



# Neurobiology and Therapeutic Potential of $\alpha 5$ -GABA Type A Receptors

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$\alpha 5$  subunit containing GABA type A receptors (GABA<sub>A</sub>Rs) have long been an enigmatic receptor subtype of interest due to their specific brain distribution, unusual surface localization and key role in synaptic plasticity, cognition and memory. These receptors are uniquely positioned to sculpt both the developing and mature hippocampal circuitry due to high overall expression and a distinct peak within the critical synapse formation period during the second postnatal week. Unlike the majority of other GABA<sub>A</sub>Rs, they exhibit both receptor clustering at extrasynaptic sites *via* interactions with the radixin scaffold as well as synaptic sites *via* gephyrin, thus contributing respectively to tonic currents and synaptic GABAergic neurotransmission.  $\alpha 5$  GABA<sub>A</sub>R signaling can be altered in neurodevelopmental disorders including autism and mental retardation and by inflammation in CNS injury and disease. Due to the unique physiology and pharmacology of  $\alpha 5$  GABA<sub>A</sub>Rs, drugs targeting these receptors are being developed and tested as treatments for neurodevelopmental disorders, depression, schizophrenia, and mild cognitive impairment. This review article focuses on advances in understanding how the  $\alpha 5$  subunit contributes to GABA<sub>A</sub>R neurobiology. In particular, I discuss both recent insights and remaining knowledge gaps for the functional role of these receptors, pathologies associated with  $\alpha 5$  GABA<sub>A</sub>R dysfunction, and the effects and potential therapeutic uses of  $\alpha 5$  receptor subtype targeted drugs.

**Keywords:** GABA A receptor, alpha 5 subunit, autism, cognition, memory, development, negative and positive allosteric modulators

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## INTRODUCTION

### Structure, Distribution and Composition

GABA type A receptors (GABA<sub>A</sub>Rs) are heteropentameric ligand-gated chloride (Cl<sup>-</sup>) ion channels typically composed of two  $\alpha$  ( $\alpha 1$ -6), two  $\beta$  ( $\beta 1$ -3), and one  $\gamma$  ( $\gamma 1$ -3) or  $\delta$  subunit (**Figure 1A**). The common structure of individual subunits consists of a large extracellular N-terminus (NT), four transmembrane  $\alpha$ -helices (M1-4) and a barely extruding extracellular C-terminus (CT). The conserved hydrophobic M domains are connected by small regions with a larger cytoplasmic domain between M3 and M4 (CD) that mediates interactions with intracellular proteins critical for receptor trafficking and surface localization (**Figure 1B**). Receptors can contain two different  $\alpha$  or  $\beta$  subunits that are arranged in a counterclockwise configuration of  $\gamma$ - $\beta$ - $\alpha$ - $\beta$ - $\alpha$  (**Figure 1C**). The two  $\alpha\beta$  NT interfaces form GABA binding sites composed of the

principal (+) side of the  $\beta$  subunit and the complementary  $\alpha$  subunit (–) side, while a single  $\alpha+(1, 2, 3 \text{ or } 5)/\gamma 2$ -interface generates the primary binding site for benzodiazepines, which are allosteric positive modulators of the GABA<sub>A</sub>R and an important clinical sedative-hypnotic-anxiolytic drug class. Several recent high resolution cryo-electron microscopy studies have provided unprecedented structural information for GABA<sub>A</sub>R (Phulera et al., 2018; Zhu et al., 2018; Laverty et al., 2019; Masiulis et al., 2019), advancing understanding of receptor architecture, principles of assembly, and binding of various ligands: GABA, bicuculline (antagonist), picrotoxin (channel blocker), and benzodiazepines. The channel properties, subcellular localization and pharmacological sensitivity of a GABA<sub>A</sub>R are defined by the subunit composition. While  $\alpha 5$  containing GABA<sub>A</sub>Rs makeup only approximately 5% of the total receptor population in the brain, they are highly expressed in both the hippocampus and olfactory bulb. They represent close to 25% of all hippocampal GABA<sub>A</sub>R (Olsen and Sieghart, 2009) and are particularly abundant in CA1 and CA3. In the olfactory bulb, over a third of the neurons in the internal granule cell layer have  $\alpha 5$  GABA<sub>A</sub>Rs (Sur et al., 1999), although the function here is unknown.  $\alpha 5$  GABA<sub>A</sub>Rs are also expressed in the spinal cord, where they contribute to presynaptic inhibitory control over sensory-motor transmission (Lucas-Osma et al., 2018) and are also implicated in resolution of hyperalgesia (Perez-Sanchez et al., 2017). Other brain regions where these receptors are found at lower levels include the cortex, subiculum, hypothalamus, sympathetic preganglionic neurons, and amygdala (Martin et al., 2009a).

