MASTERARBEIT | MASTER'S THESIS

Titel | Title

Novel Compounds Targeting β1-Containing GABAA Receptors: Identification and Pharmacological Characterization of Subtype-Selective Modulators

verfasst von | submitted by

Celina Haller BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Magistra pharmaciae (Mag. pharm.)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 605
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Pharmazie
Betreut von | Supervisor:	Ass.-Prof. Mag. Dr. Sophia Khom-Steinkellner

Danksagung

Mein besonderer Dank gilt Ass.-Prof. Mag. Dr. Sophia Khom-Steinkellner für die kompetente und engagierte Betreuung meiner Masterarbeit. Ihre Geduld, motivierenden Worte und stetige Hilfsbereitschaft haben maßgeblich zum Gelingen dieser Arbeit beigetragen. Ich bin dankbar, dass ich die Möglichkeit bekommen habe in ihrem Labor so viel zu lernen, sowohl in fachlicher als auch in persönlicher Hinsicht.

Ebenso möchte ich mich bei der ganzen Arbeitsgruppe für die kollegiale Zusammenarbeit, die gegenseitige Unterstützung und die entstandenen Freundschaften bedanken. Mein besonderer Dank gilt dabei Laureta Gashi, Susanne Kaspar, Aya Jawish und Husna Bibi Daneshyar, mit denen ich den Großteil meiner Zeit verbringen durfte. Für die geduldige Einschulung zu Beginn meiner Tätigkeit danke ich insbesondere Angelika Doppler und Yuki Bacowsky.

Darüber hinaus bedanke ich mich bei Dr. Dominik Schnalzer sowie bei Assoz.-Prof. Dipl.-Chem. Dr. Nina Schützenmeister für die Bereitstellung der Testsubstanzen. Ein weiterer Dank gilt Peter Höflich und Natasha Deutsch für die zuverlässige Versorgung mit Milli-Q® Wasser.

Ebenso möchte ich meiner Familie danken, die mir über die Jahre hinweg immer den Rücken gestärkt und stets an mich geglaubt hat. Ihre beständige Ermutigung hat mir diesen Weg wesentlich erleichtert. Ein weiteres Dankeschön geht an meine Freundinnen und Freunde, die mir in herausfordernden Phasen mit motivierenden Worten und wohltuender Ablenkung zur Seite gestanden sind.

Nicht zuletzt danke ich meinem Partner, Martin, für seine große Geduld, sein Verständnis und seine unermüdliche Unterstützung in allen Phasen meines Studiums.

Zusammenfassung

Hintergrund: $GABA_A$-Rezeptoren regulieren die inhibitorische Neurotransmission im Gehirn der Säugetiere und sind aus 5 Untereinheiten aufgebaut. Insgesamt sind 19 $GABA_A$-Rezeptor-Untereinheiten bekannt, die unterschiedliche Verteilungsmuster und Expressionslevel im Gehirn aufweisen. Diese Vielfalt ermöglicht die Bildung zahlreicher $GABA_A$-Rezeptor-Subtypen mit unterschiedlichen physiologischen und pharmakologischen Eigenschaften. Einige davon, wie jene, die β1-Untereinheiten enthalten, sind bislang nur unzureichend charakterisiert. Durch die Identifizierung und Entwicklung subtypspezifischer $GABA_A$-Rezeptor-Modulatoren könnten neue Tools entstehen, um die regionale Verteilung und die physiologische Funktion dieser Subtypen gezielt zu untersuchen.

Zielsetzung: Die Ziele meiner Masterarbeit umfassten i.) die Identifikation β1-Untereinheit-selektiver $GABA_A$-Rezeptor-Modulatoren aus einer Serie von Pyrazolochinolinon (PQ) - Verbindungen, gefolgt von deren detaillierter pharmakologischer Charakterisierung sowie ii.) die Untersuchung potenzieller Off-Target-Effekte (d. h. Interaktionen mit dem häufigsten $GABA_A$-Rezeptor-Subtyp) einer Reihe von Naturstoffderivaten.

Materialien und Methoden: $GABA_A$-Rezeptor-Subtypen (α1β2γ2, α1β2, α1β3, α1β1) wurden in *Xenopus laevis* Oozyten exprimiert und die Effekte der Substanzen auf GABA-induzierte Ströme (I_{GABA}) oder die spontane Aktivität von β1- und β3-Homopentameren mittels der zwei-Mikroelektroden-Spannungsklemmtechnik untersucht.

Ergebnisse: Meine Experimente liefern weitere Evidenz für die β1-selektive $GABA_A$-Rezeptor-Modulation durch die kürzlich identifizierten Substanzen aus der PQ-Serie. Weiters zeigten die von mir erhobenen Daten, dass die selektiven Substanzen nicht an β1- oder β3-Homopentameren sowie den *in vivo* häufiger vorkommenden $GABA_A$-Rezeptor-Subtypen (α1β2 und α1β2γ2) wirksam sind. Zudem zeigten meine Daten, dass nahezu alle Verbindungen der zweiten getesteten Serie I_{GABA} an α1β2γ2-Rezeptoren nicht signifikant modulieren mit Ausnahme einer Substanz, die unerwarteterweise eine funktionelle β1-selektive Modulation unabhängig von der Benzodiazepin-Bindungsstelle sowie eine direkte Aktivierung des Rezeptors in Abwesenheit von GABA zeigte.

Diskussion: Die Identifizierung und detaillierte Charakterisierung neuer subtypselektiver Modulatoren könnte helfen die *in vivo* Rolle der bislang wenig untersuchten β1-haltigen $GABA_A$-Rezeptoren besser zu verstehen. Diese könnten, aufgrund ihrer vermutlich geringen Expressionslevel und ihrer begrenzten regionalen Verteilung im Gehirn, wichtige Angriffspunkte für zukünftige pharmakologische Therapien darstellen.

