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α 6-Containing GABA_A Receptors: Functional Roles and Therapeutic Potentials

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Abstract—GABA_A receptors containing the α 6 subunit are highly expressed in cerebellar granule cells and less abundantly in many other neuronal and peripheral tissues. Here, we for the first time summarize their importance for the functions of the cerebellum and the nervous system. The cerebellum is not only involved in motor control but also in cognitive, emotional, and social behaviors. α 6 β γ 2 GABA_A receptors located at cerebellar Golgi cell/granule cell synapses enhance the precision of inputs required for cerebellar timing of motor activity and are thus involved in cognitive processing and adequate responses to our environment. Extrasynaptic α 6 β δ GABA_A receptors regulate the amount of information entering the cerebellum by their tonic inhibition of granule cells, and their optimal functioning enhances input filtering or contrast. The complex roles of the cerebellum in multiple brain functions can be compromised by genetic or neurodevelopmental causes that lead to a hypofunction of cerebellar α 6-containing GABA_A receptors. Animal models mimicking neuropsychiatric phenotypes

suggest that compounds selectively activating or positively modulating cerebellar α 6-containing GABA_A receptors can alleviate essential tremor and motor disturbances in Angelman and Down syndrome as well as impaired prepulse inhibition in neuropsychiatric disorders and reduce migraine and trigeminal-related pain via α 6-containing GABA_A receptors in trigeminal ganglia. Genetic studies in humans suggest an association of the human GABA_A receptor α 6 subunit gene with stress-associated disorders. Animal studies support this conclusion. Neuroimaging and post-mortem studies in humans further support an involvement of α 6-containing GABA_A receptors in various neuropsychiatric disorders, pointing to a broad therapeutic potential of drugs modulating α 6-containing GABA_A receptors.

Significance Statement— α 6-Containing GABA_A receptors are abundantly expressed in cerebellar granule cells, but their pathophysiological roles are widely unknown, and they are thus out of the mainstream of GABA_A

ABBREVIATIONS: ADHD, attention deficit hyperactivity disorder; ANT, alcohol-nontolerant; CGRP, calcitonin gene-related peptide; Compound 6/PZ-II-029, 7-methoxy-2-(4-methoxyphenyl)-2H-pyrazolo[4,3-c]quinolin-3(5H)-one; DCN, deep cerebellar nuclei; DK-I-56-1, (7-methoxy-2-(4-(methoxy-d3)phenyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one); fMRI, functional magnetic resonance imaging; furosemide, 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid; GABA_AR, GABA_A receptor; *GABRA6*, human GABA_A receptor α 6 subunit gene; GAD65, glutamate decarboxylase 65 kDa; GAD67, glutamate decarboxylase 67 kDa; GAT-1, GABA transporter 1; GIT1, G-protein-coupled receptor kinase-interacting protein-1; HPA, hypothalamic-pituitary-adrenal; miRNA, microRNA; NTG, nitroglycerine; OCD, obsessive-compulsive disorder; PAM, positive allosteric modulator; PC, Purkinje cell; PCP, phencyclidine; PPI, prepulse inhibition; Ro15-1788/flumazenil, ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,4]benzodiazepine-3-carboxylate; Ro15-4513, ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate; RSA, respiratory sinus arrhythmia; Slitrk1, SLIT and NTRK-like family, member 1; SNP, single nucleotide polymorphism; TG, trigeminal ganglia; TGVS, trigemino-vascular system; THIP/gaboxadol, 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridine-3-ol; UTR, untranslated region.

receptor research. Anatomical and electrophysiological evidence indicates that these receptors have a crucial function in neuronal circuits of the cerebellum and the nervous system, and experimental, genetic, post-mortem,

and pharmacological studies indicate that selective modulation of these receptors offers therapeutic prospects for a variety of neuropsychiatric disorders and for stress and its consequences.

I. GABA_A Receptors

GABA_A receptors (GABA_ARs) are the major inhibitory neurotransmitter receptors in the brain and are the site of action of a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, anesthetics, neuroactive steroids, and convulsants (Sieghart, 1995). These drugs allosterically modulate the function of GABA_ARs via distinct binding sites (Sieghart, 2015; Puthenkalam et al., 2016), and from their effects on animal and human behavior it can be concluded that GABA_ARs are involved in modulating anxiety, cognition, vigilance, memory, and motor functions. GABA_ARs are ligand-gated chloride channels that are formed by five subunits. A total of 6 α , 3 β , 3 γ , δ , ϵ , π , θ , and 3 ρ subunits as well as several alternatively spliced isoforms of some of these subunits have been identified in the mammalian nervous system (Olsen and Sieghart, 2008). Each one of these subunits exhibits a distinct regional and cellular distribution in the brain (Wisden et al., 1992; Fritschy and Mohler, 1995; Pirker et al., 2000). Varying the combination of up to five different subunits in a single GABA_AR subtype results in an enormous possible heterogeneity of GABA_AR subtypes (Barnard et al., 1998).

The majority of GABA_ARs are composed of two α subunits, two β subunits, and one γ subunit ($\alpha\beta\gamma$ receptors). In these receptors, alternating α and β subunits are connected by a γ subunit (Tretter et al., 1997; Baumann et al., 2002; Laverty et al., 2019) (Fig. 1). In contrast to the $\alpha\beta\gamma$ receptors, the subunit stoichiometry and arrangement of receptors containing δ , ϵ , π , or θ subunits have not been unequivocally identified (Minier and Sigel, 2004; Bollan et al., 2008; Has and Chebib, 2018).

II. GABA_A Receptors Containing $\alpha 6$ Subunits ($\alpha 6$ GABA_ARs)

The $\alpha 6$ subunit of GABA_ARs was first cloned from rat cerebellum in 1990 (Luddens et al., 1990). In situ hybridization (Laurie et al., 1992; Persohn et al., 1992) and immunohistochemical studies (Gutierrez et al., 1996; Pirker et al., 2000) revealed that the $\alpha 6$ subunit is located predominantly in cerebellar granule cells (Fig. 2A) and that its location and protein sequence are highly conserved in man, rat, mice, goldfish, and chicken, suggesting that receptors containing this subunit are of fundamental importance for cerebellar function (Bahn et al., 1996). In the meantime, however, $\alpha 6$ subunits were also identified in multiple other neuronal and non-neuronal tissues, although at a much lower abundance (Fig. 2A; Table 1). The function of $\alpha 6$ GABA_AR subunits in most of these tissues currently is not known.

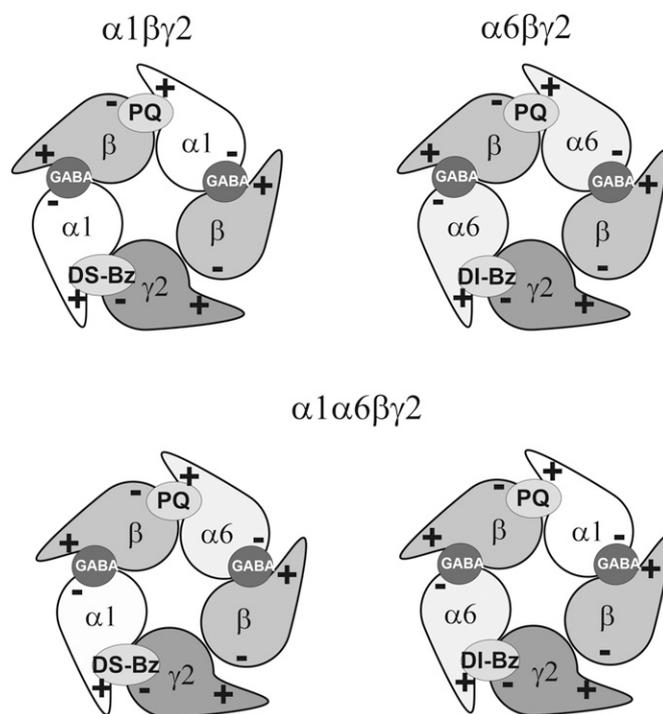


Fig. 1. GABA_AR subunit composition and arrangement. The majority of GABA_ARs is composed of two α subunits, two β subunits, and one γ subunit that form a central anion channel with the indicated subunit arrangement when viewed from above. The two α and β subunits can be identical or different. The upper left image depicts the experimentally verified subunit arrangement for the major GABA_AR subtype composed of $\alpha 1\beta\gamma 2$ subunits, and the other three panels depict $\alpha 6$ -containing arrangements that have been shown to exist in cerebellar granule cells (Scholze et al., 2020). Each subunit has a principal (+) and a complementary (-) side. The + side contains the large loop C in the extracellular domain that is part of the pharmacological binding sites formed by the interfaces of two subunits. This loop C is schematically depicted as the pointed end of each subunit. The two GABA binding sites are formed by the two $\beta +/\alpha -$ interfaces. The benzodiazepine binding site (Bz) is formed by the $\alpha +/\gamma 2 -$ interface. Receptors containing $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits form diazepam-sensitive benzodiazepine sites (DS-Bz). Receptors containing $\alpha 4$ or $\alpha 6$ subunits form diazepam-insensitive benzodiazepine binding sites (DI-Bz). Pyrazoloquinolinones (PQs) bind to two distinctive interfaces: to the benzodiazepine binding sites of all $\alpha\beta\gamma 2$ receptors with high affinity as silent binders (antagonists, similar to flumazenil) and to the PQ sites of these receptors formed by the $\alpha +/\beta -$ interfaces as modulators. $\alpha 6$ GABA_AR-selective pyrazoloquinolinones, such as Compound 6 (PZ-II-029), exert their positive modulatory action via the PQ site at the $\alpha 6 +/\beta -$ interface. The δ -containing $\alpha 6\beta\delta$ and $\alpha 1\alpha 6\beta\delta$ receptors presumably have a similar stoichiometry and subunit arrangement as the respective $\gamma 2$ -containing receptors, but this is still a matter of controversy.

So far, the subunit composition of $\alpha 6$ GABA_ARs has been investigated in the cerebellum, only (Jechlinger et al., 1998). Results indicated that 45% of GABA_ARs in the cerebellum contained $\alpha 6$ subunits and that $\alpha 6$ GABA_ARs in the rat cerebellum are predominantly composed of $\alpha 6\beta\gamma 2$ (32%), $\alpha 1\alpha 6\beta\gamma 2$ (37%), $\alpha 6\beta\delta$ (14%), or $\alpha 1\alpha 6\beta\delta$ (15%) combinations (Fig. 1). Other experiments indicated that 10%, 51%, or 21% of $\alpha 6$ receptors

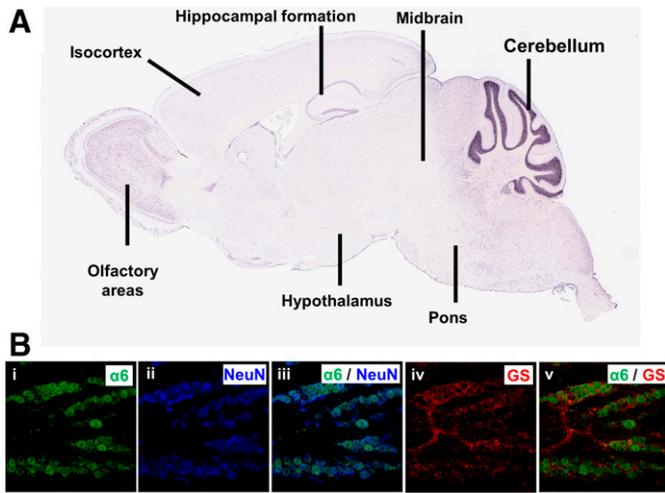


Fig. 2. GABA_A $\alpha 6$ subunit expression in the mouse brain and trigeminal ganglia. (A) In situ hybridization of a parasagittal mouse whole brain section for *Gabra6* mRNA, obtained from Allen Brain Atlas Data Portal by Allen Institute for Brain Science (<https://mouse.brain-map.org/experiment/show/308055734>). *Gabra6* expression is high in cerebellar granule cells but low in olfactory areas, isocortex, hippocampal formation, hypothalamus, midbrain, and pons. (B) Immunohistochemistry demonstrating GABA_A $\alpha 6$ subunit expression in neurons and satellite glial cells of TG in mice. Micrographs are representative mouse TG sections triple immunofluorescence-stained with antibodies against the $\alpha 6$ subunit of GABA_A receptors (B-i, B-iii, and B-v, green), NeuN (B-ii and B-iii, blue, neuronal marker), and glutamine synthetase (GS, B-iv and B-v, red, satellite glial cells marker). Modified from Tzeng et al. (2021). Neurons and satellite glial cells are organized in bands or clusters within the ganglion. The spaces between the clusters are filled by nerve fibers, microglia, and macrophages (Legradi et al., 2020).

contained homogeneous $\beta 1$, $\beta 2$, or $\beta 3$ subunits, respectively, whereas two different β subunits were present in 18% of all $\alpha 6$ receptors (Jechlinger et al., 1998). Overall, these data were confirmed in a second study that compared the total GABA_AR composition in the cerebellum of mice and rats and investigated the abundance of minor GABA_AR subtypes in the cerebellum (Poltl et al., 2003).

Cerebellar granule cells express $\alpha 1$, $\alpha 6$, $\beta 2$, $\beta 3$, $\gamma 2$, and δ subunits abundantly, and the $\alpha 1$, $\alpha 6$, $\beta 2/3$, and $\gamma 2$ subunits are concentrated at postsynaptic sites of many GABAergic Golgi synapses, demonstrating that both $\alpha 1$ and $\alpha 6$ subunits are involved in synaptic transmission in the same synapse (Nusser et al., 1998). These could be pentameric $\alpha 1\beta\gamma 2$ and $\alpha 6\beta\gamma 2$ receptors sitting side by side in the same synapse or pentameric $\alpha 1\alpha 6\beta\gamma 2$ receptors containing two different α subunit types. The actual existence of $\alpha 1\alpha 6\beta\gamma 2$ or $\alpha 1\alpha 6\beta\delta$ receptors is supported by several lines of evidence, resulting in their classification as “existing with high probability” (Olsen and Sieghart, 2008; Scholze et al., 2020) (Fig. 1).

However, Golgi synapses immunopositive for only one of the α subunits were also found (Nusser et al., 1996). Receptors composed of the $\alpha 1$, $\alpha 6$, $\beta 2/3$, and $\gamma 2$ subunits are not only present at synapses but also present extrasynaptically at lower concentrations. δ subunits could not be detected in synaptic junctions, although they were abundantly present in the extrasynaptic dendritic and

somatic membranes (Nusser et al., 1998). $\alpha 6$, $\beta 2/3$, and $\gamma 2$ but not $\alpha 1$ and δ subunits were also concentrated in some glutamatergic mossy fiber synapses, where they are colocalized with AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (Nusser et al., 1996, 1998). The extrasynaptic $\alpha 6\beta 2/3\delta$ and $\alpha 1\alpha 6\beta 2/3\delta$ receptors could thus mediate tonic inhibition, whereas the synaptic $\alpha 1\beta 2/3\gamma 2$, $\alpha 6\beta 2/3\gamma 2$, and $\alpha 1\alpha 6\beta 2/3\gamma 2$ receptors could mediate phasic inhibition (Nusser et al., 1998; Stell and Mody, 2002).

The $\alpha 6$ subunit confers unique characteristics to GABA_ARs. Electrophysiological investigations in *Xenopus laevis* oocytes indicated that GABA exhibited an up to 14-fold higher potency for activating recombinant $\alpha 6\beta\gamma 2$ than for $\alpha 1\beta\gamma 2$ receptors, whereas it exhibited an up to 50-fold further increase in potency for $\alpha 6\beta\delta$ compared with $\alpha 6\beta\gamma 2$ receptors, depending on the type of β subunit within the receptors. For $\alpha 1\beta 3\gamma 2L$: $\alpha 6\beta 3\gamma 2L$: $\alpha 6\beta 3\delta$ receptors, the potency ratio was 156: 23.3: 0.44 μM (Karim et al., 2013). However, the potency ratios were different in other recombinant expression systems because of possible differences in the receptor subunit composition, regulation by endogenous neurosteroids or Zn^{2+} , or other factors, such as phosphorylation states (Mortensen et al., 2012). Thus, in mouse fibroblast L929 cells, the potency ratio of $\alpha 1\beta 3\gamma 2L$: $\alpha 6\beta 3\gamma 2L$: $\alpha 6\beta 3\delta$ receptors for submaximal concentrations of GABA was 10: 3: 0.3 μM (Saxena and Macdonald, 1996) (Fig. 3A), whereas in human embryonic kidney 293 cells it was 2.1: 0.17: 0.17 μM (Mortensen et al., 2012), indicating that GABA potency was comparable for $\alpha 6\beta 3\gamma 2L$ and $\alpha 6\beta 3\delta$ receptors in the latter expression system. $\alpha 6\beta\gamma 2$ and $\alpha 6\beta\delta$ receptors exhibited a slower rise time and a longer decay time than $\alpha 1\beta\gamma 2$ receptors (Bianchi et al., 2002) (Fig. 3B). The rise and decay times of $\alpha 1\alpha 6\beta\gamma 2$ were different from that of $\alpha 1\beta\gamma 2$ and $\alpha 6\beta\gamma 2$ receptors but closer to

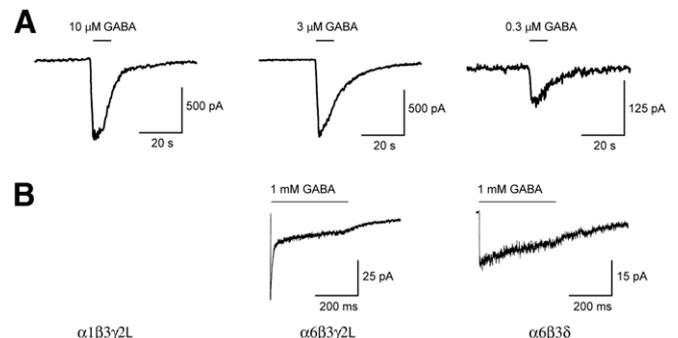


Fig. 3. Representative GABA-evoked currents of recombinant GABA_ARs. (A) Whole-cell currents elicited by respective submaximal concentrations of GABA recorded from mouse fibroblast L929 cells transfected with $\alpha 1\beta 3\gamma 2L$, $\alpha 6\beta 3\gamma 2L$, and $\alpha 6\beta 3\delta$ GABA_A receptor subunits. Modified from Saxena and Macdonald (1996). (B) Concentration jump experiments in the outside-out patch configuration induced by a saturating GABA concentration (1 mM) recorded from human embryonic kidney 293T cells transfected with $\alpha 6\beta 3\gamma 2L$ and $\alpha 6\beta 3\delta$ GABA_A receptor subunits. Modified from Bianchi et al. (2002). Horizontal bars above traces indicate the duration of GABA application.

those of $\alpha 1\beta\gamma 2$ receptors (Saxena and Macdonald, 1996; Tia et al., 1996). The unique properties of $\alpha 6\text{GABA}_A\text{Rs}$ that are grossly different from those of other GABA_AR subtypes (Mortensen et al., 2012; Karim et al., 2013) support the conclusion that these receptors have important physiologic functions in regulating the activity of neurons especially at low GABA concentrations.

