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The α1 Subunit in Sickness and in Health: Properties of the Most Predominant $GABA_A$ Receptor Subunit and Implications of Its Dysfunction in Human Epilepsy

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The aim of this review is to highlight the role of the a1 subunit of the $GABA_A$ receptor under normal conditions and to examine the consequences of its dysfunction in the context of epilepsy. First, background information relevant to $GABA_A$ receptors is discussed, followed by a summary of the biophysical properties conferred by the a1 subunit and its developmental expression patterns. Next, key findings from a1 subunit knockout mice are reviewed. Lastly, the important role of the a1 subunit in regulating inhibitory tone in the CNS is highlighted by examining consequences of mutations in the a1 subunit implicated in generalized human epilepsy syndromes.

Keywords *GABA, $GABA_A$ receptor, heterogeneity, alpha-1 subunit, epilepsy*

Introduction

$GABA_A$ receptors ($GABA_A$Rs) are a family of chloride-selective, ligand-gated ion channels that mediate the majority of fast inhibition in the adult central nervous system[1]. $GABA_A$Rs belong to a larger superfamily of ligand-gated ion channels called Cys-loop receptors, which also includes nicotinic acetylcholine receptors, glycine receptors, and serotonin type III receptors[2,3]. The $GABA_A$R gene family is comprised of at least 19 different subunits which are classified by sequence homology into 8 subtypes. These subunits can assemble in various combinations to produce functional $GABA_A$Rs: α1-6, β1-3, γ1-3, δ, ε, π, θ, and ρ1-3[4]. Based on the large number of different $GABA_A$R subunits, there are seemingly myriad different subunit combinations possible, although only a subset of these theoretical combinations has been identified *in vivo*[5]. The cache of possible subunit combinations coupled with differing spatial and temporal expression patterns provides considerable structural and functional heterogeneity to $GABA_A$Rs. Distinct subunit combinations produce distinct receptor isoforms which display highly variable properties throughout development and in adulthood[6–9]. This topic will be discussed in further detail below.

The vast majority of $GABA_A$Rs exist as a combination of two α subunits, two β subunits, and either a γ or δ[5,10] subunit arranged as shown in **Figure 1A-B**. The ε, π, and ρ1-3 subunits are far less common and are generally positioned in place of the γ or δ subunit, while the θ subunit can assume the position of the β subunit. The morphology of each $GABA_A$R subunit includes several characteristic features. A large extracellular domain at the N-terminus contains the characteristic disulfide bridge between two cysteine residues that creates the "Cys-loop" for which the receptor family is named. There are four helical transmembrane domains termed M1-M4, with the M2 of each subunit lining the ion pore. conntecting M3 and M4, there is a large intracellular domain, and beyond M4 is a very small extracellular C-terminal domain (**Figure 1C**)[1,2,11].

Full activation of $GABA_A$Rs requires the binding of two molecules of the neurotransmitter γ-amino butyric acid (GABA)—one at each α/β subunit interface[12,13]. Upon activation of the receptor, the channel opens and chloride (Cl^-) flows down its electrochemical gradient through the pore. In the mature brain, this leads to an influx of Cl^-, which causes the membrane potential of the cell to hyperpolarize and thus inhibits the generation of action potentials.

As previously mentioned, the wide variety of $GABA_A$R isoforms supports extensive functional heterogeneity, perhaps best demonstrated by the existence of two distinct forms of $GABA_A$R-mediated inhibition: tonic and phasic[14]. Tonic inhibition is mediated by extrasynaptically localized $GABA_A$Rs largely comprised of a δ subunit with an α4 and/or an α6 subunit[15], though some extrasynaptic $GABA_A$Rs containing α5 and lacking a δ subunit are known to func-

