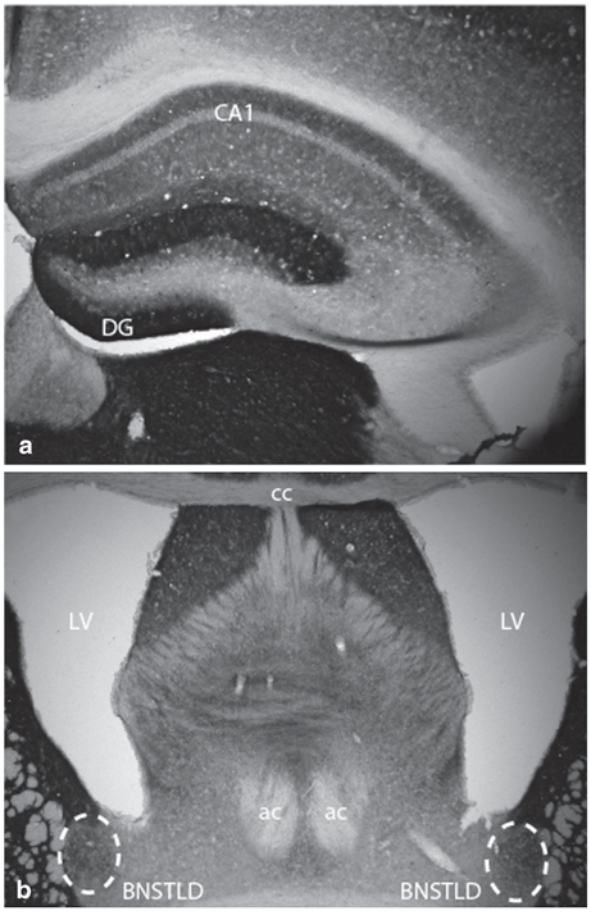

Fig. 12.1 $GABA_AR$ δ subunit expression in the dentate gyrus and *BNST*. Representative sections demonstrating immunoreactivity for the $GABA_AR$ δ subunit in the hippocampus (**a**) and the lateral subdivision of the *BNST* (**b**). Note the intense expression of the $GABA_AR$ δ subunit in the dentate gyrus molecular layer (*DG*) and moderate expression in the lateral subdivision of the *BNST* (*circled*, *BNSTLD*). *CA1* CA1 pyramidal cell layer; *DG* dentate gyrus; *cc* corpus callosum; *LV* lateral ventricle; *ac* anterior commissure; *BNSTLD* lateral subdivision of the bed nucleus of the stria terminalis.

view, see Herman et al. 1989; Jacobson 2005). The hippocampus also expresses a high density of extrasynaptic $GABA_AR$ receptors (Sperk et al. 1997) with the majority of $GABA_AR$ subunits abundantly expressed, with the exception of the α6 and γ1 subunit (Pirker et al. 2000). The $GABA_AR$ α1, α2, α4, α5, β1–3, and γ2 subunits are all detected in the hippocampus (Pirker et al. 2000). Extrasynaptic α5-containing $GABA_ARs$ are highly expressed in the CA1 region of the hippocampus and play a critical role in regulating the activity of these neurons (Glykys et al. 2008). Extrasynaptic $GABA_AR$ δ subunit-containing receptors are highly expressed in dentate gyrus granule cells (DGGCs; Sperk et al. 1997) and play a critical role in the regulation of these neurons (Stell et al. 2003; Fig. 12.1). Although it is clear that extrasynaptic $GABA_ARs$ are expressed in the hippocampus, the role of these receptors in the hippocampal regulation of the HPA axis remains unclear. Interestingly, there are changes in the expression of extrasynaptic $GABA_ARs$ in the hippocampus

following stress (Maguire and Mody 2007), which could potentially impact the regulation of the stress response.

Hippocampal neurogenesis has also been implicated in the regulation of the HPA axis (Anacker and Pariante 2012; Schloesser et al. 2009). A genetic mouse model with deficiencies in adult hippocampal neurogenesis exhibits a dysregulation of the HPA axis (Schloesser et al. 2009). Furthermore, stress and glucocorticoids inhibit adult neurogenesis (Gould et al. 1997; Tanapat et al. 1998) via actions on glucocorticoid receptors (Anacker et al. 2011a), demonstrating a link between stress and neurogenesis. Interestingly, extrasynaptic $GABA_A$Rs containing the α4, a known parter of the $GABA_A$R δ subunit, have been implicated in adult hippocampal neurogenesis (Duveau et al. 2011). However, $GABA_A$R δ subunit-containing receptors do not appear to play a role in regulating hippocampal neurogenesis (Duveau et al. 2011). Consistent with the role of the GABAergic control of neurogenesis on HPA axis function, loss of the $GABA_A$ receptor γ2 subunit in the adult forebrain results in reduced hippocampal neurogenesis and hyperexcitability of the HPA axis (Earnheart et al. 2007). Therefore, these findings suggest that neurogenesis may also play a role in the negative feedback mechanism of HPA axis regulation and highlight the importance of GABAergic inhibition in the hippocampus on HPA axis regulation.

12.3.2.2 Prefrontal Cortex

The prefrontal cortex exerts complex regulatory control over the HPA axis via region-specific connections to the BNST, amygdala, nucleus of the solitary tract, peri-PVN, and other hypothalamic regions (Radley et al. 2009; for review, see Jankord and Herman 2008), all regions which control HPA axis function. Extrasynaptic δ subunit-containing $GABA_A$Rs are expressed in the prefrontal cortex (Maldonado-Aviles et al. 2009). The α4 subunit, which often partners extrasynaptically with the δ subunit, is also expressed in the prefrontal cortex (Maldonado-Aviles et al. 2009). Thus, extrasynaptic $GABA_A$Rs likely regulate the activity of neurons in the prefrontal cortex which exerts indirect control over the HPA axis. Interestingly, neurogliaform cells in the neocortex have been demonstrated to bulk release GABA via a nonsynaptic mechanism (Olah et al. 2007, 2009; Tamas et al. 2003; see Chap. 10), which likely involves actions on extrasynaptic $GABA_A$Rs (Olah et al. 2009). These data suggest that specialized subsets of interneurons in the cortex target extrasynaptic $GABA_A$Rs which likely regulate the activity of these neurons. Furthermore, there is a high density of glucocorticoid receptors in the prefrontal cortex, suggesting that the prefrontal cortex may be a site of negative feedback regulation of the HPA axis (for review, see Cullinan et al. 1993; Herman et al. 2003, 2005). Clearly there is a role for extrasynaptic $GABA_A$Rs in the regulation of neurons in the prefrontal cortex; however, the impact on HPA axis function is complicated by the numerous indirect connections from this region onto parvocellular neurosecretory neurons in the PVN.

12.3.2.3 BNST

Unlike the limbic and cortical regions which exert indirect regulatory control over the HPA axis, the BNST relays limbic information directly to the PVN (Choi et al. 2007). Interestingly, there appear to be subdomains within the BNST which uniquely process stressful stimuli and limbic information to impact HPA axis function (Choi et al. 2007). Furthermore, both excitatory and inhibitory information flows through the BNST to influence the HPA axis. The neurons within the BNST express an abundance of extrasynaptic $GABA_AR$ subunits, including the α4, α5, and δ subunits (Pirker et al. 2000) (Fig. 12.1), suggesting that extrasynaptic $GABA_ARs$ play a role in the regulation of these neurons and, thereby, indirectly influence HPA axis function. Consistent with the role of extrasynaptic $GABA_ARs$, neurons in the BNST have been demonstrated to be under tonic GABAergic inhibition (Egli and Winder 2003), which is likely mediated by extrasynaptic $GABA_ARs$ although the exact subtypes have not been identified. Due to the limited number of studies investigating the role of extrasynaptic $GABA_ARs$ in the BNST, the role of these receptors in the regulation of neurons in the BnST and the indirect impact on HPA axis function remains largely unclear.

12.3.2.4 Amygdala

The amygdala has limited direct projections to the PVN (for review, see Jankord and Herman 2008) and, therefore, the amygdala primarily influences HPA axis function via indirect connections via the BNST, hippocampus, preoptic area, the nucleus of the solitary tract (NTS), and other hypothalamic regions (for review, see Jankord and Herman 2008). Overall, the amygdala is primarily thought to activate the HPA axis. Neurons in the amygdala express a large number of $GABA_AR$ subunits, including the α1, α2, α3, α4, α5, β1–3, γ2, γ3, and δ subunits (Pirker et al. 2000). Cell bodies and dendrites in the amygdala express robust immunoreactivity for the α1, β2, and γ2 subunits (Pirker et al. 2000), suggesting that phasic GABAergic inhibition plays a role in the regulation of these neurons. In addition, there is expression of the extrasynaptic α5 and δ subunits in this region suggesting that these neurons may also be regulated by tonic GABAergic inhibition. However, these extrasynaptic $GABA_AR$ subunits are not highly expressed in the amygdala (Fritschy and Mohler 1995). These neurons do, however, exhibit robust tonic GABAergic inhibition (Marowsky et al. 2012), which has been proposed to be mediated by extrasynaptic receptors containing the α3 subunit (Marowsky et al. 2012). The regulation of both basolateral (BLA) nucleus and lateral (LA) nucleus principal neurons by extrasynaptic $GABA_AR$-mediated tonic inhibition suggests that this inhibitory control may impact the role of the amygdala on other systems, including the HPA axis. These data suggest that extrasynaptic $GABA_ARs$ may exert control over HPA axis function via indirect connections from the amygdala. In addition, the amygdala expresses a high density of glucocorticoid receptors (Arriza et al. 1988; Aronsson et al. 1988; Ahima and Harlan 1990) as well as a lower density

of MRs (Arriza et al. 1988), implicating the amygdala in the negative feedback regulation of the HPA axis.

12.4 GABAergic Control of CRH Neurons

CRH neurons are regulated by GABAergic inhibition (see Sect. 12.2 GABAergic control of HPA axis) and numerous $GABA_AR$ subunits have been identified in the PVN (see Sect. 12.3.1 $GABA_AR$ subunit expression in CRH neurons); however, very few studies have investigated the synaptic GABAergic regulation of CRH neurons (for review, see Levy and Tasker 2012). The limited number of studies investigating the synaptic GABAergic regulation of CRH neurons is due, at least in part, to the difficulty in identifying this subset of neurons within the PVN (for review, see Levy and Tasker 2012). Parvocellular neurosecretory neurons have been identified by location, size, morphology, and electrophysiological properties (Luther et al. 2002; for review, see Levy and Tasker 2012). However, these approaches are insufficient to definitively identify CRH neurons from other parvocellular neurosecretory neurons in this region. For this reason, the generation of mouse models which express green fluorescent protein (GFP) specifically in CRH neurons has been extremely useful (Alon et al. 2009; Sarkar et al. 2011). Due to the limited number of studies specifically assessing the regulation of CRH neurons, this section will review the data on GABAergic control of parvocellular neurosecretory neurons as well.

Neurons in the PVN are regulated by both tonic and phasic GABAergic inhibition (Park et al. 2007; Sarkar et al. 2011). Using CRH-GFP reporter mice, it has been confirmed that CRH neurons are regulated by both tonic and phasic GABAergic inhibition (Sarkar et al. 2011). These neurons are sensitive to changes in extracellular levels of GABA (Park et al. 2009), likely due to the actions on extrasynaptic $GABA_AR$s. The impact of extracellular GABA levels on HPA axis function is evident in the decreased circulating corticosterone levels in mice deficient in the GABA transporter, GAT1 (*GAT1*$^{-/-}$ mice; Liu et al. 2007). The tonic GABAergic inhibition in CRH neurons is mediated by extrasynaptic δ subunit-containing receptors, which is evident from the loss of tonic inhibition in CRH neurons from mice deficient in the $GABA_AR$ δ subunit (*Gabrd*$^{-/-}$ mice; Sarkar et al. 2011). The role of δ subunit-containing $GABA_AR$s in the regulation of parvocellular neurosecretory neurons are consistent with the sensitivity of these neurons to the $GABA_AR$ δ subunit-preferential agonist, THIP (Park et al. 2007). In addition, CRH neurons are sensitive to modulation by neurosteroids via actions on extrasynaptic $GABA_AR$ δ subunit-containing receptors (Sarkar et al. 2011). These data convincingly demonstrate that CRH neurons are regulated by extrasynaptic $GABA_AR$s. Furthermore, these data demonstrate a clear role for the $GABA_AR$ δ subunit in the regulation of CRH neurons. Unfortunately, due to the limited number of studies investigating the synaptic GABAergic regulation of these neurons, it is unclear what other $GABA_AR$ subtypes might be involved.

The robust tonic and phasic GABAergic regulation of CRH neurons suggests that activation and restoration of HPA axis function requires tight regulation of GABAergic inhibition. Naturally, one would assume that increasing GABAergic signaling on CRH neurons would decrease HPA axis activity, whereas decreasing GABAergic signaling onto CRH neurons would relieve these neurons from inhibitory constraint. However, in reality, the GABAergic regulation of CRH neurons and, thus, the HPA axis is not that straightforward. The next section will focus on the complex regulation of GABAergic control of CRH neurons following stress.

12.5 Alterations in GABAergic Control of CRH Neurons Following Stress

It is clear that GABAergic inhibition plays a role in the regulation of CRH neurons and, thus, the HPA axis (see Sect. 12.2 GABAergic control of HPA axis, Sect. 12.3 $GABA_AR$ subunit expression and HPA axis regulation, and Sect. 12.4 GABAergic control of CRH neurons). This section will review how GABAergic control of the HPA axis is altered following stress. Alterations in the GABAergic control of the HPA axis have been demonstrated following both acute and chronic stress and are thought to mediate changes in HPA axis responsiveness.

12.5.1 Acute Stress

12.5.1.1 Acute Stress-Induced Changes in GABAergic Inhibition in CRH Neurons

Stress activates the GABAergic neurons controlling the HPA axis (Bowers et al. 1998; Campeau and Watson 1997), which is thought to be involved in termination of the stress response (for review, see Lopez et al. 1999). Consistent with the stress-induced activation of the GABAergic constraint onto CRH neurons, corticosterone increases the number of GABAergic synaptic contacts on parvocellular neurosecretory neurons in the PVN (Miklos and Kovacs 2002). Acute restraint stress increases the levels of GABA in the hypothalamus (Yoneda et al. 1983) and, consistent with increased GABA production, increased GAD activity has been observed following acute restraint stress (Yoneda et al. 1983). These data suggest that there may be changes in GABAergic signaling following acute stress.

Although these studies suggest alterations in GABAergic signaling in the PVN following acute stress, very few studies have focused on specific changes in $GABA_AR$ subtypes in this region. The evidence that acute stress increases benzodiazepine binding in the hypothalamus (Wilson and Biscardi 1994) suggests that there are changes in $GABA_AR$ subunit composition and/or expression following acute stress. Given the alterations in $GABA_AR$ subunit expression following acute

stress in other brain regions (see Sect. 12.5.1.2 Acute stress-induced changes in GABAergic inhibition in brain regions controlling the HPA axis), it is plausible that similar changes occur in the parvocellular neurosecretory neurons controlling the HPA axis.

Despite the limited studies investigating changes in $GABA_AR$ subunit expression in the PVN following acute stress, alterations in GABAergic inhibition of parvocellular neurosecretory neurons have been demonstrated in the PVN following stress (for review, see Mody and Maguire 2011). Acute restraint stress decreases the frequency of miniature inhibitory synaptic currents (mIPSCs) recorded in parvocellular neurosecretory neurons (Verkuyl et al. 2005). These changes in mIPSC frequency can be induced with either exogenous corticosterone treatment *in vivo* or acute treatment with corticosterone *in vitro* (Verkuyl et al. 2005), demonstrating that glucocorticoids induce alterations in GABAergic control of parvocellular neurosecretory neurons.