Early pharmacological analysis indicated rat and human hippocampal  $\alpha 5$  GABA<sub>A</sub>Rs have  $\alpha 5\beta 3\gamma 2$  characteristics (Sur et al., 1998). However, sequential immunoprecipitation from hippocampal tissue identified that  $\alpha 1/\alpha 5$  heteromers constitute approximately 9% of the  $\alpha 1$  GABA<sub>A</sub>Rs and  $\alpha 2/\alpha 5$  heteromers constitute about 20% of the  $\alpha 2$  population in the hippocampus (Araujo et al., 1999; del Río et al., 2001). More recent mass spectrometry analysis of affinity purified  $\alpha 5$  GABA<sub>A</sub>Rs from mouse hippocampus supported association of  $\alpha 5$  with  $\alpha 1-3$ ,  $\beta 1-3$  and both  $\gamma 2S$  and  $\gamma 2L$  isoforms (Ju et al., 2009). A recent comparison of  $\alpha 5\beta 1-3\gamma 2L$  GABA<sub>A</sub>Rs in HEK cells co-cultured with neurons revealed robust inhibitory postsynaptic currents (IPSCs) with slow decay rates and isoform-specific effects of pharmacological inhibitors (Chen et al., 2017). Importantly, in mixed alpha subunit GABA<sub>A</sub>Rs there appears to be preferential assembly of  $\alpha 5$  and  $\gamma 2$  together, generating a benzodiazepine binding site with  $\alpha 5$  subunit pharmacology (Araujo et al., 1999; del Río et al., 2001). Thus for a mixed  $\alpha 5$  GABA<sub>A</sub>R, the other alpha subunit is essentially pharmacologically inactive for benzodiazepines and other alpha/gamma subunit interface binding drugs (i.e., the “Z-drugs” for insomnia treatment zolpidem, zopiclone, zaleplon). Mutation of the  $\alpha 5$  subunit H105 residue, a key alpha subunit residue required for forming the benzodiazepine binding site with the  $\gamma 2$  subunit, led to repositioning of  $\alpha 5$  H105R subunits into the pharmacologically inactive alpha subunit location (Balic et al., 2009). Interestingly, our recent mass spectrometry analysis

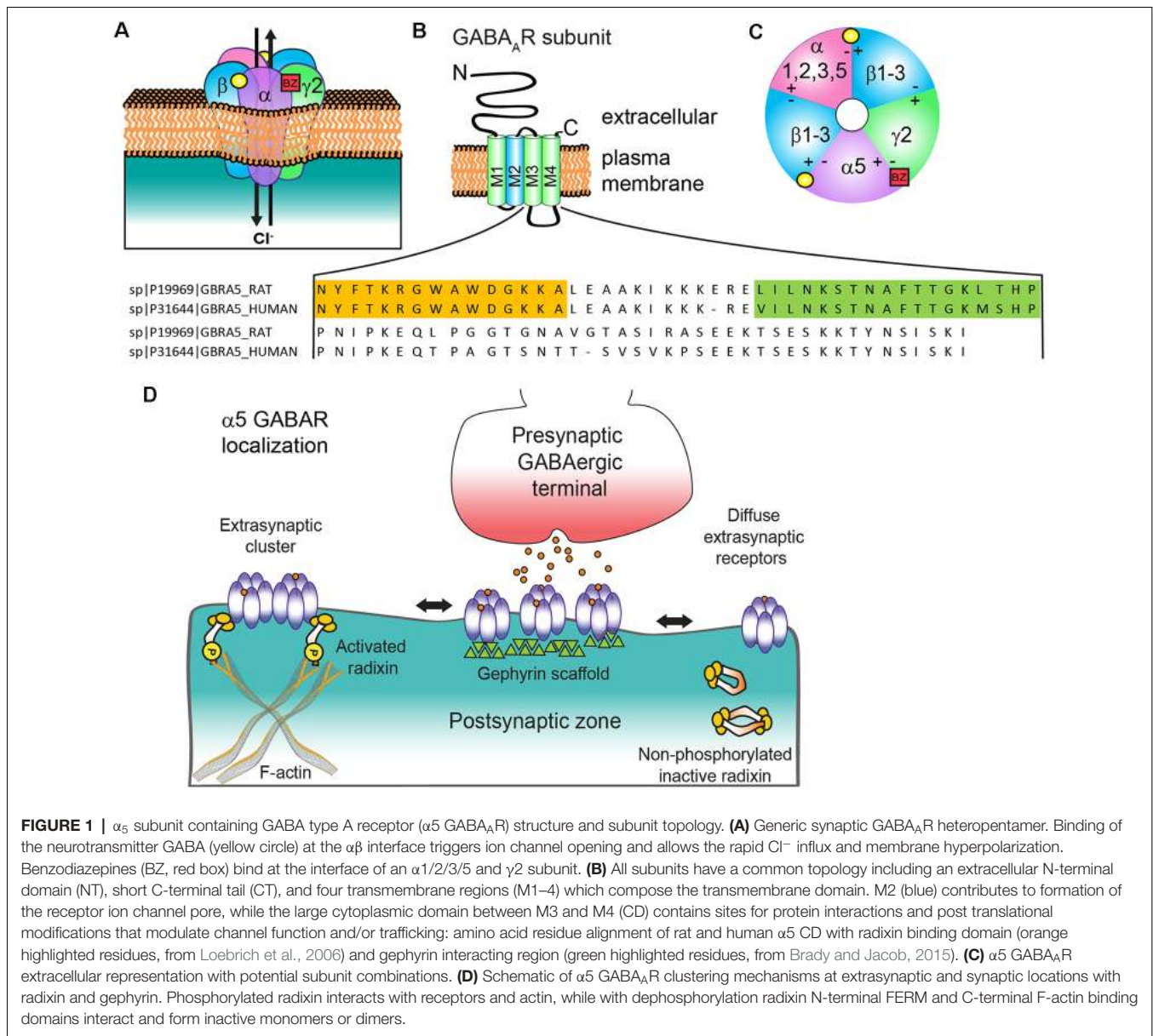
identified a specific increase in  $\alpha 5\beta 2$  containing receptors in the cortex following diazepam injection, consistent with benzodiazepine exposure leading to modification of GABA<sub>A</sub>R composition and potentially drug effects through  $\alpha 5$  plasticity (Lorenz-Guertin et al., 2019).