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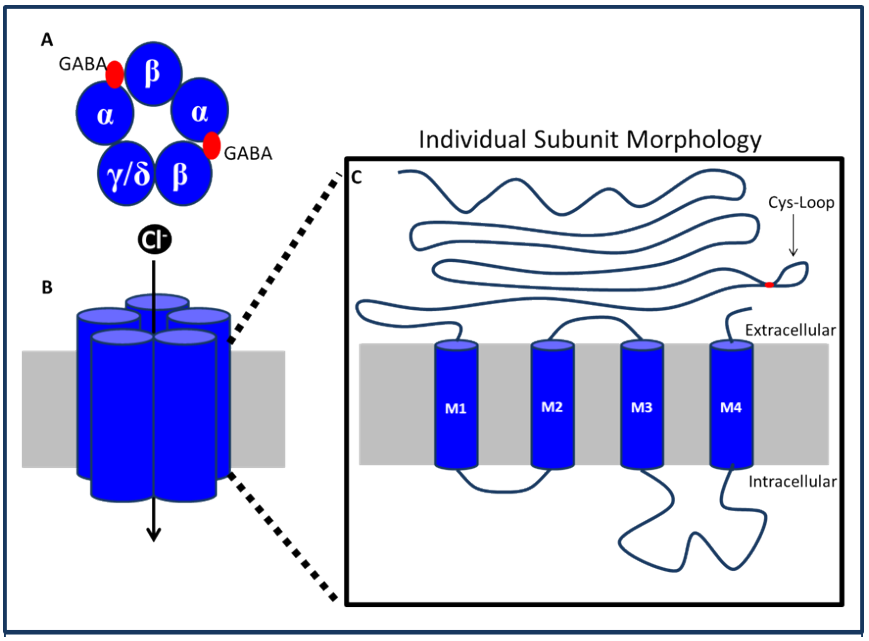

Figure 1: *Structure of $GABA_A$ receptors.* A: Top down view of typical subunit arrangement with GABA binding site indicated by red ovals. B: Side view of receptor depicting chloride flowing through the pore. C: Morphology of an individual subunit.

tion extrasynaptically as well[16]. Extrasynaptic $GABA_A$Rs are continuously activated by low levels (1M) of ambient GABA typically overflowing from the synaptic cleft. The properties of these receptors are conducive to persistent extrasynaptic activation, as they are highly sensitive to GABA, activate relatively slowly, and desensitize minimally[17]. The functional role of tonic inhibition seems to be the regulation of neuronal excitability. Conversely, phasic inhibition is mediated by synaptic $GABA_A$Rs, which most often contain a γ subunit with α1, α2, and/or α3 subunits. Unlike their extrasynaptic counterparts, synaptic $GABA_A$Rs are transiently activated by much higher (1mM) concentrations of GABA released from the presynaptic neuron into the synaptic cleft. In contrast to extrasynaptic $GABA_A$Rs, synaptic $GABA_A$Rs mediate tonic inhibition to allow for fast transmission of a fleeting signal and are thus less sensitive to GABA, activate rapidly, and desensitize extensively[18]. The short-lived activation of these receptors produces a transient inhibitory post-synaptic current (IPSC) which-translates presynaptic GABA release into a post-synaptic signal[19,20]. The remainder of this review will focus on the most predominant subtype of synaptic receptors: those containing the α1 subunit. The distinct receptor properties and known roles of the α1 subunit, as well as important findings from α1 subunit knockout mice and recently discovered epilepsy-associated mutations in the α1 subunit are discussed below.

Biophysical Properties of the α1 Subunit Relative to Other Synaptic α Subunits

The α1 subunit is the most predominant α subunit in the adult brain and is most often assembled into the α1β2γ2 $GABA_A$R isoform comprising 60% of all $GABA_A$Rs[8]. Further heterogeneity exists among synaptic receptors. The specific α subtype influences biophysical properties of $GABA_A$Rs including GABA sensitivity and the rates of activation, deactivation, and desensitization[21–25]. The primary strategy employed to determine the contribution of each α subunit to a particular property is to express different α subunits with the same β and γ subunit partners in heterologous cells and explore the properties of interest. One such property, GABA sensitivity, is defined by the concentration of GABA that is required to produce a given response. A common measure of GABA sensitivity is the concentration of GABA required to elicit a half-maximal response in a given receptor subtype, known as the EC50. A low EC50 indicates higher sensitivity and vice versa. The activation rate of a particular $GABA_A$R is the rate at which receptor current increases from 10% to 90% of the maximal or peak current, and the deactivation rate is the rate at which current amplitude decreases after GABA is removed.