The GABA_AR agonist THIP (4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridine-3-ol, gaboxadol, Fig. 4) is less potent than GABA at $\alpha\beta\gamma$ receptors but approximately 10 times more potent at δ -containing than at $\gamma 2\text{S}$ containing receptors (Brown et al., 2002; Meera et al., 2011). In contrast to GABA that is a partial agonist at δ -containing receptors (Bianchi and Macdonald, 2003; Meera et al., 2011), THIP is a full agonist at these receptors and thus elicits a markedly larger response than GABA [but see also the discussion of this topic in (Sieghart and Savic, 2018)]. Both $\alpha 6\beta 3\gamma 2$ and $\alpha 6\beta 3\delta$ receptors as well as $\alpha 1\alpha 6\beta\gamma 2$ and $\alpha 1\alpha 6\beta\delta$ receptors also are modulated by neurosteroids, and this modulation is stronger for $\alpha 6\beta 3\delta$ than for $\alpha 6\beta 3\gamma 2$ receptors (Bianchi and Macdonald, 2003).

$\alpha 6\beta\gamma 2$ receptors, like $\alpha 4\beta\gamma 2$ receptors, are diazepam-insensitive $\alpha\beta\gamma$ GABA_ARs (Luddens et al., 1990) (Fig. 1) and are also insensitive to a variety of other benzodiazepine-site ligands (Sieghart, 1995). These receptors can be positively modulated by the imidazobenzodiazepines Ro15-1788 (flumazenil) and Ro15-4513 (Korpi et al., 2007; Ramerstorfer et al., 2010) as well as by some but not all β -carbolines (Sieghart, 1995; Ramerstorfer et al., 2010) and by the flavonoid hispidulin (Fig. 4) (Kavvadias

et al., 2004; Chiou et al., 2018). Other nonbenzodiazepine positive allosteric modulators (PAMs) of GABA_ARs , such as loreclezole or etomidate (Fig. 4), are also able to modulate $\alpha 6\beta\gamma 2$ receptors, but these compounds also modulate the activity of $\alpha 1\text{-}5\beta\gamma 2$ GABA_ARs (Wafford et al., 1994; Belelli et al., 1997). However, some pyrazoloquinolinones, such as PZ-II-029 (Compound 6), LAU159 (8-chloro-2-(3-methoxyphenyl)-2H-pyrazolo[4,3-c]quinolin-3(5H)-one), and LAU463 (7-bromo-2-(4-methoxyphenyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one) (Fig. 4) that are high affinity silent modulators (antagonists) at the benzodiazepine site of $\alpha 1\text{-}6\beta\gamma 2$ receptors, are also highly selective PAMs of $\alpha 6\beta\gamma 2$ and $\alpha 6\beta\delta$ GABA_ARs (Fig. 5) by acting via a second binding site at the extracellular $\alpha 6 + \beta 2/3$ - interface of these receptors (Fig. 1) (Ramerstorfer et al., 2011; Varagic et al., 2013; Chiou et al., 2018; Treven et al., 2018; Simeone et al., 2019). In addition, the diuretics amiloride (Fisher, 2002) and furosemide (Korpi et al., 1995; Wafford et al., 1996; Korpi and Luddens, 1997) are relatively selective allosteric antagonists at $\alpha 6\beta\gamma 2$, $\alpha 1\alpha 6\beta\gamma 2$, $\alpha 6\beta\delta$, and $\alpha 1\alpha 6\beta\delta$ receptors (Fig. 4) (Sigel et al., 2006; Sieghart and Savic, 2018). Additional compounds preferentially modulating these receptors are discussed by Sieghart and Savic (2018).

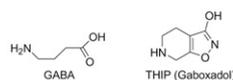
A. $\alpha 6\text{GABA}_A\text{R}$ Functions in the Cerebellum

The cerebellar cortex consists of a folded sheet of microcircuitry reiterated on a vast scale (Fig. 6). The same circuitry in different areas of the cerebellum probably computes similar operations linked to different parts of the brain (Wisden et al., 2009). After receiving specific sensory and contextual information, this circuitry probably measures or predicts time intervals for motor activity required for controlling movement of the respective body parts.

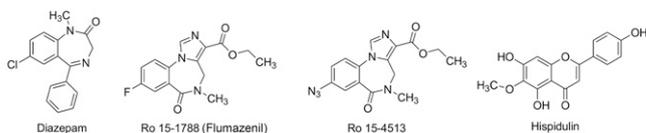
The cerebellar cortex has two excitatory inputs, the mossy fibers and the climbing fibers. Both use glutamate as a transmitter. Mossy fibers provide the inputs that elicit the coordinated movement programs, and climbing fibers provide the error signals that lead to an improved motor program and might work as a timer for motor activity (D'Angelo et al., 2013). The only output of the cerebellar cortex is mediated via the GABAergic Purkinje cells (PCs) that provide the results of the cerebellar program calculations as inhibitory signals to the deep cerebellar nuclei (DCN) (Pugh and Raman, 2009) via $\alpha 1\beta\gamma 2$ GABA_ARs (Laurie et al., 1992) (Fig. 6).

As discussed above, $\alpha 6\text{GABA}_A\text{Rs}$ are located at Golgi cell-granule cell synapses and extrasynaptic sites. The synaptic $\alpha 1\beta\gamma 2$ receptors together with synaptic $\alpha 6\beta\gamma 2$ and $\alpha 1\alpha 6\beta\gamma 2$ receptors mediate phasic inhibition, whereas the extrasynaptic $\alpha 6\beta\delta$ or $\alpha 1\alpha 6\beta\delta$ receptors as well as extrasynaptically located $\alpha 6\beta\gamma 2$ and $\alpha 1\alpha 6\beta\gamma 2$ receptors mediate tonic inhibition of granule cells (Nusser et al., 1998). Granule cells are excitatory interneurons in the cerebellar cortex that receive excitatory

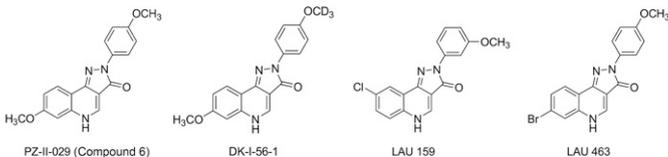
GABA site ligands



BZ site ligands



$\alpha 6\text{GABA}_A\text{R}$ -selective PAMs (BZ + PQ site ligands)



Ligands at transmembrane sites

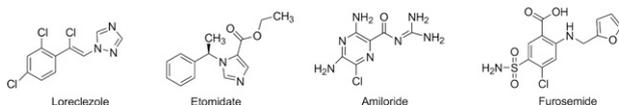
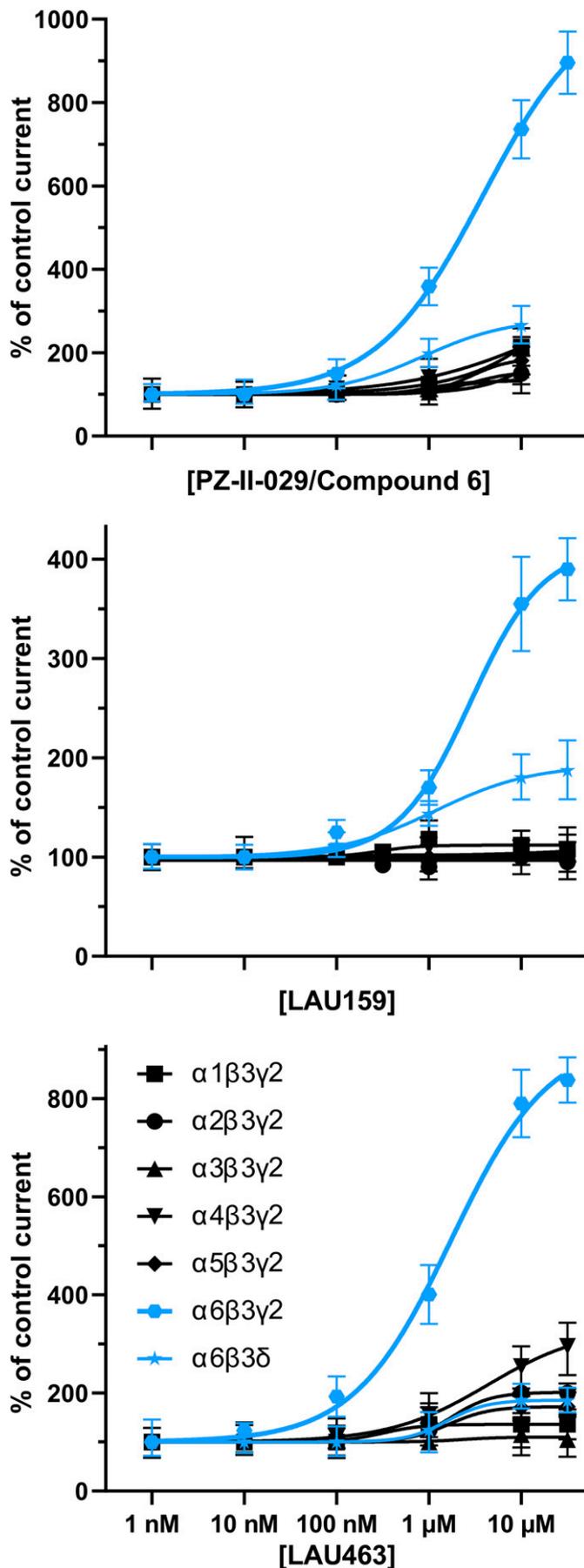


Fig. 4. Chemical structures of GABA_AR ligands. BZ, benzodiazepine.



inputs from mossy fibers and are inhibited by Golgi cells. The synaptic contacts of mossy fiber terminals, granule cell dendrites, and Golgi cells axon and dendrites are enwrapped into a glial sheet, forming the cerebellar glomerulus (Mapelli et al., 2014) (Fig. 6). Each glomerulus is characterized by one mossy fiber rosette and dendrites of several granule cells and Golgi cells as well as Golgi cell axons. By confining the space around the granule cell and Golgi cell compartments, the glomerulus provides optimal conditions for GABA released from Golgi cells to also activate extrasynaptic receptors at the granule cell dendrites as well as $\alpha 6\beta\gamma 2$ receptors at some of the excitatory mossy fiber synapses (Nusser et al., 1996), possibly limiting the excitation mediated by these synapses and thus sharpening their excitatory signal. Each granule cell dendrite contacts different glomeruli, receiving inputs and information from different mossy fibers. The axons of granule cells ascend in the molecular layer, where they originate the parallel fibers that form excitatory synapses on PCs and Golgi cells. Golgi cells can thus directly inhibit granule cells in a feedforward loop when activated by mossy fibers within the glomeruli. They also can inhibit granule cells in a feedback loop after Golgi cell activation via the ascending axons and parallel fibers of granule cells.

While granule cell depolarization begins, the increasing activity of the feedforward and feedback inhibition via Golgi cells modulates their response. GABA release from the presynaptic terminal of Golgi cells activates $\alpha 1$ - and in part $\alpha 6$ -containing receptors in the postsynaptic density (Nusser et al., 1998). $\alpha 1\beta\gamma 2$ receptors open and close rapidly in contrast to the $\alpha 6\beta\gamma 2$ receptors. Whereas $\alpha 1\beta\gamma 2$ receptors control granule cell responses in a narrow, approximately 10-millisecond band, $\alpha 6\beta\gamma 2$ receptors show a broader, approximately 50-millisecond tuning (Mapelli et al., 2014; Nieuwenhuis et al., 2014). Inhibition by GABA reduces the intensity and duration of granule cell responses without significantly changing the precision of the first spike (Nieuwenhuis et al., 2014). The longer inhibition time elicited by $\alpha 6\beta\gamma 2$ receptors thus better separates each incoming signal from other incoming signals. In addition, Golgi cells cause a broad lateral inhibition of granule cells that generates

Fig. 5. Concentration-response curves of $\alpha 6$ GABA_AR-selective ligands. Curves are reproduced based on published (Varagic et al., 2013; Chiou et al., 2018; Knutson et al., 2018; Treven et al., 2018), (PCT/US2016/035761) and unpublished data for PZ-II-029/Compound 6 (top), 8-chloro-2-(3-methoxyphenyl)-2H-pyrazolo[4,3-c]quinolin-3(5H)-one (LAU159) (middle), and 7-bromo-2-(4-methoxyphenyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one) (LAU463) (bottom). Data for $\alpha 6\beta\gamma 2$ and $\alpha 6\beta 3\delta$ are presented in blue. At 100% of control current, the response equals that of the GABA-evoked current in the absence of compounds. All measurements were performed with GABA concentrations eliciting 3%–5% and ~10%–20% of maximum GABA-elicited current for $\gamma 2$ - and δ -containing receptors, respectively. Modulation of $\alpha 6$ -containing receptors by the indicated compounds at 100 nM was already stronger than that of receptors containing other α subunits. Deuterated derivatives, such as DK-I-56-1, behave like the respective parent compounds in concentration-response investigations at different receptor subtypes (Knutson et al., 2018).

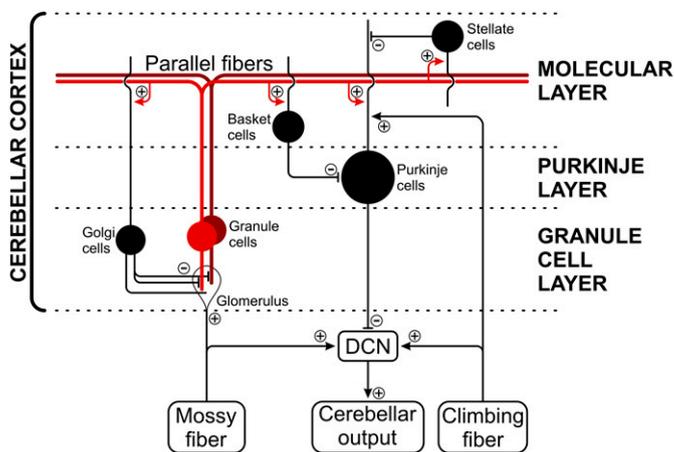


Fig. 6. Simplified schematic representation of cerebellar microcircuitry. Excitatory terminals are indicated by arrowheads and a plus sign, whereas inhibitory terminals are represented by blunt T-ends and a minus sign. The mossy fiber terminal structure that is known as glomerulus is depicted as drop-shaped terminal ending. Granule cells with their schematic processes are emphasized by red color.

dense clusters of granule cell activity organized in center-surround structures, implementing combinatorial operations of multiple mossy fiber inputs (D'Angelo et al., 2013). This allows generation of granular layer coherent oscillations and resonance, induction of mossy fiber–granule cell long-term potentiation and long-term depression, and spatio-temporal reconfiguration of granular layer activity (Nieus et al., 2014). However, long-term potentiation and long-term depression occur at several excitatory and inhibitory synapses in the main cerebellar subcircuits and regulate the function of granule cells, PCs, and DCN cells (Mapelli et al., 2015).

Although the dynamic nature of cerebellar computations and the exceptional temporal sensitivity of cerebellar circuits require high-precision phasic inhibitory mechanism, tonic inhibition at Golgi cell–granule cell synapses provides at least 97% of the inhibition of granule cells (Hamann et al., 2002). Tonic inhibition reduces the fraction of granule cells activated by mossy fiber inputs and can set the level of neuronal excitability at the mossy fiber–granule cell synapse (Hamann et al., 2002; Mapelli et al., 2014). The excitability of granule cells can additionally be regulated by GABA tonically released from glial cells and acting on cell bodies, axons, and parallel fibers of granule cells (Lee et al., 2010). Such processes might be involved in enhancing pattern recognition in Purkinje cells, for instance by distinguishing between important (strong stimulus) and nonimportant (weak stimulus) information. A perfectly balanced function of the extrasynaptic $\alpha 6\text{GABA}_A\text{Rs}$ also is essential because their overactivity (or overstimulation by drugs such as ethanol) can disrupt the synchronous firing of granule cells, causing disturbances in motor programs, whereas an underactivity can provide problems

in the cognitive performance and stress-related and social behaviors (Rudolph et al., 2020).