## CELLULAR AND CIRCUIT LOCALIZATION

### Subcellular Localization

Controversies regarding  $\alpha 5$  GABA<sub>A</sub>R subcellular localization in the literature have mirrored debates about its functional impact on GABAergic neurotransmission. Due to their initial identification as a key generator of hippocampal tonic current (Caraiscos et al., 2004; Glykys and Mody, 2006; Bonin et al., 2007),  $\alpha 5$  GABA<sub>A</sub>Rs were generally considered extrasynaptic receptors, despite earlier evidence for synaptic clustering on dendrites and the axon initial segment (Brünig et al., 2002; Christie and de Blas, 2002; Serwanski et al., 2006).  $\alpha 5$  GABA<sub>A</sub>Rs predominantly mediate tonic inhibition in hippocampal CA3 and CA1 pyramidal neurons, cortical neurons (layer 5) and are contributors to tonic inhibition in dentate gyrus granule cells (Glykys et al., 2008; Herd et al., 2008). Immunocytochemistry indicates an extensive extrasynaptic presence of  $\alpha 5$  GABA<sub>A</sub>Rs (Brünig et al., 2002; Crestani et al., 2002). However, this receptor subtype is unique in displaying surface clustering at extrasynaptic locations rather than a uniformly diffuse extrasynaptic distribution. Regions within the large cytoplasmic domain between M3 and M4 regulate subcellular clustering of  $\alpha 5$  GABA<sub>A</sub>Rs *via* interactions with radixin and gephyrin scaffolds (Figure 1D). Extrasynaptic clustering is mediated by radixin, an ezrin/radixin/moesin (ERM) family member that links actin to the plasma membrane (Loebrich et al., 2006). Phosphorylated radixin scaffolds  $\alpha 5\beta 2$  receptors to the actin cytoskeleton, ultimately reducing diffusion rates and concentrating channel activity away from axon terminals (Hausrat et al., 2015). Treatment with GABA promotes radixin phosphorylation and retention of  $\alpha 5$  GABA<sub>A</sub>Rs extrasynaptically, while AMPA, a ligand for ionotropic glutamatergic GluA type receptors, leads to dephosphorylation, an increase in synaptic  $\alpha 5$ -subunit receptors and an increase in slowly decaying miniature IPSCs (mIPSCs). Further support for the specific contribution of  $\alpha 5$  GABA<sub>A</sub>Rs to slowly decaying IPSCs is seen in early neurodevelopment during the switch from  $\alpha 5$  to  $\alpha 1$  and  $\alpha 3$  subunit expression (Pangratz-Fuehrer et al., 2016). Important areas of further investigation include assessment of the level and role of  $\alpha 5$  GABA<sub>A</sub>Rs associated with radixin or gephyrin in the developing and adult brain and plasticity mechanisms regulating these interactions.