The desensitized state of the receptor is a high-affinity state in which GABA is bound but the ion channel is closed. The desensitization rate is the rate at which the response diminishes in continued presence of GABA. Each of the biophysical properties defined above influence the shape and time course of $GABA_A$R-mediated IPSCs. Thus, the identity of the α subunit within the receptor in large part

Table 1: Relative Biophysical Properties Conferred by Synaptic α Subunits	
GABA Sensitivity	α1 > α2 > α3
Activation Rate	α2 > α1 > α3
Deactivation Rate	α1 > α2 > α3
Desensitization Rate	α1 = α2 > α3

Table 1: *Biophysical properties by synaptic α subunits.*

dictates the properties of inhibitory currents. The biophysical properties of α1 containing $GABA_A$Rs relative to other synaptic α subunits are summarized in **Table 1**[21–24].

Interestingly, the properties of $GABA_A$R mediated IPSCs are known to change throughout development[26,27]—namely the decay kinetics which are heavily influenced by the deactivation and desensitization rates of the $GABA_A$Rs[28,29]. In fetal and early postnatal development, $GABA_A$R-mediated IPSCs decay relatively slowly; later in development, the IPSCs decay much more rapidly. The timing of this change coincides with the timing of a well-established developmental change in $GABA_A$R α subunit expression[6,30,31]. Early in development, the α2 and α3 subunits predominate, but soon after birth their expression begins to wane while the expression of the α1 subunit steadily increases to become the most abundant α subunit by postnatal day 12 in mice[6]. A comparison of juvenile and mature $GABA_A$R-mediated IPSCs is shown in **Figure 2**. Given that the identity of the α subunit impacts IPSC properties, it is feasible that the developmental changes in α subunit expression and IPSC decay kinetics are causally linked. Indeed, it has been shown that in mice lacking the α1 subunit juvenile IPSC kinetics persist into adulthood[32–34]. The functional role of this developmental switch in α subunit expression and the concomitant change in IPSC kinetics is currently not well understood.

Findings From α1 Subunit Knockout Mice

In 2001, transgenic mice lacking the α1 subunit of the $GABA_A$R exhibited a 50-60% decrease in the total number of $GABA_A$Rs in the brain[33,35]. Consistent with this finding, the expression of the β2/3 and γ2 subunits—the most common binding partners of the α1 subunit—is also decreased in α1 knockout mice[36,37]. Given that the α1 subunit is the most abundant subunit and its loss results in a loss of the majority of $GABA_A$Rs in the brain, it is very surprising that these animals are viable and lack any obvious phenotypic abnormalities aside from a slight handling-induced tremor. The fact that the mice are overtly normal could suggest that changes occur within the $GABA_A$R system to compensate for the loss of α1. Indeed, post-transcriptional increases in the expression of the other α subunits have been observed[36,37], but the nature and extent of the compensation seems to vary among brain regions and has not been systematically quantified in the entire brain.

One study suggests that neurons upregulate the subunits they normally express rather than expanding their subunit repertoire[36], which is consistent with a post-transcriptional mode of upregulation. The consequences of these compensatory changes are not completely understood, but α1 knockout mice fail to develop mature IPSC kinetics[32–34] and exhibit a lower threshold for pharmacologically induced seizures[38]. This phenotype could indicate a decrease in inhibitory tone, although it was reported that α1 knockout mice did not experience spontaneous seizures[35]. However, it is important to note that these conclusions were drawn by visual inspection only rather than EEG analysis. Certain types of seizures, such as absence seizures, result in very subtle alterations in behavior that are difficult to detect even in a human, much less in a mouse. Thus it is possible that these animals did have seizures but did not display any easily detectable seizure behavior.

The α1 Subunit and Epilepsy

Because $GABA_A$Rs are the primary source

Electroencephalogram (EEG):
Method to measure electrical activity of the brain using electrodes on the surface of the scalp.

Missense Mutation:
Non-synonymous point mutation in which the identity of a single nucleotide is changed resulting in a codon that codes for an amino acid that differes from that of the WT protein at that particular location.