Together, these data depict contradictory changes in GABAergic inhibition following acute stress. For example, studies investigating markers of GABAergic inhibition demonstrate an increase in expression of enzymes required for GABA synthesis, increased number of GABAergic synaptic contacts, and increased binding to $GABA_ARs$ whereas the electrophysiological data demonstrate decreased GABAergic signaling onto parvocellular neurosecretory neurons in the PVN. The role of GABAergic control of CRH neurons following stress is further complicated by recent studies suggesting that GABAergic inhibition may switch from inhibitory to excitatory signaling following acute stress (Hewitt et al. 2009; Sarkar et al. 2011).

Inhibitory actions of GABA require the maintenance of low intracellular chloride levels, which is accomplished by the K^+/Cl^- co-transporter, KCC2, in the adult brain (Rivera et al. 1999, 2005; Payne et al. 2003). Acute restraint stress results in a shift in the equilibrium potential for GABA (E_{GABA}) in parvocellular neurosecretory neurons due to a collapse in the chloride gradient resulting in compromised GABAergic inhibition (Hewitt et al. 2009; Sarkar et al. 2011). These changes in GABAergic control of parvocellular neurosecretory neurons likely result from the dephosphorylation and downregulation of KCC2, which regulates the surface expression and function of KCC2 (Lee et al. 2007, 2011). Decreased KCC2 expression results in excitatory actions of GABA on CRH neurons following acute stress (Sarkar et al. 2011). These data suggest that following acute stress there is a switch from inhibitory to excitatory actions of GABA on CRH neurons which may play a role in HPA axis regulation (see also Sect. 12.6 The neurosteroid regulation of the HPA axis).

Clearly, GABA plays an important role in the regulation of the HPA axis at the level of CRH neurons in the PVN (for review, see Mody and Maguire 2011). Changes in GABA levels, $GABA_AR$ subunit expression, GABAergic inhibition, and a switch from inhibitory to excitatory actions of GABA have been demonstrated in the PVN following acute stress and are thought to play a role in regulating the physiological response to stress. In addition to changes in GABAergic control of the parvocellular neurosecretory neurons at the apex of HPA axis control, there are

also numerous changes in GABAergic inhibition in other brain regions controlling the HPA axis following acute stress.

12.5.1.2 Acute Stress-Induced Changes in GABAergic Inhibition in Brain Regions Controlling the HPA Axis

In contrast to the limited number of studies investigating changes in the GABAergic control of parvocellular neurosecretory neurons following acute stress, there have been a larger number of studies investigating acute stress-induced changes in GABAergic inhibition in other brain regions known to regulate HPA axis function. Changes in GABAergic signaling following acute stress have been observed in brain regions controlling the HPA axis, including the hippocampus, prefrontal cortex, BNST, and amygdala (for review, see Cullinan et al. 2008).

Hippocampus

Changes in GABAergic inhibition following acute stress are suggested by increased [^{3}H]GABA binding in the forebrain (Akinci and Johnston 1993; Wilson and Biscardi 1994; Skerritt et al. 1981; for review, see Skilbeck et al. 2010), suggesting alterations in $GABA_A$ receptor expression. Consistent with alterations in GABAergic signaling, GAD65 and GAD67 expression is increased in the hippocampus following acute stress (Hasler et al. 2010; Bowers et al. 1998). However, decreased levels of GABA have been observed in the hippocampus following acute stress (de Groote and Linthorst 2007). Changes in specific $GABA_A$Rs subunits have also been demonstrated in brain regions controlling the HPA axis following acute stress. For example, there is a decreased expression of the $GABA_A$R α1 subunit (Zheng et al. 2007) and the $GABA_A$R γ2 subunit in the hippocampus following acute stress (Maguire and Mody 2007), suggesting stress-induced alterations in the receptors mediating phasic GABAergic inhibition. Consistent with stress-induced alterations in phasic GABAergic inhibition, GR agonists rapidly increase the frequency and amplitude of sIPSCs in CA1 pyramidal neurons (Hu et al. 2010). In addition, acute restraint stress alters the expression of extrasynaptic $GABA_A$Rs. Following acute stress, there is an increase in the expression of extrasynaptic δ subunit-containing $GABA_A$Rs (Maguire and Mody 2007) and an increase in tonic GABAergic inhibition in the dentate gyrus granule cells (Maguire and Mody 2007). These data demonstrate alterations in GABAergic inhibition in the hippocampus following acute stress. Given the important role for extrasynaptic $GABA_A$Rs in the regulation of hippocampal excitability (Coulter and Carlson 2007), it is likely that acute stress-induced changes in the regulation of the hippocampus indirectly impact HPA axis function.

Prefrontal Cortex

Acute stress has been demonstrated to impair the function of the prefrontal cortex (for review, see Arnsten 2009), which has been proposed to result from depressed GABAergic inhibition (Rodriguez Manzanares et al. 2005). Consistent with stress-induced impairments in GABAergic inhibition, reductions in GABA levels have been observed in the prefrontal cortex following acute stress (Hasler et al. 2010). CRH has been shown to modulate GABAergic transmission in the prefrontal cortex (Tan et al. 2004). In contrast, corticosterone treatment suppresses GABA release in the prefrontal cortex, which has been suggested to play a role in the termination of the stress response (Acosta et al. 1992). In addition to changes in GABA levels, there is also evidence for alterations in $GABA_A$Rs. Acute stress increases benzodiazepine binding in the cortex (Motohashi et al. 1993), suggesting that there are changes in the expression of synaptic $GABA_A$Rs. There is a decrease in the expression of the $GABA_A$R α1 subunit in the prefrontal cortex following acute stress (Zheng et al. 2007). The limited amount of data available suggests that there are alterations in GABAergic transmission in the prefrontal cortex following acute stress. Therefore, these changes may alter the control of the HPA axis mediated by the prefrontal cortex.

BNST

GABAergic neurons in the BNST are activated following acute stress (Radley et al. 2009). However, few studies have investigated changes in GABAergic inhibition in the BNST following stress. Alterations in the expression of the enzymes responsible for GABA synthesis suggest changes in GABAergic signaling in the BNST following stress. Following acute stress, GAD65 and GAD67 expression are increased in the BNST, which indirectly regulates the HPA axis (Hasler et al. 2010). Additional studies are required to investigate changes in GABAergic inhibition in the BNST following acute stress and the implications for the regulation of the HPA axis.

Amygdala

The amygdala plays a critical role in the processing of stressful information (for review, see Roozendaal et al. 2009). Thus, it is reasonable that stress would impact the activity of neurons in this region. Stress-induced synaptic plasticity in the amygdala has been proposed to be mediated by GABAergic inhibition (Mahan and Ressler 2012; for review, see Roozendaal et al. 2009). Depressed GABAergic inhibition was observed in neurons in the basolateral amygdala following acute stress (Rodriguez Manzanares et al. 2005). However, acute stress has been demonstrated to activate a specific subset of neurons, including parvalbumin-positive interneurons, in the basolateral amygdala (Reznikov et al. 2008), which is associated with an increase in GABA efflux (Reznikov et al. 2009). Clearly, changes in GABAergic inhibition in the amygdala have been noted following acute stress. However, the

significance of these changes on stress reactivity remains unclear. Further studies are required to make sense of the role of GABAergic inhibition on the function of the amygdala following stress.

Collectively, these data demonstrate changes in GABAergic inhibition following acute stress in brain regions relevant to control of the HPA axis. The numerous changes in GABAergic inhibition documented in multiple brain regions make it difficult to interpret the net effect of these changes on stress reactivity and HPA axis function.

12.5.2 Chronic Stress

The data above highlight changes in GABAergic inhibition in brain regions controlling the HPA axis following acute stress. Similarly, chronic stress also alters the GABAergic control of parvocellular neurosecretory neurons in the PVN as well as altering GABAergic inhibition in other brain regions exerting control, either directly or indirectly, on the HPA axis, including the hippocampus, prefrontal cortex, BNST, and amygdala. This section will focus on changes in extrasynaptic $GABA_A$Rs following prolonged stress that impacts HPA axis regulation.

12.5.2.1 Chronic Stress-Induced Changes in GABAergic Inhibition in CRH Neurons

Changes in GABAergic inhibition following chronic stress are suggested by the increase in [^{3}H]GABA binding in the forebrain (for review, see Skilbeck et al. 2010), suggesting alterations in $GABA_A$ receptor expression. In contrast to the sparse number of studies investigating changes in GABAergic inhibition onto CRH neurons following acute stress, there is ample evidence that chronic stress alters GABAergic input onto parvocellular neurosecretory neurons in the PVN. For instance, chronic stress increases GAD65 expression in the PVN, peri-PVN, and other hypothalamic regions (Bowers et al. 1998), suggesting alterations in GABAergic signaling.

Alterations in the GABAergic control of parvocellular neurosecretory neurons in the PVN are thought, at least in part, to be due to changes in $GABA_A$R subunit expression in these neurons. Consistent with alterations in $GABA_A$R subunit expression in the PVN, there is decreased benzodiazepine binding in the hippocampus and hypothalamus following chronic stress (Weizman et al. 1990). Removal of glucocorticoids by adrenalectomy prevents the stress-induced alterations in benzodiazepine binding, suggesting that glucocorticoids mediate this effect (Weizman et al. 1990). These binding studies suggest that there are alterations in specific $GABA_A$R subtypes following chronic stress. In fact, decreased expression of the β1 and β2 subunits of the $GABA_A$R was observed in the PVN following chronic stress (Cullinan 2000). In parvocellular neurons, only expression of the extrasynaptic

Table 12.3 Chronic stress-Induced Alterations in $GABA_A$Receptor subunit expression in the PVN following stress

$GABA_A$R subunit	Expression in PVN	References
α1	NC	Verkuyl, 2004
α2	ND	
α3	NC	Verkuyl, 2004
α4	increase, NC	Verkuyl, 2004; Serra, 2006
α5	increase	Verkuyl, 2004
α6	NC	Verkuyl, 2004
β1	decrease, NC	Cullinan, 2000; Verkuyl, 2004
β2	decrease, NC	Cullinan, 2000; Verkuyl, 2004
β3	NC	Cullinan, 2000; Verkuyl, 2004
γ1	NC	Verkuyl, 2004
γ2	NC	Verkuyl, 2004
γ3	NC	Verkuyl, 2004
δ	increase, decrease	Verkuyl, 2004; Serra, 2006

Changes in $GABA_A$R subunit expression has been demonstrated following chronic stress. Interestingly, extrasynaptic α5 and α subunit expression is increased following chronic stress (red box)
NC no change, ND not determined

α5 and δ subunits was found to be altered following chronic stress (Verkuyl et al. 2004). These data implicate extrasynaptic $GABA_A$Rs in the regulation of parvocellular neurosecretory neurons following chronic stress. However, the impact on GABAergic control of these neurons following chronic stress remains unclear since the expression of the $GABA_A$R α5 subunit is increased while the δ subunit is decreased (Verkuyl et al. 2004).

There is also evidence of functional changes in GABAergic inhibition onto parvocellular neurosecretory neurons in the PVN following chronic stress. Chronic stress has been demonstrated to decrease mIPSC frequency in parvocellular neurons (Joels et al. 2004; Verkuyl et al. 2004), which can be reproduced by exogenous administration of elevated levels of glucocorticoids (Joels et al. 2004; Verkuyl et al. 2004). Both chronic stress and chronic exposure to elevated glucocorticoid levels suppress GABAergic inputs onto parvocellular neurosecretory neurons (Joels et al. 2004; Verkuyl et al. 2004). These data suggest that glucocorticoids mediate the changes in GABAergic control of parvocellular neurosecretory neurons following chronic stress (Table 12.3).

In contrast to the changes in GABAergic inhibition following chronic stress, which are thought to be mediated by elevated levels of glucocorticoids, chronic removal of glucocorticoids also alters GABAergic control of parvocellular

neurosecretory neurons in the PVN. For instance, chronic removal of glucocorticoids as a result of adrenalectomy (ADX) increases GABAergic constraint of the HPA axis. Adrenalectomy increases benzodiazepine (De Souza et al. 1986; Goeders et al. 1986) and muscimol (Majewska et al. 1985) binding in the hypothalamus, which is restored with glucocorticoid replacement (Goeders et al. 1986). In addition, adrenalectomy increases the number of inhibitory synaptic contacts in the PVN (Miklos and Kovacs 2002). Consistent with an increase in the number of synaptic contacts and benzodiazepine binding, the mIPSC frequency is increased in parvocellular neurosecretory neurons following adrenalectomy (Verkuyl and Joels 2003). Corticosterone replacement in adrenalectomized animals restores mIPSC frequency back to control levels (Verkuyl and Joels 2003). The increased mIPSC frequency as a result of adrenalectomy could be a result of either increased presynaptic GABA release or an increased number of GABAergic synapses (Miklos and Kovacs 2002).

Together, these data suggest that either prolonged elevations or removal of glucocorticoids can alter the inhibitory constraint of the HPA axis. These studies highlight that there are changes in the GABAergic regulation of the HPA axis in the face of altered glucocorticoid exposure, which may occur at the level of parvocellular neurosecretory neurons in the PVN. In addition to alterations in GABAergic control of parvocellular neurosecretory neurons which may mediate the effects of chronic stress on HPA axis function, there is also ample evidence of changes in GABAergic inhibition in other brain regions controlling the HPA axis following chronic stress.

12.5.2.2 Chronic Stress-Induced Changes in GABAergic Inhibition in Brain Regions Controlling the HPA Axis

There is a more robust literature documenting changes in GABAergic control of brain regions known to exert control over the HPA axis, such as the hippocampus, prefrontal cortex, BNST, and amygdala. The majority of these studies have focused on changes in GABAergic inhibition in the hippocampus following chronic stress.

Hippocampus

Alterations in GABAergic inhibition in the hippocampus following chronic stress are suggested by a decrease in the number of parvalbumin-positive interneurons in the hippocampus (Czeh et al. 2005; Hu et al. 2010). In contrast, chronic stress increases GAD67 expression in the CA3 region of the hippocampus and the dentate gyrus (Bowers et al. 1998). Numerous changes in $GABA_AR$ subunit composition have been observed under conditions of altered glucocorticoid levels or following chronic stress. Chronic exposure to stress levels of corticosterone decreased $GABA_AR$ α1, α2, and β1 subunit expression in the hippocampus, whereas, β2, β3, and γ2 expression is increased (Orchinik et al. 1995). In contrast, chronic depletion of corticosterone as a result of adrenalectomy decreases transcript expression

of α1, α2, and γ2 subunits in the hippocampus while increasing the transcript expression of the $GABA_AR$ β2 subunit (Orchinik et al. 1994). Alterations of $GABA_AR$ subunit transcript expression are restored by exogenous treatment with corticosterone (Orchinik et al. 1994). Following chronic stress, there is an increase in β2 expression in all hippocampal subfields (Cullinan and Wolfe 2000) and a downregulation of the $GABA_AR$ β3 subunit (Cullinan and Wolfe 2000). Given that the $GABA_AR$ β3 subunit is a likely partner with the α4 and δ subunits (Jones et al. 1997; Sur et al. 1999), these changes likely reflect changes in extrasynaptic $GABA_ARs$ following chronic stress. However, chronic social isolation stress increases the expression of the extrasynaptic $GABA_AR$ α4 and δ subunits in the hippocampus (Serra et al. 2006). Consistent with the importance of GABAergic inhibition in brain regions controlling the HPA axis, hyperexcitability of the HPA axis has been observed in a mouse model with forebrain-specific deficits in $GABA_AR$ γ2 subunit expression (Earnheart et al. 2007). Changes in $GABA_AR$ subunit expression in the hippocampus following chronic stress are also associated with functional changes in GABAergic inhibition. The frequency of sIPSCs is decreased in dentate gyrus granule cells in animals subjected to chronic stress (Holm et al. 2011). In contrast, the frequency and amplitude of sIPSCs were shown to be increased in CA1 pyramidal neurons following chronic restraint stress (Hu et al. 2010). In addition, following chronic stress, there is a significant increase in the THIP-mediated tonic current (Holm et al. 2011), suggesting a chronic stress-induced upregulation of extrasynaptic $GABA_ARs$. Consistent with changes in extrasynaptic $GABA_ARs$ following chronic stress, social isolation also increases tonic GABAergic inhibition in DGGCs (Serra et al. 2006). These data suggest that there are alterations in extrasynaptic $GABA_A$ receptor-mediated inhibition following chronic stress in brain regions which exert control over the HPA axis.