Functional studies indicate the  $\alpha 5$  subunit is also important for phasic events including: spontaneous inhibitory postsynaptic currents (sIPSCs), evoked IPSCs (eIPSCs) and GABA<sub>slow</sub> IPSCs (Collinson et al., 2002; Prenosil et al., 2006; Zarnowska et al., 2009; Vargas-Caballero et al., 2010). Consistent with a synaptic role for  $\alpha 5$  GABA<sub>A</sub>Rs, we demonstrated that the  $\alpha 5$  subunit directly interacts with the gephyrin synaptic scaffold, with approximately half of surface  $\alpha 5$  GABA<sub>A</sub>Rs being synaptically



localized throughout the first 3 weeks of circuit development (Brady and Jacob, 2015). Single particle tracking studies measured reduced diffusion of surface  $\alpha 5$  GABA<sub>A</sub>Rs at synapses (Renner et al., 2012) and similar to other synaptic receptors,  $\alpha 5$  GABA<sub>A</sub>Rs showed an increase in diffusion with negative modulator DMCM treatment (Lévi et al., 2015). Further studies are needed to determine both acute and prolonged effects of  $\alpha 5$  preferring GABA<sub>A</sub>R drugs on receptor diffusive properties and surface stability.

### Cell Type and Input-Specific Expression

$\alpha 5$  GABA<sub>A</sub>Rs show input-specific synaptic localization and function in different brain regions both for pyramidal cells and interneurons. Recent work demonstrates preferential localization of  $\alpha 5$  GABA<sub>A</sub>Rs to inhibitory synapses on dendrites

of somatostatin-expressing interneurons in CA1 that are targeted by vasoactive intestinal peptide and calretinin-positive interneurons (Magnin et al., 2019). Somatostatin interneurons and NO-synthase-positive neurogliaform cells target  $\alpha 5$  GABA<sub>A</sub>Rs on dendrites of hippocampal CA1 pyramidal neurons to generate slow IPSCs (Schulz et al., 2018). Importantly, these outward-rectifying  $\alpha 5$ -GABA<sub>A</sub>Rs generate a greater hyperpolarizing current at slightly depolarized membrane potentials, thereby having a large impact on NMDA-receptor-activation and action potential firing in pyramidal neurons. In the cortex, pyramidal cells exhibit dendritically localized  $\alpha 5$  GABA<sub>A</sub>Rs at sites innervated by bitufted interneurons (an SST positive neuron class; Ali and Thomson, 2008). A recent human and mouse prefrontal cortex gene expression study determined that the majority of  $\alpha 5$  GABA<sub>A</sub>Rs are in pyramidal

cells, followed by parvalbumin interneurons (Hu et al., 2018). Interestingly,  $\alpha$ 5 GABA<sub>A</sub>R mRNA was uniquely expressed in human SST interneurons, albeit at a low level. As deficits in both GABAergic signaling and SST signaling (Fuchs et al., 2017) have been identified as contributors to major depressive disorder, this data suggests positive modulation of  $\alpha$ 5 GABA<sub>A</sub>R could be therapeutic by multiple mechanisms. It is clear that improving understanding of GABA<sub>A</sub>R subtype subcellular (extrasynaptic vs. synaptic) and circuit-specific localization and function are critical areas of current research and future pharmacological development (reviewed in Engin et al., 2018).

## FUNCTIONAL ROLE OF $\alpha$ 5 GABA<sub>A</sub>Rs

### Neuronal Excitability, Learning and Memory

Genetic and pharmacological studies in rodents demonstrate that  $\alpha$ 5 GABA<sub>A</sub>Rs are key in learning and memory processes (reviewed in Martin et al., 2009a). The two primary mouse models used in studying the  $\alpha$ 5 GABA<sub>A</sub>R contribution to cognitive processes are the  $\alpha$ 5 subunit knockout mice (*Gabra5*<sup>-/-</sup>) and the  $\alpha$ 5H105R point mutation mice. Although originally generated to render  $\alpha$ 5 receptors insensitive to benzodiazepines,  $\alpha$ 5H105R mice also have a 25% decrease in hippocampal  $\alpha$ 5 protein level (Crestani et al., 2002). As described earlier, *Gabra5*<sup>-/-</sup> mice showed a reduction in diverse types of phasic GABA<sub>A</sub>R currents and the tonic current. Behaviorally, the increased excitability of *Gabra5*<sup>-/-</sup> hippocampal pyramidal neurons was correlated with improved performance in a spatial learning behavior (Collinson et al., 2002), though later studies were not able to replicate this result (Cheng et al., 2006; Martin et al., 2009b). However, both *Gabra5*<sup>-/-</sup> and  $\alpha$ 5H105R mice show enhanced trace fear conditioning, a hippocampal learning task, while performing similarly to wild-type mice in a cued fear conditioning assay, which relies on the amygdala, hippocampus, and cortex (Crestani et al., 2002; Martin et al., 2009b). Long-term potentiation (LTP), the cellular correlate of learning and memory, is constrained by GABA<sub>A</sub>R-mediated inhibition. *Gabra5*<sup>-/-</sup> mice showed a reduced threshold for LTP induction with 10–20 Hz stimulation (Martin et al., 2010). In addition, *Gabra5*<sup>-/-</sup> mice showed greater power of kainate-induced gamma frequency oscillations (Towers et al., 2004), and knockout of delta and  $\alpha$ 5 subunits led to spontaneous gamma oscillations in CA3 (Glykys et al., 2008). Gamma oscillations occur in a range of cognitive states including memory processing, are thought to support neural coding of environmental information and are disturbed in some psychiatric disorders (reviewed in Lisman and Buzsáki, 2008). In summary, a reduction in  $\alpha$ 5 inhibition may improve learning and memory through enhanced neuronal firing and network oscillatory activity.