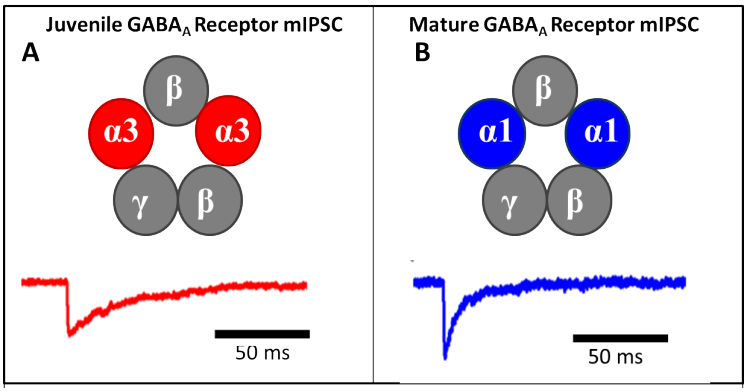

Figure 2: *Developmental change in α subunit expression and IPSC kinetics.* A: Juvenile α3 containing receptor with much slower IPSC delay kinetics. B: Mature α1 containing receptor with fast IPSC decay kinetics.

Endoplasmic Reticulum Associated Degradation:
A cellular process that marks misfolded proteins within the ER for ubiquitination followed by degradation via the proteasome.

Nonsense Mediated Decay:
Cellular mechanism to detect nonsense mutations (premature stop codons) and prevent translation of truncated proteins by degrading the mutant mRNA.

of inhibition in the central nervous system, it is not surprising that several mutations in various GABA$_A$R subunits have been identified in patients with idiopathic generalized epilepsy (IGE) syndromes such as childhood absence epilepsy and juvenile myoclonic epilepsy[39–41]. Epilepsy is a large collection of syndromes diagnosed upon the occurrence of two or more unprovoked seizures. Epilepsy may be classified as IGE if the cause is thought to be genetic and the seizures appear to involve the entire brain simultaneously with no obvious focal origin. Most IGEs are thought to be multigenic, which renders developing animal models fairly difficult. However, the identification of monogenic forms of IGE has permitted the generation of animal models based on human disease-associated mutations. To date, there have been four mutations identified specifically in the α1 subunit in human patients suffering from idiopathic generalized epilepsy (IGE.) Two of these mutations, K353delins18X and D219N[42], were only recently reported and have not been fully characterized. The other two however, A322D[43] and S326fs328X[44], have been extensively studied.

α1(K353delins18X) Mutation

The α1(K353delins18X) mutation was identified in 1 unaffected and 3 affected individuals with IGE exhibiting generalized tonic-clonic seizures. These seizures manifest as a sudden tensing of skeletal muscles followed by rapid contractions and relaxations resulting in characteristic convulsions[45]. This mutation involves the insertion of 25 base pairs into intron 10 which interrupts splicing and causes the retention of intron 10 in the transcript. The inclusion of intron 10 leads to an 18-amino acid insertion into the protein as well as the truncation of the fourth transmembrane domain due to a premature stop codon. Work in heterologous expression systems revealed that the protein is localized to the ER with complete loss of cell surface expression. In agreement with these findings, GABA-mediated currents were absent in these cells[42]. The fate of the mutant protein and the mechanism by which the α1(K353delins18X) mutation leads to epilepsy is yet to be determined.

α1(D219N) Missense Mutation

The D219N missense mutation was identified in 4 of 5 affected individuals with IGE in a French-Canadian family exhibiting IGE or febrile seizures(FS)—so aptly named due to their coincidence with fever[45]. Two of the four individuals with FS also reported a single generalized tonic clonic seizure. Studies conducted in heterologous expression systems indicate that surface expression of the mutant subunit is reduced as compared to WT α1 subunit, consistent with observations of decreased GABA-evoked peak current amplitudes. Additionally, α1(D219N) subunit-containing receptors were shown to desensitize more rapidly than WT α1 subunit-containing receptors[42]. Further studies characterizing the effects of this mutation both *in vitro* and *in vivo* will be required to elucidate the mechanism by which it promotes the development of epilepsy.