Abstract

Background: $GABA_A$ receptors regulate inhibitory neurotransmission in the mammalian brain and are composed of five subunits. In total, 19 $GABA_A$ receptor subunits, with varying regional distribution patterns and expression levels throughout the brain, have been identified. This variety enables the formation of multiple $GABA_A$ receptor subtypes with diverse physiological and pharmacological properties.

As some subtypes, such a β1-containing subtypes, remain relatively underexplored, it is of special interest to identify modulators that specifically target these receptors. Such modulators could serve as molecular tools to further investigate their physiological function.

Aims: The aims of my thesis included i.) identification of novel β1-subunit-selective $GABA_A$ receptor modulators followed by their in-depth pharmacological characterization from a series of pyrazoloquinolinone compounds as well as ii.) investigation of potential off-target effects (i.e. interaction with the most abundant $GABA_A$ receptor subtype) by a series of natural product derived compounds.

Materials and Methods: $GABA_A$ receptors (α1β2γ2, α1β2, α1β3, α1β1) were expressed in *Xenopus laevis* oocytes and compound effects on either GABA-induced chloride currents (I_{GABA}) or spontaneous activity of β1 or β3 homopentamers were investigated using the two-microelectrode voltage clamp technique.

Results: My experiments provided further evidence for the β1-selective modulation of $GABA_A$ receptors by the recently identified compounds and excluded interactions with β1 or β3 homomers as well as the more abundant α1β2 and α1β2γ2 subtypes. Furthermore, my data showed that nearly all compounds from the second tested series did not significantly enhance I_{GABA} at α1β2γ2 receptors, except for one compound that unexpectedly exhibited functional β1-selective modulation independent of the benzodiazepine binding site, as well as direct receptor activation in the absence of GABA.

Discussion: My data may aid in further investigating and characterizing the hitherto underexplored β1-containing $GABA_A$ receptors *in vivo*. These may represent important targets for pharmacological agents, due to their assumedly low abundance and restricted regional distribution in the brain.

Inhaltsverzeichnis

1. Gamma-aminobutyric acid

After its initial discovery in plants and in the mammalian brain in the 1950s, the important role of gamma-aminobutyric acid (GABA) in neurotransmission was recognized in the 1960s by Roberts and Heidelbergin (Sarasa et al., 2020; Smart and Stephenson, 2019). Nowadays, it is considered the major inhibitory neurotransmitter in the mammalian brain and together with the primary excitatory neurotransmitter glutamate, GABA regulates the balance between excitatory and inhibitory signaling in the central nervous system (CNS). Thus, any dysbalance is associated with the emergence and symptoms of various neurological diseases and disorders including depression, epilepsy and anxiety (Sears and Hewett, 2021).

In the CNS, GABA mainly binds to its associated postsynaptic receptors, where ionotropic GABA type A ($GABA_A$)-receptors mediate fast and metabotropic GABA type B ($GABA_B$)-receptors mediate slow synaptic transmission. They are involved in the regulation of mood, anxiety and panic and also play a significant role in memory processing, seizure activity and pain modulation (Sarasa et al., 2020). Outside the brain, for instance in pancreatic tissue, GABA has been shown to exert anti-oxidative, anti-inflammatory and thus organ-protective effects (Gajić Bojić et al., 2025; Sarasa et al., 2020).

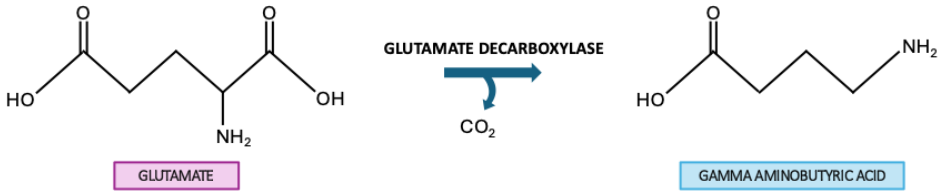

Figure 1: GABA synthesis. Glutamate acts as a precursor for GABA synthesis. The reaction is catalyzed by glutamate decarboxylase. Own illustration based on (Sarasa et al., 2020)

Glutamate, the main excitatory neurotransmitter of the nervous system, serves as a precursor for the synthesis of GABA (**Figure 1**). The decarboxylation process is catalyzed by glutamate decarboxylase (GAD), an enzyme that requires pyridoxal-5-phosphate as a cofactor. There are two major isoforms of GAD: GAD65 is mainly found in nerve terminals and is responsible for activity-dependent GABA release required for neurotransmission, while GAD67 is predominantly expressed in neuronal soma. GAD67 is associated with the regulation of redox processes and synaptogenesis (Sarasa et al., 2020; Sears and Hewett, 2021).

After its synthesis, GABA is loaded into synaptic vesicles by the vesicular GABA transporter (VGAT), a process that is dependent on both proton gradient and electrochemical potential across the vesicular membrane. As outlined before, upon release, GABA can act on either ionotropic or metabotropic receptors (Sears and Hewett, 2021). Since both glutamate and GABA are not enzymatically degraded/inactivated in the synaptic cleft, their efficient re-uptake is a critical part in maintaining the excitatory/inhibitory balance. The extracellular basal concentrations of neurotransmitters must be kept low, to ensure that synaptic signaling is distinct and discernible. In case of glutamate, the reuptake is especially important, as prolonged receptor stimulation may lead to excitotoxicity (Sears and Hewett, 2021; Zhou and Danbolt, 2013). GABA is cleared from the extracellular space by specific GABA-transporters (GATs) which are part of the solute carrier 6 (Slc6) family and act as Na^+- Cl^-- Symporters, utilizing electrochemical gradients to drive transport. Four GATs have been characterized. Among them, GAT1, GAT2 and GAT3 are high affinity transporters, while the betaine-GABA transporter (BGT1) exhibits low affinity for GABA. GAT1 and GAT3 are the most abundant in the brain and have the greatest affinity for GABA. In contrast, GAT2 and BGT1 are primarily found in leptomeningeal cells and blood vessels, and thus play only a minor role in synaptic GABA reuptake (Andersen and Schousboe, 2023; Sears and Hewett, 2021).