### Development

In contrast to their inhibitory role in the mature nervous system, GABA<sub>A</sub>Rs can promote excitation in newly forming circuits, allowing chloride efflux to produce membrane

depolarization which promotes calcium entry, dendritic outgrowth, synaptogenesis and unsilencing of glutamatergic synapses (reviewed in Ben-Ari et al., 2007).  $\alpha$ 5 GABA<sub>A</sub>Rs are particularly well positioned to sculpt early hippocampal circuit development due to exceptionally high expression that peaks in the first two postnatal weeks (Liu et al., 1998; Ramos et al., 2004; Yu et al., 2014; Bader et al., 2017), and receptor localization at both extrasynaptic and synaptic sites. During the first postnatal week, tonic  $\alpha$ 5 currents enhance cell excitability and synaptic activity, facilitating the induction of giant depolarizing potentials, which are important for early network maturation (Ben-Ari, 2002; Marchionni et al., 2007). Importantly, GABAergic activation of circuit formation also occurs with newborn neurons integrating into networks in the adult mammalian brain *in vivo* (Ge et al., 2006). A few *in vitro* pharmacological and genetic studies have supported the role of  $\alpha$ 5 GABA<sub>A</sub>Rs in dendritic development. Cultured hippocampal neurons treated with an  $\alpha$ 5-specific negative allosteric modulator (NAM; RY-80) exhibited decreased dendritic arborization and reduced expression of the AMPA type glutamate receptor GluA2 subunit (Giusi et al., 2009). To investigate the role of  $\alpha$ 5 GABA<sub>A</sub>Rs in emerging circuits, we genetically manipulated  $\alpha$ 5 binding to gephyrin, increasing or decreasing the ratio of extrasynaptic/synaptic  $\alpha$ 5 GABA<sub>A</sub>Rs (Brady and Jacob, 2015). Interestingly, reducing synaptic  $\alpha$ 5 GABA<sub>A</sub>Rs promoted dendritic outgrowth at the expense of dendritic spine maturation in hippocampal neurons. Consistent with these findings, recent work showed that single-cell deletion of *Gabra5* in adult-born dentate gyrus granule cells caused severe alterations of migration and dendrite development (Deprez et al., 2016). Further research is needed to elucidate the specific role of the  $\alpha$ 5 subunit in dendritic architecture, both during development and in adult neurogenesis.

### Genetic Disorders with Altered $\alpha$ 5 GABA<sub>A</sub>R Neurotransmission

While acute reduction in  $\alpha$ 5 GABA<sub>A</sub>Rs has shown potential for improving cognition and memory, further studies both in mouse models and human patients link long term reduction with significant pathologies. Reduced  $\alpha$ 5 GABA<sub>A</sub>R levels, function or protein interactions have been observed in patients with neurodevelopmental disorders including intellectual disability, epilepsy and autism. Common conditions among these disorders include cognitive impairments, increased anxiety, autism-related behaviors, sleep disorders and epilepsy susceptibility. Analogous behavioral changes and pathologies are observed in mouse models including *Gabra5*<sup>-/-</sup> mice (Zurek et al., 2016; Mesbah-Oskui et al., 2017), Fragile X syndrome model mice (*Fmr1*<sup>-/-</sup> mice, Bakker and Oostra, 2003), and other mouse models of ASD (reviewed in Kazdoba et al., 2016). *Fmr1*<sup>-/-</sup> mice show downregulation of  $\alpha$ 5 GABA<sub>A</sub>R and a deficit in tonic inhibition (Curia et al., 2009). Subsequent studies of  $\alpha$ 5H105R mice identified behavioral changes including hyperactivity and impaired encoding of object location memories (Hauser et al., 2005; Prut et al., 2010), although some behavioral changes may be attributed to subunit ordering rearrangements in a mixed alpha subunit GABA<sub>A</sub>R (see earlier, Composition).