α1(A322D) Missense Mutation

The A322D missense mutation was identified in 8 affected individuals within a large French-Canadian family suffering from a type of IGE called juvenile myoclonic epilepsy (JME). Myoclonic seizures are characterized by sudden, brief, involuntary jerks of the arms or legs[45]. This mutation is autosomal dominant and results in the insertion of a charged aspartate residue in place of a highly conserved alanine within the M3 domain[39–41,43]. Experiments in heterologous cells indicate that this mutation disrupts the insertion of M3 into the lipid bilayer which results in its retention in the endoplasmic reticulum (ER) and subsequent partial degradation through ER-associated degradation[39–41,46] (ERAD). However, the mutant subunit is not completely degraded, as total and surface mutant protein is detectable, albeit at significantly lower levels than the WT α1 subunit[39–41,46]. It has also been postulated that the α1(A322D) mutation exerts a dominant negative effect by oligomerizing with and trapping WT subunits in the ER which are then subject to ERAD[39,47]. Consistent with reduced surface expression, the peak GABA-evoked current through receptors containing the α1(A322D) subunit was reduced 88%[47]. In the mutant receptors, open time of the channel was considerably reduced. Additionally, α1(A322D) subunit-containing receptors exhibited substantially reduced sensitivity to GABA with a nearly 100-fold increase in the GABA EC50[39,40]. It would be particularly enlightening to study the effects of this mutation *in vivo* and to that end, our group is in the process of generating an α1(A322D) knock-in mouse line.

α1(S326fs328X) Frameshift Mutation

The α1(S326fs328X) mutation is an autosomal dominant *de novo* mutation identified in a patient with childhood absence epilepsy. In contrast to the previously mentioned seizure types, absence seizures are not associated with any sort of convulsions or motor movements. Rather, they are characterized by sudden brief lapses in consciousness often accompanied by a blank stare[45]. A single base pair deletion leads to a frameshift and premature termination codon (PTC) in the eighth exon which corresponds to the third transmembrane domain of the protein[44]. The PTC has been shown to induce nonsense mediated decay (NMD) of the mutant mRNA, albeit incomplete. Mutant mRNA that escapes NMD is subsequently translated into truncated protein which is retained in the ER and subjected to ERAD[48,49]. Thus, the α1(S326fs328X) mutant subunit is not incorporated into the cell membrane and GABA-evoked currents are absent[44,48].

Based on these findings, it is thought that the epilepsy phenotype is a result of haploinsufficiency in the WT α1 gene. This raises the intriguing possibility that heterozygous α1 knockout animals could serve as a model for this disease. Indeed, our group recently revealed through EEG analysis that heterozygous α1 knockout mice do in fact experience seizures, though they are absence seizures rather than convulsive seizures[50]. This may explain why it was previously reported that α1 knockout mice did not exhibit an epileptic phenotype as mentioned above. The seizures were greatly attenuated by treatment with ethosuximide (ETX), the most commonly prescribed drug for absence seizures in human patients. These findings represent a novel model of absence epilepsy based on a mutation identified in a human epilepsy patient.

Conclusions

$GABA_A$Rs are a heterogeneous population of receptors and their properties are heavily influenced by their α subunit composition. The α1 subunit is the most predominant subunit in the adult brain and is largely responsible for the maintenance of inhibitory tone in the CNS. As evidenced by the consequences of the dysfunction or loss of the α1 subunit, it also seems to be involved in epilepsy susceptibility. The transgenic α1(A322D) knock-in and α1 knockout mouse lines that our group focuses on represent the first mouse models of genetic epilepsy based on mutations in the α1 subunit identified in human epilepsy patients. These two mutations were both identified in patients suffering from generalized epilepsy, but their seizure phenotypes were distinct. Based on our preliminary analyses, the epileptic phenotypes of the two mouse lines also differ. By dissecting the similarities and differences in pathogenesis behind these two models, we aim to identify common themes among all generalized epilepsies and also delineate important differences that contribute to distinct disease manifestations. While epilepsy is an exceptionally complicated and heterogeneous condition, the advent of animal models reflecting specific disease-associated mutations in $GABA_A$Rs represents a promising and riveting new avenue for advancing our understanding of the pathogenesis of generalized epilepsy.

References

1. Sieghart, W. Structure , Pharmacology , and Function of GABA A Receptor Subtypes. *Advances in Pharmacology* **54**, 231-263 (2006).

2. Connolly, C. & Wafford, K. The Cys-Loop Superfamily of Ligand-gated Ion Channels: The Impact of Receptor Structure and Function. *Biochem Soc Trans* **32**, 529-534 (2004).

3. Unwin, N. Neurotransmitter action: opening of ligand-gated ion channels. *Cell* **72,** 31-41 (1993).

4. Sieghart, W. & Sperk, G. Subunit composition, distribution and function of GABA(A) receptor subtypes. *Current Topics in Medicinal Chemistry* **2**, 795-816 (2002).