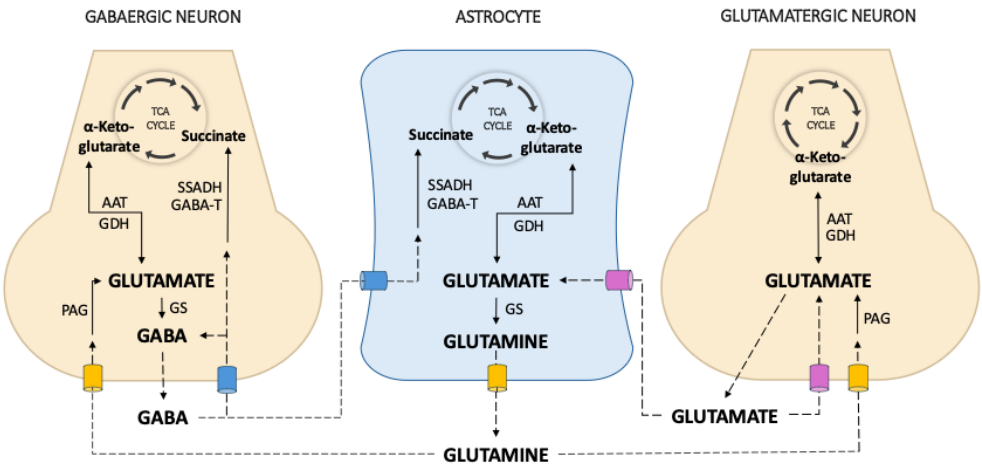

Figure 2: The glutamate/GABA-glutamine-cycle – GABA is recycled through a process called the GABA shunt. After its degradation to α - Ketoglutarate it is converted to glutamate and subsequently to glutamine, which acts as a precursor for either GABA or glutamate synthesis. Own illustration based on (Andersen and Schousboe, 2023)

After reuptake into presynaptic terminals, GABA can be directly recycled and stored in synaptic vesicles, and used again for neurotransmitter release (Sears and Hewett, 2021). Alternatively, after uptake into neurons and, primarily, astrocytes, it can be irreversibly degraded, via a

metabolic pathway known as the GABA shunt. First, GABA is converted into succinic semialdehyde by GABA-transaminase (GABA-T) and then into succinate by succinic semialdehyde dehydrogenase (SSADH) (Andersen et al., 2024; Bak et al., 2006). Succinate then enters the tricarboxylic acid (TCA) cycle, where it is further metabolized to alpha-ketoglutarate and subsequently to glutamate. Finally, glutamate acts as a precursor for glutamine-synthesis (Bak et al., 2006).

This synthesis is not only a crucial component of astrocytic energy-metabolism, but also an integral part of GABA recycling, known as glutamate/GABA-glutamine-cycle, which is shown in **Figure 2** (Andersen, 2025). In addition to metabolizing GABA to glutamine via the TCA-cycle, astrocytes also take up extracellular glutamate and convert it into glutamine. In both pathways, the key enzyme is glutamine-synthetase. The newly synthesized glutamine is then released into the extracellular space and taken up by either GABAergic or glutamatergic neurons. Within neurons, glutamine is converted back to glutamate by the enzyme phosphate-activated glutaminase (PAG). The resulting glutamate can then be directly used as a neurotransmitter or further converted into GABA, as previously described. The TCA cycle is therefore essential for sustaining neurotransmitter recycling by providing key metabolic intermediates (Andersen and Schousboe, 2023; Bak et al., 2006).

2. GABA-activated receptors

For a long time, it was assumed that GABAergic inhibition was only dependent on a single type of receptor. However, decades of research have revealed that two distinct types of receptors are involved. Ionotropic $GABA_A$ receptors mediate fast inhibitory signaling by forming ligand-gated chloride channels, allowing Cl^- to enter the neuron and causing rapid hyperpolarization of the postsynaptic membrane, as will be discussed in detail in chapter 3.2. In contrast, metabotropic $GABA_B$ receptors regulate slow inhibitory transmission. These receptors belong to the class of $G_{i/o}$-coupled protein receptors and modulate ion-channel activity via intracellular signaling pathways. This mechanism will be further described in chapter 2.2. Both receptor types are essential for maintaining the balance between excitation and inhibition in the central nervous system (Shaye et al., 2021; Sigel and Steinmann, 2012; Smart and Stephenson, 2019).

2.1. Ionotropic GABA receptors

Following the discovery of GABA and its role as a neurotransmitter, efforts to identify and characterize its receptors began. One of the earliest significant findings was the antagonistic effect of bicuculline on GABA action, demonstrated by Curtis and colleagues (Johnston, 2013; Smart and Stephenson, 2019). Extensive research followed, leading to the discovery of additional antagonists and modulators, including anesthetics and benzodiazepines and finally, using benzodiazepine-affinity columns, the first $GABA_A$ receptor subunits, the α and β subunit, were identified and subsequently characterized and studied in detail (Sigel et al., 1982; Smart and Stephenson, 2019). Since receptors composed of only α and β subunits were found to be insensitive to benzodiazepines, it was hypothesized, that at least one additional subunit is required. This assumption was confirmed with the discovery of the γ subunit, which significantly advanced research in $GABA_A$ receptor pharmacology and structure (Smart and Stephenson, 2019).