The most commonly reported loci of chromosomal abnormalities in ASD patients are found in the q11.2–13 region on chromosome 15 (Hogart et al., 2010). Among the genes in this region are the  $\alpha 5$ ,  $\beta 3$ , and  $\gamma 3$  subunits. An autism patient exome study identified mutations including  $\alpha 5$ G113A (NT),  $\alpha 5$ V204I (NT) and mutations in the extrasynaptic anchor radixin: T516I, P471T, D197H, A496V (Zurek et al., 2016). Exome sequencing of sporadic genetic epilepsy patients identified  $\alpha 5$ V204I (NT),  $\alpha 5$ W280R (M1),  $\alpha 5$ S402A (CD) and  $\alpha 5$ P453L (CT) mutations (Hernandez et al., 2016). Recombinant studies of these mutant  $\alpha 5\beta 3\gamma 2$  GABA<sub>A</sub>Rs indicated no pronounced changes in surface or total  $\alpha 5$  levels, while functional deficiencies ranged from reduced currents and gating defects to altered channel activation and deactivation. A V294L (M2, pore-lining helix) mutation identified in a patient with severe early-onset epilepsy and developmental delay showed receptors with 10 times greater GABA sensitivity, although maximal GABA currents were reduced by increased receptor desensitization (Butler et al., 2018). An autism patient pilot PET imaging study with the  $\alpha 5$  preferring tracer [11C]Ro15-4513 identified reduced  $\alpha 5$  binding across multiple brain regions (Mendez et al., 2013), while another recent study showed changes in a GABA-sensitive perceptual task without differences in binding (Horder et al., 2018). As both studies were without genetic information, this suggests further testing with patient stratification by exome data could provide greater insight. Despite being a genetically heterogeneous disorder, the potential utility for mechanism-based GABA<sub>A</sub>R pharmacologic treatment with ASDs is supported by shared pathologies both in patients and related mouse models.

## $\alpha 5$ GABA<sub>A</sub>R THERAPEUTICS

NAMs that selectively reduce  $\alpha 5$  GABA<sub>A</sub>R function have been heavily pursued for the potential development of cognitive enhancing or “smart” drugs. The following are a selection of  $\alpha 5$  GABA<sub>A</sub>R NAMs: L-655,708,  $\alpha 5$ IA, Ro15-4513, MRK-016, RO4938581, and RY-80 (reviewed in Clayton et al., 2015; Sieghart and Savic, 2018). Importantly,  $\alpha 5$  NAMs did not exhibit the convulsant or pro-convulsant activity of more general alpha subunit NAMs, had good oral bioavailability and easily crossed the blood brain barrier (reviewed in Atack, 2011). In contrast to NAMs which act *via* the GABA<sub>A</sub>R benzodiazepine binding site, S44819 was recently identified as a competitive antagonist of GABA at  $\alpha 5$  GABA<sub>A</sub>R and showed similar pro-cognitive effects as NAMs: blocking  $\alpha 5$ -GABA<sub>A</sub>R tonic current, enhancing LTP, reversing scopolamine-induced impairment of spatial working memory and enhancing object recognition memory (Ling et al., 2015; Etherington et al., 2017). Finally, recent evidence for beneficial effects of positive allosteric modulators (PAMs) in aged brain cognition, autism, depression and schizophrenia has bolstered  $\alpha 5$  PAM drug development. A selection of  $\alpha 5$  preferring PAMs includes SH-053-R-CH3-2’F, MP-III-022, and GL-II-73 (Sieghart and Savic, 2018; Prevot et al., 2019). Potential therapeutic applications for  $\alpha 5$  preferring NAMs and PAMs are discussed below with a focus on CNS specific uses (Table 1),

**TABLE 1** | Summary table of  $\alpha 5$  subunit containing GABA type A receptor ( $\alpha 5$  GABA<sub>A</sub>R) targeted drugs and potential utility.

Drug type	Reduce $\alpha 5$ GABA <sub>A</sub> R activity (NAM or competitive antagonist)	Increase $\alpha 5$ GABA <sub>A</sub> R activity (PAM)
Compound	L-655, 708, $\alpha 5$ IA, Ro15-4513, MRK-016, RO4938581, RY-80, S44819 (competitive antagonist)	SH-053-R-CH3-2’F, MP-III-022, Compound 44, GL-II-73
Therapeutic potential	Procognition/smart drugs	Mild cognitive impairment in aging
	Neurodevelopmental disorders with excessive GABAergic neurotransmission	Neurodevelopmental disorders with insufficient inhibitory tone
	Inflammation induced mild cognitive impairment	Depression
	Post-anesthesia memory blockade	Schizophrenia

*This includes drugs that can reduce  $\alpha 5$  GABA<sub>A</sub>R activity [negative allosteric modulators (NAMs) and the competitive antagonist S44819] and positive allosteric modulators (PAMs) that enhance  $\alpha 5$  GABA<sub>A</sub>R activity. Representative compounds and therapeutic potential are listed.*

although important remaining questions exist for both *in vivo* specificity and receptor subtype selectivity as recently reviewed (Sieghart and Savic, 2018).