Granule cells, in addition to activating PCs and Golgi cells, also generate feedforward and lateral inhibition of PCs by activating stellate cells (at the dendrites of PCs) and basket cells (at the soma of PCs), respectively (Fig. 6). Feedforward inhibition limits the excitation of PCs, whereas lateral inhibition reduces the activity of those PCs that fire asynchronously. Spike synchrony of PCs requires coactivation of several discrete groups of granule cells to overcome lateral inhibition by basket and stellate cells. This lateral inhibition also narrows the duration of granule cell–mediated excitation to 1–2 milliseconds, an effect that would be expected to increase the precision of PC firing in response to excitation (Person and Raman, 2012).

PCs represent the major output neurons of the cerebellar cortex, providing an inhibitory control on the DCN. DCN neurons spontaneously fire tens of action potentials per second in vivo when animals are not engaged in cerebellar behavior (Pugh and Raman, 2009) possibly to maintain ongoing muscle tone. Due to their specific ion channels, even a short excitation, possibly via mossy fibers that not only activate granule cells but also the respective DCN neurons, can drive DCN neurons into a depolarization block. Their firing usually resumes only after an active hyperpolarization likely induced by the inhibitory GABAergic input from PCs (Pugh and Raman, 2009). This behavior possibly allows DCN neurons to resume firing in synchrony with PCs.

Accumulated evidence indicates that subsets of PCs synchronize their firing during behaviors that require the cerebellum, for instance in a learned motor task in rats, and the increase of synchrony is time-locked to movement (Heck et al., 2007). The synchronous inhibitory input from PCs sets the timing and rate of action potentials produced by DCN cells, thereby relaying information out of the cerebellum (Person and Raman, 2012). Then, changing spatiotemporal patterns of PC activity allows different subsets of inhibitory neurons to control cerebellar output at different times.

B. Role of the Cerebellum in the Function of the Brain

The cerebellum is involved in sensorimotor functions regulating the movement of all muscles in the body, including those controlling emotional facial expressions and speech, and is thus involved in all our communication with and all our reactions to the surrounding world. For that, information from all our senses is analyzed and integrated in the appropriate brain regions, eliciting context-specific motor programs in the motor cortex that are conveyed to the cerebellum via mossy fibers. These motor programs are constantly adapted and optimized by incoming sensory information via climbing fibers to fit to the

TABLE 1
Distribution of α6-subunits in the nervous system and in peripheral tissues

Region	Experimental Subjects	Detection Method	Reference
Central nervous system			
Cerebellum	Human post-mortem study (schizophrenia and control)	RT-qPCR	Bullock et al. (2008)
	Rats (Long Evans)	RT-qPCR	Bullock et al. (2009)
	Rats (undefined)	ISH	Laurie et al. (1992)
	Rats (Albino)	ISH	Persohn et al. (1992)
	Rats (Sprague-Dawley)	Northern blot	Mhatre and Ticku (1992)
	Rats (Sprague-Dawley)	IHC	Pirker et al. (2000)
	Mice (C57BL/6J)	ISH	Gutierrez et al. (1996)
	α6 ^{-/-} Mice (C57BL/6)	LacZ- <i>Gabra6</i>	Varecka et al. (1994)
	Mice, chicken, and fish	ISH	Lein et al. (2007)
		Autoradiography	Jones et al. (1997)
Cochlear nuclei/ Cochlear root nuclei	Rats (Wistar)	ISH	Campos et al. (2001)
	Rats (Sprague-Dawley)	IHC	Gutierrez et al. (1996)
Cortex (prefrontal)	Mice (C57BL/6J)	ISH	Varecka et al. (1994)
	α6 ^{-/-} Mice (C57BL/6)	LacZ- <i>Gabra6</i>	Lein et al. (2007)
	Rats (Wistar)	RT-PCR	Jones et al. (1997)
	Human post-mortem study (AUD and control)	RT-qPCR	Gomez-Nieto et al. (2008)
Cortex (superior frontal)	Rats (Long Evans)	RT-qPCR	Gatta et al. (2021)
	Human post-mortem study (autism and control)	RT-qPCR	Agrawal and Dwivedi (2020)
Hippocampus	Rats (Sprague-Dawley)	Western blot	Fatemi et al. (2014)
	α6 ^{-/-} Mice (C57BL/6)	RT-qPCR, IHC, Western blot	Yang et al. (2016)
Inferior colliculus (dorsal)	Mice (C57BL/6J)	LacZ- <i>Gabra6</i>	Jones et al. (1997)
Infralimbic area	Mice (C57BL/6J)	ISH	Lein et al. (2007)
Olfactory nerve	Rats (Sprague-Dawley)	IHC	Gutierrez et al. (1996)
Olfactory cortex (peduncular area, taenia tecta)	Mice (C57BL/6J)	ISH	Lein et al. (2007)
Orbital layer	Mice (C57BL/6J)	ISH	Lein et al. (2007)
Periaqueductal gray	Alcol P rats (Wistar)	RNA seq	McClintick et al. (2016)
Parabrachial nucleus	Mice (C57BL/6J)	ISH	Lein et al. (2007)
Raphe nucleus (dorsal)	Alcol P rats (Wistar)	RNA seq	McClintick et al. (2015)
Retina (inner plexiform layer)	Rats (Sprague-Dawley)	IHC	Gutierrez et al. (1996)
Spinal cord	Rats (Sprague-Dawley) (dorsal horn, layer II)	IHC	Gutierrez et al. (1996)
	Turtles (motor neurons)	RT-PCR, IHC, Western blot	Gomez-Nieto et al. (2008)
	Rats (Sprague-Dawley) (V _c -C ₁)	IHC	Andres et al. (2014)
Striatum (ventral)	Mice (C57BL/6J)	Western blot	Kramer and Bellinger (2013)
	D ₃ R ^{-/-} mice	RT-PCR	Kramer and Bellinger (2014)
Substantia nigra	α6 ^{-/-} Mice (C57BL/6)	LacZ- <i>Gabra6</i>	Leggio et al. (2015)
Thalamus (geniculate nuclei)	α6 ^{-/-} Mice (C57BL/6)	LacZ- <i>Gabra6</i>	Jones et al. (1997)
Peripheral nervous system			
Cochlea	Mice (CBA/J)	RT-PCR	Drescher et al. (1993)
	Rats (Wistar)	IHC	Yamamoto et al. (2002)
Trigeminal ganglia	(spiral ganglion and organ)		
	Rats (Sprague-Dawley)	IHC and RT-PCR	Hayasaki et al. (2006)
	Rats (Sprague-Dawley)	IHC and qPCR	Puri et al. (2011)
	Rats (Sprague-Dawley)	IHC	Puri et al. (2012)
		Western blot	Kramer and Bellinger (2013)
			Kramer and Bellinger (2014)
Vestibular end-organs	Rats (Wistar)	IHC	Fan et al. (2018)
	Rats (Wistar)	RNA seq	Kogelman et al. (2017)
	Mice (ICR)	IHC	Tzeng et al. (2021)
	Rats (Sprague-Dawley)	RT-PCR	Rezaee et al. (1999)
Peripheral tissues			
T lymphocytes	Human blood samples	RT-qPCR	Dionisio et al. (2011)
	Jurkat cells (human T lymphocyte cell line)	RT-qPCR	Mendu et al. (2012)
	Rats (BB strain) (CD4 ⁺ and CD8 ⁺)	RT-qPCR	Alam et al. (2006)
	Rats (Wistar) (CD4 ⁺ and CD8 ⁺)	RT-qPCR	Mendu et al. (2011)
Liver	Human resections	Western blot	Mendu et al. (2012)
	Rats (Sprague-Dawley)	IHC	Bautista et al. (2014)
Lung alveolar (type I and II)	Rats (Sprague-Dawley)	RT-qPCR	Jin et al. (2006)
Pancreatic α-cells	Rats (Wistar)	RT-qPCR	Jin et al. (2013)
Placenta and uterus	Rats (Wistar)	RT-PCR	Akinci and Schofield (1999)

α6^{-/-} mice, GABA_A receptor α6 subunit knockout mice; Alcol P rats, alcohol-preferring rats; autoradiography, [³H]Ro15-4513; AUD, alcohol use disorder; BB strain, congenic Biobreeding strain; CBA/J, a strain of Agouti mice; CD4⁺: CD4⁺ T lymphocytes; CD8⁺: CD8⁺ T lymphocytes; D₃R^{-/-} mice, dopamine D₃ receptor knockout mice; ICR, Institute of Cancer Research; IHC, immunohistochemistry; ISH, in situ hybridization; LacZ-*Gabra6*, LacZ expression driven from the *Gabra6* locus detected by β-galactosidase staining; Northern blot, Northern blotting for mRNA; PCR, polymerase chain reaction; qPCR, quantitative PCR; RNA seq, RNA sequencing; RT-PCR, reverse-transcription PCR; RT-qPCR, quantitative reverse-transcription PCR; V_c-C₁, trigeminal nucleus caudalis and upper cervical region; Western blot, Western blotting for protein.

individual requirements. The cerebellum is thus also involved in attention and cognitive performance (Mannarelli et al., 2019; Schmahmann et al., 2019) as well as in language and emotional processing (Sacchetti et al., 2009; De Smet et al., 2013) and in controlling the reward circuitry and social behaviors (Wagner et al., 2017; Carta et al., 2019).

The cerebellum forms reciprocal, closed-loop circuits with much of the cerebral cortex as well as subcortical structures and contains repeating processing modules, the function of which is driven by the input the module receives (Schmahmann, 1991; D'Mello and Stoodley, 2015). Motor coordination (anterior cerebellum) and cognitive functioning (posterior cerebellum) are mediated by different parts of the cerebellum. Thus, connections between PCs in the posterior lateral cerebellum to DCN neurons, which are distinct from those controlling motor activity, convey the information from the cerebellar cortex to the prefrontal cortex via the thalamus (Caligiore et al., 2016). Lesions in the "limbic cerebellum" (vermis and fastigial nucleus) cause a "cerebellar cognitive affective syndrome" (Schmahmann and Sherman, 1998) that involves disorders of attentional control, emotional control, and social skills as seen in patients with autism spectrum (D'Mello and Stoodley, 2015) and psychosis spectrum disorders (Schmahmann et al., 2007). The cerebellum also has extensive connections with the ventral tegmental area (Carta et al., 2019), which is involved in the reward circuitry, and the basal ganglia, providing the neural basis for a cerebellar involvement in Parkinson disease, dystonia, Tourette syndrome, and addiction as well as in normal basal ganglia function, such as reward-related learning (Caligiore et al., 2016).

Relative to other regions of the brain, the human cerebellum undergoes enormous growth between 24 and 40 weeks postconception, increasing approximately 5-fold in volume and over 30-fold in surface area. Although this rapid cerebellar growth slows postnatally, neural differentiation and growth of axonal inputs and outputs continue throughout the first postnatal year. This substantial prenatal growth continued postnatally renders the cerebellum especially vulnerable to developmental disruptions and damage (D'Mello and Stoodley, 2015). Abnormalities in cerebellar structure, connectivity, and function have been identified in patients with neurodevelopmental disorders like autism spectrum disorder (Shevelkin et al., 2014; D'Mello and Stoodley, 2015), schizophrenia (Parker et al., 2013; Peters et al., 2016), and dyslexia (Person and Raman, 2012) and might thus be causatively related to changes in emotional, cognitive, and social behaviors of these patients.

From the above description of the structure and function of the cerebellum, the involvement of $\alpha 6$ GABA_ARs in neuropsychiatric disorders can easily be delineated. Synaptic and extrasynaptic $\alpha 6$ GABA_ARs are of central

importance for the functioning of the cerebellum. A significant disturbance in their expression or function might thus not only result in motor incoordination, ataxia, dystonia, and epilepsy but also contribute to neuropsychiatric disorders.

III. $\alpha 6$ GABA_ARs and Animal Models of Neuropsychiatric Disorders

A. $\alpha 6$ GABA_ARs and Animal Models of Angelman Syndrome

Angelman syndrome (Table 2) is a neurodevelopmental disorder that is most frequently caused by a maternal deletion of the chromosome 15q11-q13 region, which includes the causative *UBE3A* gene encoding a ubiquitin E3 ligase (Roden et al., 2010). The major clinical manifestations consist of severe developmental delay, speech impairment, epilepsy, movement and balance problems, and characteristic behavior, such as paroxysmal laughter (Egawa et al., 2012). Abnormal movement and balance are largely attributed to cerebellar ataxia. No effective therapeutic strategies have yet been elucidated.

Recently, a study on a *UBE3A*-deficient mouse model of Angelman syndrome (Egawa et al., 2012) demonstrated that the deletion of the ubiquitin E3 ligase, which controls the degradation of GABA transporter 1 (GAT-1), leads to a surplus of GAT-1 and decreased GABA concentrations in the extrasynaptic space of cerebellar granule cells. Reduced tonic inhibition of granule cells causes abnormal firing of PCs and ataxia. Low doses of THIP (gaboxadol), which preferentially activates extrasynaptic $\alpha 6\beta 3\delta$ receptors, improved the abnormal PC firing and reduced cerebellar ataxia in these mice (Egawa et al., 2012).

B. $\alpha 6$ GABA_ARs and Animal Models of Down Syndrome

Down syndrome (Table 2) is due to the inheritance of an additional copy of all or part of chromosome 21 (trisomy 21) and is characterized by intellectual disability and impaired motor control. Lack of coordinated movement, poor balance, and unclear speech imply dysfunctions of the cerebellum, which is known to be reduced in volume in Down syndrome (Table 2). The principal cause of the smaller cerebellum is a diminished number of granule cells. The Ts65Dn mouse, the most widely investigated animal model of Down syndrome, was generated by triplication of a region of the mouse chromosome 16, which makes it trisomic for approximately half of the orthologous protein-coding genes and a subset of non-protein-coding RNAs located on the long arm of the human chromosome 21 (Hsa21). Ts65Dn mice replicate the reduced number and density of cerebellar granule cells that are characteristic for Down syndrome (Szemes et al., 2013). Using the Ts65Dn mouse model of Down syndrome, it was demonstrated that the tonic GABA_A

TABLE 2
Therapeutic potentials of α6GABA_AR PAMs based on patient studies and animal models of neuropsychiatric disorders

Disorder	Patient Study/Animal Model	Pathophysiological Changes	Effects of α6GABA _A R PAMs
Angelman syndrome	Post-mortem (human)	Cerebellar atrophy, PC, and granule cells loss and lower cerebellar GABA levels (Jay et al., 1991).	# THIP (gaboxadol), a δGABA _A R agonist, reduced PC abnormal firing and reduced cerebellar ataxia in <i>Ube3a</i> -deficient mice (Egawa et al., 2012).
	Gene association/post-mortem (human)	Functional deficit of <i>UBE3A</i> -encoded protein (Kishino et al., 1997; Lalande and Calciano, 2007).	
	<i>Ube3a</i> -deficient mice	Reduced tonic inhibition of cerebellar GCs causes abnormal PC firing and cerebellar ataxia (Egawa et al., 2012).	* α6GABAR PAMs may achieve similar therapeutic effects in <i>Ube3a</i> -deficient mice and patients with Angelman syndrome.
Down syndrome	Postmortem (human)	Reduced cerebellar volume and fewer cerebellar GCs (Aylward et al., 1997; Baxter et al., 2000).	
	MRI (human)	Smaller brain volume with disproportionately smaller cerebellum (Pinter et al., 2001).	
	Ts65Dn mouse model	Smaller tonic GABA currents, unchanged α6 subunits, and increased excitability in cerebellar GCs (Szemes et al., 2013).	* THIP and α6GABA _A R PAMs may restore tonic GABA transmission in cerebellar GCs and alleviate motor deficits in Ts65Dn mice and in patients with Down syndrome.
Essential tremor	Postmortem (human)	Cerebellar and PC abnormalities (Louis and Faust, 2020).	# THIP suppressed harmaline-induced tremor in wild-type but not in <i>Gabra6</i> ^{-/-} or <i>Gabrd</i> ^{-/-} mice (Handforth et al., 2018), suggesting an involvement of α6βδ GABA _A Rs.
	fMRI (human)	Excessive cerebellar activity (Sharifi et al., 2014).	
	Cerebellar PC VGAT deletion mice	Onset of induced tremor in wild-type mice coincident with rhythmic PC firing. Tremorgenic agents had no effects in mice with impaired PC output (Brown et al., 2020).	# α6GABA _A R PAMs (Compound 6 and LAU 463 and their deuterated derivatives DK-I-56-1 and DK-I-58-1, respectively) suppressed harmaline-induced action tremor in mice in a manner prevented by intracerebellar furosemide (Huang et al., 2021), suggesting an involvement of cerebellar α6GABA _A Rs.
Tic disorders	MRI (human)	Reduced cerebellar gray matter volume (Tobe et al., 2010; Sigurdsson et al., 2020).	# <i>C. inermis</i> leaf juice reduced intractable motor tics in a patient (Fan et al., 2009).
	[¹¹ C]flumazenil PET (human)	Widespread abnormalities of GABA _A Rs in the brain, increase of GABA _A Rs in bilateral cerebellum (Lerner et al., 2012).	# This herb's extract (Chen et al., 2012) and a bioactive constituent, hispidulin, (Huang et al., 2015), reduced tic-associated responses in mice. Hispidulin acted as an α6GABA _A R PAM (Liao et al., 2016).
	PPI test (human)	PPI deficit in TS children (Swerdlow et al., 2001).	# α6GABA _A R-selective PAM DK-I-56-1 reduced tic behaviors and PPI deficits in mouse models of tic disorders (Cadeddu et al., 2021).
ADHD	MRI (human)	Smaller superior cerebellar vermis (Mackie et al., 2007).	* α6GABA _A R PAMs may restore tonic GABA inhibition in cerebellar GCs and reduce ADHD phenotypes.
	fMRI (human)	Fronto-parieto-cerebellar deficits (Hart et al., 2012).	
	PPI test (human)	PPI deficit in patients with ADHD (Ornitz et al., 1992).	# α6GABA _A R PAMs (Compound 6, hispidulin, Ro15-4513, loreclezole) significantly reversed PPI deficits in mice induced with methamphetamine (Chiou et al., 2018).
	Gene association	<i>GITI</i> SNP is associated with ADHD phenotypes (Won et al., 2011).	
	<i>Git1</i> -knockout mice	Reduced tonic GABA currents in cerebellar GC and ADHD-like behaviors (Kim et al., 2017).	# DK-I-56-1 reversed PPI deficits in animal models of tic disorders (Cadeddu et al., 2021).
Obsessive-compulsive disorder	MRI (human)	Significant cerebellar enlargement (Pujol et al., 2004; Tobe et al., 2010; Brooks et al., 2016).	# α6GABA _A R PAMs (Compound 6, hispidulin, Ro15-4513, loreclezole) significantly reversed PPI deficits in mice induced with methamphetamine (Chiou et al., 2018).
	fMRI (human)	Decreased functional connectivity between the cerebral cortex and cerebellum in executive control and emotion-processing networks (Anticevic et al., 2014; Xu et al., 2019).	# DK-I-56-1 reversed PPI deficits in animal models of tic disorders (Cadeddu et al., 2021).
	PPI test (human)	PPI deficit (Swerdlow et al., 1993; Ahmari et al., 2012).	
Huntington disease	Post-mortem (Human)	Cerebellar atrophy and loss of PCs correlate with motor deficits (Rub et al., 2013; Singh-Bains et al., 2019).	# α6GABA _A R-selective PAMs might eliminate PPI deficits (Chiou et al., 2018; Cadeddu et al., 2021) and possibly also reduce motor symptoms of Huntington disease.