Further complicating the role of stress in neural circuits controlling the HPA axis, chronic stress and chronic administration of stress hormones induce damage and functional deficits in brain regions involved in HPA axis regulation, particularly limbic regions (for review, see Joels et al. 2004; McEwen 2001) including the hippocampus. Decreased hippocampal volume, reduced neurogenesis, and dendritic atrophy are consequences of long-term glucocorticoid exposure and chronic stress (for review, see Joels et al. 2004; McEwen 2001). Thus, chronic stress and/or long-term exposure to glucocorticoids may indirectly alter HPA axis function via inducing hippocampal damage.

Prefrontal Cortex

Following chronic stress, GAD activity is increased and GABA turnover is increased in the frontal cortex (Otero Losada 1988). Interestingly, this same study observed a reduction in the levels of GABA in frontal cortex following chronic stress (Otero Losada 1988). Reduced neuronal uptake of [^{3}H]GABA was observed in the prefrontal cortex following chronic stress (Acosta et al. 1992). In addition, alterations in $GABA_A$ receptor expression have been observed with decreased [^{3}H] SR95531 binding in the prefrontal cortex following chronic stress (Gruen et al.

1995). Similarly, chronic stress alters the action of benzodiazepines on prefrontal cortex function (Finlay 1995). These data suggest that alterations in GABAergic inhibition may occur in the prefrontal cortex following stress. However, to our knowledge, the role of extrasynaptic $GABA_A$Rs has not been examined.

In addition to potential changes in GABAergic inhibition in the prefrontal cortex, chronic stress also induces structural changes which alter the role of the prefrontal cortex on HPA axis regulation. These changes are similar to those that occur in the hippocampus, including dendritic shortening and decreased spine density (for review, see McEwen et al. 2012) which may also impact HPA axis regulation.

BNST

There are alterations in the relative expression of the enzymes responsible for GABA synthesis (GAD65/GAD67) following chronic stress in the BNST, another brain region known to control HPA axis activity (Ventura-Silva et al. 2012). For example, GAD65 expression is increased in the BNST following chronic stress (Christiansen et al. 2011). In addition to the potential increased GABA synthesis, increased expression of $GABA_A$Rs has been observed in the BNST following chronic stress (Ventura-Silva et al. 2012). These data suggest that there may be alterations in GABAergic inhibition in the BNST following chronic stress. Further studies are required to fully appreciate the scope and function of changes in GABAergic inhibition in the BNST on HPA axis regulation and stress reactivity.

Amygdala

Chronic stress induces hyperexcitability of principal neurons in the amygdala (Rosenkranz et al. 2010) which may be due to impairments in GABAergic inhibition. Neurons in the amygdala are activated by elevated levels of stress mediators, resulting in a reduction in GABAergic inhibition (for review, see Roozendaal et al. 2009). However, the majority of these studies rely on exogenous application of stress mediators making it difficult to interpret the relationship to chronic stress. Interestingly, following chronic stress, the positive modulation of GABAergic inhibition is suppressed (Braga et al. 2003), demonstrating compromised GABAergic inhibition in the amygdala. The plasticity of GABAergic inhibition in the amygdala may play a role in the regulation of the HPA axis following chronic stress (for review, see Jankord and Herman 2008).

Taken together, these data demonstrate changes in GABAergic inhibition following chronic stress in brain regions relevant to HPA axis regulation. However, similar to the changes observed following acute stress, the numerous changes in GABAergic inhibition observed in numerous brain regions make it difficult to interpret the net effect of these changes on stress reactivity and HPA axis function. Given the importance of GABAergic inhibition on HPA axis function, GABA modulators, such as neurosteroids, also have the potential to impact HPA axis function.

12.6 Neurosteroid Regulation of the HPA Axis

Under basal conditions, neurosteroids have been demonstrated to exhibit anxiolytic properties (Bitran et al. 1995; Crawley et al. 1986) suggesting that they may impact stress reactivity. Indeed, neurosteroids have been implicated in stress regulation (for review, see Gunn et al. 2011). The levels of steroid hormones and neurosteroids are elevated in response to stress (Barbaccia et al. 1996b, a; Purdy et al. 1991; Reddy and Rogawski 2002). Stress increases circulating levels of the stress-derived neurosteroid, 5α-pregnane-3α,21-diol-20-one (THDOC), from 1–5 to 15–30 nM (Reddy and Rogawski 2002; for review, see Reddy 2003), reaching levels which can act directly on $GABA_A$Rs to both potentiate the effects of GABA (Barbaccia et al. 1996b, a; Purdy et al. 1991) and alter $GABA_A$R subunit expression (Maguire and Mody 2007, 2009). Extrasynaptic $GABA_A$Rs are uniquely sensitive to modulation by neurosteroids. Neurosteroids act preferentially at extrasynaptic δ subunit-containing receptors enhancing the tonic component of GABAergic inhibition (Houston et al. 2012; Stell et al. 2003; Belelli et al. 2002; Wohlfarth et al. 2002). Although neurosteroids are more efficacious at extrasynaptic δ subunit-containing $GABA_A$Rs, they can also potentiate the effects of GABA at receptors containing most isoforms. Interestingly, although the δ subunit confers increased neurosteroid sensitivity, the binding site for neurosteroids has been identified on the α/β interface of the $GABA_A$R complex (Hosie et al. 2006, 2009). Thus, neurosteroids potentially have the ability to alter HPA axis function via actions on $GABA_A$Rs. For example, increased neurosteroid levels following acute stress exert a negative feedback onto the HPA axis decreasing corticosterone and ACTH levels (Owens et al. 1992; Patchev et al. 1994). However, it remains unclear where neurosteroids exert their control over HPA axis function given that CRH neurons are sensitive to neurosteroid modulation (Sarkar et al. 2011) as are neurons in many brain regions indirectly controlling the HPA axis (Wilson and Biscardi 1997).

12.6.1 Neurosteroid Modulation of CRH Neurons

Neurosteroid regulation of CRH neurons is suggested by the evidence that neurosteroids induce CRH synthesis and release at the level of the hypothalamus (Naert et al. 2007). Under basal conditions, microinjection of THDOC into the PVN rapidly decreases circulating corticosterone levels (Sarkar et al. 2011). The enzymes responsible for neurosteroidogenesis are expressed in the PVN (Li et al. 1997; Eechaute et al. 1999; Gao et al. 2002; Saalmann et al. 2007), suggesting that there may be local neurosteroid production and action in the PVN. Long-term exposure to neurosteroids has been shown to decrease CRH expression in vitro (Budziszewska et al. 2010) further implicating neurosteroids in the regulation of CRH neurons. However, until recently, it was unclear whether neurosteroids directly regulate CRH neurons. Recent data demonstrate a role for neurosteroid potentiation of $GABA_A$R δ subunit-containing receptors on CRH

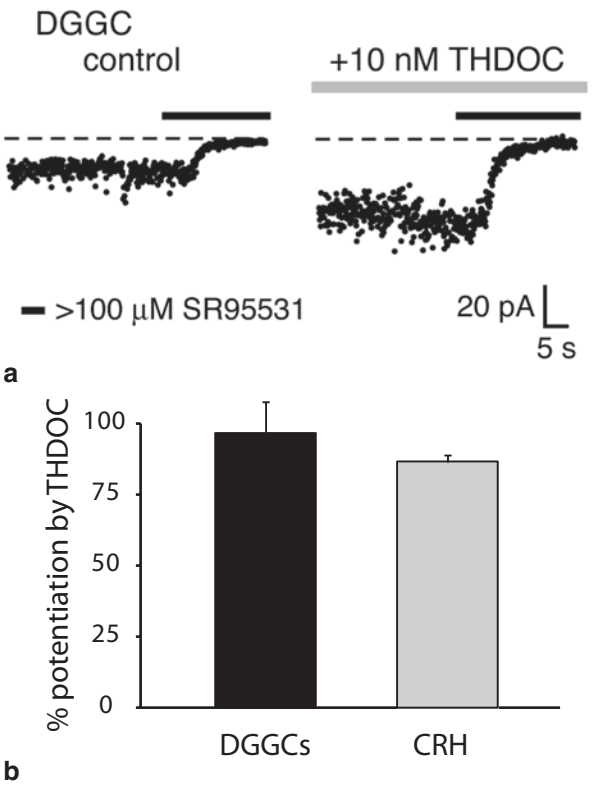

Fig. 12.2 Neurosteroid potentiation of GABAergic inhibition in *CRH* neurons and *DGGCs*. **a** Representative traces of the tonic GABAergic inhibition in dentate gyrus granule cells (*DGGCs*) in the presence of nACSF or 10 nM *THDOC*. Each dot represents the average holding current every 100 ms before and after the addition of *SR95531* (*black line*). The difference between the average holding current after the addition of *SR95531* (*dotted line*) and the average holding current in nACSF is attributed to the tonic GABAergic current. **b** The percent potentiation by 10 nM *THDOC* is similar for *DGGCs* and *CRH* neurons. (These data represented in this figure were adapted with permission from Sarkar et al. 2011; Stell et al. 2003)

neurons in the regulation of the HPA axis (Sarkar et al. 2011). CRH neurons in the PVN are sensitive to neurosteroid modulation (Fig. 12.2). Tonic GABAergic inhibition, which is mediated by extrasynaptic $GABA_A$Rs, is increased in the presence of the neurosteroid THDOC (10nM; Sarkar et al. 2011; Fig. 12.2). It is clear that the neurosteroid sensitivity of CRH neurons is mediated by $GABA_A$R δ subunit-containing receptors since this modulation is absent in CRH neurons from *Gabrd*$^{-/-}$ mice (Sarkar et al. 2011). These data are consistent with previous findings demonstrating changes in $GABA_A$R δ subunit expression in parvocellular neurons in the PVN following stress (Verkuyl et al. 2004) suggesting that these receptors play a role in stress reactivity. The activity of CRH neurons is also modulated by neurosteroids. Under basal conditions, the firing rate of CRH neurons is decreased in the presence of THDOC (Sarkar et al. 2011). The impact of neurosteroids on the activity of CRH neurons is mediated by the effects on $GABA_A$R δ subunit-containing receptors, since the effect of neurosteroids is abolished in *Gabrd*$^{-/-}$ mice (Sarkar et al. 2011).

Interestingly, following stress, CRH neurons still exhibit modulation by neurosteroids acting on extrasynaptic $GABA_A$Rs but the net effect of this regulation is dramatically altered. The inhibitory actions of GABA require the maintenance of the chloride gradient which is accomplished by KCC2 in the adult brain (Rivera et al.

1999, 2005; Payne et al. 2003). Following acute stress there is a dephosphorylation of KCC2 residue Ser940 which controls the function and surface expression of the transporter, leading to downregulation of total KCC2 levels, causing a collapse in the chloride gradient, resulting in the excitatory actions of GABA on CRH neurons (Sarkar et al. 2011). Under basal conditions, THDOC decreases the firing rate of CRH neurons; however, following acute stress, THDOC increases the firing rate of CRH neurons by potentiating the excitatory actions of GABA on these neurons (Sarkar et al. 2011). These effects are abolished in *Gabrd*$^{-/-}$ mice demonstrating that they are mediated by extrasynaptic $GABA_AR$ δ subunit-containing receptors (Sarkar et al. 2011). These data clearly demonstrate a role for extrasynaptic $GABA_ARs$ in the neurosteroid regulation of CRH neurons both under basal conditions and following acute restraint stress. In addition to the effects of neurosteroids on CRH neurons in the regulation of HPA axis activity, neurons in other brain regions that regulate the HPA axis are also sensitive to neurosteroid modulation.

12.6.2 Neurosteroid Modulation in Brain Regions Controlling the HPA Axis

Enzymes involved in neurosteroidogenesis have been identified in brain regions which exert indirect control over the HPA axis including the hippocampus, prefrontal cortex, BNST, and amygdala (Agis-Balboa et al. 2006). These data suggest that local neurosteroid synthesis may play a role in the regulation of neurons in these brain regions, thereby indirectly influencing the regulatory effects of these brain regions on HPA axis function.

12.6.2.1 Hippocampus

The enzymes responsible for neurosteroid synthesis, such as 5α-reductase and 3α-hydroxysteroid dehydrogenase (3α-HSD), are detected in the multiple hippocampal subfields (Agis-Balboa et al. 2006) suggesting that local neurosteroid synthesis may play a role in regulating neuronal excitability in these areas. The expression of 5α-reductase and 3α-HSD is intense in the dentate gyrus, CA1 and CA2 regions of the hippocampus, whereas expression in CA3 is weak (Agis-Balboa et al. 2006). In addition to the subregion specificity in the expression of enzymes responsible for neurosteroid synthesis, there are also subregion-specific differences in neurosteroid sensitivity in the hippocampus. There is a high density of extrasynaptic $GABA_AR$ δ subunit-containing receptors, which confer neurosteroid sensitivity, in the dentate gyrus (Stell et al. 2003). Low nanomolar concentrations of neurosteroids potentiate extrasynaptic $GABA_ARs$, increasing the tonic GABAergic inhibition in dentate gyrus granule cells (Stell et al. 2003; Fig. 12.2). Higher concentrations of neurosteroids potentiate the phasic component of GABAergic inhibition, mediated by synaptic $GABA_ARs$, in dentate gyrus granule cells (Stell et al. 2003). Although the expression of these receptors is low in other hippocampal subregions, including

the CA1 and CA3 regions, neurons in these regions are still modulated by higher concentrations of neurosteroids acting on the synaptic $GABA_A$Rs (Stell et al. 2003). These data support the notion that neurosteroid modulation of extrasynaptic $GABA_A$Rs in the hippocampus can alter hippocampal excitability and thus influence the extent of hippocampal control of other systems including the HPA axis.

In addition to their effect on $GABA_A$Rs, neurosteroids may also impact hippocampal function by altering adult hippocampal neurogenesis. Hippocampal neurogenesis has been demonstrated to play a role in the regulation of the HPA axis (Schloesser et al. 2009; Anacker et al. 2011b). Conversely, stress and glucocorticoids inhibit adult neurogenesis (Gould et al. 1997; Tanapat et al. 1998). These findings suggest that neurogenesis may also play a role in the negative feedback mechanism of HPA axis regulation. Extrasynaptic $GABA_A$Rs, which are sensitive to neurosteroid modulation, have been demonstrated to play a role in hippocampal neurogenesis (Duveau et al. 2011). Neurosteroids have been identified as endogenous regulators of neurogenesis (for review, see Charalampopoulos et al. 2008), which may represent another mechanism whereby neurosteroids can impact HPA axis function.