## NAM $\alpha 5$ GABA<sub>A</sub>R Therapeutic Applications

### Pro-cognition

The ability of  $\alpha 5$  preferring NAMs to enhance learning and memory in rodents provided crucial evidence for the importance of  $\alpha 5$  GABA<sub>A</sub>Rs in these processes (Chambers et al., 2002, 2003; Street et al., 2004). The  $\alpha 5$  NAM L-655,708, which shows approximately 50–100-fold selectivity for  $\alpha 5$  GABA<sub>A</sub>Rs, reduced tonic inhibition, enhanced LTP, improved performance in the Morris water maze and generated spontaneous gamma oscillations in the CA3 region of the hippocampus (Caraiscos et al., 2004; Atack et al., 2006; Glykys et al., 2008). However anxiogenic activity and pharmacokinetics (reviewed in Atack, 2011) prevented its use in humans. Although  $\alpha 5$ IA was non-anxiogenic and reduced ethanol-induced learning impairment in young volunteers, prolonged use was prevented by high dose renal toxicity (Atack, 2010). MRK-016 showed pro-cognitive efficacy and was non-anxiogenic; poor compound tolerance in the elderly stopped further clinical development (Atack et al., 2009). Efforts to develop clinically successful  $\alpha 5$  NAM are ongoing.

### Developmental Disorders

Down syndrome mice (Ts65Dn) show cognitive impairment due to excessive GABAergic inhibition. Acute treatment with  $\alpha 5$ IA reversed deficits in novel object recognition and spatial learning and was able to restore deficits of immediate early genes expression during memory processing (Braudeau et al., 2011). Although Ts65Dn mice show no major changes in  $\alpha 5$  GABA<sub>A</sub>R levels (Deidda et al., 2015), growing evidence indicates increased  $\alpha 5$  GABA<sub>A</sub>R activity is an important

pathological component, as genetic ablation of  $\alpha$ 5 GABA<sub>A</sub>Rs partially rescues learning, LTP and neuromorphological changes (Vidal et al., 2018). Furthermore, a recent study revealed a specific increase in GABA<sub>A</sub>R dendritic inhibition in Ts65Dn mice that led to reduced NMDAR activation and impaired LTP that could be restored with  $\alpha$ 5 NAM treatment (Schulz et al., 2019). *Rdx*<sup>-/-</sup> mice have increased GABAergic inhibition *via* enhanced  $\alpha$ 5 synaptic levels, impaired short-term memory and a reversal learning deficit, with the latter being improved with  $\alpha$ 5IA treatment (Hausrat et al., 2015). The subsequently identified  $\alpha$ 5 NAM RO4938581, with high affinity and efficacy at  $\alpha$ 5 GABA<sub>A</sub>Rs vs.  $\alpha$ 1–3 GABA<sub>A</sub>Rs (Ballard et al., 2009), demonstrated efficacy in Ts65Dn mice at improving spatial memory, reversing LTP deficits, and restoring neurogenesis while reducing both hyperactivity and the enhanced density of hippocampal GABAergic boutons (Martínez-Cué et al., 2013). Although these pharmacological successes led to a Phase II clinical trial for a related compound RG1662 (Hoffman-La Roche) in Down syndrome patients, the trial did not meet the primary and secondary endpoints of improved cognition and function.

### Inflammation Induced Mild Cognitive Impairment and Post Anesthesia Memory Blockade

Increased systemic inflammation caused by pathological events such as stroke, infection, and traumatic brain injury is associated with memory problems during recovery from the initial insult. In an acute inflammation model, increased tonic  $\alpha$ 5 GABA<sub>A</sub>R current and surface levels *via* P38 MAPK signaling was central to generating inflammation induced memory deficits (Wang et al., 2012). Importantly, these inflammation induced memory impairments were absent in *Gabra5*<sup>-/-</sup> mice and could be blocked by treatment with the  $\alpha$ 5 NAMs L-655,708 or MRK-016. Similarly, following stroke injury, tonic inhibition is increased in the peri-infarct zone, and L-655,708 treatment from 3-days post-stroke increases functional recovery (Clarkson et al., 2010). *Gabra5*<sup>-/-</sup> mice also exhibited improved motor recovery post-stroke. Sustained upregulation of  $\alpha$ 5 GABA<sub>A</sub>Rs is also indicated in memory blockade following anesthesia (Zurek et al., 2014). Both the injectable anesthetic etomidate and the inhaled anesthetic isoflurane increase  $\alpha$ 5 GABA<sub>A</sub>R tonic conductance, promoting the amnesic properties of these drugs (Cheng et al., 2006; Martin et al., 2009b; Saab et al., 2010). Pharmacological inhibition of  $\alpha$ 5 GABA<sub>A</sub>Rs reduces anesthetic potentiation of GABA<sub>A</sub>Rs (Lecker et al., 2013) and restores recognition memory in mice after anesthesia. Recent investigation of age-dependent efficacy of L-655,708 showed that  $\alpha$ 5 NAM treatment prior or following anesthesia restored spatial learning and memory in young rats, while aged rats only showed improvement with  $\alpha$ 5 NAM treatment prior to anesthesia (Zhao et al., 2019). Importantly, low dose isoflurane downregulated  $\alpha$ 5 mRNA in aging hippocampal neurons but upregulated  $\alpha$ 5 mRNA in neurons from young animals. This suggests different approaches will be needed to improve post anesthesia memory blockade in young vs. aged populations.