As mentioned before, $GABA_A$ receptors play a key role in mediating neuronal inhibition and maintaining the excitatory/inhibitory balance within the central nervous system. Two forms of inhibition can be distinguished: phasic and tonic inhibition. Phasic inhibition occurs through the binding of GABA to postsynaptic $GABA_A$ receptors, resulting in the generation of rapid, transient inhibitory postsynaptic currents (IPSCs), which allow for fast and precise control of neuronal activity. In contrast, tonic inhibition is mediated by extrasynaptic $GABA_A$ receptors. Due to their high affinity for GABA, these receptors respond to low ambient concentrations of GABA in the extracellular space, generating an ongoing slight hyperpolarization that prevents hyperexcitability (Farrant and Nusser, 2005; Smart and Stephenson, 2019).

2.2. Metabotropic GABA-activated receptors

While studying the effect of GABA on noradrenaline release in the heart, another type of GABA-activated receptor was discovered by Norman Bowery and his colleagues. In contrast to the previously described $GABA_A$ receptors, these receptors were bicuculline-insensitive, and baclofen acted on them in an agonistic manner, similar to GABA. As a result, they were classified as a distinct, novel receptor type – $GABA_B$ receptors. It was later discovered that these receptors are metabotropic and belong to the class C G protein–coupled receptors (GPCRs), coupled to Gi/o proteins (Shaye et al., 2021; Smart and Stephenson, 2019).

$GABA_B$ receptors always function as heterodimers consisting of both a GABAB1 and a GABAB2 subunit. Neither subunit alone can initiate effective signaling. In addition, two isoforms of the GABAB1 subunit have been described, GABAB1a and GABAB1b. GABAB1a

contains two N-terminal sushi domains (SD1 and SD2), which are absent in GABAB1b. These domains are believed to play a role in the axonal targeting of the GABAB1a subunit, whereas both isoforms can be found in dendritic compartments (Biermann et al., 2010; Shaye et al., 2021).

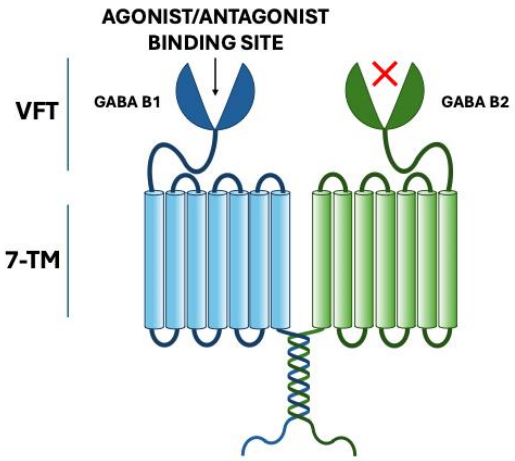

Figure 3: Schematic representation of the $GABA_B$ receptor. Own illustration based on (Xu et al., 2014)

The so-called venus flytrap (VFT) domains are located extracellularly in the N-terminal region of both $GABA_B$ subunits. Each VFT consists of two lobes, LB1 and LB2, which together form the orthosteric binding site by enclosing the ligand in between (Shaye et al., 2021). Despite their structural similarity, the VFTs of the two subunits differ significantly in function as only the VFT of GABAB1 possesses ligand-binding capability. In contrast, the VFT of GABAB2 does not bind GABA but is believed to play a key role in receptor activation by transducing conformational changes from the extracellular to the intracellular receptor domain and thereby enhancing the receptor´s response to agonists (Frangaj and Fan, 2018). Linker regions connect the VFTs to the transmembrane (7TM) domains, which consist of 7 α-helices. The 7TM domain of the GABAB2 subunit is primarily responsible for coupling to G-proteins. However, a recent study by Mao et al. revealed that a major part of the TM7 in both GABAB1 and GABAB2 is a phospholipid on the extracellular site of the domains. This phospholipid not only blocks access to potential allosteric binding sites but also plays a crucial role in maintaining the structural integrity of the receptor (Mao et al., 2020). A schematic representation of the $GABA_B$ receptor is shown in **Figure 3**.

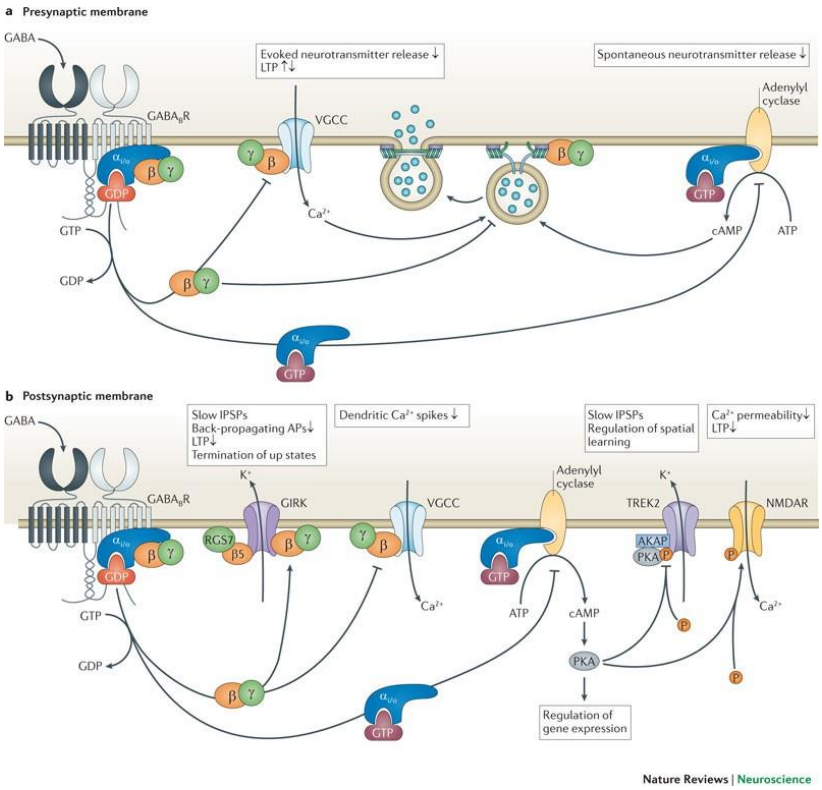

Figure 4: $GABA_B$ receptor signal transduction. (**a**) Mechanism of action and signal transduction of $GABA_B$ receptor at the presynaptic membrane. (**b)** Mechanism of action and signal transduction of $GABA_B$ receptor at the presynaptic membrane (Gassmann and Bettler, 2012, Reproduced with permission from Springer Nature)