5. Mckernan, R.M. & Whiting, P.J. Which GABA A receptor subtypes really occur in the brain? *Trends in Neuroscience* **19**, 139-143 (1996).

6. Laurie, D.J., Wisden, W. & Seeburg, P.H. The Distribution of Thirteen GABA (A) Receptor Subunit mRNAs in the Rat Brain III: Embryonic and Postnatal Development. *The Journal of Neuroscience* **12**, 4151-4172 (1992).

7. Olsen, R.W. & Sieghart, W. GABA A receptors: subtypes provide diversity of function and pharmacology. *Neuropharmacology* **56**, 141-8 (2009).

8. Möhler, H. GABA(A) receptor diversity and pharmacology. *Cell and Tissue Research* **326**, 505-16 (2006).

9. Hevers, W. & Lüddens, H. The diversity of GABAA receptors: Pharmacological and electrophysiological properties of GABAA channel subtypes. *Molecular Neurobiology* **18**, 35-86 (1998).

10. Baumann, S.W., Baur, R. & Sigel, E. Subunit arrangement of gamma-aminobutyric acid type A receptors. *The Journal of Biological Chemistry* **276**, 36275-80 (2001).

11. Macdonald, R.L. & Olsen, R.W. GABAA receptor channels. *Annual Review of Neuroscience* **17**, 569-602 (1994).

12. Baumann, S.W., Baur, R. & Sigel, E. Individual properties of the two functional agonist sites in GABA(A) receptors. *The Journal of Neuroscience* **23**, 11158-66 (2003).

13. Kash, T.L., Jenkins, A., Kelley, J.C., Trudell, J.R. & Harrison, N.L. Coupling of agonist binding to channel gating in the GABA(A) receptor. *Nature* **421**, 272-5 (2003).

14. Mody, I. Distinguishing between GABA(A) receptors responsible for tonic and phasic conductances. *Neurochemical Research* **26**, 907-13 (2001).

15. Sur, C., Farrar, S., Kerby, J., Whiting, P., Atack, J., McKernan, R. Preferential Coassembly of Alpha 4 and Delta Subunits of the GABAA Receptor in Rat Thalamus. *Molecular pharmacology* **56**, 110-115 (1999).

16. Caraiscos, V.B., Elliott, E., You-Ten, K., Cheng, V., Belelli, D., Newell, J., Jackson, M., Lambert, J., Rosahl, T., Wafford, K., MacDonald, J., Orser, B. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 3662-7 (2004).

17. Mtchedlishvili, Z. & Kapur, J. High-Affinity , Slowly Desensitizing GABA A Receptors Mediate Tonic Inhibition in Hippocampal Dentate Granule Cells. *Molecular Pharmacology* **69**, 564-575 (2006).

18. Haas, K.F. & Macdonald, R.L. GABA A receptor subunit gamma2 and delta subtypes confer unique kinetic properties on recombinant GABA A receptor currents in mouse fibroblasts. *Journal of Physiology* **514**, 27-45 (1999).

19. Farrant, M. & Nusser, Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nature Reviews Neuroscience* **6**, 215-29 (2005).

20. Nusser, Z., Sieghart, W. & Somogyi, P. Segregation of Different GABA A Receptors to Synaptic and Extrasynaptic Membranes of Cerebellar Granule Cells.*Journal of Neuroscience* **18**, 1693-1703 (1998).

21. Gingrich, K.J., Roberts, W. a & Kass, R.S. Dependence of the GABAA receptor gating kinetics on the alpha-subunit isoform: implications for structure-function relations and synaptic transmission. *The Journal of Physiology* **489**, 529-43 (1995).

22. Eyre, M.D., Renzi, M., Farrant, M. & Nusser, Z. Setting the Time Course of Inhibitory Synaptic Currents by Mixing Multiple GABAA Receptor Alpha Subunit Isoforms. *Journal of Neuroscience* **32**, 5853-5867 (2012).

23. Picton, A.J. & Fisher, J.L. Effect of the alpha subunit subtype on the macroscopic kinetic properties of recombinant GABA(A) receptors. *Brain Research* **1165**, 40-9 (2007).

24. Lavoie, a M., Tingey, J.J., Harrison, N.L., Pritchett, D.B. & Twyman, R.E. Activation and deactivation rates of recombinant GABA(A) receptor channels are dependent on alpha-subunit isoform. *Biophysical Journal* **73**, 2518-26 (1997).