(continued)

TABLE 2—Continued

Disorder	Patient Study/Animal Model	Pathophysiological Changes	Effects of $\alpha 6$ GABA _A R PAMs
	MRI (human)	Aberrant cerebellar diffusion and reduced cerebellar volume associated with impaired motor function and increased psychiatric symptoms (Rees et al., 2014).	
	PPI test (human)	PPI deficit in patients with Huntington disease (Swerdlow et al., 1995).	
Schizophrenia	Post-mortem (human)	Loss of cerebellar PCs (Maloku et al., 2010). Increased $\alpha 6$ subunit expression in the superior frontal cortex (Fatemi et al., 2017). Decreased <i>GAD67</i> , <i>GAD65</i> , and <i>GAT-1</i> and increased <i>GABRA6</i> mRNA in the cerebellum. (Fatemi et al., 2005; Bullock et al., 2008). However, no change in <i>GABRA6</i> mRNA and protein expression in the cerebellum (Fatemi et al., 2013).	# $\alpha 6$ GABA _A R-selective PAMs (Compound 6, hispidulin, Ro15-4513, loreclezole) rescued PPI deficits (Chiou et al., 2018). # Compound 6, DK-I-56-1, and hispidulin rescued social withdrawal and cognitive deficits (Chiou et al., 2019; Mouri et al., 2020) in mouse models mimicking schizophrenia.
	MRI (human)	Volume reductions in posterior superior vermis as well as in frontal and temporal lobe, thalamus, and left cerebellar hemisphere, supporting the model of cognitive dysmetria in schizophrenia (Volz et al., 2000; Okugawa et al., 2002). Reduced cerebellar volume associated with negative and psychotic symptom duration and psychosocial impairment (Wassink et al., 1999).	
	fMRI (human)	Impaired PFC-cerebellum functional connectivity associated with negative symptoms (Brady et al., 2019).	
	PPI test (human)	PPI deficit (Braff et al., 1978; San-Martin et al., 2020).	
	PET (human)	Dysfunctional prefrontal thalamic-cerebellar circuitry (Andreasen et al., 1996).	
	Gene association (Human)	<i>GABRA6</i> rs3219151 C>T associated with schizophrenia susceptibility (Gong et al., 2013). 5q34 mutations are associated with schizophrenia and cause lower <i>GABRA6</i> expression (Petryshen et al., 2005).	
	PCP-chronically treated rats	Decreased <i>GAD65</i> and <i>GAD67</i> and increased <i>Gabra6</i> mRNA at cerebellar Golgi-granule synapses (Bullock et al., 2009).	
Stress-associated disorders	MRI (adolescents with early-life stress)	Reduced cerebellar gray matter volume (Walsh et al., 2014; Lim et al., 2018).	* $\alpha 6$ GABA _A R PAMs may attenuate functional deficits in the cerebellum and reduce symptoms of stress-associated disorders.
	fMRI (adults with early-life stress)	Reduced blood flow and neuronal activity in vermis (Anderson et al., 2002).	
	MRI (pediatric PTSD)	Reduced cerebellar volume (De Bellis et al., 2015).	
	MRI (adult PTSD)	Reduced cerebellar volume (Baldacara et al., 2012).	
	PPI test (human)	PPI deficits in patients with premenstrual dysphoric disorder (Kask et al., 2008). PPI deficits in women with PTSD (Pineles et al., 2016) and in trauma-affected refugees with PTSD (Meteran et al., 2019).	
	Gene association	<i>GABRA6</i> rs3219151 T carriers had greater responses to stress (Uhart et al., 2004; Lynch et al., 2015). <i>GABRA6</i> rs3219151 T male carriers had higher cortisol levels and higher abdominal obesity (Rosmond et al., 2002). <i>GABRA6</i> rs3811995 C carriers had higher incidents of externalizing behaviors and substance use (Trucco et al., 2016).	
	Cerebellar GCs <i>Gabrd</i> -KO mice	Increased stress-related behaviors (Rudolph et al., 2020).	
Anxiety disorders	PPI test (human)	PPI deficit in patients with panic disorder (Ludewig et al., 2002).	# $\alpha 6$ GABA _A R PAMs (Compound 6, hispidulin, Ro15-4513, loreclezole) significantly reversed PPI deficits in mice induced with methamphetamine (Chiou et al., 2018).
	Gene association (human)	<i>GABRA6</i> rs3219151 T carriers had higher anxiety, depression, and suicide risk after recent negative life events (Gonda et al., 2017).	

(continued)

TABLE 2—Continued
Pathophysiological Changes

Disorder	Patient Study/Animal Model	Pathophysiological Changes	Effects of α6GABA _A R PAMs
		<i>GABRA6</i> rs3219151 C carriers associated with panic disorder, but T carriers had stronger reactions to a dynamic fearful face (Inoue et al., 2015). Panic disorder–associated miRNA reduced <i>GABRA6</i> expression in a Luciferase-reporter assay (Muinos-Gimeno et al., 2011). Increased anxiety-related behaviors (Rudolph et al., 2020).	# DK-I-56-1 reversed PPI deficits in animal models of tic disorders (Cadeddu et al., 2021). * α6GABA _A R PAMs may attenuate functional deficits in cerebellum and reduce symptoms of anxiety and panic disorders.
Depression/bipolar disorder	Post-mortem (human) MRI (human) Gene association (human) Adolescent rats with maternal separation Hippocampal <i>Gabra6</i> -KD rats	Decreased cerebellar GAD65 and GAD67 (depression/bipolar disorder) (Fatemi et al., 2005). Loss of cerebellar PCs in bipolar disorder (Maloku et al., 2010). Increased α6 subunits in the lateral cerebellum (major depression) (Fatemi et al., 2013). Increased α6 subunits in the superior frontal cortex (bipolar disorder) (Fatemi et al., 2017). Reduced cerebellar volume (bipolar disorder) (Baldacara et al., 2012). <i>GABRA6</i> rs3219151 C-allele associated with major depression (Inoue et al., 2015). <i>GABRA6</i> rs3811993 missense variant associated with bipolar disorder (Ament et al., 2015). Decreased <i>Gabra6</i> mRNA and α6 subunits in the hippocampus and depressive-like behavior (Yang et al., 2016). Inducing depressive-like behaviors (Yang et al., 2016).	* α6GABA _A R PAMs might compensate low GABA levels in cerebellum and ameliorate some symptoms of these disorders. * Increased α6 subunits in depression/bipolar patients might be an incomplete compensatory change due to impaired GABAergic transmission. α6GABA _A R PAMs might compensate α6GABA _A R deficits and ameliorate depressive symptoms.
Autism spectrum disorder	Postmortem (human) MRI (human) PPI (human)	Reduced cerebellar PC size and number (Fatemi et al., 2002a; Whitney et al., 2008). Decreased GAD65 and GAD67 in parietal and cerebellar cortices (Fatemi et al., 2002b; Yip et al., 2007). Fewer α6, β2, δ, and γ2 subunits in the superior frontal cortex (Fatemi et al., 2014). Reduced cerebellar gray matter in vermis associated with social and interaction deficits (Riva et al., 2013; D’Mello et al., 2015). PPI deficit in children/adolescents but not adults (Cheng et al., 2018).	* α6GABA _A R PAMs may compensate for reduced GABA and α6GABA _A R levels and attenuate symptoms in autism spectrum disorders. # α6GABA _A R PAMs (Compound 6, hispidulin, Ro15-4513, loreclezole) significantly reversed PPI deficits in mice induced with methamphetamine (Chiou et al., 2018). # DK-I-56-1 reduced tic-like jerks in animal models of tic disorders (Cadeddu et al., 2021).
Alcohol use disorder	Postmortem (chronic excessive alcoholics) Gene association (human) Repeated ethanol-treated rats	Cerebellar atrophy (Torvik and Torp, 1986). Reduced PC volume but increased granular layer in the cerebellum (Andersen, 2004). Decreased cerebellar δ, but unchanged α6 protein and decreased PFC α6 and δ mRNA (Gatta et al., 2017; Gatta et al., 2021). α6Ser385 carriers in offspring of alcoholics had the lowest alcohol sensitivity during adolescence (Iwata et al., 1999) and were more vulnerable to become alcoholics later on (Schuckit et al., 1999; Hu et al., 2005). <i>GABRA6</i> rs3219151 T allele carriers were significantly associated with alcohol dependence (Loh et al., 1999; Sander et al., 1999; Radel et al., 2005; Sahni et al., 2019). Alcohol-dependent patients carrying the <i>GABRA6</i> rs3219151 TT or CC alleles differentially respond to naltrexone or acamprostate treatment, respectively (Ooteman et al., 2009). Increased cerebellar α6 but decreased α1 protein (Mhatre and Ticku, 1992; Morrow et al., 1992).	* Cerebellar changes in humans and rats suggest that α6GABA _A R PAMs may have some effects also in this disorder. Because of the multiple changes in other receptors and proteins, these effects are difficult to predict. * Alcohol-dependent patients carrying the <i>GABRA6</i> rs3219151 CC alleles might benefit from treatment with α6GABA _A R PAMs.

(continued)

TABLE 2—Continued

Disorder	Patient Study/Animal Model	Pathophysiological Changes	Effects of $\alpha 6$ GABA _A R PAMs
	Alcohol-preferring rats	Increased $\alpha 6$ and δ but decreased $\gamma 2$ mRNAs in the periaqueductal gray and dorsal raphe nucleus (McClintick et al., 2015, 2016).	
Trigeminal-related pain	TMJ inflammation (rats)	CFA induced stronger TMJ pain responses in rats with reduced TG <i>Gabra6</i> expression (Puri et al., 2012).	* $\alpha 6$ GABA _A R PAMs may reduce TMJ or trigeminal-related orofacial pain.
	Trigeminal neuropathic pain (rats)	Infraorbital nerve ligation induced trigeminal neuropathy (Vasovic et al., 2019).	# $\alpha 6$ GABA _A R-selective PAM DK-I-56-1 suppressed infraorbital nerve ligation-induced nociceptive responses (Vasovic et al., 2019).
Migraine	TGVS-activated rats	Capsaicin (<i>i.c.</i>) induces central and peripheral sensitization in the TGVS (Fan et al., 2018).	# $\alpha 6$ GABA _A R-selective PAMs (Compound 6, Ro15-4513, loreclezole) reduced migraine-related peripheral and central sensitization of TGVS. These effects were mimicked by topiramate and inhibited by systemic furosemide (Fan et al., 2018).
	Nitroglycerin-repeatedly treated mice	Decreased GAD65, unchanged GAT1, and unchanged $\alpha 6$ GABA _A Rs in trigeminal ganglia (Tzeng et al., 2021).	# $\alpha 6$ GABA _A R-selective PAM (DK-I-56-1) abolished and prevented migraine-like grimaces in a manner mimicked by sumatriptan and topiramate, respectively, and blocked by systemic furosemide (Tzeng et al., 2021).

$\alpha 6$ GABA_AR, $\alpha 6$ subunit-containing GABA_A receptor; ADHD, attention deficit hyperactivity disorder; CFA, complete Freund's adjuvant; GAD65, glutamate decarboxylase 65 kDa; GAD67, glutamate decarboxylase 67 kDa; GAT-1, GABA transporter 1; GC, granule cell; GIT1/Git1, G-protein-coupled-receptor kinase-interacting protein-1; KO, knockout; *i.c.*, intracisternally; ICR, Institute of Cancer Research; KO, knockout; MRI, magnetic resonance imaging; fMRI, functional magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NTG, nitroglycerin; PAM, positive allosteric modulator; PC, Purkinje cells; PET, positron emission tomography; PFC, prefrontal cortex; PTSD, post-traumatic stress disorder; PPI, prepulse inhibition; SNP, single nucleotide polymorphism; TG, trigeminal ganglia; TGVS, trigemino-vascular system; THIP, 4,5,6,7-tetrahydroisothiazolo-[5,4-c]pyridin-3-ol (gaboxadol); TMJ, temporomandibular joint; TS, Tourette syndrome; VGAT, vesicular GABA transporter; wt, wild type; *, hypothesis to be proven; #, hypothesis proven.

receptor current in granule cells is smaller than in wild-type mice and that Ts65Dn granule cells are thus more excitable (Szemes et al., 2013). Enhancing tonic inhibition of granule cells might thus improve coordinated movement in this mouse model as well as in patients with Down syndrome (Szemes et al., 2013).

C. $\alpha 6$ GABA_ARs and Animal Models of Essential Tremor

Essential tremor (Table 2) is the most common movement disorder. It is characterized by action tremor manifested with involuntary rhythmic contractions and relaxations of certain muscle groups in one or more body parts and involves an oscillatory network that includes the cerebellum, thalamus, motor cortex, and brainstem, as shown by magnetoencephalography (Schnitzler et al., 2009). The cause of essential tremor currently is not known. Post-mortem studies in individuals with essential tremor identified abnormalities in the cerebellum, which primarily affected PCs, basket cells, and climbing fibers (Louis and Faust, 2020). Functional magnetic resonance imaging (fMRI) studies revealed excessive cerebellar activity in patients with essential tremor (Sharifi et al., 2014).

Harmaline is a β -carboline neurotoxin that can induce action tremor in multiple species, including humans, in a frequency range of 8–16 Hz (Martin et al., 2005). Harmaline affects many types of receptors, ion channels, and gap junctions and likely affects the activity of multiple cell populations in the brain (Paterson

et al., 2009; Jiménez-Jiménez et al., 2020). Evidence accumulated that harmaline-induced action tremor involves synchronous rhythmic firing in the inferior olive nucleus, which provides a major input to cerebellar PCs via climbing fibers, strongly suggesting an involvement of PCs in tremor generation (Brown et al., 2020; Handforth and Lang, 2021). Experiments in awake behaving mice indicated that the onset of harmaline-induced tremor was coincident with rhythmic PC firing, and harmaline-induced tremor was blocked by genetically silencing of cerebellar PC output. Finally, mimicking harmaline-induced PC firing pattern by optogenetic PC stimulation reproduced the tremors induced by harmaline (Brown et al., 2020). Since Golgi cells control cerebellar activity by releasing GABA onto synaptic and extrasynaptic $\alpha 6$ GABA_ARs on cerebellar granule cells, it was investigated whether THIP (gaboxadol), which preferentially activates extrasynaptic $\alpha 6\beta\delta$ GABA_ARs, would suppress harmaline-induced tremor in mice (Handforth et al., 2018). Whereas THIP reduced harmaline-induced tremor in the respective littermates, it did not in $\alpha 6^{-/-}$ or $\delta^{-/-}$ mice, suggesting that cerebellar $\alpha 6\beta\delta$ GABA_ARs mediate THIP-induced tremor reduction (Handforth et al., 2018). Consistently, $\alpha 6$ GABA_AR-selective PAMs significantly suppressed harmaline-induced tremor activity in mice as did propranolol, a clinically used pharmacotherapy for essential tremor. Importantly, intracerebellar microinjection of furosemide, an $\alpha 6$ GABA_AR antagonist, prevented the antitremor effect of $\alpha 6$ GABA_AR-selective PAMs but not that of propranolol (Huang et al., 2021).