In contrast to $GABA_A$ receptors, which mediate fast synaptic signaling, $GABA_B$ receptors are involved in the regulation of slow synaptic transmission (Möhler and Benke, 2001; Shaye et al., 2021). Released GABA can bind to $GABA_B$ receptors located on both pre- and postsynaptic membranes, resulting in a combined inhibitory effect. On the presynaptic side (shown in **Figure 4a**), GABA binding activates the $G_{\alpha i/o}$ pathway, leading to a decrease of cyclic adenosine monophosphate (cAMP) levels, by inhibiting adenylyl cyclase. This in turn reduces vesicular fusion and neurotransmitter release. In GABAergic neurons, presynaptic $GABA_B$ receptors function as auto-receptors and are a fundamental part in the negative feedback regulation of GABA, by terminating its release. In contrast, in non-GABAergic neurons they act as hetero-receptors and inhibit the release of other neurotransmitters, such as glutamate, through hyperpolarization of the cellular membrane. Moreover, the $G_{\beta\gamma}$ subunit of the G-protein inhibits voltage-gated Ca^{2+}-channels (VGCC), also leading to the reduction of presynaptic neurotransmitter release. Postsynaptically (shown in **Figure 4b**), the same $G_{\beta\gamma}$ subunit additionally activates G-protein-gated inwardly rectifying K^+-channels (GIRK), causing K^+-efflux and leading to membrane hyperpolarization (Gassmann and Bettler, 2012; Möhler and Benke, 2001).

Due to their critical role in slow synaptic inhibition and thus controlling synaptic networks, $GABA_B$ receptors have become an increasingly studied target for the treatment of various neuropsychiatric diseases including those involving dysregulated nociception, cognitive function, mood regulation or addictive diseases (Delaney and Crane, 2016; Domi et al., 2021; Sarasa et al., 2020).

3. $GABA_A$ receptors

3.1. Structure of the $GABA_A$ receptor

$GABA_A$ receptors belong to the family of ligand-gated ion channels (LGIC) composed of five subunits forming an ion-conducting pore (Olsen and Sieghart, 2008).

Each subunit features an extracellular domain (ECD), composed of an N-terminal α-helix and ten β-strands. This domain contains the Cys-loop, a conserved motif across all members of the family of Cys-loop receptors, located between the β6 and β7 strand, which is critical for ligand binding. The transmembrane domain (TMD) is composed of 4 α-helices (M1-M4) with distinct functions. Specifically, the M2 segments from all five subunits form the ion conducting pore, comprising two distinct gates, that regulate ion flux. One gate is located in the center of the pore and is responsible for desensitization, the other gate is found closer to the cytosolic opening. The large intracellular loop, located between M3 and M4, represents a site for receptor phosphorylation, protein-protein interactions and modulation of channel properties. In addition, small cavities within the TMD provide further possible binding sites for small molecules. Finally, each subunit comprises in a short extracellular C-terminal domain (Kim and Hibbs, 2021; Olsen and Sieghart, 2008; Sigel and Steinmann, 2012; Zhu et al., 2018). A schematic representation of the $GABA_A$ receptor subunit is shown in **Figure 5A**.

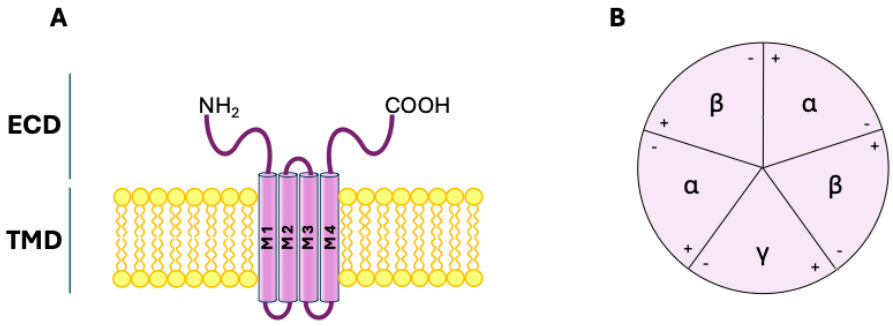

Figure 5: (**A**) Schematic representation of a $GABA_A$ receptor subunit. (**B**) Arrangement of the subunits from top view. Own illustration based on (Ghit et al., 2021)

Theoretically, based on the existence of 19 distinct $GABA_A$ receptor subunits, numerous subunit combinations and thus distinct $GABA_A$ receptor subtypes are theoretically possible (Olsen and Sieghart, 2009). However, only a limited number of subunit assemblies are expressed and functional *in vivo*. The most common $GABA_A$ receptor subtype is composed of two homologous α, two homologous β and one γ subunits, arranged counterclockwise around the pore in the order γ - β - α - β - α (**Figure 5B**) (Sigel and Steinmann, 2012). Other subunits like ε and δ can replace γ in certain brain regions (Chuang and Reddy, 2018; Olsen and Sieghart, 2008; Zhou et al., 2025).

As a pentameric ligand gated ion channel, the $GABA_A$ receptor belongs to the Cys-loop receptor superfamily, named after the conserved loop in the extracellular domain. Other members of this family include glycine receptors, nicotinic acetylcholine receptors (nAChR) and serotonin type 3 receptors (5-HT3Rs). Owing to the high degree of structural conservation among these receptors, comparative modeling approaches had been traditionally used to predict the architecture of $GABA_A$ receptor subunits. These models had provided insight not only into the arrangement of subunits, but also into important structural features of ligand binding sites and transmembrane domains (Ernst et al., 2003; Olsen and Sieghart, 2009).