12.6.2.2 Prefrontal Cortex

The enzymes required for local neurosteroidogenesis are expressed in the prefrontal cortex; however, the expression of 5α-reductase and 3α-HSD is restricted to principal neurons in the cortex and not expressed in cortical GABAergic interneurons or glial cells (Agis-Balboa et al. 2006). Interestingly, the expression of these enzymes is reduced following chronic stress (Dong et al. 2001; Matsumoto et al. 2003; Pinna et al. 2003; Serra et al. 2000; for review, see Graziano 2011). These data suggest that local neurosteroid synthesis may play a role in neuronal regulation and that this regulation may be altered following stress. In addition to the presence of neurosteroidogenic enzymes, extrasynaptic $GABA_A$Rs which are sensitive to neurosteroid modulation are also expressed in the prefrontal cortex. Extrasynaptic $GABA_A$R α4 and δ subunits are expressed in the prefrontal cortex (Maldonado-Aviles et al. 2009), suggesting that these neurons may be regulated by neurosteroid-sensitive tonic GABAergic inhibition. The presence of $GABA_A$R δ subunit-containing $GABA_A$Rs in the prefrontal cortex suggests that these neurons are regulated by low nanomolar concentrations of neurosteroids. In addition, higher concentrations of neurosteroids potentially regulate neuronal excitability of principal neurons in the prefrontal cortex via actions on synaptic $GABA_A$Rs. Neurosteroid modulation of neurons in the prefrontal cortex may, thereby, indirectly impact HPA axis function.

12.6.2.3 BNST

The majority of neurosteroidogenic enzymes, including 5α-reductase and 3α-HSD, are absent from the BNST (Do Rego et al. 2009). However, a separate study

demonstrated high aromatase immunoreactivity in a densely packed group of neurons in the BNST (Jakab et al. 1993). Due to the fact that all the enzymes required for neurosteroidogenesis have not been identified in this region and the limited number of studies, it is difficult to evaluate the potential role of local neurosteroid synthesis in this region. Extrasynaptic $GABA_AR$ subunits have been identified in the BNST, including the α4, α5, and δ subunits (Pirker et al. 2000). The high expression of the $GABA_AR$ δ subunit, which confers sensitivity to neurosteroid modulation, suggests that neurosteroids have the potential to play a role in the regulation of these neurons and, thereby, indirectly influence HPA axis function. However, more detailed studies are required to investigate the role of neurosteroids on $GABA_AR$ regulation in the BNST.

12.6.2.4 Amygdala

The different nuclei in the amygdala also exhibit regional differences in the expression of the neurosteroidogenic enzymes, 5α-reductase and 3α-HSD (Agis-Balboa et al. 2006). The expression of these neurosteroidogenic enzymes is weak in the central and basolateral nuclei, whereas there is intense staining for these enzymes in the basolateral amygdala (BLA; Agis-Balboa et al. 2006). These data suggest that there may be regional differences in neurosteroid actions on $GABA_ARs$ within the amygdala. However, the ability of neurosteroids to modulate GABAergic inhibition in the amygdala may be limited by the low expression of extrasynaptic $GABA_AR$ subunits, including the δ subunit, which mediate tonic GABAergic inhibition and neurosteroid sensitivity (Fritschy and Mohler 1995). These data suggest that these neurons may not be regulated by low nanomolar concentrations of neurosteroids. However, these neurons are likely modulated by higher concentrations of neurosteroids acting on synaptically localized $GABA_ARs$. However, the extent of neurosteroid modulation in the amygdala and the impact on HPA axis function remains unclear.

Collectively, these findings demonstrate that brain regions known to exert control over the HPA axis (see Sect 12.3.2 $GABA_AR$ subunit expression in brain regions controlling the HPA axis) are regulated by neurosteroids. Thus, in addition to the direct neurosteroid regulation of CRH neurons, neurosteroid modulation of $GABA_ARs$ may also indirectly affect HPA axis function by regulating neuronal excitability in brain regions known to control the HPA axis.

12.6.3 Neurosteroids and HPA Axis Suppression During Pregnancy

The activity of the HPA axis is dramatically altered during pregnancy and the postpartum period. During pregnancy, circulating levels of both CRH and cortisol rise. Placental CRH production and decreased presence of circulating CRH binding

protein is thought to drive increased cortisol levels throughout pregnancy (for review, see Kammerer et al. 2006). Interestingly, although the basal circulating levels of CRH and cortisol increase throughout pregnancy, the stress-induced cortisol secretion is blunted during pregnancy (de and Buitelaar 2005). For example, during pregnancy, women lose the cortisol response to an acute stressor (Kammerer et al. 2002) and the CRH-stimulated ACTH release is blunted in pregnant women (Schulte et al. 1990). This is significant because it suggests that the diurnal release of cortisol and the stress-induced release of cortisol are controlled by independent regulatory mechanisms which may be able to be teased apart. Recent studies suggest that the suppression of the HPA axis during pregnancy (Brunton et al. 2008; Brunton and Russell 2011) is mediated by neurosteroids (Brunton et al. 2009; Brunton and Russell 2008). Treatment with the neurosteroidogenesis inhibitor, finasteride, restores normal HPA axis responsiveness (Brunton et al. 2009). These data suggest that neurosteroids may play a role in HPA axis suppression during pregnancy. Although the role of extrasynaptic $GABA_A$Rs has not been directly examined, it is tempting to implicate these receptors in HPA axis suppression during pregnancy given the notable role of these receptors in conferring neurosteroid sensitivity in CRH neurons and other brain regions controlling the HPA axis.

Concluding Remarks

This review clearly demonstrates a role for extrasynaptic $GABA_A$Rs in the regulation of the HPA axis at the level of CRH neurons as well as in other brain regions controlling HPA axis function, including the hippocampus, prefrontal cortex, BNST, and amygdala. Given the widespread anatomical distribution of extrasynaptic $GABA_A$Rs and the differing impact of these numerous brain regions on the regulation of the HPA axis, it is difficult to interpret the net effect of these receptors on HPA axis function. Furthermore, the neurosteroid sensitivity of extrasynaptic $GABA_A$Rs provides another level of complexity for the role of these receptors in the regulation of the stress response. Despite the complex role of extrasynaptic $GABA_A$Rs in the regulation of the HPA axis, it is evident that these receptors influence HPA axis function. Our limited knowledge of the role of extrasynaptic $GABA_A$Rs is largely due to the sparse number of studies investigating the synaptic regulation of the HPA axis, which is gaining considerable interest in the scientific community (Levy and Tasker 2012; Wamsteeker and Bains 2010), let alone the limited number of studies directly investigating the role of extrasynaptic receptors in the regulation of the HPA axis. Additional studies are required to fully appreciate the contribution of extrasynaptic $GABA_A$Rs on the regulation of the HPA axis.

Stress is known to either worsen or trigger illnesses ranging from the common cold to cancer (for review, see Cohen 2007). Furthermore, hyperexcitability of the HPA axis has been implicated in the pathophysiology of numerous disorders, including depression (for review, see Lloyd and Nemeroff 2011; Pariante and Lightman 2008). Therefore, insights into the regulation of the HPA axis may identify

novel targets for therapeutic intervention, such as extrasynaptic $GABA_A$Rs. Very few studies have investigated which $GABA_A$R subtypes play a role in the regulation of the HPA axis. However, this book chapter has highlighted the importance of GABAergic inhibition on HPA axis function. Additional studies are required to fully appreciate the mechanisms regulating HPA axis function. However, we are optimistic that HPA axis modulation may be a feasible therapeutic target. Given the evidence that there may be independent mechanisms regulating basal glucocorticoid secretion and stress-induced glucocorticoid (Kammerer et al. 2006; Sarkar et al. 2011), we support revisiting HPA axis modulation as a therapeutic target.

Acknowledgments J.M. is funded by NS073574.

References

Acosta GB, Otero Losada ME, Rubio MC (1992) Changes in GABAergic function after chronic chemical stress. Gen Pharmacol 23:241–244

Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A (2006) Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. Proc Natl Acad Sci U S A 103:14602–14607

Ahima RS, Harlan RE (1990) Charting of type II glucocorticoid receptor-like immunoreactivity in the rat central nervous system. Neuroscience 39:579–604

Akinci MK, Johnston GA (1993) Sex differences in acute swim stress-induced changes in the binding of MK-801 to the NMDA subclass of glutamate receptors in mouse forebrain. J Neurochem 61:2290–2293

Alon T, Zhou L, Perez CA, Garfield AS, Friedman JM, Heisler LK (2009) Transgenic mice expressing green fluorescent protein under the control of the corticotropin-releasing hormone promoter. Endocrinology 150:5626–5632

Anacker C, Pariante CM (2012) Can adult neurogenesis buffer stress responses and depressive behaviour? Mol Psychiatry 17:9–10

Anacker C, Zunszain PA, Carvalho LA, Pariante CM (2011a) The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology 36:415–425

Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, Price J, Pariante CM (2011b) Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Mol Psychiatry 16:738–750

Arnsten AF (2009) Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci 10:410–422

Aronsson M, Fuxe K, Dong Y, Agnati LF, Okret S, Gustafsson JA (1988) Localization of glucocorticoid receptor mRNA in the male rat brain by in situ hybridization. Proc Natl Acad Sci U S A 85:9331–9335

Arriza JL, Simerly RB, Swanson LW, Evans RM (1988) The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. Neuron 1:887–900

Barbaccia ML, Concas A, Roscetti G, Bolacchi F, Mostallino MC, Purdy RH, Biggio G (1996a) Stress-induced increase in brain neuroactive steroids: antagonism by abecarnil. Pharmacol Biochem Behav 54:205–210

Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH, Biggio G (1996b) Time-dependent changes in rat brain neuroactive steroid concentrations and GABA(A) receptor function after acute stress. Neuroendocrinology 63:166–172

Belelli D, Casula A, Ling A, Lambert JJ (2002) The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. Neuropharmacology 43:651–661

Bitran D, Shiekh M, Mcleod M (1995) Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain gaba(A) receptors. J Neuroendocrinol 7:171–177
Boudaba C, Szabo K, Tasker JG (1996) Physiological mapping of local inhibitory inputs to the hypothalamic paraventricular nucleus. J Neurosci 16:7151–7160
Bowers G, Cullinan WE, Herman JP (1998) Region-specific regulation of glutamic acid decarboxylase (GAD) mRNA expression in central stress circuits. J Neurosci 18:5938–5947
Braga MF, Aroniadou-Anderjaska V, Manion ST, Hough CJ, Li H (2003) Stress impairs [alpha]1A adrenoceptor-mediated noradrenergic facilitation of GABAergic transmission in the basolateral amygdala. Neuropsychopharmacology 29:45–58
Brunton PJ, Russell JA (2008) Attenuated hypothalamo-pituitary-adrenal axis responses to immune challenge during pregnancy: the neurosteroid opioid connection. J Physiol 586:369–375
Brunton PJ, Russell JA (2011) Allopregnanolone and suppressed hypothalamo-pituitary-adrenal axis stress responses in late pregnancy in the rat. Stress 14:6–12
Brunton PJ, Russell JA, Douglas AJ (2008) Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis during pregnancy and lactation. J Neuroendocrinol 20:764–776
Brunton PJ, McKay AJ, Ochedalski T, Piastowska A, Rebas E, Lachowicz A, Russell JA (2009) Central opioid inhibition of neuroendocrine stress responses in pregnancy in the rat is induced by the neurosteroid allopregnanolone. J Neurosci 29:6449–6460
Buckingham JC (1979) Corticotrophin releasing factor. Pharmacol Rev 31:253–275
Budziszewska B, Zajac A, Basta-Kaim A, Leskiewicz M, Steczkowska M, Lason W, Kacinski M (2010) Effects of neurosteroids on the human corticotropin-releasing hormone gene. Pharmacol Rep 62:1030–1040
Campeau S, Watson SJ (1997) Neuroendocrine and behavioral responses and brain pattern of c-fos induction associated with audiogenic stress. J Neuroendocrinol 9:577–588
Charalampopoulos I, Remboutsika E, Margioris AN, Gravanis A (2008) Neurosteroids as modulators of neurogenesis and neuronal survival. Trends Endocrinol Metab 19:300–307
Choi DC, Furay AR, Evanson NK, Ostrander MM, Ulrich-Lai YM, Herman JP (2007) Bed nucleus of the stria terminalis subregions differentially regulate hypothalamic-pituitary-adrenal axis activity: implications for the integration of limbic inputs. J Neurosci 27:2025–2034
Christiansen AM, Herman JP, Ulrich-Lai YM (2011) Regulatory interactions of stress and reward on rat forebrain opioidergic and GABAergic circuitry. Stress 14:205–215
Cohen S J-D (2007) PSychological stress and disease. JAMA 298:1685–1687
Coulter DA, Carlson GC (2007) Functional regulation of the dentate gyrus by GABA-mediated inhibition. Prog Brain Res 163:235–243
Crawley JN, Glowa JR, Majewska MD, Paul SM (1986) Anxiolytic activity of an endogenous adrenal steroid. Brain Res 398:382–385
Cullinan WE (2000) GABA(A) receptor subunit expression within hypophysiotropic CRH neurons: a dual hybridization histochemical study. J Comp Neurol 419:344–351
Cullinan WE, Wolfe TJ (2000) Chronic stress regulates levels of mRNA transcripts encoding beta subunits of the GABA(A) receptor in the rat stress axis. Brain Res 887:118–124
Cullinan WE, Herman JP, Watson SJ (1993) Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. J Comp Neurol 332:1–20
Cullinan WE, Ziegler DR, Herman JP (2008) Functional role of local GABAergic influences on the HPA axis. Brain Struct Funct 213:63–72
Czeh B, Simon M, van der Hart MG, Schmelting B, Hesselink MB, Fuchs E (2005) Chronic stress decreases the number of parvalbumin-immunoreactive interneurons in the hippocampus: prevention by treatment with a substance P receptor (NK1) antagonist. Neuropsychopharmacology 30:67–79
Dallman MF, Akana SF, Jacobson L, Levin N, Cascio CS, Shinsako J (1987) Characterization of corticosterone feedback regulation of ACTH secretion. Ann N Y Acad Sci 512:402–414
de Groote L, Linthorst AC (2007) Exposure to novelty and forced swimming evoke stressor-dependent changes in extracellular GABA in the rat hippocampus. Neuroscience 148:794–805

de Kloet ER, Vreugdenhil E, Oitzl MS, Joels M (1998) Brain corticosteroid receptor balance in health and disease. Endocr Rev 19:269–301

De Souza EB, Goeders NE, Kuhar MJ (1986) Benzodiazepine receptors in rat brain are altered by adrenalectomy. Brain Res 381:176–181

de Weerth C, Buitelaar JK (2005) Cortisol awakening response in pregnant women. Psychoneuroendocrinology 30:902–907

Decavel C, van den Pol AN (1990) GABA: a dominant neurotransmitter in the hypothalamus. J Comp Neurol 302:1019–1037

Decavel C, van den Pol AN (1992) Converging GABA- and glutamate-immunoreactive axons make synaptic contact with identified hypothalamic neurosecretory neurons. J Comp Neurol 316:104–116

Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, Tsutsui K, Tonon MC, Pelletier G, Vaudry H (2009) Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. Front Neuroendocrinol 30:259–301

Dong E, Matsumoto K, Uzunova V, Sugaya I, Takahata H, Nomura H, Watanabe H, Costa E, Guidotti A (2001) Brain 5alpha-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. Proc Natl Acad Sci U S A 98:2849–2854