D. α 6GABA_ARs and Animal Models of Tic Disorders

In a case report, a pediatric patient with intractable motor tic disorders was found to respond well to an herbal remedy consisting of the leaf juice of *Clerodendrum inerme* (L.) Gaertn (*C. Inerme*) without apparent hepatotoxicity or renal toxicity (Fan et al., 2009). Tic disorders (Table 2), including Tourette syndrome, an idiopathic spectrum of tic disorders, are attributed to an over-reactive dopaminergic system in the cortico-thalamic-striatal circuit (Singer, 2005). Using methamphetamine-induced hyperlocomotion in mice, a hyperdopaminergic behavior model, the flavonoid hispidulin (6-methoxy-4,5,7-trihydroxyflavonoid) (Fig. 4) was identified as a constituent of the leaf extract that alleviated methamphetamine-induced hyperlocomotion (Chen et al., 2012; Huang et al., 2015).

Hispidulin exhibits a micromolar affinity for the benzodiazepine site of human GABA_ARs and is a PAM of recombinant GABA_ARs composed of the α 1 β 2 γ 2S, α 2 β 2 γ 2S, α 3 β 2 γ 2S, α 5 β 2 γ 2S, or α 6 β 2 γ 2S subunits (Kavvadias et al., 2004). The alleviating effect of hispidulin on methamphetamine-induced hyperlocomotion in mice (Liao et al., 2016) was mimicked by compounds modulating α 6GABA_ARs, including Compound 6 (PZ-II-029), the selective α 6GABA_AR PAM (Fig. 4), but not by diazepam and was prevented by an intracerebellar microinjection of furosemide, a selective allosteric α 6GABA_ARs antagonist, suggesting that hispidulin acts by positively modulating α 6GABA_ARs (Liao et al., 2016).

Compound 6 also significantly reduced stereotypic behaviors in mice with intrastriatal SLIT and NTRK-like family, member 1 (*Slitrk1*) gene silencing, an animal model of Tourette syndrome [Wu CY, (2016) Master Thesis, Graduate Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan; Du et al., Soc Neurosci 2017, Prog. No. 690.01, November 11-15, 2017, Washington DC, USA]. The *Slitrk1* gene is the mouse version of the human *SLITRK1* gene that codes for a transmembrane and signaling protein, which is responsible for synapse regulation and presynaptic differentiation in the brain. It has been linked to the formation of excitatory synapses. Among all adult striatal neurons, *Slitrk1* was reported to be only expressed in cholinergic interneurons (Stillman et al., 2009) that were lost in post-mortem brains of patients with Tourette syndrome (Kataoka et al., 2010). Mutations in the *SLITRK1* gene have been linked to obsessive-compulsive spectrum disorders, such as Tourette syndrome (O'Rourke et al., 2009), obsessive-compulsive disorder (OCD) (Ozomaro et al., 2013), and trichotillomania (Chattopadhyay, 2012).

In addition, an involvement of α 6GABA_ARs in tic-related behaviors was supported by a study indicating that DK-I-56-1 (Fig. 4), the deuterated derivative of Compound 6 (Knutson et al., 2018), significantly

reduced tic-like jerks in the D1CT-7 mouse model of Tourette syndrome (Cadeddu et al., 2021). The D1CT-7 mice are a transgenic line generated through the attachment of a neuropotentiating cholera toxin to the D1 dopamine receptor promoter. These mice display tic-like manifestations consisting of brief, sudden axial jerks (Cadeddu et al., 2021).

In addition to tic attacks, patients with Tourette syndrome also manifest sensorimotor gating deficits, which may contribute to their premonitory urges (Leckman et al., 1993) and can be measured by the disruption in the prepulse inhibition (PPI) of the startle response (Swerdlow et al., 2001). Exposing D1CT-7 mice to brief stressors dramatically increased their tic-like manifestations and elicited PPI deficits (Cadeddu et al., 2021). Similarly, striatal *Slitrk1*-silenced mice also exhibited a haloperidol-sensitive PPI disruption (Du et al., 2017; Soc Neuroscience 2017, Prog. No. 690.01, November 11–15, 2017, Washington DC, USA). This supports the validity of these two animal models and the hyperdopaminergic status in Tourette syndrome, as implicated in patients because of abnormal function of cortico-striatal-thalamo-cortical circuits (Leckman and Riddle, 2000). Currently, it is not known how the striatal dopaminergic overactivity in Tourette syndrome can be modulated by α 6GABA_AR PAMs via reducing cerebellar granule cell activity.

PPI disruptions are also manifested in patients with several other neuropsychiatric disorders (Table 2), such as attention deficit/hyperactivity (ADHD) disorder (Ornitz et al., 1992), OCD (Swerdlow et al., 1993), Huntington disease (Swerdlow et al., 1995), schizophrenia (Braff et al., 1978), and autism spectrum disorder (Cheng et al., 2018), to name only a few of them.

E. α 6GABA_ARs and Animal Models of Schizophrenia

Especially in patients with schizophrenia, PPI disruption is a well known endophenotype manifestation (Braff et al., 1978). PPI disruption can be induced by methamphetamine or the N-methyl-D-aspartate (NMDA) channel blockers ketamine, phencyclidine (PCP), and MK-801 (dizocilpine) based on hyperdopaminergic and hypoglutamatergic hypotheses of schizophrenia, respectively (Geyer and Moghaddam, 2002). Using these PPI disruption models in mice, it was demonstrated that hispidulin but not diazepam rescued disrupted PPI when applied either via an intraperitoneal injection or a bilateral intracerebellar microinjection (Chiou et al., 2018). The effect of systemic hispidulin on methamphetamine-disrupted PPI could be mimicked by Compound 6, the α 6GABA_AR-selective PAM, and its analogs (PCT/US2016/035761). Effects of hispidulin and Compound 6 were prevented by an intracerebellar microinjection of furosemide, suggesting that cerebellar α 6GABA_ARs are the targets of hispidulin and Compound 6 (Chiou et al., 2018). Since hispidulin, in contrast to Compound 6,

could not modulate $\alpha 6\beta 3\delta$ receptors, these data suggest that PAMs of cerebellar $\alpha 6\beta \gamma 2$ receptors can rescue disrupted PPI (Chiou et al., 2018).

Interestingly, maternal separation stress in mice carrying the mutated schizophrenia risk gene, *DISC1* (Disrupted in Schizophrenia 1), can induce PPI impairment and social withdrawal, mimicking positive and negative symptoms, respectively, in schizophrenia (Niwa et al., 2013). Hispidulin reduced both behavioral phenotypes in these mice and in chronic PCP-treated mice (Mouri et al., 2020). The $\alpha 6$ GABA_AR-selective PAMs Compound 6 and DK-I-56-1 also reduced PPI impairment, social withdrawal, and cognitive impairment in chronic PCP-treated mice (Chiou et al., 2019, poster at the Soc Neurosci 2019, No. 645.14/B55, Chicago, IL, USA) and might thus be able to ameliorate the three core symptom dimensions of schizophrenia, positive symptoms, negative symptoms, and cognitive impairment.

F. $\alpha 6$ GABA_ARs and Animal Models of Stress-Associated Disorders

1. $\alpha 6$ GABA_ARs, Stress Response, Anxiety-Like Behaviors, and Social Deficits. A *Gabrd* knockout in cerebellar granule cells of mice caused a replacement of $\alpha 6\beta \delta$ by $\alpha 6\beta \gamma 2$ and $\alpha 6\beta$ receptors (Tretter et al., 2001). This resulted in a decreased tonic inhibition and enhanced excitability of granule cells and a differential activation of cerebellar output regions as well as of many cortical and subcortical brain areas involved in cognition, anxiety-like behaviors, social deficits, and stress responses, specifically in females (Rudolph et al., 2020). *Gabrd* knockout also resulted in deficits of maternal behaviors, which is relevant to disorders like postpartum depression. In contrast, motor functions were unaffected in these mice (Rudolph et al., 2020). PAMs of $\alpha 6\beta \delta$ GABA_ARs may thus attenuate all these behaviors elicited by a decreased tonic inhibition of granule cells.

2. $\alpha 6$ GABA_ARs and Stress-Induced Depressive Behavior. An essential role of hippocampal $\alpha 6$ GABA_ARs in maternal separation stress-induced adolescent depressive behaviors was demonstrated in rats (Yang et al., 2016). $\alpha 6$ GABA_ARs are expressed in low abundance on hippocampal interneurons of naive adolescent rats, as indicated by immunohistochemistry. Maternal separation stress from postnatal days 2–15 significantly reduced $\alpha 6$ and $\alpha 2$ but not $\alpha 1$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\gamma 2$, or δ expression in the hippocampus, and this was paralleled by an anhedonia-like depressive behavior. Such a depressive behavior was also elicited in young naive control animals by a knock-down of $\alpha 6$ expression in the hippocampus (Yang et al., 2016). These results indicated that $\alpha 6$ GABA_ARs are key modulators of hippocampal synaptic transmission, and malfunctioning of these receptors likely plays a crucial role in the pathophysiology of maternal separation-induced depression in rats.

3. $\alpha 6$ GABA_ARs and Stress-Induced Attention Deficit and Hyperactivity. The maternal separation stress in rats also led to an increased locomotor activity, as shown by a longer travel distance in an open field test (Yang et al., 2016). A similar phenomenon was observed by Kwak et al. (2009), in which maternal separation after P14 caused hyperactivity, as indicated by increased movement in an open field. The hyperactive profile was similar to the findings in an animal model of ADHD, as characterized by poor concentration, increased motor activity, and behavioral impulsivity (Womersley et al., 2015).

G-protein-coupled receptor kinase-interacting protein-1 (GIT1) has been reported to be associated with ADHD in humans (Won et al., 2011), and the genetic deletion of GIT1 resulted in ADHD-like behaviors in mice (Kim et al., 2017) (Table 2). Moreover, a decreased glial GABA intensity was observed in the cerebellum of GIT1-knockout mice, which led to reduced tonic GABA currents in cerebellar granule cells, which are likely mediated by $\alpha 6\beta \delta$ receptors (Kim et al., 2017). Overactive cerebellar granule cells might thus contribute to the symptoms of ADHD.

G. $\alpha 6$ GABA_ARs and Animal Models of Adverse Ethanol Effects

Ethanol is a PAM at most GABA_AR subtypes, including $\alpha 6\beta \gamma 2$ and $\alpha 6\beta \delta$ receptors, explaining its anxiolytic, sedative-hypnotic, anticonvulsant, and motor incoordinating properties (Kumar et al., 2004). In addition, ethanol exhibits multiple actions at other ion channels, neurotransmitter receptors, and transporters in the brain (Korpi et al., 2015).

1. $\alpha 6$ GABA_ARs and the Ethanol Antagonist Ro15-4513. The imidazobenzodiazepine Ro15-4513 (Fig. 4) is a negative allosteric modulator at $\alpha 1\beta \gamma 2$, $\alpha 2\beta \gamma 2$, $\alpha 3\beta \gamma 2$, and $\alpha 5\beta \gamma 2$ receptors and a PAM of the diazepam-insensitive $\alpha 4\beta \gamma 2$ and $\alpha 6\beta \gamma 2$ receptors (Mohler et al., 1984; Sieghart et al., 1987; Luddens et al., 1990; Hevers and Luddens, 1998). It was demonstrated to be a potent antagonist of the muscimol-induced and ethanol-stimulated ³⁶Cl⁻ uptake into brain vesicles and also blocked the anticonflict activity of low doses of ethanol as well as the behavioral intoxication observed at higher doses of ethanol (Suzdak et al., 1986). The structural analog Ro15-1788 (flumazenil, Fig. 4) could block the antiethanol effects of Ro15-4513 but not the ethanol effects itself. The antiethanol effect of Ro15-4513 could also not be blocked by other negative allosteric modulators acting via the benzodiazepine site of GABA_ARs, leading to the conclusion that the antiethanol effect of Ro15-4513 might be mediated by a unique interaction with GABA_ARs (Suzdak et al., 1986).

2. $\alpha 6$ GABA_ARs and Ethanol-Induced Motor Incoordination. This unique interaction site was tentatively identified by the finding that ethanol impairs motor

coordination by enhancing tonic inhibition at cerebellar $\alpha 6\beta 3\delta$ receptors (Hanchar et al., 2006). The ethanol action at $\alpha 6\beta 3\delta$ receptors was proposed to be mediated by a novel high-affinity binding site for ethanol, Ro15-4513, and Ro15-1788 located at the $\alpha 4/\alpha 6+\beta 3$ - subunit interface of $\alpha 4/\alpha 6\beta 3\delta$ receptors at a position homologous to the classic benzodiazepine binding site at the $\alpha 4/\alpha 6+\gamma 2$ - interface (Hanchar et al., 2006; Wallner et al., 2014). Low concentrations of ethanol could inhibit [³H]Ro15-4513 binding to this site, and this [³H]Ro15-4513 binding could also be inhibited by Ro15-1788 but not by diazepam. It was hypothesized that the bulky azido group of Ro15-4513 might overlap with the alcohol binding site, explaining the antiethanol action of Ro15-4513 and the finding that Ro15-1788 cannot inhibit the ethanol effects in rats (Hanchar et al., 2006).

However, only some (Sundstrom-Poromaa et al., 2002; Wei et al., 2004; Fleming et al., 2007; Mody et al., 2007) but not other groups (Borghese et al., 2006; Botta et al., 2007; Korpi et al., 2007; Baur et al., 2009) could confirm the high potency of ethanol for modulation of recombinant $\alpha 4/\alpha 6\beta 3\delta$ receptors. For a critical discussion of this topic, see (Kumar et al., 2004; Krystal et al., 2006; Wallner and Olsen, 2008; Wallner et al., 2014).

3. *$\alpha 6$ GABA_ARs and the Alcohol-Nontolerant Rats.* The selectively bred alcohol-nontolerant (ANT) rats, in contrast to the alcohol-tolerant rats, carried the point mutation $\alpha 6R100Q$, in which a glutamine replaced the arginine at position 100 of the $\alpha 6$ subunit. The mutated $\alpha 6(Q100)\beta 2\gamma 2$ receptors exhibited a novel high-affinity benzodiazepine binding site, and their function could thus be potentiated by diazepam. The additional modulation by diazepam of a mutated diazepam-insensitive receptor explained the impairments of postural reflexes by diazepam observed in ANT rats (Korpi et al., 1993).

The high ethanol sensitivity of the ANT rats that voluntarily avoid ethanol was then explained by the finding that the already high ethanol sensitivity of recombinant $\alpha 6\beta 3\delta$ receptors was even further increased in $\alpha 6(Q100)\beta 3\delta$ receptors (Hanchar et al., 2005; Wallner et al., 2014).

4. *$\alpha 6$ Subunit Expression Changes on Chronic Ethanol Administration.* Consistent with the modulatory action of ethanol on multiple ion channels, neurotransmitter receptors, and transporters in the brain (Korpi et al., 2015), chronic ethanol administration changed the expression of literally hundreds of different receptors and proteins and also differentially altered the expression of $\alpha 6$ and δ GABA_AR subunit mRNAs and peptides in different brain regions of rodents (Kumar et al., 2004; McClintick et al., 2015, 2016).

In the periaqueductal gray, which is involved in processing pain, fear, and anxiety, repeated binge-like

alcohol drinking by adolescent alcohol-preferring rats caused an increased expression of $\alpha 6$ and δ and a decrease of $\gamma 2$ and other GABA_AR subunit mRNAs (McClintick et al., 2016).

Serotonin neurons in the dorsal raphe nucleus modulate the effects of ethanol on dopamine release in the shell of the nucleus accumbens, which is directly associated with the rewarding effects of ethanol consumption (Koob, 2013). In the dorsal raphe nucleus, repeated alcohol binges across adolescence of alcohol-preferring rats led to increased $\alpha 6$ and δ mRNAs, whereas $\gamma 2$ mRNA and seven other GABA_AR mRNAs were decreased (McClintick et al., 2015).

In the cerebellum, $\alpha 6$ subunit mRNA and peptide levels were increased, whereas $\alpha 1$ subunit peptide and mRNAs were decreased, after 6 days of ethanol administration in rats (Mhatre and Ticku, 1992; Morrow et al., 1992).

H. $\alpha 6$ GABA_ARs and Animal Models of Trigeminal Nerve-Related Pain

1. *$\alpha 6$ GABA_ARs and Trigeminal Ganglia.* $\alpha 6$ GABA_ARs are also expressed in some sensory neurons (Gutierrez et al., 1996), including trigeminal ganglia (TG) (Hayasaki et al., 2006; Puri et al., 2012; Fan et al., 2018; Tzeng et al., 2021) (Fig. 2B), but not in dorsal root ganglia (Kogelman et al., 2017). Immunofluorescent studies performed in adult rats demonstrated that the $\alpha 6$ subunit was expressed not only in >74% of small, medium, and large TG neurons but also in satellite glia cells (Puri et al., 2012; Fan et al., 2018). The subunit composition of $\alpha 6$ GABA_ARs within TG neurons and glia is currently unclear, but both $\alpha 6\beta 2/\beta 3\gamma 2$ and $\alpha 6\beta 2/\beta 3\delta$ receptors might be present there (Hayasaki et al., 2006).

In TG, GABA is synthesized by glutamate decarboxylase 65 (GAD65) in neurons and accumulated in both neurons and glia (Hayasaki et al., 2006; Tzeng et al., 2021). Cell bodies of mammalian sensory ganglia are generally devoid of synaptic contacts, but exogenous GABA can induce Cl⁻ currents in all TG neurons examined (Hayasaki et al., 2006). It was thus hypothesized that frequently firing sensory neurons can lead to elevated extracellular K⁺ concentrations during repolarization of neurons between action potentials, which might induce GABA release from satellite glia and neurons (Minchin, 1975; Hayasaki et al., 2006; Fan et al., 2018). The high GABA sensitivity and long open probability of $\alpha 6$ GABA_ARs at TG neurons (Mortensen et al., 2012; Karim et al., 2013) might thus facilitate an efficient feedback inhibition of firing TG neurons (Fan et al., 2018).