Yet, cryo-electron microscopy (cryo-EM) marked a breakthrough in the structural analysis of $GABA_A$ receptors, beginning with the characterization of a β3-homomeric receptor, discovered by Miller and Aricescu in 2014. Although this structure lacked a functional GABA binding site, it provided valuable insights into the general architecture of the receptor (Miller and Aricescu, 2014). Following cryo-EM studies succeeded in resolving the structures of heteropentameric $GABA_A$ receptors composed of α, β and γ subunits and offered critical information on subunit

assembly and the molecular arrangement of ligand binding sites (Kim and Hibbs, 2021; Laverty et al., 2019; Zhou et al., 2025).

The two GABA binding sites are located between the β^+ and α^- interface. On the β^+ side, the binding pocket is formed by loops A, B and C, the α^- side contributes via loops D, E and F (Ernst et al., 2003; Olsen and Sieghart, 2009). These loops contain aromatic residues, like α1F64, which create an aromatic box, providing a favorable environment for ligand binding. The β2Y205 residue in loop C engages in cation-π interactions with the positively charged amine group of GABA. Additional electrostatic interactions involve β2Y97 and β2E155, while β2T202 forms a hydrogen bond. The carboxyl group of GABA establishes a salt bridge with α1R67. These molecular interactions not only ensure the specificity and strength of GABA binding but also play a crucial role in initiating the conformational changes that lead to channel opening and subsequent neuronal inhibition (Kim and Hibbs, 2021; Laverty et al., 2019; Masiulis et al., 2019).

3.2. Function of the $GABA_A$ receptor

GABA binding to the β^+/α^- interface leads to conformational change, resulting in a rotation in the extracellular domain of the subunits. This movement is transduced to the transmembrane domains, resulting in the opening of the ion pore (Masiulis et al., 2019; Sallard et al., 2021). The opening involves displacement of the activation gate, located within the M2 transmembrane helices that line the pore, allowing ions to flow along an electrochemical gradient (Kim and Hibbs, 2021; Sallard et al., 2021).

Additionally, a gate near the intracellular opening of the pore contributes to receptor desensitization (Kim and Hibbs, 2021). This state is induced by a prolonged or very high exposure to GABA and prevents exceeding inhibitory signaling. In this state, the pore remains closed, despite the presence of an agonist. Often, a refractory period follows, during which the receptor is unresponsive to any GABA binding. A typical GABA mediated current is shown in **Figure 6**.

Finally, to terminate the signaling, GABA is spontaneously released from the binding site, returning the receptor to its resting position (Sallard et al., 2021). As a result, the $GABA_A$ receptor cycles through multiple conformational states, resting (closed), activated (open) and desensitized (agonist-bound but closed), during activation. (Kim and Hibbs, 2021).

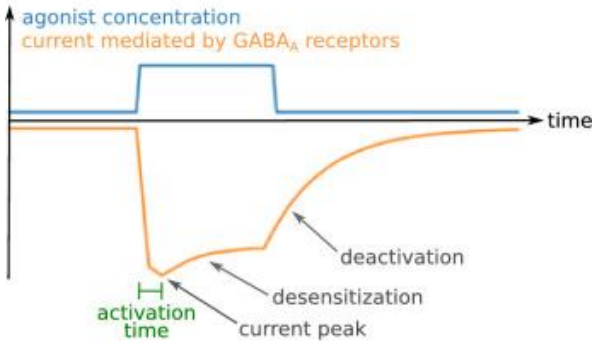

Figure 6: Typical GABA mediated current (Sallard et al., 2021, licensed under CC BY 4.0, https://creativecommons.org/licenses/by/4.0/)

Usually, activation of the $GABA_A$ receptor leads to an influx of Cl^- into the neuron, resulting in hyperpolarization of the membrane and decreased neuronal excitability. However, $GABA_A$ receptors are also permeable to other ions, such as bicarbonate (HCO_3^-), albeit to a lesser extent. Notably, the permeability of HCO_3^- is physiologically relevant, as its electrochemical gradient typically favors an outward flux (Sallard et al., 2021).

The direction of the ion flux through $GABA_A$ receptors strongly depends on the relationship between the ion´s equilibrium potential and the membrane potential. In mature neurons, the intracellular chloride concentration is typically kept below its electrochemical equilibrium mainly due to the activity of K^+-Cl^- cotransporter (KCC2), which extrudes Cl^- from the cell (Kilb, 2021). This creates a Cl^- gradient that favors Cl^- influx upon $GABA_A$ receptor activation, resulting in membrane hyperpolarization. In parallel, HCO_3^- flows outward and, in a much lesser extent, exerts a depolarizing influence. Under pathological conditions, such as during epileptic seizures, this balance can shift. The prolonged $GABA_A$ receptor activation may lead to intracellular Cl^- accumulation, reversing the Cl^- gradient. As a result, Cl^- and HCO_3^- efflux dominate, leading to depolarization rather than hyperpolarization. In this context, $GABA_A$-mediated signaling becomes excitatory instead of inhibitory (Kilb, 2021; Sallard et al., 2021).

In addition, during neuronal development, $GABA_A$-receptors also mediate depolarization, thus excitatory signaling. This is due to the low expression of KCC2, while the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) expression is high. NKCC1 maintains elevated intracellular Cl^- concentrations and therefore leads to an outwardly directed ion flux upon GABA stimulation. This depolarizing effect is important for neuronal growth and synaptogenesis in immature neurons. During maturation NKCC1 expression decreases, while KCC2 is upregulated, thereby reducing intracellular Cl^- levels, which leads to the shift from mainly excitatory to inhibitory GABAergic signaling (Colombi et al., 2024; Kaila et al., 2014; Schulte et al., 2018).