Duveau V, Laustela S, Barth L, Gianolini F, Vogt KE, Keist R, Chandra D, Homanics GE, Rudolph U, Fritschy JM (2011) Spatiotemporal specificity of GABAA receptor-mediated regulation of adult hippocampal neurogenesis. Eur J Neurosci 34:362–373

Earnheart JC, Schweizer C, Crestani F, Iwasato T, Itohara S, Mohler H, Luscher B (2007) GABAergic control of adult hippocampal neurogenesis in relation to behavior indicative of trait anxiety and depression states. J Neurosci 27:3845–3854

Eechaute WP, Dhooge WS, Gao CQ, Calders P, Rubens R, Weyne J, Kaufman JM (1999) Progesterone-transforming enzyme activity in the hypothalamus of the male rat. J Steroid Biochem Mol Biol 70:159–167

Egli RE, Winder DG (2003) Dorsal and ventral distribution of excitable and synaptic properties of neurons of the bed nucleus of the stria terminalis. J Neurophysiol 90:405–414

Fenelon VS, Herbison AE (1995) Characterisation of GABAA receptor gamma subunit expression by magnocellular neurones in rat hypothalamus. Brain Res Mol Brain Res 34:45–56

Fritschy JM, Mohler H (1995) GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359:154–194

Finlay JM, Zigmond MJ, Abercrombie ED (1995) Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: Effects of diazepam. Neuroscience 64(3):619–628

Gao CQ, Dhooge WS, Kaufman JM, Weyne JJ, Eechaute WP (2002) Hypothalamic 5 alpha-reductase and 3 alpha-oxidoreductase activity in the male rat. J Steroid Biochem Mol Biol 80:91–98

Glykys J, Mann EO, Mody I (2008) Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? J Neurosci 28:1421–1426

Goeders NE, De Souza EB, Kuhar MJ (1986) Benzodiazepine receptor GABA ratios: regional differences in rat brain and modulation by adrenalectomy. Eur J Pharmacol 129:363–366

Gould E, Mcewen BS, Tanapat P, Galea LA, Fuchs E (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J Neurosci 17:2492–2498

Graziano P (2011) Neurosteroid biosynthesis upregulation: a novel promising therapy for anxiety disorders and PTSD, Anxiety Disorders, Prof. Vladimir Kalinin (Ed.), ISBN: 978-953-307-592-1, In Tech, DOI: 10.5772/18472. Available from: http://www.intechopen.com/books/anxiety-disorders/neurosteroid-biosynthesis-upregulation-a-novel promising-therapy-for-anxiety-disorders-and-ptsd

Gruen RJ, Wenberg K, Elahi R, Friedhoff AJ (1995) Alterations in GABAA receptor binding in the prefrontal cortex following exposure to chronic stress. Brain Res 684:112–114

Gunn BG, Brown AR, Lambert JJ, Belelli D (2011) Neurosteroids and GABA(A) receptor interactions: a focus on stress. Front Neurosci 5:131

Hasler G, van der Veen JW, Grillon C, Drevets WC, Shen J (2010) Effect of acute psychological stress on prefrontal GABA concentration determined by proton magnetic resonance spectroscopy. Am J Psychiatry 167:1226–1231

Herman JP, Cullinan WE (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20:78–84

Herman JP, Patel PD, Akil H, Watson SJ (1989) Localization and regulation of glucocorticoid and mineralocorticoid receptor messenger RNAs in the hippocampal formation of the rat. Mol Endocrinol 3:1886–1894

Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE (2003) Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front Neuroendocrinol 24:151–180

Herman JP, Mueller NK, Figueiredo H (2004) Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. Ann N Y Acad Sci 1018:35–45

Herman JP, Ostrander MM, Mueller NK, Figueiredo H (2005) Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Prog Neuropsychopharmacol Biol Psychiatry 29:1201–1213

Hewitt SA, Wamsteeker JI, Kurz EU, Bains JS (2009) Altered chloride homeostasis removes synaptic inhibitory constraint of the stress axis. Nat Neurosci 12:438–443

Hill MN, Tasker JG (2012) Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic-pituitary-adrenal axis. Neuroscience 204:5–16

Holm MM, Nieto-Gonzalez JL, Vardya I, Henningsen K, Jayatissa MN, Wiborg O, Jensen K (2011) Hippocampal GABAergic dysfunction in a rat chronic mild stress model of depression. Hippocampus 21:422–433

Hosie AM, Wilkins ME, da Silva HM, Smart TG (2006) Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. Nature 444:486–489

Hosie AM, Clarke L, da SH, Smart TG (2009) Conserved site for neurosteroid modulation of GABA A receptors. Neuropharmacology 56:149–154

Houston CM, McGee TP, Mackenzie G, Troyano-Cuturi K, Rodriguez PM, Kutsarova E, Diamanti E, Hosie AM, Franks NP, Brickley SG (2012) Are extrasynaptic GABAA receptors important targets for sedative/hypnotic drugs? J Neurosci 32:3887–3897

Hu W, Zhang M, Czeh B, Flugge G, Zhang W (2010) Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. Neuropsychopharmacology 35:1693–1707

Jacobson L (2005) Hypothalamic-pituitary-adrenocortical axis regulation. Endocrinol Metab Clin North Am 34:271–292, vii

Jacobson L, Sapolsky R (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr Rev 12:118–134

Jakab RL, Horvath TL, Leranth C, Harada N, Naftolin F (1993) Aromatase immunoreactivity in the rat brain: gonadectomy-sensitive hypothalamic neurons and an unresponsive “limbic ring” of the lateral septum-bed nucleus-amygdala complex. J Steroid Biochem Mol Biol 44:481–498

Jankord R, Herman JP (2008) Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. Ann N Y Acad Sci 1148:64–73

Joels M, Karst H, Alfarez D, Heine VM, Qin Y, van RE, Verkuyl M, Lucassen PJ, Krugers HJ (2004) Effects of chronic stress on structure and cell function in rat hippocampus and hypothalamus. Stress 7:221–231

Jones A, Korpi ER, McKernan RM, Pelz R, Nusser Z, Makela R, Mellor JR, Pollard S, Bahn S, Stephenson FA, Randall AD, Sieghart W, Somogyi P, Smith AJ, Wisden W (1997) Ligand-gated ion channel subunit partnerships: GABAA receptor alpha6 subunit gene inactivation inhibits delta subunit expression. J Neurosci 17:1350–1362

Kammerer M, Adams D, Castelberg BV, Glover V (2002) Pregnant women become insensitive to cold stress. BMC Pregnancy Childbirth 2:8

Kammerer M, Taylor A, Glover V (2006) The HPA axis and perinatal depression: a hypothesis. Arch Womens Ment Health 9:187–196

Larsen PJ, Seier V, Fink-Jensen A, Holst JJ, Warberg J, Vrang N (2003) Cocaine- and amphetamine-regulated transcript is present in hypothalamic neuroendocrine neurones and is released to the hypothalamic-pituitary portal circuit. J Neuroendocrinol 15:219–226

Lee HH, Walker JA, Williams JR, Goodier RJ, Payne JA, Moss SJ (2007) Direct protein kinase C-dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter KCC2. J Biol Chem 282:29777–29784

Lee HH, Deeb TZ, Walker JA, Davies PA, Moss SJ (2011) NMDA receptor activity downregulates KCC2 resulting in depolarizing GABA(A) receptor-mediated currents. Nat Neurosci 14:736–743

Levy BH, Tasker JG (2012) Synaptic regulation of the hypothalamic-pituitary-adrenal axis and its modulation by glucocorticoids and stress. Front Cell Neurosci 6:24

Li X, Bertics PJ, Karavolas HJ (1997) Regional distribution of cytosolic and particulate 5alpha-dihydroprogesterone 3alpha-hydroxysteroid oxidoreductases in female rat brain. J Steroid Biochem Mol Biol 60:311–318

Liu GX, Cai GQ, Cai YQ, Sheng ZJ, Jiang J, Mei Z, Wang ZG, Guo L, Fei J (2007) Reduced anxiety and depression-like behaviors in mice lacking GABA transporter subtype 1. Neuropsychopharmacol 32:1531–1539

Lloyd RB, Nemeroff CB (2011) The role of corticotropin-releasing hormone in the pathophysiology of depression: therapeutic implications. Curr Top Med Chem 11:609–617

Lopez JF, Akil H, Watson SJ (1999) Neural circuits mediating stress. Biol Psychiatry 46:1461–1471

Luther JA, Daftary SS, Boudaba C, Gould GC, Halmos KC, Tasker JG (2002) Neurosecretory and non-neurosecretory parvocellular neurones of the hypothalamic paraventricular nucleus express distinct electrophysiological properties. J Neuroendocrinol 14:929–932

Maguire J, Mody I (2007) Neurosteroid synthesis-mediated regulation of GABA(A) receptors: relevance to the ovarian cycle and stress. J Neurosci 27:2155–2162

Maguire J, Mody I (2009) Steroid hormone fluctuations and GABA(A)R plasticity. Psychoneuroendocrinology 34(Suppl 1):S84–S90

Mahan AL, Ressler KJ (2012) Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. Trends Neurosci 35(1):24–35

Majewska MD, Bisserbe JC, Eskay RL (1985) Glucocorticoids are modulators of GABAA receptors in brain. Brain Res 339:178–182

Maldonado-Aviles JG, Curley AA, Hashimoto T, Morrow AL, Ramsey AJ, O'Donnell P, Volk DW, Lewis DA (2009) Altered markers of tonic inhibition in the dorsolateral prefrontal cortex of subjects with schizophrenia. Am J Psychiatry 166:450–459

Marowsky A, Rudolph U, Fritschy JM, Arand M (2012) Tonic inhibition in principal cells of the amygdala: a central role for alpha3 subunit-containing GABAA receptors. J Neurosci 32:8611–8619

Matsumoto K, Nomura H, Murakami Y, Taki K, Takahata H, Watanabe H (2003) Long-term social isolation enhances picrotoxin seizure susceptibility in mice: up-regulatory role of endogenous brain allopregnanolone in GABAergic systems. Pharmacol Biochem Behav 75:831–835

Mcewen BS (2001) Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. Ann N Y Acad Sci 933:265–277

Mcewen BS, Eiland L, Hunter RG, Miller MM (2012) Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. Neuropharmacology 62:3–12

Miklos IH, Kovacs KJ (2002) GABAergic innervation of corticotropin-releasing hormone (CRH)-secreting parvocellular neurons and its plasticity as demonstrated by quantitative immunoelectron microscopy. Neuroscience 113:581–592

Mody I, Maguire J (2011) The reciprocal regulation of stress hormones and GABA(A) receptors. Front Cell Neurosci 6:4

Motohashi N, Okamoto Y, Osada M, Yamawaki S (1993) Acute swim stress increases benzodiazepine receptors, but not GABAA or GABAB receptors, in the rat cerebral cortex. Neurochem Int 23:327–330

Naert G, Maurice T, Tapia-Arancibia L, Givalois L (2007) Neuroactive steroids modulate HPA axis activity and cerebral brain-derived neurotrophic factor (BDNF) protein levels in adult male rats. Psychoneuroendocrinology 32:1062–1078

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

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

Orchinik M, Weiland NG, Mcewen BS (1994) Adrenalectomy selectively regulates GABAA receptor subunit expression in the hippocampus. Mol Cell Neurosci 5:451–458.

Orchinik M, Weiland NG, Mcewen BS (1995) Chronic exposure to stress levels of corticosterone alters GABAA receptor subunit mRNA levels in rat hippocampus. Brain Res Mol Brain Res 34:29–37

Otero Losada ME (1988) Changes in central GABAergic function following acute and repeated stress. Br J Pharmacol 93:483–490

Owens MJ, Ritchie JC, Nemeroff CB (1992) 5 alpha-pregnane-3 alpha, 21-diol-20-one (THDOC) attenuates mild stress-induced increases in plasma corticosterone via a non-glucocorticoid mechanism: comparison with alprazolam. Brain Res 573:353–355

Pariante CM, Lightman SL (2008) The HPA axis in major depression: classical theories and new developments. Trends Neurosci 31:464–468

Park JB, Skalska S, Son S, Stern JE (2007) Dual GABAA receptor-mediated inhibition in rat presympathetic paraventricular nucleus neurons. J Physiol 582:539–551

Park JB, Jo JY, Zheng H, Patel KP, Stern JE (2009) Regulation of tonic GABA inhibitory function, presympathetic neuronal activity and sympathetic outflow from the paraventricular nucleus by astroglial GABA transporters. J Physiol 587:4645–4660

Patchev VK, Shoaib M, Holsboer F, Almeida OF (1994) The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience 62:265–271

Payne JA, Rivera C, Voipio J, Kaila K (2003) Cation-chloride co-transporters in neuronal communication, development and trauma. Trends Neurosci 26:199–206

Pinna G, Dong E, Matsumoto K, Costa E, Guidotti A (2003) In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proc Natl Acad Sci U S A 100:2035–2040

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815–850

Purdy RH, Morrow AL, Moore PH, Paul SM (1991) Stress-induced elevations of gamma-aminobutyric-acid type-A receptor-active steroids in the Rat-Brain. Proc Natl Acad Sci U S A 88:4553–4557

Radley JJ, Gosselink KL, Sawchenko PE (2009) A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response. J Neurosci 29:7330–7340

Reddy DS (2003) Is there a physiological role for the neurosteroid THDOC in stress-sensitive conditions? Trends Pharmacol Sci 24:103–106

Reddy DS, Rogawski MA (2002) Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. J Neurosci 22:3795–3805

Reul JM, de Kloet ER (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinol 117:2505–2511

Reul JM, de Kloet ER (1986) Anatomical resolution of two types of corticosterone receptor sites in rat brain with in vitro autoradiography and computerized image analysis. J Steroid Biochem 24:269–272

Reznikov LR, Reagan LP, Fadel JR (2008) Activation of phenotypically distinct neuronal subpopulations in the anterior subdivision of the rat basolateral amygdala following acute and repeated stress. J Comp Neurol 508:458–472

Reznikov LR, Reagan LP, Fadel JR (2009) Effects of acute and repeated restraint stress on GABA efflux in the rat basolateral and central amygdala. Brain Res 1256:61–68

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K (1999) The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251–255

Rivera C, Voipio J, Kaila K (2005) Two developmental switches in GABAergic signalling: the K+-Cl- cotransporter KCC2 and carbonic anhydrase CAVII. J Physiol 562:27–36

Rodriguez Manzanares PA, Isoardi NA, Carrer HF, Molina VA (2005) Previous stress facilitates fear memory, attenuates GABAergic inhibition, and increases synaptic plasticity in the rat basolateral amygdala. J Neurosci 25:8725–8734

Roland BL, Sawchenko PE (1993) Local origins of some GABAergic projections to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J Comp Neurol 332:123–143

Roozendaal B, Mcewen BS, Chattarji S (2009) Stress, memory and the amygdala. Nat Rev Neurosci 10:423–433

Rosenkranz JA, Venheim ER, Padival M (2010) Chronic stress causes amygdala hyperexcitability in rodents. Biological Psychiatry 67:1128–1136

Saalmann YB, Kirkcaldie MT, Waldron S, Calford MB (2007) Cellular distribution of the GABAA receptor-modulating 3alpha-hydroxy, 5alpha-reduced pregnane steroids in the adult rat brain. J Neuroendocrinol 19:272–284

Sarkar J, Wakefield S, Mackenzie G, Moss SJ, Maguire J (2011) Neurosteroidogenesis is required for the physiological response to stress: role of neurosteroid-sensitive GABAA receptors. J Neurosci 31:18198–18210

Schloesser RJ, Manji HK, Martinowich K (2009) Suppression of adult neurogenesis leads to an increased hypothalamo-pituitary-adrenal axis response. Neuroreport 20:553–557