25. Böhme, I., Rabe, H. & Lüddens, H. Four amino acids in the alpha subunits determine the gamma-aminobutyric acid sensitivities of GABAA receptor subtypes. *The Journal of Biological Chemistry* **279**, 35193-200 (2004).

26. Okada, M., Onodera, K., Van Renterghem, C., Sieghart, W. & Takahashi, T. Functional correlation of GABA(A) receptor alpha subunits expression with the properties of IPSCs in the developing thalamus. *The Journal of Neuroscience* **20**, 2202-8 (2000).

27. Dunning, D., Hoover, C., Soltesz, I., Smith, M., Dowd, D., Maguеresse, C., Alfonso, J., Khodosevich, K., Martín, Á., Bark, C., Monyer, H. GABA A Receptor Mediated Miniature Postsynaptic Currents and α -Subunit Expression in Developing Cortical Neurons.*The Journal of Neurophysiology* **82:** 3286-3297 (1999).

28. Jones, M.V. & Westbrook, G.L. Desensitized states prolong GABAA channel responses to brief agonist pulses. *Neuron* **15**, 181-91 (1995).

29. Ortinski, P.I., Lu, C., Takagaki, K., Fu, Z. & Vicini, S. Expression of distinct alpha subunits of GABAA receptor regulates inhibitory synaptic strength. *Journal of Neurophysiology* **92**, 1718-27 (2004).

30. Hashimoto, T., Nguyen, Q., Rotaru, D., Keenan, T., Arion, D., Beneyto, M., Gonzalez-Burgos, G., Lewis, D.. Protracted developmental trajectories of GABAA receptor alpha1 and alpha2 subunit expression in primate prefrontal cortex. *Biological Psychiatry* **65**, 1015-23 (2009).

31. Fritschy, J.-M., Paysan, J., Enna, A. & Mohler, H. Switch in the Expression of Rat GABAA-Receptor Subtypes During Postnatal Development: An lmmunohistochemical Study. *Journal of Neuroscience* **14**, 5302-5324 (1994).

32. Bosman, L.W.J., Heinen, K., Spijker, S. & Brussaard, A.B. Mice lacking the major adult GABAA receptor subtype have normal number of synapses, but retain juvenile IPSC kinetics until adulthood. *Journal of Neurophysiology* **94**, 338-46 (2005).

33. **Vicini, S., Ferguson, C., Prybylowski, K., Kralic, J., Morrow, L., Homanics, G. . GABA(A) receptor alpha1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. The Journal of Neuroscience 21, 3009-16 (2001).**

This paper represents the generation of the original transgenic GABAaR α1 subunit knockout mouse line. We use the heterozygous animals from this line in our lab as a model for studying the pathogenesis of absence epilepsy.

34. Goldstein, P., Elsen, F., Ying, S., Ferguson, C., Homanics, G., Harrison, N. Prolongation of hippocampal miniature inhibitory postsynaptic currents in mice lacking the GABA(A) receptor alpha1 subunit. *Journal of Neurophysiology* **88**, 3208-17 (2002).

35. Sur, C., Wafford, K., Reynolds, D., Hadingham, K., Bromidge, F., Macaulay, A., Collinson, N., Meara, G., Howell, O., Newman, R., Myers, J., Atack, J., Dawson, G., McKernan, R., Whiting, P., Rosahl, T. Loss of the major GABA(A) receptor subtype in the brain is not lethal in mice. *The Journal of Neuroscience* **21**, 3409-18 (2001).

36. Kralic, J., Sidler, C., Parpan, F., Homanics, G., Morrow, A., Fritschy, J. Compensatory alteration of inhibitory synaptic circuits in cerebellum and thalamus of gamma-aminobutyric acid type A receptor alpha1 subunit knockout mice. *The Journal of Comparative Neurology* **495**, 408-21 (2006).

37. Kralic, J., Korpi, E., Buckley, T., Homanics, G., Morrow, A. Molecular and Pharmacological Characterization of GABA A Receptor Alpha 1 Subunit Knockout Mice. *The Journal of Pharmacology and Experimental Therapeutics* **302**, 1037-1045 (2002).