3.3. Subtypes and Subunits of the $GABA_A$ receptor

As previously mentioned, 19 different $GABA_A$ receptor subunits have been cloned and characterized. These include α1–6, β1–3, γ1–3, δ, ε, θ, and π, which primarily assemble into classic heteropentameric $GABA_A$ receptors, as well as ρ1-3, which form mostly homopentameric $GABA_A$-ρ-receptors. For several subunits alternative splice variants have been identified, which allows for even more subtype diversity. (Olsen and Sieghart, 2008; Sallard et al., 2021). However, differences in receptor activity have only been observed for the two splice variants, γ2L and γ2S, of the γ2 subunit (Sallard et al., 2021).

The heteropentameric $GABA_A$ receptors are predominantly composed of two homologous α, two homologous β and one additional subunit, typically γ, δ, ε. Although, in theory, more than 2000 subunit combinations are possible, only a limited number of functionally relevant subtypes have been identified *in vivo* (Möhler, 2006; Smart and Stephenson, 2019).

Electrophysiological studies have also demonstrated the existence of functional receptors, composed exceptionally of α and β subunits (Kasaragod et al., 2022; Mortensen et al., 2012; Mortensen and Smart, 2006; Smart and Stephenson, 2019). Furthermore, there is evidence that heterologous arrangements within one subunit class can occur. For example, receptors containing both α1 and α6 subunits along with β and γ subunits have been reported (Olsen and Sieghart, 2008). Similarly, recent cryo-electron microscope studies revealed receptor compositions with heterologous α and β subunits (Sun et al., 2023; Zhou et al., 2025). Homopentameric receptors composed of homologous β3 subunits have been observed as well, however, their in vivo existence is still uncertain (Miller and Aricescu, 2014; Tretter and Moss, 2008; Yip et al., 2013).

With an occurrence of ~60%, the α1β2γ2 subtype is the most abundant $GABA_A$ receptor in the brain and is found in nearly all brain regions. α2- and α3-containing receptors are typically assembled with β2 and γ2 subunits (Chuang and Reddy, 2018; Möhler, 2006). For example, the α2β3γ2 subtype is highly expressed in hippocampal pyramidal neurons whereas the α3β3γ2 subtype is enriched in cholinergic neurons of the basal forebrain (Pirker et al., 2000). Receptors containing α4 and α6 subunits are less abundant (~5%) and are found in combination with β1-3, γ2 and especially δ subunits. The α4 subunit is mainly expressed in the thalamus and dentate gyrus, while the α6 subunit is predominantly localized in cerebellar granule cells. These subunits often co-assemble with the δ subunit, which is typically expressed in extrasynaptic receptors, mediating tonic inhibition. Receptors containing the α5 subunit are largely restricted to the hippocampus, where they account for about 15-20% of

$GABA_A$ receptors and play an important role in cognitive processing and learning. The γ2 subunit is especially relevant pharmacologically, as only receptors containing γ2 are highly sensitive to classical benzodiazepines (Chuang and Reddy, 2018; Möhler, 2006). While receptors containing the γ1, γ3, ε, θ, and π subunit also exist in various combinations, their pharmacological properties are less well understood and seem to be less relevant in therapeutic contexts (Olsen and Sieghart, 2008).

The $GABA_A$-ρ-receptor forms a homopentameric anion channel, which typically consists exclusively of ρ-subunits (ρ1- ρ3). It is predominantly expressed in retinal neurons where it mediates chloride currents that are smaller in amplitude and more sustained than those of conventional retinal $GABA_A$ receptors. Due to their higher affinity for GABA and their slower desensitization kinetics, their inhibitory signaling may contribute to visual processing, but their physiological function is still not well understood (Enz and Cutting, 1998; Naffaa et al., 2017).

The different subunits and subtypes of the $GABA_A$ receptor not only differ in their regional distribution within the brain but also in their physiological functions. They also determine the pharmacological effects of specific agents, such as benzodiazepines. For instance, the α1 subunit has been mainly associated with mediating the sedative and hypnotic effects of benzodiazepines, such as diazepam (Möhler, 2006). Moreover, evidence suggests that the α2 subunit not only highly contributes to the anxiolytic effects of benzodiazepines but also to the regulation of non-rapid eye movement (NREM) sleep, indicating a role in sleep initiation and maintenance (Möhler, 2006). In addition, extrasynaptic $GABA_A$ receptors, particularly those containing the δ subunit, are thought to play a key role in shaping sleep architecture. This is supported by electroencephalogram (EEG) changes observed in mice, treated with δ-subunit selective modulators (Möhler, 2006; Winsky-Sommerer, 2009).

Additionally α3 containing subtypes have been shown to exert strong control over the dopaminergic system (Yee et al., 2005). Extrasynaptic receptors containing α5 and α6 subunits significantly impact locomotor function by mediating tonic inhibition (Möhler, 2006; Sieghart et al., 2022). Due to their dense extrasynaptic expression in the hippocampus, α5-containing receptors are also critically involved in synaptic plasticity and memory processing (Magnin et al., 2019; Möhler, 2006). This was demonstrated by showing improved cognitive performance in mice treated with a selective partial inverse agonist, targeting α5 containing receptors (Möhler, 2006). Moreover, the α5 subunit is implicated in the regulation of anxiety-related behavior (Magnin et al., 2019).

The different β subunits also influence the pharmacological profiles of $GABA_A$ receptors. β2 and β3 subunits are particularly involved in mediating the immobilizing and hypnotic effects of general anesthetics such as etomidate and propofol, as demonstrated in genetically modified

mouse models (Jurd et al., 2003; Möhler, 2006). The β1 subunit is less abundant in the brain, and fewer receptor subtypes containing this subunit have been characterized. However, salicylidene salicylhydrazide has been identified as negative allosteric modulator that binds selectively to β1-containing receptors (Olsen and Sieghart, 2008). Moreover, compounds of the pyrazoloquinolinone class have shown β1 subunit selectivity (Simeone et al., 2017)., and derivatives of those compounds will be studied in detail in this thesis.