2. *$\alpha 6$ GABA_ARs and Animal Models of Trigeminal Neuropathic Pain.* Trigeminal neuropathic pain is a continuous and burning pain that involves the orofacial region and can be induced by a surgical incision of the facial skin or oral mucosa or the extraction of a

tooth. The activation of nociceptive endings in the orofacial tissues by a noxious stimulus results in activation of A- δ and C-fibers, which have their cell bodies in the TG and conduct the information on quality, location, intensity, and duration of pain into the central nervous system (Sessle, 2011). Since only 11% of patients with painful traumatic trigeminal neuropathy had significant pain reduction in response to the available pharmacotherapy by off-label drugs (Haviv et al., 2014; Benoliel et al., 2016), new treatment options are required for this disorder.

It has been demonstrated that a 30% reduction of TG $\alpha 6$ subunits caused hyperalgesia to temporomandibular joint inflammation (Puri et al., 2012; Kramer and Bellinger, 2013). Positive modulation of $\alpha 6$ GABA_ARs may thus reduce pain elicited by TG activation, such as trigeminal neuropathic pain. This was confirmed in a rat model by the finding that the highly $\alpha 6$ GABA_AR-selective pyrazoloquinolinone DK-I-56-1 but not a related pyrazoloquinolinone that did not modulate $\alpha 6$ GABA_ARs inhibited the development and reduced the established trigeminal neuropathic pain (Vasovic et al., 2019). These data for the first time demonstrated that PAMs of $\alpha 6$ GABA_ARs might represent a mechanistically novel treatment option for trigeminal neuropathic pain (Knutson et al., 2018; Vasovic et al., 2019).

3. $\alpha 6$ GABA_ARs and Animal Models of Migraine. TG are also involved in the pathogenesis of migraine. The ophthalmic branch of trigeminal sensory nerves receives peripheral inputs from dural vessels and sends central projections to the trigeminal cervical complex, which subsequently projects to higher-order pain centers (Bae et al., 2004; Goadsby, 2007). Activation of this trigemino-vascular system (TGVS) via both peripheral and central sensitization and releasing calcitonin gene-related peptide (CGRP) is considered an essential neuropathogenic mechanism of migraine (Goadsby et al., 1988).

Using intracisternally injected capsaicin, an established rat model of TGVS activation mimicking pathologic changes in migraine, it was investigated whether $\alpha 6$ GABA_AR PAMs can counteract both peripheral and central effects of capsaicin in rats (Fan et al., 2018). Capsaicin is an agonist of vanilloid 1 type of transient receptor potential channels that are coexpressed with CGRP and substance P in small- and medium-sized unmyelinated TG neurons (Caterina and Julius, 2001; Bae et al., 2004). Vanilloid 1 type of transient receptor potential channel activation in TG causes the release of CGRP and induces vasodilation and neurogenic inflammation within the meninges in experimental animals, mimicking migraine (Meents et al., 2010). Capsaicin thus elevated CGRP immunoreactivity in TG (indicating its induced synthesis) and depleted CGRP-immunoreactivity (indicating its induced CGRP release) in the

dura mater. Centrally, intracisternally applied capsaicin activates the trigeminal cervical complex by increasing the number of c-Fos immunoreactivity in these neurons. The $\alpha 6$ GABA_AR-selective PAM, Compound 6 (PZ-II-029), significantly attenuated both central and peripheral capsaicin-induced changes in the TGVS (Fan et al., 2018). All effects of Compound 6 were mimicked by topiramate, a clinically effective antimigraine agent, but not by diazepam. The brain-impermeable furosemide antagonized the peripheral but not central effects of Compound 6, suggesting that $\alpha 6$ GABA_ARs in TG, which are located outside the blood-brain barrier (Eftekhari et al., 2015), are the action target of Compound 6.

This was the first study indicating that positive modulation of $\alpha 6$ GABA_ARs in TG can inhibit nociceptive activation of the TGVS (Fan et al., 2018). In another migraine-mimicking model, TGVS activation was induced in mice via repeated injections of nitroglycerin (NTG) (Pradhan et al., 2014), a nitric oxide donor known to induce migraine in humans (Demartini et al., 2019). Repeated NTG injections induced a significant elevation in the grimace score in mice, a validated behavioral readout of spontaneous pain in rodents (Langford et al., 2010). Immunofluorescent staining of TG showed a significant downregulation of GAD65 but no change in GAT-1 or $\alpha 6$ subunits in repeated NTG- but not saline-treated mice (Tzeng et al., 2021). The $\alpha 6$ GABA_AR-selective PAMs Compound 6 and DK-I-56-1 significantly aborted migraine-like grimaces in these mice in a manner prevented by furosemide (Tzeng et al., 2021). This study indicates that hypofunction of GABAergic transmission may be the underlying cause of migraine-like responses in mice and supports the concept that $\alpha 6$ GABA_ARs in TG are a novel target for migraine therapy (Tzeng et al., 2021).

IV. The Human GABA_A Receptor $\alpha 6$ Subunit Gene and Neuropsychiatric Phenotypes

The human GABA_A receptor $\alpha 6$ subunit gene (*GABRA6*) is located at the human chromosome 5q34 together with the genes for the $\alpha 1$, $\beta 2$, $\gamma 2$, and π subunits (Fig. 7) (Olsen and Sieghart, 2008). A variety of genetic studies indicate an association of *GABRA6* variants and neuropsychiatric phenotypes.

So far, 491 unique variants of the *GABRA6* transcription unit have been identified and registered in the Ensembl database derived from the 1000genomes project, but only a few of these variants have been used in genetic studies so far. Each variant has a unique Reference Single Nucleotide Polymorphism (SNP) (rs) number (Holmes et al., 2020), which provides a stable variant notation for mutation and polymorphism analysis (https://www.ncbi.nlm.nih.gov/snp/docs/RefSNP_about/). In Table 3, important information on *GABRA6* variants that so far have been reported in genetic studies is summarized, and Fig. 7

provides an overview on the localization of the variants that have been investigated.

A. GABRA6 Variants and Epilepsy

Experimental and clinical observations suggest that epilepsy may result from an imbalance of excitatory and inhibitory neurotransmitter systems, ultimately leading to a decreased net inhibition. So far, genetic variants of GABA_AR subunit genes encoding the α 1, α 6, β 2, β 3, γ 2, δ , and ϵ subunits have been reported to be associated with human epilepsy (Hirose, 2014; Markus et al., 2020).

The *GABRA6* missense variant, rs373363000 C>T, where the Arg46 of the α 6 subunit was mutated to Trp46 [α 6(R46W); Table 3], was identified as a susceptibility gene that may contribute to the pathogenesis of childhood absence epilepsy (Dibbens et al., 2009). The α 6(R46W) mutation is located at the α 6+ interface of GABA_ARs and thus contributes to the α 6+ β -, α 6+ γ -, and α 6+ δ - subunit interfaces in assembled receptors. The α 6(R46W) mutation impaired assembly and gating of both α 6(R46W) β 2 γ 2L and α 6(R46W) β 2 δ receptors and substantially reduced the current density of both receptors. The α 6(R46W) mutation could thus increase seizure susceptibility through a reduction of α 6 β 2 γ 2 and α 6 β 2 δ receptor expression and function (Hernandez et al., 2011).

Two other genetic studies indicated that the *GABRA6* rs3219151 T>C variants at the 3'untranslated region (UTR) of *GABRA6* at chromosome 5q33.34 (Fig. 7) can influence epilepsy susceptibility (see Table 3 for divergent amino acid or nucleotide numbering in different publications). In a North Indian population comprising 401 epilepsy patients and 202 healthy controls, the frequencies of the CC genotypes were found to be significantly higher in patients with epilepsy than in control subjects (Kumari et al., 2011). These results were confirmed in a South Indian population of 310 patients with idiopathic generalized epilepsy and 310 healthy controls. The C variant of *GABRA6* rs3219151 might thus be a risk factor for the development of idiopathic generalized epilepsy (Prasad et al., 2014).

A direct contribution of α 6GABA_ARs to disorders associated with the *GABRA6* rs3219151 C variants is supported by the finding that the 3'UTR of mRNAs is the binding target of microRNAs (miRNAs) and RNA binding proteins that regulate mRNA levels in specific subcellular regions and determine the intensity of gene repression (Koulentaki and Kouroumalis, 2018). Binding of miRNAs to the 3'UTR cause mRNA cleavage or translation repression and thus elicit a reduced gene expression. If the C allele of this polymorphism is a risk factor for generalized epilepsy (Kumari et al., 2011; Prasad et al., 2014), it is reasonable to assume that the C allele might have contributed to a novel miRNA binding site and thus have caused a reduced expression of α 6GABA_ARs in the cerebellum or other brain areas. Impaired motor coordination

in the cerebellum could contribute to epileptic seizures elicited by other causes.

This conclusion is supported by the finding that the *GABRA6* rs3219151 T>C SNP is targeted by at least four miRNAs that regulate gene expression (Gong et al., 2013; Gonda et al., 2017). Two of the four miRNAs were predicted to bind at either *GABRA6* rs3219151 T>C allele, whereas one only bound to allele T and another one only bound to allele C. Three of these miRNAs are expressed in the cerebellum and adrenal gland (Gonda et al., 2017). Since miRNAs can interact with either the T- or the C-allele or with both alleles, depending on the regional and cellular miRNA expression, different symptoms of neuropsychiatric disorders can be associated with carriers of either the T- or C-allele or both alleles.

B. GABRA6 Variants and Stress

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenomedullary system, which are regulated by neuronal pathways, including the GABAergic system. The GABA system plays a key role in the HPA-axis downregulation in response to stress as demonstrated by the strong inhibitory effect on the HPA axis by alprazolam, an anxiolytic benzodiazepine enhancing GABA_AR signaling (Giordano et al., 2006; Gonda et al., 2019). Stress and its glucocorticoid component represent a major vulnerability factor for somatic health problems, alcoholism, depression, anxiety disorders, and cognitive impairment. Approximately 60% of the variance in glucocorticoid levels may be attributable to genetic differences (Lynch et al., 2015).

1. *GABRA6 Variants, Stress, and Heart Rate.* In a longitudinal study (Lynch et al., 2015), 186 high-risk urban children from New York were recruited at age 4. The presence or absence of child maltreatment was determined, as was the amount of crime that occurred in their neighborhood during the previous year. At age 9, the children were brought to the laboratory where their physiologic responses [i.e., changes in the amplitude of respiratory sinus arrhythmia (RSA) to a video-induced cognitive challenge] was assessed, and their DNA samples were collected for subsequent genotyping. RSA is an index of autonomic cardiac regulation and can be indirectly assessed by measuring the high-frequency component of heart rate variability. High levels of RSA and its reliable suppression or reduction are positive indices of social and emotional regulation. Thus, withdrawal of the vagal brake in response to challenge shifts autonomic regulation of the heart from parasympathetic to sympathetic control to provide the cardiac and metabolic outputs necessary for behavioral mobilization. When the challenge has passed, vagal pathways restore parasympathetic control of the heart to promote calm states and allow for social engagement. Low levels of RSA and its

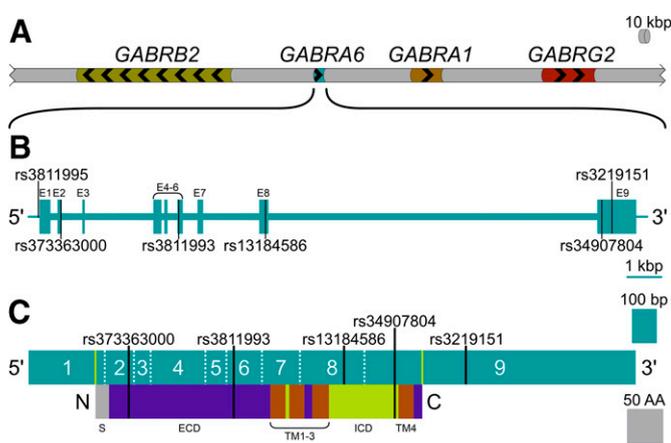


Fig. 7. 5q34 locus of GABA_AR genes, *GABRA6* gene, and localization of the variants: (A) GABA_AR genes in the 5q34 locus are depicted to scale reflecting their respective sizes, with arrows showing the direction of the reading frame. The π subunit encoding gene *GABRP* is located nearby on the 5q35.1 locus (on the right side of *GABRG2*). The scale is shown on the right-hand side above the scheme. kbp, kilo base pairs. (B) Enlarged view of *GABRA6* with nine exons (E1–E9, boxes), eight introns, and two (5'- and 3'-) flanking regions. The scale is indicated on the right-hand side below the scheme. All variants listed in Table 3 are denoted with black lines. Rs3811995 is part of the 5'-upstream sequence and as such is not transcribed. (C) Mature mRNA and the respective protein precursor are depicted schematically and to a scale wherein one codon equals one amino acid. In the mRNA scheme (upper part of the panel), the relative positions of start and stop codons are marked by yellow lines, exon boundaries by white dashed lines, and the transcribed variants in black lines as in (B). The exon numbers are written in white. In the protein precursor (lower part of the panel), the nonsynonymous variants are indicated by extended black lines. The color code on the protein refers to the localization: gray for the signal peptide (S), purple for all extracellular domains (ECD), dark red for the four transmembrane helices (TM1–4), and green for all intracellular domains (ICD). Note that rs3219151 is part of the 3'-untranslated region on exon 9, and Rs13184586 is a synonymous variant that does not cause a replacement of an amino acid. The 5'- and 3'-untranslated regions of the mRNA are important for many processes pertaining to mRNA structure, fate, and translation. The scale bars above and below the schemes indicate the mRNA and protein sizes, respectively. bp, base pairs; AA, amino acids.

unreliable modulation appear to increase the risk for difficulties in social and emotional regulation and in some cases may contribute to psychopathology. Multiple studies support the link between aberrant RSA reactivity patterns and risk for both internalizing (anxiety, doubts, compulsive behavior) and externalizing problems (inadequate behavior, aggression) (Lynch et al., 2015).

In this study, *GABRA6* rs3219151 T>C variant carriers exhibited different stress responses (Lynch et al., 2015). TT- or TC-carriers had significantly higher cortisol and blood pressure responses to social stressors than CC-carriers. Among CC-carriers, only nonmaltreated children demonstrated the ability to modulate RSA in response to challenge. In contrast, both nonmaltreated and maltreated children carrying TT- or TC-alleles were able to modulate their RSA levels in response to challenge. It was concluded that homozygosity for the C-allele of *GABRA6* rs3219151 may contribute to risk for psychopathology among neglected and multiply maltreated children due to their inappropriate reactions to stress (Lynch et al., 2015). A

reduced expression of $\alpha 6$ GABA_ARs in certain brain regions caused by the miRNA binding site of the C allele of *GABRA6* rs3219151 (see above) might thus have resulted in a reduced inhibition via $\alpha 6$ GABA_ARs and could explain this risk for psychopathology.

2. *GABRA6* Variants, Stress, and Obesity. In a study on 284 unrelated Swedish men exposed to perinatal stress at the end of the second world war, evidence was provided that cortisol secretion and abdominal obesity are associated with the *GABRA6* rs3219151 T>C variants (Rosmond et al., 2002). T-allele carriers had a significantly higher waist-to-hip ratio and abdominal sagittal diameter compared with CC-carriers. TT-carriers had significantly higher mean salivary cortisol levels and had significantly higher diurnal cortisol secretion than TC-carriers. These findings suggest that T-allele carriers exhibit a predisposition to hypercortisolism and perhaps abdominal obesity (Rosmond et al., 2002).

3. *GABRA6* Variants, Stress, and Personality Traits. An association of the *GABRA6* rs3219151 T-allele and hypercortisolism was also supported by another study (Uhart et al., 2004). In an association study with 56 healthy subjects, the T-allele carriers were found to have greater stress responses, including the elevation of adrenocorticotrophic hormone, cortisol, and blood pressure than CC-carriers in the Trier Social Stress test. T-allele carriers exhibited a personality tending to “extraversion behavior” (Uhart et al., 2004) that takes pleasure in activities involving social interactions and community tasks, whereas individuals with “introversion behavior” prefer to spend time alone rather than engage in social interactions.

4. *GABRA6* Variants, Stress, Externalizing Behavior, and Substance Use. Another SNP of *GABRA6*, the 5'UTR *GABRA6* rs3811995 C>T variant (Table 3), was reported to be associated with externalizing behavior and risk of adolescent substance use (Trucco et al., 2016). Subjects were collected from the Michigan longitudinal study, including 487 adolescents from 292 families with high risk of substance use, mostly of European American ancestry. Their temperament traits “behavioral control” and “resiliency” at ages 9–11 were assessed using interviewer ratings, and externalizing behaviors at ages 12–14 were assessed using teacher ratings. Substance uses including maximum alcoholic beverages/24 hours, and frequency of past year cigarette and marijuana use were self-reported at ages 15–17. Results indicated that those with the *GABRA6* rs3811995 CC genotype had lower behavioral control and greater externalizing behavior and were associated with a higher liability of substance use (Trucco et al., 2016).

Mutations in the 5'UTR of a gene can potentially affect the process of transcription, whereby RNA polymerase is recruited to the gene. Variation in the DNA sequence may alter the affinities of existing protein-

stress-associated suicide risk is elevated in T-allele carriers (Gonda et al., 2017). Considering that the *GABRA6* rs3219151 T-allele might be associated with extraversion behavior that requires high motivation and decisiveness, it might possibly also facilitate the last impulse for suicidal behavior as a reaction to a hopeless situation.