Schulte HM, Weisner D, Allolio B (1990) The corticotrophin releasing hormone test in late pregnancy: lack of adrenocorticotrophin and cortisol response. Clin Endocrinol (Oxf) 33:99–106

Serra M, Pisu MG, Littera M, Papi G, Sanna E, Tuveri F, Usala L, Purdy RH, Biggio G (2000) Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. J Neurochem 75:732–740

Serra M, Mostallino MC, Talani G, Pisu MG, Carta M, Mura ML, Floris I, Maciocco E, Sanna E, Biggio G (2006) Social isolation-induced increase in alpha and delta subunit gene expression is associated with a greater efficacy of ethanol on steroidogenesis and GABA receptor function. J Neurochem 98:122–133

Skerritt JH, Trisdikoon P, Johnston GA (1981) Increased GABA binding in mouse brain following acute swim stress. Brain Res 215:398–403

Skilbeck KJ, Johnston GA, Hinton T (2010) Stress and GABA receptors. J Neurochem 112:1115–1130

Sperk G, Schwarzer C, Tsunashima K, Fuchs K, Sieghart W (1997) GABA(A) receptor subunits in the rat hippocampus.1. Immunocytochemical distribution of 13 subunits. Neuroscience 80:987–1000

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABA(A) receptors. Proc Natl Acad Sci U S A 100:14439–14444

Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, McKernan RM (1999) Preferential coassembly of alpha 4 and delta subunits of the gamma-aminobutyric acid(A) receptor in rat thalamus. Mol Pharmacol 56:110–115

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

Tan H, Zhong P, Yan Z (2004) Corticotropin-releasing factor and acute stress prolongs serotonergic regulation of GABA transmission in prefrontal cortical pyramidal neurons. J Neurosci 24:5000–5008

Tanapat P, Galea LA, Gould E (1998) Stress inhibits the proliferation of granule cell precursors in the developing dentate gyrus. Int J Dev Neurosci 16:235–239

Tasker JG, Dudek FE (1993) Local inhibitory synaptic inputs to neurones of the paraventricular nucleus in slices of rat hypothalamus. J Physiol 469:179–192
Tasker JG, Di S, Malcher-Lopes R (2005) Rapid central corticosteroid effects: evidence for membrane glucocorticoid receptors in the brain. Integr Comp Biol 45:665–671
Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress responses. Nat Rev Neurosci 10:397–409
Ventura-Silva AP, Pego JM, Sousa JC, Marques AR, Rodrigues AJ, Marques F, Cerqueira JJ, Almeida OF, Sousa N (2012) Stress shifts the response of the bed nucleus of the stria terminalis to an anxiogenic mode. Eur J Neurosci 36(10):3396–3406
Verkuyl JM, Joels M (2003) Effect of adrenalectomy on miniature inhibitory postsynaptic currents in the paraventricular nucleus of the hypothalamus. J Neurophysiol 89:237–245
Verkuyl JM, Hemby SE, Joels M (2004) Chronic stress attenuates GABAergic inhibition and alters gene expression of parvocellular neurons in rat hypothalamus. Eur J Neurosci 20:1665–1673
Verkuyl JM, Karst H, Joels M (2005) GABAergic transmission in the rat paraventricular nucleus of the hypothalamus is suppressed by corticosterone and stress. Eur J Neurosci 21:113–121
Wamsteeker JI, Bains JS (2010) A synaptocentric view of the neuroendocrine response to stress. Eur J Neurosci 32:2011–2021
Watts AG (2005) Glucocorticoid regulation of peptide genes in neuroendocrine CRH neurons: a complexity beyond negative feedback. Front Neuroendocrinol 26:109–130
Weizman A, Weizman R, Kook KA, Vocci F, Deutsch SI, Paul SM (1990) Adrenalectomy prevents the stress-induced decrease in in vivo [3H]Ro15-1788 binding to GABAA benzodiazepine receptors in the mouse. Brain Res 519:347–350
Wilson MA, Biscardi R (1994) Sex differences in GABA/benzodiazepine receptor changes and corticosterone release after acute stress in rats. Exp Brain Res 101:297–306
Wilson MA, Biscardi R (1997) Influence of gender and brain region on neurosteroid modulation of GABA responses in rats. Life Sci 60:1679–1691
Wohlfarth KM, Bianchi MT, Macdonald RL (2002) Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. J Neurosci 22:1541–1549
Yoneda Y, Kanmori K, Ida S, Kuriyama K (1983) Stress-induced alterations in metabolism of gamma-aminobutyric acid in rat brain. J Neurochem 40:350–356
Zheng G, Zhang X, Chen Y, Zhang Y, Luo W, Chen J (2007) Evidence for a role of GABAA receptor in the acute restraint stress-induced enhancement of spatial memory. Brain Res 1181:61–73

Chapter 13
Tonic $GABA_A$-Receptor-Mediated Inhibition in Fragile-X Syndrome: A Cause of Dysfunction or a Pathway for a Cure?

Brandon S. Martin and Molly M. Huntsman

Abstract Persistent conductance through tonically active extrasynaptic $GABA_A$ receptors provides dynamic and powerful regulation of neuronal networks. The adverse effects of reduced inhibitory function of this receptor system range from mood disorders and anxiety to epilepsy. Converging lines of evidence support the hypothesis that alterations in cellular excitability are a common mechanism underlying neurodevelopmental disorders including those in the autism spectrum. Fragile X syndrome (FXS) is a leading genetic cause of intellectual disability and a leading monogenetic cause of autism. Several aspects of the behavioral phenotype in both human and mouse models point to excitatory/inhibitory imbalances in key brain regions. While the prevailing theory implicates excessive glutamatergic signaling, recent evidence suggests that cellular and behavioral excitability may also derive from dysfunction in inhibitory neurotransmission. This chapter will highlight specific examples of defective and reduced $GABA_A$-receptor-mediated tonic inhibitory neurotransmission as a contributing factor to the hyperexcitable phenotype observed in FXS.

Keywords Intellectual disability · Amygdala · Homeostasis · GABA · Inhibitory tone · GABA neuron

13.1 Introduction

13.1.1 Fragile X Syndrome is a Single Gene Disorder

Fragile X syndrome (FXS) is a leading genetic cause of intellectual disability affecting approximately one in 3600 individuals (Hagerman et al. 2009). In

M. M. Huntsman (✉)
Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences and Department of Pediatrics, School of Medicine, University of Colorado, Anschutz Medical Campus, C238, 12850 E. Montview Building V20, Room 3121, Aurora, CO 80045, USA
e-mail: molly.huntsman@UCDenver.edu

B. S. Martin
Children's National Medical Center, Washington, DC, USA

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5_13

addition to intellectual disabilities, FXS is associated with numerous other emotional and behavioral disorders including hyperactivity, anxiety, autism, and epilepsy (Hagerman et al. 2009). A substantial proportion of individuals meet the Diagnostic and Statistical Manual of Mental Disorders-fourth edition (DSM-IV) criteria for either autism or autism spectrum disorder (ASD), which makes FXS one of the leading genetic causes of autism (Hagerman et al. 2009; Kaufmann et al. 2004; Belmonte and Bourgeron 2006). FXS is linked to the expansion of an unstable cytosine-guanine-guanine (CGG) tri-nucleotide repeat in the regulatory (5′UTR) region of the *Fmr1* gene located on the X-chromosome. Moderate increases in CGG repeats at this locus (~45–200 base pairs vs. the normal CGG average ~30 base pairs) lead to a "premutation" phenotype resulting in distinctive clinical features such as primary ovarian insufficiency and fragile X-associated tremor/ataxia syndrome or FXTAS (Hagerman et al. 2009; Kenneson et al. 2001). This condition occurs in a proportionally larger population of individuals than does the full syndrome (one in 130–250 females and one in 250–800 males; Hagerman et al. 2009). When the expansion reaches greater than 200 CGG repeats, silencing of the *Fmr1* gene occurs (Hagerman et al. 2009) because of hypermethylation that prevents access of transcriptional machinery. This restriction leads to extreme reduction or elimination of the protein product fragile X mental retardation protein (FMRP). Consequently, the severity of the FXS behavioral and physical manifestations is linked to the amount of this FMRP reduction (Loesch et al. 2004). The primary animal model for FXS was generated in mouse by deletion of the *Fmr1* gene via homologous recombination resulting in a total lack of FMRP protein in the animal (Consortium 1994). *Fmr1* knockout (KO) mice display many of the same behavioral alterations and neuroanatomical deficiencies found in the human condition. At the behavioral level, these phenotypes include hyperactivity, anxiety, and social abnormalities (Bakker and Oostra 2003; McNaughton et al. 2008). On the cellular level, *Fmr1* KO mice also exhibit elongated spines on the dendrites of excitatory neurons in multiple brain regions similar to that reported in postmortem human brain tissue (Bagni and Greenough 2005; Olmos-Serrano et al. 2010; McKinney et al. 2005; Irwin et al. 2001; Comery et al. 1997; Braun and Segal 2000). Primary intracellular studies from *Fmr1* KOs reveal that FMRP is a regulator of protein translation and mRNA trafficking (Bassell and Warren 2008) and therefore plays a critical role in processes associated with mRNA transport and new protein synthesis required for mechanisms of synaptic plasticity (Kelleher et al. 2004). Activity of group I metabotropic glutamate receptors (Gp I mGluRs)—a powerful pathway for activity regulated protein synthesis (Weiler and Greenough 1993)—is a major target of FMRP regulation. Therefore, the loss of FMRP results in excessive activation of mechanisms downstream of Gp I mGluRs, most notably those involved in synaptic plasticity such as long-term depression (LTD). For instance, in hippocampal circuits of the *Fmr1* KO mouse LTD is "exaggerated" because the loss of FMRP prevents control of AMPA receptor retraction in response to Gp I mGluR activation (Bassell and Warren 2008; Huber et al. 2002). These results and those of numerous other related studies form the basis of "the mGluR theory" of FXS, which centrally implicates excessive glutamatergic signaling via Gp I

mGluRs in multiple cellular, synaptic, and behavioral phenotypes of FXS in both humans and animal models. In fact, reduction of GpI mGluR activity with mGluR antagonist treatment can rescue some, but importantly not all cellular and behavioral phenotypes of the disorder (Bear et al. 2004).

13.1.2 Deficiencies of the GABAergic System in FXS

While there is strong evidence in support of excessive glutamatergic-mediated signaling via Gp I mGluRs, a number of recent studies identify deficient GABAergic inhibitory transmission as a contributor to FXS phenotypes. Complementary deficits in these two receptor systems support a prevalent causal theory for many of the symptoms of FXS including autism—hyperexcitability and excitatory/inhibitory imbalance. Several marked symptoms of FXS including anxiety, autistic behaviors, epilepsy, and cognitive impairment likely stem from imbalances in neural circuits (Berry-Kravis et al. 2010; McNaughton et al. 2008; Cordeiro et al. 2011; Grigsby et al. 2007). In fact, many of the FXS comorbid disorders such as anxiety and epilepsy can be corrected with GABAergic agonists aimed to reduce hyperexcitability and maintain excitatory/inhibitory balance (Heulens et al. 2012; Olmos-Serrano et al. 2011; D'Hulst and Kooy 2007). Therefore, the GABAergic system remains a potentially viable, underexplored complement to FXS treatments aimed at excessive mGluR signaling.

Over the past 10 years, numerous studies have reported extensive alterations in critical components of the GABAergic system in behaviorally relevant forebrain regions such as the amygdala, cortex, and hippocampus in FXS (El Idrissi et al. 2005; Centonze et al. 2008; Chang et al. 2008; Olmos-Serrano et al. 2010; D'Hulst et al. 2006, 2009). FMRP is broadly expressed in GABAergic inhibitory interneuron populations throughout development, indicating that it is involved in normal interneuron maturation and function (Olmos-Serrano et al. 2010; Feng et al. 1997; Schütt et al. 2009). The loss of FMRP function presents many problems for presynaptic GABA release including the expression of scaffolding proteins, enhanced suppression of inhibition by endocannabinoid modulation, changes in levels of glutamic acid decarboxylase (GAD65/67, the enzyme required to synthesize GABA), decreases in GABA transporter expression (GAT1), decreases in enzymes for GABA catabolism (GABA-T and SSADH), and decrease in excitatory drive of parvalbumin-positive GABAergic interneurons (Schütt et al. 2009; Zhang and Alger 2010; Gibson et al. 2008; Adusei et al. 2010; D'Hulst et al. 2009; Olmos-Serrano et al. 2010; Liao et al. 2008; Curia et al. 2009; El Idrissi et al. 2005). Studies indicate that postsynaptically there are broad decreases in $GABA_A$ receptor subunits and postsynaptic GABAergic components such as gephyrin (El Idrissi et al. 2005; Curia et al. 2009; Adusei et al. 2010; D'Hulst et al. 2009). Importantly, the severities of these various pre- and postsynaptic deficits in FXS are region specific and therefore may have diverse effects on FXS phenotypes (Gibson et al. 2008; Zhang and Alger 2010; Olmos-Serrano et al. 2010; Paluszkiewicz et al. 2011).

Broad reductions of GABA synthesis and release affect the strength of synaptic inhibitory transmission that may contribute to hyperexcitability in brain areas relevant to FXS. However, this reduction in GABA availability spills over to affect GABA concentration in the extrasynaptic space regulating a powerful GABAergic tonic conductance that can significantly affect excitability and excitatory/inhibitory balance of a neuronal network. Accordingly, several independent lines of evidence implicate defective tonic GABAergic conductance in multiple brain regions in FXS. First and most strikingly relevant to FXS, the FMRP binds to the δ subunit of $GABA_A$ receptors, a major extrasynaptic subunit in FXS-relevant regions such as the cortex, hippocampus, and amygdala (Miyashiro et al. 2003; Dictenberg et al. 2008). In all three of these regions δ subunit expression (mRNA, protein, or both) is reduced (D'Hulst et al. 2006; Curia et al. 2009). The subiculum of the hippocampus shows additional reductions in mRNA and protein of another common extrasynaptic $GABA_A$ receptor participating in tonic conductance, the α5-subunit-containing receptor (Curia et al. 2009). Electrophysiological recordings also reveal reduced total tonic currents in pyramidal cells in this area. Despite this early evidence that the GABAergic system and tonic GABAergic conductance in particular may be affected in FXS, detailed electrophysiological studies examining the state of excitatory/inhibitory balance and the involvement of the GABAergic system in maintaining that balance have not been extensively conducted in key brain regions such as the cortex. However, recent studies have investigated tonic GABAergic conductance deficits in the FXS amygdala and the role of that conductance in affecting hyperexcitability.

13.2 Tonic Inhibition in the Amygdala and FXS

The amygdala is implicated in many of the symptoms of FXS including epilepsy, hyperactivity, autism, and social anxiety (Truitt et al. 2007, 2009; Sanders and Shekhar 1995). Moreover, the structure's involvement in the control of fear processing is well defined (Shaban et al. 2006; Likhtik et al. 2008; Sah and Westbrook 2008; Ehrlich et al. 2009; Poulos et al. 2009). Nonetheless, until recently, physiological studies on this highly integrated subcortical structure had not been performed in a model of FXS. Studies show that in the basolateral nucleus of the amygdala (BLA) of the *Fmr1* KO mouse model of FXS there are reductions in GABAergic inhibitory efficacy that include reduced GABA production and release, decreased frequency and amplitude of action-potential (AP)-dependent and independent inhibitory postsynaptic currents (IPSCs), and decreased tonic inhibitory tone. These deficits occur in association with increased excitability of principal excitatory projection neurons (PNs) as indicated by a reduced threshold to fire action potentials in *Fmr1* KO PNs compared to wild type (WT) (Olmos-Serrano et al. 2010). PNs make up approximately 70–80 % of the neurons in the BLA, receive a diverse array of sensory cortical and thalamic inputs (for review, see Sah et al. 2003), and project to and thereby regulate the output of the central nucleus (CeN) of the amygdala. The CeN

subsequently projects to and regulates downstream structures such as the hypothalamus, the cranial nerve nuclei, the locus coeruleus, and the bed nucleus of the stria terminalis that are involved in producing innate and conditioned fear responses. Therefore, increased excitability of PNs in the *Fmr1* KO BLA likely has considerable implications on proper information processing, input/output of the amygdala, and regulation of subsequent behavioral/physiological responses to stimuli that underlie the observed behavioral phenotypes of FXS.