## PAM $\alpha$ 5 GABA<sub>A</sub>R Therapeutic Applications

### Neurodevelopmental Disorders

Mouse models of neurodevelopmental disorders that present with insufficient inhibitory tone show improvement with positive modulators of GABA<sub>A</sub>R signaling. In the *Scn1a*<sup>+/-</sup> mouse model of Dravet syndrome, a severe childhood epileptic encephalopathy syndrome with hyperactivity and autism behaviors, abnormal social behaviors and fear memory deficits were rescued following treatment with a benzodiazepine, clonazepam (Han et al., 2014). In an ASD mouse model with reduced GABA<sub>A</sub>R-mediated inhibition, the BTBR T+tf/J mouse, the  $\alpha$ 2,3 and 5 PAM L-838,417, improved deficits in social interaction, repetitive behaviors, and spatial learning (Han et al., 2014).

### Mild Cognitive Impairment in Aging

Although  $\alpha$ 5 GABA<sub>A</sub>R NAMs enhance memory in young rodents, it appears positive modulation may be more therapeutic in aging brains impaired by excess activity. Particularly in disorders such as Alzheimer's which are hallmarked by overexcitation (Ambrad Giovannetti and Fuhrmann, 2019), enhanced cognition may be achieved with reducing pathological excitability, as observed with the FDA approved NMDAR antagonist memantine. Furthermore, there is growing evidence for a general decline in GABAergic inhibitory tone in aging humans, monkeys and rodents (Rozycka and Liguz-Leczna, 2017; Lissemore et al., 2018). From this newer perspective, an  $\alpha$ 5 GABA<sub>A</sub>R PAM focused approach (Compound 44) identified improved hippocampal-dependent memory in aged rats with cognitive impairment (Koh et al., 2013).

### Depression and Schizophrenia

Another important unmet need where  $\alpha$ 5 GABA<sub>A</sub>Rs PAM pharmacotherapy may be applicable is in the development of new fast-acting anti-depressant drugs. Most current antidepressants act on the monoaminergic systems, and are only moderately therapeutically efficacious after dosing for several weeks. Significant evidence links GABAergic deficits with major depressive disorders (MDD) (Luscher et al., 2011). Investigation of anti-depressant activity of the  $\alpha$ 5 PAM SH-053-2'F-R-CH3 showed stress reduction in female mice both as an acute and chronic treatment (Piantadosi et al., 2016). Although male mice did not respond to PAM treatment, they also failed to show the upregulation of *Gabra5* gene expression following unpredictable chronic mild stress seen in female mice. This particular PAM was also able to reverse pathological increases in dopaminergic activity in the MAM-model of schizophrenia (Gill et al., 2011). GL-II-73 a recently developed  $\alpha$ 5 preferring PAM showed anxiolytic and antidepressant efficacy, reversing stress-induced and age-related working memory deficits both in male and female mice (Prevot et al., 2019). Somewhat contradictory to this data and the GABA deficit hypothesis of MDD,  $\alpha$ 5 NAM have also shown rapid antidepressant actions in mice, potentially *via* ketamine like mechanisms of disinhibition (Fischell et al., 2015; Zanos et al., 2017).

## CONCLUSION

Due to the unique physiology and pharmacology of  $\alpha 5$  GABA<sub>A</sub>Rs, these receptors are being targeted and tested as treatments for neurodevelopmental disorders, mild cognitive impairment, depression and schizophrenia. The recent cryo-EM studies of heteropentameric synaptic GABA<sub>A</sub>Rs and binding of GABA, antagonists, and benzodiazepines should further advance  $\alpha 5$  subtype specific structure-based drug design. Despite the progress in understanding of  $\alpha 5$  GABA<sub>A</sub>R neurobiology, comparatively little is understood regarding mechanisms that regulate  $\alpha 5$  GABA<sub>A</sub>R trafficking, stability, and both synaptic and extrasynaptic clustering. Furthermore, understanding of  $\alpha 5$  GABA<sub>A</sub>R plasticity occurring from endogenous signaling

mechanisms and from drug treatments in the developing, mature and aging brain will be needed to effectively and safely advance therapeutic application of  $\alpha 5$  GABA<sub>A</sub>R preferring drugs.

## AUTHOR CONTRIBUTIONS

TJ prepared the figure, table and wrote the manuscript.

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**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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