C. *GABRA6* Variants and Mood Disorders

1. *GABRA6* Variants and Neuroticism. A tendency to experience negative affect, as measured by the neuroticism component of the Neuroticism, Extraversion, and Openness Personality Inventory, is a trait marker for major depression. A serotonin transporter promoter polymorphism (5-HTTLPR) has been extensively studied in neuroticism and several psychiatric disorders. A study with 384 subjects who completed the Neuroticism, Extraversion, and Openness Personality Inventory demonstrated that the 5-HTTLPR short allele and the rs34907804 $\alpha 6$ Pro385 allele were associated with higher neuroticism scores (Sen et al., 2004).

2. *5q34 GABA_AR Gene Cluster and Depression*. After a linkage study identified the chromosomal area 5q34 as a susceptibility region for mood disorders (Edenberg et al., 1997), in the mouse a similar location on chromosomes was demonstrated to be associated with depression-like behaviors based on the forced-swim and tail-suspension tests (Yoshikawa et al., 2002).

Later, a study on 203 Japanese patients with mood disorder (Yamada et al., 2003) demonstrated significant associations of *GABRA1* and *GABRA6* polymorphisms with mood disorders in females but not males. The female T-allele carriers of the *GABRA6* rs3219151 variant were more affected by unipolar or bipolar disorders than female controls (Yamada et al., 2003).

3. *GABRA6* Variants and Harm Avoidance. In a study investigating 937 Spanish individuals, the TT-carriers of the *GABRA6* rs3219151 T>C polymorphism presented slightly higher scores for harm avoidance than C-allele carriers (Arias et al., 2012). This is consistent with the more active extraversion behavior of T-allele carriers.

4. *GABRA6* Variants, Major Depression, and Panic Disorder. Associations of *GABRA6* rs3219151 variants with major depression and panic disorder were studied in 280 and 189 Japanese patients, respectively (Inoue et al., 2015). The C-allele was significantly associated with major depression and panic disorder in genotype and allele analyses, which is consistent with the more introverted behavior of the C-allele carriers. However, in panic disorder patients exposed to a dynamic fearful face during fMRI scanning, responses in the bilateral anterior cingulate and medial orbital frontal cortex were stronger in T-allele carriers than in CC-carriers (Inoue et al., 2015). This

finding is consistent with the higher stress reactivity of T-allele carriers discussed above.

A study with a Spanish cohort showed an association between nonacrophobic panic disorder and the miRNA miR-138-2. This miRNA is highly enriched in the brain, including the frontal cortex, the hippocampus, and the midbrain, and reduced, among other genes, also *GABRA6* expression by 30% (Muinos-Gimeno et al., 2011). Such a reduction could have significantly influenced the amounts of $\alpha 6$ GABA_ARs expressed in the respective brain region quite well, causing disturbances in the behavioral tasks mediated by these receptors.

5. *GABRA6* Variants and Bipolar Disorder. A genome-wide association study investigating 200 individuals from 41 families multiply affected with bipolar disorders identified several rare variants in ion channel genes that influence risk for bipolar disorder. Among these, the missense variant in *GABRA6* rs3811993 resulting in a threonine-to-methionine substitution at position 187 of the $\alpha 6$ subunit (Table 3) was identified to be significantly associated with bipolar disorder and to be among the top 0.1% most deleterious SNPs genome-wide (Ament et al., 2015).

6. *GABRA6* Variants and Schizophrenia. A meta-analysis of 20 genome-wide linkage scans in patients with schizophrenia identified the chromosome 5q23.2-q34 as the second most significant risk locus in the genome (Lewis et al., 2003). A linkage to an overlapping region at chromosome 5q31-q35 was then reported in a genome-wide scan of schizophrenia and schizoaffective depressed type families of Portuguese descent (Sklar et al., 2004). In a subsequent study (Petryshen et al., 2005), significant associations were detected between schizophrenia and 19 SNPs spanning the 5q34 GABA_AR gene cluster in a Portuguese patient sample of 321 cases and 242 controls. The majority of associated SNPs were in *GABRA1*, with some in *GABRB2*, *GABRA6*, and *GABRP* (Petryshen et al., 2005). Twenty-one haplotype blocks (sets of genetic determinants located on a single chromosome) of strong linkage disequilibrium of these SNPs were defined, spanning the whole gene cluster. Significant associations were detected with several haplotypes in the gene cluster. These findings were replicated in the second stage in an independent German family-based sample consisting of 238 parent-proband trios (Petryshen et al., 2005). A comparison of transcript levels of 32 patients with schizophrenia carrying the schizophrenia-associated *GABRA1* haplotypes and unaffected siblings of this study in peripheral leukocytes found a significantly lower patient transcript expression of *GABRA6* and genes coexpressed in haplotypes, including some synaptic and vesicle-associated genes that have been reported to be altered in schizophrenia

prefrontal cortex. These results support the involvement of the chromosome 5q34 GABA_AR gene cluster in schizophrenia and suggest that schizophrenia-associated haplotypes may alter the expression of *GABRA6* as well as that of GABA-related genes (Petryshen et al., 2005).

In a case control study carried out in 959 control subjects and 598 unrelated schizophrenia cases of Han Chinese origin (Gong et al., 2013), it was demonstrated that *GABRA6* rs3219151 C>T was significantly associated with schizophrenia susceptibility. According to Table 2 in Gong et al. (2013), the C-allele was associated with an increased risk for schizophrenia. Assuming that the C-allele of *GABRA6* rs3219151 causes a reduced expression of the *GABRA6* gene as discussed above, these data are consistent with the (Petryshen et al., 2005) study, indicating that a reduced expression of α 6GABA_ARs might be associated with schizophrenia.

D. *GABRA6* Variants and Alcohol-Associated Disorders

1. *GABRA6* Variants, Alcohol, and Benzodiazepine Sensitivity. Children of alcoholics are at a high risk of alcoholism. In a study investigating the *GABRA6* rs34907804 (α 6Pro385Ser) variant in 51 adult Caucasian offspring of alcoholics, the sensitivity to diazepam was assessed by using diazepam-induced changes in two eye movement measures: peak saccadic velocity and average smooth pursuit gain. The α 6Ser385 carriers were less sensitive to diazepam and alcohol (Iwata et al., 1999). The same conclusion was supported by another study with 100 patients (Hoffman et al., 2002). The α 6Ser385 mutation did not affect baseline sedation and anxiety but significantly decreased the anxiolytic effect of low-dose midazolam (Hoffman et al., 2002). These findings support the assumption that α 6GABA_ARs may modulate anxiety within specific regions of the central nervous system as suggested in (Leggio et al., 2015).

In a pilot study it was investigated whether a low level of response to alcohol in children of alcoholics predicts future alcohol abuse and dependence (Schuckit et al., 1999). Results showed that subjects with the α 6Ser385 genotype were significantly more vulnerable to become alcoholics than the α 6Pro385 genotype and a genotype of a serotonin transporter polymorphism. All subjects with combined “low level of response to alcohol at age 20” and the α 6Ser385 genotype had developed alcoholism and demonstrated the lowest alcohol sensitivity during adolescence overall (Schuckit et al., 1999).

In a subsequent study, the authors strengthened the support for a relationship between the serotonin transporter polymorphism and α 6Ser385 alleles to “low level of response to alcohol” and to alcoholism in a prospectively studied cohort (Hu et al., 2005). The α 6Ser385 mutation might thus reduce ethanol

sensitivity in children of alcoholics (Iwata et al., 1999), but enhance their vulnerability for alcoholism in later life.

2. *5q34 GABA_AR Gene Cluster and Alcohol Dependence*. Multiple association studies suggest a role for the GABA_AR gene cluster on chromosomal 5q34 in alcohol-associated disorders. Thus, in 108 patients and 58 controls from Scotland, it was demonstrated that nearly 70% of alcohol-dependent subjects with Korsakoff psychosis carried the T-allele at *GABRA6* rs3219151 T>C and the C-allele at the BanI RFLP of the *GABRB2*, whereas 62% of patients and 45% of controls carried these alleles (Loh et al., 1999).

This finding could not be confirmed by another study investigating 349 German alcohol-dependent subjects and 182 controls, but the frequency of the T-allele carriers of the *GABRA6* rs3219151 T>C variants was significantly increased in dissocial alcoholics compared with the controls (Sander et al., 1999). Nevertheless, in a study with 162 male Korean alcoholics and 172 controls, there was a significant association between genetic polymorphisms of the *GABRA1* and *GABRA6* genes and alcoholism (Park et al., 2006).

Another study (Radel et al., 2005) conducted genotyping for 6 SNPs at the 5q34 GABA_A gene cluster in 433 Southwestern Native American and 511 Finnish alcohol-dependent subjects and unaffected individuals. Results showed an association of alcohol dependence with the *GABRA6* rs3219151 T- and the *GABRB2* 1412 T-alleles in both populations. Linkage disequilibrium mapping with haplotypes yielded evidence for an alcohol-dependence locus at the GABA_A gene cluster region in both populations. The most highly significant signals were at 3-locus haplotypes that included one or more *GABRA6* variants (Radel et al., 2005).

A study investigating 141 alcohol-dependent cases and 110 volunteers from India (Sahni et al., 2019) showed that *GABRA6* rs3219151 variants were significantly associated with age at first use and daily ethanol consumption. TT-carriers were more liable to alcohol dependence, had a lower age at first use, higher daily intake, and longer duration of dependence (Sahni et al., 2019).

3. *GABRA6* Variants and Treatment Responses of Alcoholism. In 98 male alcoholic inpatients treated with lorazepam and thiamine, craving for alcohol and food was investigated (Han et al., 2008). Body weight increased in all patients with alcohol dependence. The *GABRA6* rs3219151 T-allele carriers had greater changes in alcohol and food craving than CC-carriers. Body weight and craving for food significantly increased in T-allele carriers, and these parameters correlated with a reduced craving for alcohol (Han et al., 2008).

In a randomized double-blind, placebo-controlled study, 126 alcohol-dependent subjects were treated with either naltrexone or acamprosate. Both are effective medications, but the effect sizes are modest (Ooteman et al., 2009). Results indicated that acamprosate might be a better treatment option for CC-carriers of the *GABRA6* rs3219151 variant, whereas naltrexone outperformed acamprosate in reducing the urge to drink alcohol in TT-carriers. Both treatments displayed equal efficacy in subjects heterozygous for this polymorphism.

The finding that some genetic indicators have good matching potential in this study may be caused by their close relationship to the neurobiological disease process (Gottesman and Gould, 2003; Ooteman et al., 2009). Thus, patients carrying the C-allele might exhibit a reduced expression of $\alpha 6$ GABA_ARs, as discussed above, and might benefit from reducing the overactive glutamatergic system of the glutamatergic/GABAergic negative reinforcement system by acamprosate, whereas those carrying the T-allele might exhibit standard expression of $\alpha 6$ GABA_ARs and benefit from reducing the dopaminergic/opioidergic positive reinforcement system by the opiate antagonist naltrexone. If these considerations were correct, patients who are alcohol-dependent and carrying the C-allele of the *GABRA6* rs3219151 T>C polymorphism might benefit from the administration of $\alpha 6$ GABA_AR PAMs.

E. Possible Contribution of GABRA6 Variants to Neuropsychiatric Phenotypes

Most of the *GABRA6* variants so far used for genetic studies cause a changed expression and altered function of the *GABRA6* gene. Mutations in the coding region of the *GABRA6* gene (Table 3), such as rs373363000 ($\alpha 6$ R46W), that elicit an impaired assembly and gating of $\alpha 6$ (R46W) $\beta 2\gamma 2$ L and $\alpha 6$ (R46W) $\beta 2\delta$ receptors, result in a reduced inhibitory activity in all brain regions where these receptors are expressed and can thus directly increase seizure susceptibility (Hernandez et al., 2011). Mutations causing a replacement of an amino-acid residue of $\alpha 6$ GABA_ARs, such as rs34907804 ($\alpha 6$ Pro385Ser) or rs3811993 ($\alpha 6$ Thr187Met), can cause changes in the expression or function of receptors and thus directly contribute to the respective disorders. Mutations in the coding regions without change in protein sequence, such as rs13184586 ($\alpha 6$ Ala335Ala), or missense mutations that do not cause altered protein function might have more subtle effects or might associate the *GABRA6* gene with a disorder through their linkage with another disorder-associated mutation in their proximity.

In addition, mutations in the 5'UTR or 3'UTR can directly cause a reduced expression of the *GABRA6* gene. Mutations in the 5'UTR, such as rs3811995 C>T, can interfere with the recruitment of RNA polymerase, thus altering the specificity and kinetics of

the transcriptional process (Kumari et al., 2011). Mutations in the 3'UTR region, such as rs3219151 T>C, elicit an altered interaction with specific miRNAs resulting in a reduced expression of the *GABRA6* gene in only those brain regions expressing the respective miRNAs (Gonda et al., 2017).

Finally, mutations close to the coding region can also cause changes in the expression of $\alpha 6$ GABA_ARs, although the actual gene associated with the disorder or phenotype might be different from and located in the vicinity of the *GABRA6* gene. This is exemplified by studies demonstrating an association of the 5q34 GABA_AR gene cluster with schizophrenia in which a reduced expression of the *GABRA6* gene as well as that of other genes coexpressed in a 5q34 *GABRA1* haplotype were demonstrated (Petryshen et al., 2005).

How could the *GABRA6* gene contribute to multiple neuropsychiatric disorders or phenotypes? Genetic studies performed with the rs3219151 T>C variants seem to be most informative for answering this question because they are more abundant than those using other variants (Table 3), and they suggest that both T- and C-alleles are associated with different phenotypes of related neuropsychiatric conditions. This is consistent with the concept that most neuropsychiatric disorders are polygenic, and thus, many genes can contribute to individual symptoms and phenotypes.

Stress is a major vulnerability factor for a variety of neuropsychiatric disorders (Lynch et al., 2015), and genetic studies in humans discussed in *Section IV* suggest extensive links between *GABRA6* and stress response. T-allele carriers of the *GABRA6* rs3219151 variants exhibit higher cortisol and higher RSA levels as compared with C-allele carriers. In spite of their greater stress responses, however, high RSA levels are positive indices of social and emotional regulation (Lynch et al., 2015). Thus, T-allele carriers might be able to better compensate various stress situations than C-allele carriers, as indicated by their tendency to harm avoidance (Arias et al., 2012). In addition, their extroversion behavior might allow them to better overcome stress situations by exaggerating eating and drinking behavior in a social context, leading to obesity (Rosmond et al., 2002) or alcohol abuse (*Section IV.E.2*). In the absence of genetic, epigenetic, or environmental vulnerability factors, mild to moderate stress might not cause the development of neuropsychiatric phenotypes in T-allele carriers. But in the presence of appropriate vulnerability factors and/or depending on the specific types of stress they are exposed to (Gonda et al., 2019), T-allele carriers might develop anxiety or depression (Yamada et al., 2003; Gonda et al., 2017) or tend to bipolar disorders (Yamada et al., 2003). In contrast, C-allele carriers of the *GABRA6* rs3219151 variants exhibit lower

cortisol and RSA levels than T-allele carriers (Lynch et al., 2015), and their already reduced α 6GABA_AR expression in certain brain regions might make them more vulnerable to stress, consistent with animal experiments indicating an enhanced stress response under conditions of reduced α 6GABA_ARs expression (Yang et al., 2016; Rudolph et al., 2020). Under appropriate stress conditions they might tend to panic disorder and major depression (Inoue et al., 2015), schizophrenia (Gong et al., 2013), or seizures (Kumari et al., 2011; Prasad et al., 2014). The *GABRA6* variant-dependent expression of α 6GABA_ARs together with genetic, epigenetic, and environmental vulnerability factors might thus facilitate the development of different phenotypes of neuropsychiatric disorders. Differences in stress processing and in addiction risk have also been observed for the less studied *GABRA6* variants, adding evidence to the emerging picture that *GABRA6* variants influence stress processing.

V. Post-Mortem Changes of α 6 Subunit Expression in Neuropsychiatric Disorders

Post-mortem changes in the α 6 subunit expression in patients with neuropsychiatric disorders could have been elicited by a genetic predisposition or by life events and thus could be the cause or consequence of the disorder. Alternatively, such changes also could have been elicited by the often long-term medication of the patients.

A. α 6 Subunit Changes in Schizophrenia and Mood Disorders

Deficits in GABA signaling have been described in the cerebellum, prefrontal cortex, and limbic system in individuals with schizophrenia (Table 2). In a post-mortem study investigating lateral cerebellar hemisphere tissue of 13 males with schizophrenia and 13 matched controls, patients had significantly lower mRNA and protein levels of GAD67, GAD65, or GAT-1 and increases in *GABRA6*, and *GABRD* mRNAs (Fatemi et al., 2005; Bullock et al., 2008). These findings suggest a reduced GABAergic transmission at the Golgi-granule cell synapse and a compensatory upregulation of α 6GABA_ARs in individuals with schizophrenia (Bullock et al., 2008).

In another study (Fatemi et al., 2013), the mRNA and protein expression of 12 GABA_A subunit proteins (α 1, α 2, α 3, α 5, α 6, β 1, β 2, β 3, δ , ϵ , γ 2, and γ 3) in the lateral cerebella from 9–15 subjects with schizophrenia, 10–15 subjects with bipolar disorder, 12–15 with major depression without psychotic features, and 10–15 healthy controls were investigated. Among other findings, significant increases in protein levels of α 1, α 6, and γ 3 receptors in lateral cerebella of subjects with major depression were demonstrated with no changes in either schizophrenia or bipolar

disorder. The absence of changes in α 6 subunit expression in schizophrenia was a finding discrepant to that of Bullock et al. (2008) and was potentially explained by a different anatomic location of the cerebellar tissue used for both sets of experiments and regional differences in GABA subunit expression throughout the cerebellum (Fatemi et al., 2013).