GABAergic tonic inhibition powerfully controls cellular excitability (Brickley et al. 1996; Hamann et al. 2002; Mitchell and Silver 2003; Semyanov et al. 2003; Krook-Magnuson and Huntsman 2005; Bright et al. 2007) and may therefore affect thresholds for PN activity in the BLA. As mentioned above, PNs of the *Fmr1* KO BLA have reduced GABAergic tonic inhibitory tone. Importantly, the tonic current in these cells relies exclusively on action-potential-mediated release of GABA to bind to extrasynaptic tonically active $GABA_A$ receptors since blockade of action potentials with tetrodotoxin removes virtually all the GABAergic tonic current from both WT and *Fmr1* KO PNs. Because GABA production and release is limited in the *Fmr1* KO BLA, the availability of ligand for tonic receptors is also reduced which limits overall tonic currents. However, in addition to decreased GABA availability, *Fmr1* KO PNs also show decreased capacity for tonically active GABAergic currents when levels are assessed in the presence of saturating extrasynaptic GABA concentrations. Therefore, decreased tonic $GABA_A$ receptor expression or function likely also contributes to decreased tonic inhibitory tone in the *Fmr1* KO BLA (Olmos-Serrano et al. 2010; Martin et al. 2014).

Tonic GABAergic currents are comprised of at least three distinct $GABA_A$ receptor subtypes in the BLA (Fig. 13.1). First, the ubiquitously expressed δ-subunit-containing $GABA_A$ receptor plays a substantial role in PN tonic currents. The δ subunit is diffusely expressed throughout the amygdala (Fritschy and Mohler 1995; Pirker et al. 2000). Moreover, PNs of the BLA display δ-subunit-specific tonic currents as indicated by an increase in tonic current with application of the δ-subunit preferring GABA super agonist, 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP; ~20 pA increase; Olmos-Serrano et al. 2010; Martin et al. 2014). Importantly, this tonic component is significantly reduced in *Fmr1* KO PNs (Martin et al. 2014).

Recent evidence suggests the existence of a second, atypical tonic component in BLA PNs, the α3-subunit-containing $GABA_A$ receptor. Until recently (Marowsky et al. 2012), α3 subunits were not typically known for extrasynaptic expression (Fritschy et al. 1992; Pirker et al. 2000). However, we now know that in PNs a substantial portion of α3-subunit-containing $GABA_A$ receptors do not coexpress with the inhibitory postsynaptic marker, gephyrin, suggesting they reside outside the synapse. Furthermore, application of the α3-specific agonist TP003 induces an increase in tonic current in these cells (Marowsky et al. 2012). This specific component of tonic current in the BLA remains uninvestigated in FXS. However, given the considerable reduction in GABAergic components globally and in the BLA of *Fmr1* KO mice described above, the α3-subunit-mediated tonic current in *Fmr1* KO mice is also likely deficient.

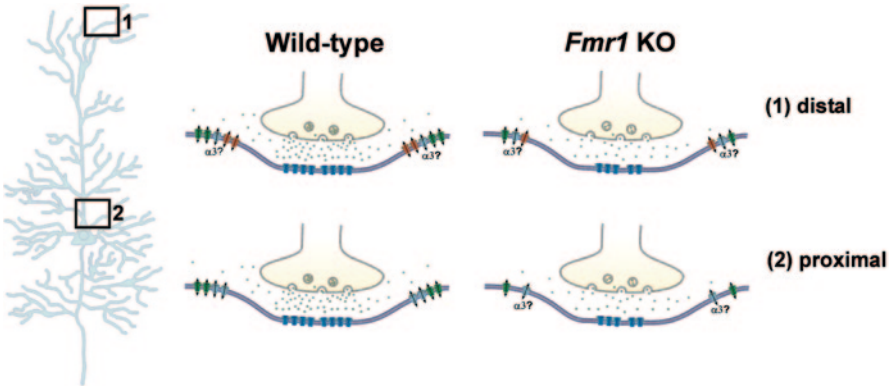

Fig. 13.1 Distribution of extrasynaptic $GABA_A$ receptors in proximal and distal regions of principal projection neurons (*PNs*) in the WT and *Fmr1* KO basolateral amygdala. In the WT situation, δ (*green*) and α3-subunit-containing receptors (*light blue*) are likely located ubiquitously in extrasynaptic membranes; whereas, α5-subunit-containing receptors (*red*) concentrate in the distal extrasynaptic compartments. Extrasynaptic $GABA_A$ receptors receive GABA from robust release of GABA from synapses that spills into the extrasynaptic space. In *Fmr1* KO PNs extrasynaptic $GABA_A$ receptors expression/function is globally reduced (the fate of α3-subunit-containing receptors is unknown). These reductions intensify when accompanied by weak production and release of GABA, thereby reducing the availability of GABA on a deficient extrasynaptic receptor population

BLA PNs express a third tonic GABAergic component common to the cortex and the hippocampus, the α5-subunit-containing receptor. This $GABA_A$ receptor subunit shows more diffuse expression in the BLA than δ-subunit-containing receptors (Fritschy and Mohler 1995), but our own experiments find that BLA PNs do have small α5-subunit-mediated tonic currents in the presence of saturating GABA concentrations revealed by blockade of α5-subunit-containing receptors with the α5-specific inverse agonist, α5ia (Martin et al. 2014). Interestingly, based on immunohistochemical and electrophysiological data (Marowsky et al. 2012; Olmos-Serrano et al. 2010; Martin et al. 2014), recent studies indicate that unlike δ- and α3-subunit-containing receptors which likely express ubiquitously in extrasynaptic membranes of PNs, α5-subunit-containing receptors are restricted to more distal extrasynaptic sites (Serwanski et al. 2006). In order to observe α5-subunit-dependent tonic currents, cesium must be included in the recording pipette to block outward potassium currents that normally reduce the ability of the patch-clamp electrode to maintain voltage control far from the somatic recording site. In the absence of cesium, distal synaptic events are not well detected (Isaacson et al. 1993; Stuart and Spruston 1998; Day et al. 2006)**.** Recordings of GABAergic currents without cesium in the pipette and therefore of mostly proximal inhibitory currents show no evidence of α5-subunit-mediated tonic current with addition of the α5-specific inverse agonist. Instead, these recordings paradoxically show *increases* in phasic IPSC efficacy (mean frequency and amplitude increase). Control experiments in the absence of action potentials reveal that this increased efficacy in

the presence of α5 subunit blockade results from the removal of a postsynaptic α5-subunit-dependent inhibitory shunt of distal synaptic events and not a presynaptic increase in GABA release. Thus, α5-subunit-containing $GABA_A$ receptors specifically perform tonic inhibition at distal sites on BLA PNs as opposed to the soma and proximal dendrites (Martin et al. 2014). Once again importantly, *Fmr1* KO PNs show decreased α5-subunit-dependent tonic currents and reduced increases in inhibitory synaptic efficacy compared to wild type with application of the α5-specific inverse agonist.

The distinct subcellular location and physiological roles of α5-subunit- versus α3- and δ-subunit-containing receptors places each receptor subtype in the position to modulate distinct aspects of PN excitation. Since α5-subunit-mediated tonic inhibition remains concentrated more in distal synaptic compartments, this mode of tonic conductance is well positioned to modulate glutamatergic synaptic plasticity (i.e., LTP and LTD) that underlies amygdala coding by restricting excitation received from specific excitatory afferents as well as phasic inhibition to distinct dendritic compartments. GABAergic inhibition has been demonstrated to be crucial for input discrimination and spike timing-dependent plasticity in neuronal networks including those of the amygdala (Jang and Kwag 2012; Shin et al. 2006). A lack of tonic GABAergic control in the dendritic compartment that exists in *Fmr1* KO PNs as a result of deficient α5-subunit-mediated tonic inhibition may therefore contribute to a generalization or weakening of distinct plastic changes necessary to appropriately code incoming sensory information and maintain proper associations between BLA afferents representing for instance the conditioned stimulus and unconditioned stimulus in the typical fear-conditioning paradigm. This deficit may also compound known ineffective plasticity mechanisms at glutamatergic synapses instigated by the loss of FMRP in these compartments (Suvrathan et al. 2010). This combined phenomenon could at least partially underlie amygdala-related phenotypes in the *Fmr1* KO mouse; particularly fear conditioning and extinction deficits and anxiety. Currently, however, no studies have addressed this possibility.

Overall PN excitability control is complementary to maintenance of proper dendritic coding. To this end, δ- and α3-subunit-containing receptors that are conspicuously positioned closer to the soma and axon initial segment compared to α5-subunit-containing receptors probably play an important role. As described above, *Fmr1* KO PNs show a hyperexcitability phenotype defined by a decreased threshold to fire action potentials. In conjunction with this phenotype, *Fmr1* KO mice demonstrate hyperactive behavior compared to their wild-type counterparts. Supplementation with THIP, the δ-subunit-specific GABAergic super agonist improves both the in vitro cellular hyperexcitable phenotype, shifting firing threshold to wild-type levels (Olmos-Serrano et al. 2010) as well as the in vivo behavioral hyperactivity by reducing hyperactive behavior close to wild-type levels when acutely injected (Olmos-Serrano et al. 2011). These results establish the efficacy of targeting at least δ-subunit-specific tonic inhibition to reduce the expression of FXS phenotypes. Consequently given the division of labor by tonic components in the amygdala (α5 vs. δ/α3), distinct pharmacological targeting of these

components may improve discrete but complementary dysfunctions in amygdala coding and excitability related to various amygdala-based dysfunctions in FXS.

13.3 Tonic Inhibitory Transmission in FXS: A Cause or a Cure?

Multiple factors likely contribute to the hyperexcitable phenotype in FXS, so it is worth asking, "Does the presence of defective tonic inhibitory transmission directly produce the phenotype or is tonic GABAergic deficiency simply a viable therapeutic target to correct that phenotype?" As of yet, the δ subunit is the only $GABA_A$ receptor subunit mRNA identified as a direct target of FMRP (Dictenberg et al. 2008; Miyashiro et al. 2003). Despite the known interaction, current studies reveal little about how FMRP modulates δ subunit mRNA translation and subsequent tonic $GABA_A$ receptor assembly. Basic alterations in $GABA_A$ receptor subunit expression may be regulated in an activity-dependent manner (Huntsman et al. 1994). If FMRP regulates δ subunit expression, the reduction of at least the δ-subunit-mediated tonic conductance in *Fmr1* KO mice could result from a failure of compensatory mechanisms to regulate changes in neuron and circuit excitability. Additionally, some recent evidence indicates that $GABA_A$ receptor subunit translation depends on the concurrent translation of compatible subunits. For instance, the loss of β2 subunit expression in mice results in decreases in expression of $GABA_A$ receptor α subunits normally assembled with β2 subunits (Sur et al. 2001). In this way, a similar misexpression of the δ subunit in FXS could downregulate translation of coassembled subunits (i.e., α4 or β1,2,3), which may then subsequently produce a cascade of alterations of additional subunit expression, tonic (i.e., α5) and/or phasic (i.e., α1). However, this possibility has not yet been directly explored.

Using the FXS BLA as an example, reduction in $GABA_A$ receptor expression related to the loss of FMRP, especially in combination with presynaptic GABA production and release deficits (Vislay et al. 2013; Olmos-Serrano et al. 2010; D'Hulst et al. 2006; Gantois et al. 2006) could cause a loss of tonic inhibitory tone that may contribute to PN hyperexcitability in FXS. However, some evidence does not fully support this conclusion. For instance the reduction of a persistent tonic inhibitory conductance does not correlate with changes in some passive membrane properties of *Fmr1* KO PNs compared to wild type. Properties such as input resistance and membrane decay constant do not show significant differences (Olmos-Serrano et al. 2010). Since tonic GABAergic conductance has been shown to heavily modulate gain in neurons (Semyanov et al. 2003; Mitchell and Silver 2003) one might expect a decrease in tonic GABAergic tone to be accompanied by an increase in membrane input resistance. However, this condition is not the case perhaps owing to compensatory expression of other channels such as potassium channels. Although input resistance does not seem to rely on the level of tonic conductance in *Fmr1* KO PNs, recent evidence from the hippocampus suggests that GABAergic tonic conductance can sometimes principally affect membrane offset independently of

gain, if those participating tonically active receptors outwardly rectify and therefore pass a greater amount of current at more depolarized (near action-potential threshold) membrane potentials (Pavlov et al. 2009). A close examination of the input/output curves of *Fmr1* KO versus wild-type PNs reveals that the slope of the curve and therefore neuronal gain appears unchanged while only the offset appears affected between genotypes (Olmos-Serrano et al. 2010). Therefore, while not yet directly tested, a persistent tonic conductance in BLA PNs could potentially outwardly rectify similarly to those currents in the hippocampus and perform the role of maintaining neuronal offset. Indeed all the possibilities described above remain speculative and changes in PN excitability in the *Fmr1* KO BLA likely result from a combination of mechanisms involving deficient phasic and tonic GABAergic inhibition as well as altered passive and active membrane properties that rely on changes in voltage activated sodium, calcium, and potassium channel expression yet to be investigated in FXS.

Regardless of the degree of cause that can be attributed to deficient tonic conductance in determining cell excitability in FXS, it remains clear that supplementation can improve FXS phenotypes making GABAergic tonic conductance a viable target for FXS treatment (Olmos-Serrano et al. 2010, 2011). GABAergic tonic conductance is many orders of magnitude stronger than phasic inhibitory conductance and therefore can more effectively and dynamically control neuronal gain and/or offset (Semyanov et al. 2003; Mitchell and Silver 2003). Therefore, specifically targeting tonic conductance can affect cell and network excitability more readily than targeting phasic inhibitory transmission. In addition, pharmacological targeting of extrasynaptic receptors may carry reduced potential for unwanted side effects by enabling targeting of a more specific $GABA_A$ receptor pool. For example, benzodiazepines are known to be effective $GABA_A$ receptor agonists that permit limited drug interactions and typically affect phasic receptors but also have efficacy at some tonic receptors (i.e., α5-subunit containing) (reviewed in Mohler et al. 2004). Benzodiazepine sensitive $GABA_A$ receptor subtypes mediate different aspects of receptor activation such as anxiolytic or sedative actions based on composition (reviewed in Mohler et al. 2004). Although $GABA_A$-receptor-subtype-specific benzodiazepines have been synthesized, a residual degree of benzodiazepine promiscuity with nontargeted $GABA_A$ receptor subtypes or unwanted effects in networks outside the region of interest contributes to often unwanted side effects related to increased somnolence, respiratory depression, and rebound symptoms such as increased anxiety when treatment is discontinued (Nemeroff 2003). Targeting extrasynaptic receptors such as the δ-subunit-containing receptor provides a more isolated pharmacological target. For example, compounds that preferentially activate these extrasynaptic $GABA_A$ receptors such as neuroactive steroids (see Chap. 10) often have a much greater specificity for extrasynaptic receptors than they have for phasic receptors (Reddy 2010). These compounds are effective at reducing hyperexcitability in FXS as demonstrated by the reduction of audiogenic seizures in mice dosed with the neuroactive steroid ganaxolone (Heulens et al. 2012). In addition, as described above, the δ-subunit preferring agonist THIP can rescue cellular and behavioral phenotypes in the *Fmr1* KO mouse (Olmos-Serrano

et al. 2010, 2011). Thus, deficient tonic conductance may not lie at the root of the disorder, but the strength of its ability to modulate neuronal networks makes tonically active $GABA_A$ receptors promising sites of therapeutic intervention for a myriad of FXS symptoms including autistic behaviors, epilepsy, and anxiety.