38. Kralic, J., O'Buckley, T., Khisti, R., Hodge, C., Homanics, G., Morrow, A. GABA(A) receptor alpha-1 subunit deletion alters receptor subtype assembly, pharmacological and behavioral responses to benzodiazepines and zolpidem. *Neuropharmacology* **43**, 685-94 (2002).

39. Macdonald, R.L., Kang, J.Q., Gallagher, M.J. & Feng, H.J. GABA A Receptor Mutations Associated with Generalized Epilepsy. *Advances in Pharmacology* **54**, 1497-1506 (2006).

40. Macdonald, R.L., Gallagher, M.J., Feng, H.-J. & Kang, J. GABA(A) receptor epilepsy mutations. *Biochemical Pharmacology* **68**, 1497-506 (2004).

41. Macdonald, R.L., Kang, J.Q. & Gallagher, M.J. Mutations in GABAA receptor subunits associated with genetic epilepsies. *The Journal of Physiology* **588**, 1861-9 (2010).

42. Lachance-Touchette, P., Brown, P., Meloche, C., Kinirons, P., Lapointe, L., Lacasse, H., Lortie, A., Carmant, L., Bedford, F., Bowie, D., Cossette, P. Novel α1 and γ2 GABAA receptor subunit mutations in families with idiopathic generalized epilepsy. *The European Journal of Neuroscience* **34**, 237-49 (2011).

43. Cossette, P., Liu, L., Brisebois, K., Dong, H., Lortie, A., Vanasse, M., Saint-Hilaire, J., Carmant, L., Verner, A., Lu, W., Wang, Y., Rouleau, G. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nature Genetics* **31**, 184-9 (2002).

44. **Maljevic, S., Krampfl, K., Cobilanschi, J., Tilgen, N., Beyer, S., Weber, Y., Schlesinger, F., Ursu, D., Melzer, W., Cossette, P., Bufler, J., Lerche, H., Heils, A. et al. A mutation in the GABA(A) receptor alpha(1)-subunit is associated with absence epilepsy. Annals of Neurology 59, 983-7 (2006).**

This paper identifies the first mutation in GABAaR α1 subunit associated with childhood absence epilepsy. This mutation is the basis for the mouse model our lab uses to study the pathogenesis of absence epilepsy.

45. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for Revised Classification of Epilepsies and Epileptic Syndromes. *Epilepsia* **30**, 389-399 (1989).

46. Gallagher, M.J., Shen, W., Song, L. & Macdonald, R.L. Endoplasmic reticulum retention and associated degradation of a GABAA receptor epilepsy mutation that inserts an aspartate in the M3 transmembrane segment of the alpha1 subunit. *The Journal of Biological Chemistry* **280**, 37995-8004 (2005).

47. Ding, L., Feng, H., Macdonald, R., Botzolakis, E., Hu, N., Gallagher, M. GABA(A) receptor alpha1 subunit mutation A322D associated with autosomal dominant juvenile myoclonic epilepsy reduces the expression and alters the composition of wild type GABA(A) receptors. *The Journal of Biological Chemistry* **285**, 26390-405 (2010).

48. **Kang, J.-Q., Shen, W. & Macdonald, R.L. Two molecular pathways (NMD and ERAD) contribute to a genetic epilepsy associated with the GABA(A) receptor GABRA1 PTC mutation, 975delC, S326fs328X. The Journal of Neuroscience 29, 2833-44 (2009).**

This paper establishes that the mutant α1 subunit is degraded, indicating that α1 haploinsufficiency may be associated with the childhood absence epilepsy phenotype. These findings justify heterozygous α1 knockout mice as a putative model for the α1(S326fs328X) mutation identified in human epilepsy patients.

49. Macdonald, R.L., Kang, J.-Q. & Gallagher, M.J. Mutations in GABAA receptor subunits associated with genetic epilepsies. *The Journal of Physiology* **588**, 1861-9 (2010).

50. **Arain, F.M., Boyd, K.L., &Gallagher, M.J. Decreased viability and absence-like epilepsy in mice lacking or deficient in the GABAA Receptor α1 Subunit. Epilepsia (2012, In Press).**

This paper validates heterozygous α1 knockout mice as a model for absence epilepsy as it shows that the mice have absence-like seizures that remit with ethosuximide, the most commonly prescribed drug for the treatment of absence seizures.

Further information: http://www.mc.vanderbilt.edu/neurology/faculty/gallagher.htm