The protein expression of 14 GABA_A receptor subunits was also investigated in the superior frontal cortex of subjects with schizophrenia, bipolar disorder, and healthy controls (Fatemi et al., 2017). Results indicated a higher expression of GABA_A α 1, α 6, and ϵ subunits and a lower expression of β 1 subunits in subjects with schizophrenia and bipolar disorders versus controls. Thus, impaired GABAergic transmission may negatively contribute to symptoms, such as anxiety or panic as well as impaired learning and information processing, all of which are disrupted in schizophrenia and bipolar disorder (Fatemi et al., 2017).

B. α 6 Subunit Changes in Autism

In a similar study, protein and mRNA levels for nine GABA_AR subunits were measured in cerebellum, superior frontal cortex, and parietal cortex of subjects with autism versus matched controls. It was demonstrated that there was a downregulation of α 6, β 2, δ , ϵ , γ 2, θ , and ρ 2 protein subunits in the superior frontal cortex of subjects with autism (Fatemi et al., 2014).

C. α 6 Subunit Changes in Alcohol Use Disorder

Subjects with excessive alcohol drinking for an average of 35 years had lower mRNA and protein levels of δ subunits but unchanged α 1 and α 6 subunits in their postmortem cerebella (Gatta et al., 2017) and lower mRNA levels of α 6, α 2, and δ subunits in the prefrontal cortex as compared with the control group (Gatta et al., 2020).

VI. Discussion

In the present review we for the first time summarize the importance of α 6GABA_ARs for the function of the cerebellum and their possible involvement in various neuropsychiatric disorders. α 6 β γ 2 and α 6 β δ receptors, because of their synaptic and extrasynaptic location at the GABAergic Golgi cell-granule cell synapse where they mediate phasic and tonic inhibition, respectively, play a crucial role in cerebellar function. Their high GABA sensitivity and long open probability make them ideally suited for sharpening input signals and thus for discriminating between multiple and complex mossy fiber inputs in cerebellum (see *Section II.A*). Because of the role of the cerebellum in motor control, mutations causing changes in the expression or function of α 6GABA_ARs can thus cause impaired motor control and increased susceptibility to

epileptic seizures in humans (Hernandez et al., 2011; Kumari et al., 2011; Prasad et al., 2014).

In addition, the substantial prenatal growth that is continued postnatally and the associated prolonged neurodevelopment render the cerebellum especially vulnerable to developmental disruptions and damage elicited by genetic or environmental causes, such as stress or ethanol exposure (D'Mello and Stoodley, 2015). For instance, alterations in cerebellar structure, connectivity, and function, have been identified by neuroimaging or postmortem studies (for references see Table 2) in patients suffering from Angelman syndrome, Down syndrome, essential tremor, tic disorders, ADHD, OCD, Huntington disease, schizophrenia, autism spectrum disorder, stress-associated disorders, depression, and alcohol use disorder. Cerebellar abnormalities also implicate changes in the expression or functional balance of $\alpha 6$ GABA_ARs. Thus, impaired motor control in animal models of Angelman syndrome (Egawa et al., 2012) or essential tremor (Handforth et al., 2018) could be alleviated by THIP, a potent agonist of extrasynaptic $\alpha 6\beta\delta$ GABA_ARs in the cerebellum (Table 2).

The cerebellum, however, is not only involved in motor regulation but also in cognitive, emotional, and social behaviors (see *Section II.B*). Depending on the exact nature and location of the genetic or developmental disruptions of the cerebellar circuits and the resulting local dysbalance of $\alpha 6$ GABA_ARs function, different brain regions will be influenced via the closed-circuit connections of the cerebellum with the whole brain, contributing to different symptoms of individual disorders.

Stress represents a major vulnerability factor not only for somatic health problems but also for alcoholism, depression, and other neuropsychiatric disorders (Lynch et al., 2015). In rodents, stress can modulate miRNA expression in pregnant females, leading to altered transcriptomic brain profiles in their offspring (Rinaldi et al., 2010; Zucchi et al., 2013). Similar miRNA alterations are also observed in human psychiatric disorders, and transcriptomic changes in the offspring include genes related to neurodevelopment and axonal guidance. miRNAs can also reduce the expression of $\alpha 6$ subunits (Gong et al., 2013; Gonda et al., 2017). In adolescent rats, chronic maternal separation stress caused a reduced $\alpha 6$ expression in the hippocampus and depressive behaviors (Yang et al., 2016) that also could be elicited in young naive control animals by $\alpha 6$ -knockdown in the hippocampus (Yang et al., 2016). Maternal separation stress also elicited ADHD-like behaviors in rodents (Kwak et al., 2009; Womersley et al., 2015; Yang et al., 2016), and a reduced tonic current at cerebellar granule cells was observed in a mouse model of ADHD (Kim et al., 2017).

Stress thus modifies epigenetic signatures linked to disease during critical periods of fetal and postnatal development (Zucchi et al., 2013). In humans, stress during pregnancy, such as family discord, divorce, or stress in early-life, such as disturbances of the infant-parent relationship, social separation, child neglect, or abuse, also can negatively influence brain development of the offspring and increase the risk of behavioral, emotional, and cognitive problems and later-onset disorders, such as ADHD, autism, and schizophrenia (Ronald et al., 2011; Gunn et al., 2015; Beversdorf et al., 2019). Thus, several prospective longitudinal studies in humans reported a significant association of antenatal anxiety or perceived stress during pregnancy with ADHD symptoms as well as autistic traits in school-aged children from community samples (Ronald et al., 2011). Although the underlying pathologic mechanisms causing ADHD currently are not known, a decreased volume of the superior cerebellar vermis was observed in MRI studies in 36 children with ADHD (Mackie et al., 2007), and a meta-analysis of fMRI studies in patients with ADHD indicated a reduced activation in areas of timing, such as the left inferior prefrontal cortex/insula, cerebellum, and left inferior parietal lobe (Hart et al., 2012).

These considerations are consistent with emerging evidence from animal and human studies suggesting that the cerebellum also plays a role in stress responses (Moreno-Rius, 2019) in fear and anxiety-related disorders (Moreno-Rius, 2018), autism spectrum disorder, schizophrenia, and addiction (Carta et al., 2019). Human postmortem studies indicated a decreased GAD65 and GAD67 expression in the cerebellar cortex and parietal cortex of autistic subjects (Fatemi et al., 2002b; Yip et al., 2007) and changes in $\alpha 6$ -subunit expression in autism, schizophrenia, and mood disorders (Table 2). Whereas $\alpha 6$ subunits were downregulated in the superior frontal cortex of subjects with autism (Fatemi et al., 2014), an upregulation of $\alpha 6$ subunits was observed in the cerebellum and superior frontal cortex of subjects with schizophrenia and mood disorders (Bullock et al., 2008; Fatemi et al., 2013, 2017). Interestingly, the $\alpha 6$ subunit changes in the cerebellum of subjects with schizophrenia were similar to those observed in rats chronically treated with the N-methyl-D-aspartate (NMDA) channel blocker PCP, which elicits schizophrenia-like symptoms in humans as well as in animal models (Bullock et al., 2009).

Stress-related psychiatric disorders, such as anxiety and major depression are strongly associated with alcohol use disorders (Rompala et al., 2018), and alcohol-dependent patients exhibit an altered stress response (Chen et al., 2020). In addition, extensive ethanol exposure of pregnant rats can elicit neurodevelopmental changes in offspring that are similar to those of adverse early life experiences in humans and

can lead to long-lasting alterations suggesting a cerebellar involvement, including deficits in fine motor skills and motor learning (Diaz et al., 2014) as well as ADHD behavior in humans and rats (Wang et al., 2020). Chronic ethanol consumption caused a compensatory upregulation of α 6GABA_AR subunits in the cerebellum of rodents (Section III.G.4) but no change in humans (Section V.C.), whereas other GABA_AR subunits were downregulated in both species, supporting the importance of α 6 subunits for the function of the brain.

All these long-lasting pathologic consequences of prenatal and antenatal stress and ethanol consumption in rodents and humans can be enhanced or reduced by genetic predisposition. Most neuropsychiatric disorders can be influenced by multiple genes, and the individual genes usually have only a small contribution to the overall expression of the disorder. Depending on the genetic variants, the phenotypes of the disorders can be different. *GABRA6* gene variants can contribute to such genetic predispositions. Multiple genetic studies in humans discussed in this review suggest an association of *GABRA6* variants with stress-induced somatic symptoms as well as with personality, temperament, perceived parenting, alcohol-associated disorders, mood disorders, and schizophrenia. Most of these studies were performed with the *GABRA6* rs3219151 T>C variant, wherein the C-allele results in a novel miRNA binding site and thus a reduced expression of α 6 subunits and hence of α 6GABA_ARs in brain regions expressing the C-allele together with its interacting miRNA. Although α 6GABA_ARs are not the main and only cause of these multigenetic disorders, a changed expression of α 6GABA_ARs could have influenced the susceptibility for these disorders.

Involvement of α 6GABA_ARs and their potential as drug targets for a variety of neuropsychiatric disorders is further supported by the recent identification of PAMs with a high functional selectivity for modulating α 6 β 2 (and α 6 β δ) receptors (Fig. 5) (Varagic et al., 2013; Chiou et al., 2018; Knutson et al., 2018) and their first application in animal models of neuropsychiatric disorders (Chiou et al., 2018; Cadeddu et al., 2021). α 6GABA_ARs expressed in TG are part of an inhibitory feedback system that is mediated by GABA released from neurons and glia cells when the TGVS is activated (Fan et al., 2018). Again, the high GABA-sensitivity of these receptors and their long open probability make them ideally suited for this function. α 6GABA_AR PAMs enhance the GABAergic feedback inhibition of TG neurons in a furosemide-sensitive way and can ameliorate not only trigeminal neuropathic pain (Vasovic et al., 2019) but also migraine pain (Fan et al., 2018; Tzeng et al., 2021) with an efficacy comparable to clinically used antimigraine agents.

In addition, systemic or intracerebellar application of α 6GABA_AR PAMs could rescue PPI deficits in animal models of tic disorders [Wu et al., 2016; Master thesis, Graduate Institute of Pharmacology, College of Medicine (Chiou LC, Adviser) p 77, National Taiwan University, Taipei, Taiwan; (Cadeddu et al., 2021)] as well as in animal models of schizophrenia in a furosemide-sensitive way (Chiou et al., 2018), supporting a mechanism of action via α 6GABA_ARs. PPI deficits are sensorimotor gating deficits that are thought to reflect abnormalities in the processing and integration of sensory and motor information (Ahmari et al., 2012). The sensory overload resulting from reduced sensorimotor gating is thought to give rise to cognitive fragmentation, attentional deficits, and some of the complex clinical symptoms associated with these disorders (Giakoumaki et al., 2008). PPI deficits not only are observed in schizophrenia (Braff et al., 1978) and tic disorders, including Tourette syndrome, but also in patients with a variety of other neuropsychiatric disorders (Table 2), such as ADHD (Ornitz et al., 1992), OCD (Swerdlow et al., 1993), Huntington disease (Swerdlow et al., 1995), premenstrual dysphoric disorder (Kask et al., 2008), post-traumatic stress disorder (Pineles et al., 2016; Meteran et al., 2019), panic disorder (Ludewig et al., 2002), nocturnal enuresis (Freitag et al., 2006), mania in bipolar disorder (Giakoumaki et al., 2007), and autism spectrum disorder (Cheng et al., 2018).

Patients suffering from schizophrenia display subtle cognitive abnormalities that may reflect a difficulty in rapidly coordinating the steps that occur in a variety of mental activities. The cerebellum may play a role in coordinating both motor and cognitive performance (Andreasen et al., 1996). In addition, a breakdown of connectivity in a specific dorsolateral prefrontal cortex-to-cerebellum network directly corresponded to negative symptom severity in schizophrenia, and restoration of network connectivity with transcranial magnetic stimulation corresponded to amelioration of negative symptoms (Brady et al., 2019). In mouse models mimicking schizophrenia, α 6GABA_AR PAMs not only rescued PPI deficits (Chiou et al., 2018) but also reduced social withdrawal and cognitive deficits (Mouri et al., 2020) (Chiou et al., 2019, Abstract and Poster No. 645.14/B55 at the Soc Neurosci Meeting 2019, Chicago, IL, USA). These findings are supported by recent evidence indicating that the reward circuit and social behavior in mice can be directly modulated by an excitatory projection from the cerebellum to the ventral tegmental area (Carta et al., 2019).

Fronto-parieto-cerebellar deficits (Hart et al., 2012) and decreased functional connectivity between the cerebral cortex, striatum, and the cerebellum (Anticevic et al., 2014; Xu et al., 2019) were also observed in ADHD and OCD, respectively. Interestingly, for all disorders with

PPI deficits, abnormalities in structure and function of the cerebellum have been reported that might also have caused a dysbalance in the expression or function of $\alpha 6$ GABA_ARs (Table 2). Such a dysbalance can explain a possible benefit of $\alpha 6$ GABA_AR PAMs in the treatment of a variety of neuropsychiatric disorders. This does not necessarily mean that sensorimotor gating deficits are exclusively caused by the cerebellum. $\alpha 6$ GABA_ARs are also expressed in the prefrontal cortex (Agrawal and Dwivedi, 2020; Gatta et al., 2021), the superior frontal cortex (Fatemi et al., 2014), or the cochlear nerve (Gomez-Nieto et al., 2008) and in many other brain regions (Table 1) that also might contribute to sensorimotor gating deficits or their amelioration by $\alpha 6$ GABA_AR PAMs.

Thus, experimental and pharmacological studies in animal models of neuropsychiatric disorders as well as genetic, neuroimaging, behavioral (PPI), and post-mortem studies in humans suggest the involvement of $\alpha 6$ GABA_ARs in a variety of neuropsychiatric disorders (Table 2). Synaptic $\alpha 6\beta\gamma 2$ receptors with their high GABA sensitivity and long open probability are required for enhancing the precision of sensory inputs and adequate timing of motor activity and thus strongly influence cognition and adequate responses to our environment. Tonic inhibition mediated by extrasynaptic $\alpha 6\beta\delta$ receptors regulate the amount of information entering the cerebellum, and their optimal function allows the brain to concentrate on really important information. This is essential in stress situations when multiple sensory informations, considerations, and emotions pointing in different directions are coming together, thus impeding a rapid and optimal response. This might be even more of a problem in disorders with suboptimal functioning of $\alpha 6$ GABA_ARs, as indicated by a disrupted PPI, that can be ameliorated by $\alpha 6$ GABA_AR PAMs. Enhancing the function of $\alpha 6\beta\gamma 2$ and $\alpha 6\beta\delta$ receptors by PAMs might improve the integration of the sensory and emotional input and increase the threshold for incoming information, thus allowing the brain to again concentrate on important information. This conclusion is supported by the recent finding that a granule cell-specific deletion of the GABA_AR δ -subunit in the cerebellum of mice resulted in a differential activation of many cortical and subcortical brain areas involved in cognition, anxiety-like, and stress-related behaviors in females (Rudolph et al., 2020). If stress can be elicited by a reduced tonic inhibition of granule cells, $\alpha 6$ GABA_AR PAMs modulating $\alpha 6\beta\delta$ receptors should alleviate stress symptoms.

$\alpha 6$ GABA_ARs thus represent novel targets for the treatment of a large variety of neuropsychiatric disorders. $\alpha 6$ GABA_ARs in the cerebellum but also in other parts of the nervous system because of their much higher GABA sensitivity and longer open probability than other GABA_AR subtypes seem to be a major hub that allows to better differentiate between incoming

signals and to partially correct various dysfunctions of the circuitries to which they contribute. The overall benefit of $\alpha 6$ GABA_ARs PAMs in these disorders cannot easily be predicted. In some disorders, such as migraine (Fan et al., 2018; Tzeng et al., 2021), tic disorders (Cadeddu et al., 2021) [Wu et al., 2016; Master thesis, Graduate Institute of Pharmacology, College of Medicine (Chiou LC, Adviser) p 77, National Taiwan University, Taipei, Taiwan], schizophrenia (Chiou et al., 2018), and essential tremor (Huang et al., 2021), such compounds in animal models seem to be as effective as currently used standard medications. In other disorders, especially multigenic disorders, these compounds might only improve some symptoms. In contrast to THIP that directly activates all $\alpha 6\beta\delta$ and $\alpha 4\beta\delta$ (Brown et al., 2002) receptors, $\alpha 6$ GABA_AR-selective PAMs only modulate $\alpha 6$ GABA_ARs involved in ongoing GABAergic transmission. This property together with their lack of modulation of other GABA_AR subtypes explains their absence of unwanted side effects, such as sedation, motor-incoordination, addictive potential, and toxicity (Knutson et al., 2018; Vasovic et al., 2019; Tzeng et al., 2021). $\alpha 6$ GABA_AR-selective PAMs can be taken orally (Tzeng et al., 2021; Mitrović et al., 2021), and their deuterated derivatives, such as DK-I-56-1, exhibit a rapid onset of action and an optimal half-life (Knutson et al., 2018). These compounds exhibiting satisfactory preclinical pharmacokinetic and safety pharmacology will open new opportunities for the treatment of a variety of neuropsychiatric disorders and might also help to better cope with stress and its consequences.

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