Conclusions

An emerging theme in neurodevelopmental disorders implicates defective GABAergic system components as a major contributor to specific cellular, circuit, and behavioral phenotypes. Studies in *Fmr1* KO mice reveal disturbances of the GABAergic system and functional inhibitory neurotransmission in a number of brain regions that are highly relevant to the FXS phenotype including the amygdala. Thus, the GABAergic system presents an important pharmacological target for the treatment of a number of the neurological manifestations of FXS. The biophysical aspects and extrasynaptic localization of tonically active $GABA_A$ receptors makes these particular GABAergic components intriguing candidates to improve function of abnormally developed networks by regulating circuit excitability and output.

Acknowledgments This work is supported by the National Institutes of Health (NIH, NS053719), Autism Speaks (MMH), FRAXA (MMH), and an Epilepsy Foundation predoctoral training fellowship (BSM).

References

Adusei DC, Pacey LK, Chen D, Hampson DR (2010) Early developmental alterations in GABAergic protein expression in fragile X knockout mice. Neuropharmacology 59:167–171

Bagni C, Greenough WT (2005) From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Nat Rev Neurosci 6:376–387

Bakker CE, Oostra BA (2003) Understanding fragile X syndrome: insights from animal models. Cytogenet Genome Res 100:111–123

Bassell GJ, Warren ST (2008) Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron 60:201–214

Bear MF, Huber KM, Warren ST (2004) The mGluR theory of fragile X mental retardation. Trends Neurosci 27:370–377

Belmonte MK, Bourgeron T (2006) Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat Neurosci 9:1221–1225

Berry-Kravis E, Raspa M, Loggin-Hester L, Bishop E, Holiday D, Bailey DB (2010) Seizures in fragile X syndrome: characteristics and comorbid diagnoses. Am J Intellect Dev Disabil 115:461–472

Braun K, Segal M (2000) FMRP involvement in formation of synapses among cultured hippocampal neurons. Cereb Cortex 10:1045–1052

Brickley SG, Cull-Candy SG, Farrant M (1996) Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of $GABA_A$ receptors. J Physiol 497(Pt 3):753–759

Bright DP, Aller MI, Brickley SG (2007) Synaptic release generates a tonic GABA(A) receptor-mediated conductance that modulates burst precision in thalamic relay neurons. J Neurosci 27:2560–2569

Centonze D, Rossi S, Mercaldo V, Napoli I, Ciotti MT, De Chiara V, Musella A, Prosperetti C, Calabresi P, Bernardi G, Bagni C (2008) Abnormal striatal GABA transmission in the mouse model for the fragile X syndrome. Biol Psychiatry 63:963–973

Chang S, Bray SM, Li Z, Zarnescu DC, He C, Jin P, Warren ST (2008) Identification of small molecules rescuing fragile X syndrome phenotypes in *Drosophila*. Nat Chem Biol 4:256–263

Comery TA, Harris JB, Willems PJ, Oostra BA, Irwin SA, Weiler IJ, Greenough WT (1997) Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proc Natl Acad Sci U S A 94:5401–5404

Consortium TD-BFX (1994) Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. Cell 78:23–33

Cordeiro L, Ballinger E, Hagerman R, Hessl D (2011) Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. J Neurodev Disord 3:57–67

Curia G, Papouin T, Seguela P, Avoli M (2009) Downregulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. Cereb Cortex 19:1515–1520

D'Hulst C, Kooy RF (2007) The $GABA_A$ receptor: a novel target for treatment of fragile X? Trends Neurosci 30:425–431

D'Hulst C, De Geest N, Reeve SP, Van Dam D, De Deyn PP, Hassan BA, Kooy RF (2006) Decreased expression of the $GABA_A$ receptor in fragile X syndrome. Brain Res 1121:238–245

D'Hulst C, Heulens I, Brouwer JR, Willemsen R, De Geest N, Reeve SP, De Deyn PP, Hassan BA, Kooy RF (2009) Expression of the GABAergic system in animal models for fragile X syndrome and fragile X associated tremor/ataxia syndrome (FXTAS). Brain Res 1253:176–183

Day M, Wang Z, Ding J, An X, Ingham CA, Shering AF, Wokosin D, Ilijic E, Sun Z, Sampson AR, Mugnaini E, Deutch AY, Sesack SR, Arbuthnott GW, Surmeier DJ (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 9:251–259

Dictenberg JB, Swanger SA, Antar LN, Singer RH, Bassell GJ (2008) A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome. Dev Cell 14:926–939

Ehrlich I, Humeau Y, Grenier F, Ciocchi S, Herry C, Luthi A (2009) Amygdala inhibitory circuits and the control of fear memory. Neuron 62:757–771

El Idrissi A, Ding XH, Scalia J, Trenkner E, Brown WT, Dobkin C (2005) Decreased GABA(A) receptor expression in the seizure-prone fragile X mouse. Neurosci Lett 377:141–146

Feng Y, Gutekunst CA, Eberhart DE, Yi H, Warren ST, Hersch SM (1997) Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. J Neurosci 17:1539–1547

Fritschy JM, Mohler H (1995) $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359:154–194

Fritschy JM, Benke D, Mertens S, Oertel WH, Bachi T, Mohler H (1992) Five subtypes of type A gamma-aminobutyric acid receptors identified in neurons by double and triple immunofluorescence staining with subunit-specific antibodies. Proc Natl Acad Sci U S A 89:6726–6730

Gantois I, Vandesompele J, Speleman F, Reyniers E, D'Hooge R, Severijnen LA, Willemsen R, Tassone F, Kooy RF (2006) Expression profiling suggests underexpression of the GABA(A) receptor subunit delta in the fragile X knockout mouse model. Neurobiol Dis 21:346–357

Gibson JR, Bartley AF, Hays SA, Huber KM (2008) Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. J Neurophysiol 100:2615–2626

Grigsby J, Brega AG, Leehey MA, Goodrich GK, Jacquemont S, Loesch DZ, Cogswell JB, Epstein J, Wilson R, Jardini T, Gould E, Bennett RE, Hessl D, Cohen S, Cook K, Tassone F, Hagerman PJ, Hagerman RJ (2007) Impairment of executive cognitive functioning in males with fragile X-associated tremor/ataxia syndrome. Mov Disord 22:645–650

Hagerman RJ, Berry-Kravis E, Kaufmann WE, Ono MY, Tartaglia N, Lachiewicz A, Kronk R, Delahunty C, Hessl D, Visootsak J, Picker J, Gane L, Tranfaglia M (2009) Advances in the treatment of fragile X syndrome. Pediatrics 123:378–390

Hamann M, Rossi DJ, Attwell D (2002) Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. Neuron 33:625–633

Heulens I, D'Hulst C, Van Dam D, De Deyn PP, Kooy RF (2012) Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. Behav Brain Res 229:244–249

Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 99:7746–7750

Huntsman MM, Isackson PJ, Jones EG (1994) Lamina-specific expression and activity-dependent regulation of seven $GABA_A$ receptor subunit mRNAs in monkey visual cortex. J Neurosci 14:2236–2259

Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, Kooy RF, Willems PJ, Cras P, Kozlowski PB, Swain RA, Weiler IJ, Greenough WT (2001) Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. Am J Med Genet 98:161–167

Isaacson JS, Solis JM, Nicoll RA (1993) Local and diffuse synaptic actions of GABA in the hippocampus. Neuron 10:165–175.

Jang HJ, Kwag J (2012) GABA(A) receptor-mediated feedforward and feedback inhibition differentially modulate hippocampal spike timing-dependent plasticity. Biochem Biophys Res Commun 427(3):466–472

Kaufmann WE, Cortell R, Kau AS, Bukelis I, Tierney E, Gray RM, Cox C, Capone GT, Stanard P (2004) Autism spectrum disorder in fragile X syndrome: communication, social interaction, and specific behaviors. Am J Med Genet 129A:225–234

Kelleher RJ, 3rd, Govindarajan A, Tonegawa S (2004) Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron 44:59–73

Kenneson A, Zhang F, Hagedorn CH, Warren ST (2001) Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. Hum Mol Genet 10:1449–1454

Krook-Magnuson EI, Huntsman MM (2005) Excitability of cortical neurons depends upon a powerful tonic conductance in inhibitory networks. Thalamus Relat Syst 3:115–120

Liao L, Park SK, Xu T, Vanderklish P, Yates JR (2008) Quantitative proteomic analysis of primary neurons reveals diverse changes in synaptic protein content in fmr1 knockout mice. Proc Natl Acad Sci U S A 105:15281–15286

Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Paré D (2008) Amygdala intercalated neurons are required for expression of fear extinction. Nature 454:642–645

Loesch DZ, Huggins RM, Hagerman RJ (2004) Phenotypic variation and FMRP levels in fragile X. Ment Retard Dev Disabil Res Rev 10:31–41

Marowsky A, Rudolph U, Fritschy JM, Arand M (2012) Tonic inhibition in principal cells of the amygdala: a central role for alpha3 subunit-containing $GABA_A$ receptors. J Neurosci 32:8611–8619

Martin BS, Corbin JG, Huntsman MM (2014) Deficient tonic GABAergic conductance and synaptic balance in the Fragile-X Syndrome Amygdala. J Neurophysiol in press

McKinney BC, Grossman AW, Elisseou NM, Greenough WT (2005) Dendritic spine abnormalities in the occipital cortex of C57BL/6 Fmr1 knockout mice. Am J Med Genet B Neuropsychiatr Genet 136B:98–102

McNaughton CH, Moon J, Strawderman MS, Maclean KN, Evans J, Strupp BJ (2008) Evidence for social anxiety and impaired social cognition in a mouse model of fragile X syndrome. Behav Neurosci 122:293–300

Mitchell SJ, Silver RA (2003) Shunting inhibition modulates neuronal gain during synaptic excitation. Neuron 38:433–445

Miyashiro KY, Beckel-Mitchener A, Purk TP, Becker KG, Barret T, Liu L, Carbonetto S, Weiler IJ, Greenough WT, Eberwine J (2003) RNA cargoes associating with FMRP reveal deficits in cellular functioning in Fmr1 null mice. Neuron 37:417–431

Mohler H, Fritschy JM, Crestani F, Hensch T, Rudolph U (2004) Specific GABA(A) circuits in brain development and therapy. Biochem Pharmacol 68:1685–1690

Nemeroff C (2003) The role of GABA in the pathophysiology and treatment of anxiety disorders. Psychopharmacol Bull 37:133–146

Olmos-Serrano JL, Paluszkiewicz SM, Martin BS, Kaufmann WE, Corbin JG, Huntsman MM (2010) Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. J Neurosci 30:9929–9938

Olmos-Serrano JL, Corbin JG, Burns MP (2011) The GABA(A) receptor agonist THIP ameliorates specific behavioral deficits in the mouse model of fragile X syndrome. Dev Neurosci 33:395–403

Paluszkiewicz S, Martin B, Huntsman M (2011) Fragile X Syndrome: the GABAergic system and circuit dysfunction. Dev Neurosci 33:349–364

Pavlov I, Savtchenko LP, Kullmann DM, Semyanov A, Walker MC (2009) Outwardly rectifying tonically active $GABA_A$ receptors in pyramidal cells modulate neuronal offset, not gain. J Neurosci 29:15341–15350

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815–850

Poulos AM, Li V, Sterlace SS, Tokushige F, Ponnusamy R, Fanselow MS (2009) Persistence of fear memory across time requires the basolateral amygdala complex. Proc Natl Acad Sci U S A 106:11737–11741

Reddy DS (2010) Neurosteroids: endogenous role in the human brain and therapeutic potentials. Prog Brain Res 186:113–137

Sah P, Westbrook RF (2008) Behavioural neuroscience: the circuit of fear. Nature 454:589–590

Sah P, Faber ES, Lopez De Armentia M, Power J (2003) The amygdaloid complex: anatomy and physiology. Physiol Rev 83:803–834

Sanders SK, Shekhar A (1995) Regulation of anxiety by $GABA_A$ receptors in the rat amygdala. Pharmacol Biochem Behav 52:701–706

Schütt J, Falley K, Richter D, Kreienkamp HJ, Kindler S (2009) Fragile X mental retardation protein regulates the levels of scaffold proteins and glutamate receptors in postsynaptic densities. J Biol Chem 284:25479–25487

Semyanov A, Walker MC, Kullmann DM (2003) GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nat Neurosci 6:484–490

Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, De Blas AL (2006) Synaptic and nonsynaptic localization of $GABA_A$ receptors containing the alpha5 subunit in the rat brain. J Comp Neurol 499:458–470

Shaban H, Humeau Y, Herry C, Cassasus G, Shigemoto R, Ciocchi S, Barbieri S, van der Putten H, Kaupmann K, Bettler B, Luthi A (2006) Generalization of amygdala LTP and conditioned fear in the absence of presynaptic inhibition. Nat Neurosci 9:1028–1035

Shin RM, Tsvetkov E, Bolshakov VY (2006) Spatiotemporal asymmetry of associative synaptic plasticity in fear conditioning pathways. Neuron 52:883–896

Stuart G, Spruston N (1998) Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. J Neurosci 18:3501–3510

Sur C, Wafford KA, Reynolds DS, Hadingham KL, Bromidge F, Macaulay A, Collinson N, O'Meara G, Howell O, Newman R, Myers J, Atack JR, Dawson GR, McKernan RM, Whiting PJ, Rosahl TW (2001) Loss of the major GABA(A) receptor subtype in the brain is not lethal in mice. J Neurosci 21:3409–3418

Suvrathan A, Hoeffer CA, Wong H, Klann, E., Chattarji S (2010) Characterization and reversal of synaptic defects in the amygdala in a mouse model of fragile X syndrome. Proc Natl Acad Sci U S A 107:11591–11596.

Truitt WA, Sajdyk TJ, Dietrich AD, Oberlin B, McDougle CJ, Shekhar A (2007) From anxiety to autism: spectrum of abnormal social behaviors modeled by progressive disruption of inhibitory neuronal function in the basolateral amygdala in Wistar rats. Psychopharmacology 191:107–118

Truitt WA, Johnson PL, Dietrich AD, Fitz SD, Shekhar A (2009) Anxiety-like behavior is modulated by a discrete subpopulation of interneurons in the basolateral amygdala. Neuroscience 160:284–294

Vislay RL, Martin BM, Olmos-Serrano L, Kratovac S, Nelson DL, Corbin JG, Huntsman MM (2013) Homeostatic responses fail to correct defective amygdala inhibitory circuit maturation in Fragile X syndrome. J Neurosci 33:7548–7558

Weiler IJ, Greenough WT (1993) Metabotropic glutamate receptors trigger postsynaptic protein synthesis. Proc Natl Acad Sci U S A 90:7168–7171.

Zhang L, Alger BE (2010) Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. J Neurosci 30:5724–5729

Index

A. C. Errington et al. (eds.), *Extrasynaptic $GABA_A$ Receptors,* The Receptors 27,
DOI 10.1007/978-1-4939-1426-5