The diversity of subtypes and subunits plays an important role in the development of new pharmacological agents for the treatment of various diseases. The existence of different subunit combinations and their differences in expression levels and localization within the brain provides the framework for the selective targeting of receptor subtypes. This approach holds continued promise for achieving the therapeutic effect while minimizing adverse/unwanted effects (Engin, 2023; Olsen and Sieghart, 2009).

3.4. Peripheral $GABA_A$ receptors

Apart from their relevance in the central nervous system, $GABA_A$ receptors are also expressed in various peripheral tissues, where they exert important regulatory and physiological functions (Gajić Bojić et al., 2025). For instance, $GABA_A$ receptors have been identified in the lungs, particularly in bronchial smooth muscle cells, where they mediate bronchodilatory effects. This could make them pharmacologically relevant and novel targets for treating asthma and chronic obstructive pulmonary disease (Forkuo et al., 2016).

In the cardiovascular system, $GABA_A$ receptors are primarily found in vascular smooth muscle, however, their precise functional role remains to be clarified (Gajić Bojić et al., 2025). Within the enteric nervous system, $GABA_A$ receptors appear to modulate intestinal motility and peristalsis and can mediate excitatory effects through depolarization of enteric neurons (Zizzo et al., 2022).

There is also growing evidence that $GABA_A$ receptors play a key role in immune regulation. They are expressed on multiple immune cell types and influence immune cell proliferation and cytokine release (Bhat et al., 2010; Jin et al., 2013). Additional immunosuppressive effects could be confirmed in the pancreas, where GABA mediates anti-apoptotic effects on β-cells by reducing the production of reactive oxygen species (ROS). $GABA_A$ receptors on β-cells are involved in insulin secretion and promote β-cell proliferation and survival (Purwana et al., 2014). In murine models of type 1 diabetes, GABA treatment prevented hyperglycemia and even reversed the disease phenotype (Soltani et al., 2011). These findings suggest, that pancreatic $GABA_A$ receptors may represent promising targets for new treatment options of

type 1 diabetes. This assumption is supported in early clinical trials, where the application of $GABA_A$ modulators in patients with type 1 diabetes led to reduced HbA1c levels and increased β-cell proliferation (Tian et al., 2017).

Although the physiological function of peripheral $GABA_A$ receptors is still not fully understood, their relevance in research increases. They may lead to novel tactics in treating several diseases, such as asthma and diabetes and could also offer an explanation for adverse effects of centrally acting drugs (Gajić Bojić et al., 2025).

4. The involvement of the GABAergic system in neurological and psychiatric diseases

As already mentioned, disturbances of the excitatory/inhibitory signaling have broad effects and may contribute to an abundance of neurological and psychiatric diseases. This includes epilepsy, autism, depression and anxiety. The GABAergic system is also relevant in alcohol use disorder, as ethanol also acts at $GABA_A$ receptors (Brickley and Mody, 2012; Bryson et al., 2023; Dharavath et al., 2023; Ghit et al., 2021). Selected diseases will be further discussed in the following chapters.

4.1. Epilepsy and neurodevelopmental disorders

Epilepsy is one of the most prevalent chronic neurological diseases and affects approximately 50 million people worldwide (Sarlo and Holton, 2021). It is characterized by recurring epileptic seizures, that result from an abnormal, synchronized neuronal hyperexcitability (Avoli et al., 2022). The dysregulation of the excitatory/inhibitory balance is considered a key factor in the pathophysiology of epileptiform diseases. Generally, during acute seizures, the excitatory effect of glutamate is enhanced, while the inhibitory effect of GABA is either diminished or insufficiently increased. This imbalance significantly contributes to the generation of ictal activity (Richardson et al., 2024; Staley, 2015).

Part of the shifted excitatory/inhibitory balance involving GABAergic transmission can be explained by a dysfunction of GABA homeostasis. This includes impairments in the synthesis, release and reuptake of GABA (Bryson et al., 2023). A variety of mutations in the solute carrier 6 member 1 (SLC6A1) family, a gene that encodes for GAT1, have been identified in recent years. These gene variants, especially the loss of function SLC6A1 variants, lead to a reduced GAT1 cell surface expression and therefore impair GABA reuptake, leading to higher

extracellular GABA levels (Mermer et al., 2021). The higher extrasynaptic levels may seem inconsistent with the notion of reduced inhibitory GABAergic transmission in epilepsy. However, this relation may be explained by the fact, that increased ambient GABA levels enhance thalamic tonic inhibition which has been associated with hypersynchrony and seizure generation in certain types of epilepsy, such as absence epilepsy (Bryson et al., 2023; Mermer et al., 2021). Additionally, temporal lobe epilepsy (TLE) has been associated with impairments of the glutamate/GABA-glutamine-cycle, potentially leading to a reduced supply of glutamine for GABA synthesis. This may result in decreased GABA mediated inhibitory transmission (Bryson et al., 2023).

Several forms of genetic epilepsy have been linked to mutations of the $GABA_A$ receptor. The γ2 subunit mutations encoded by GABRG2, being the first discovered, have been extensively examined and are related to developmental and epileptic encephalopathies, lennox-gastaut syndrom and genetic generalized epilepsies (Khazipov et al., 2014). However, in the context of this thesis, mutations of the β subunits are of particular interest. Among the β subunits, mutations in β2 and β3 have been reported most frequently, likely due to their relatively high expression levels in the CNS (Richardson et al., 2024).

Pathogenic missense mutations in the GABRB3 gene, encoding the β3 subunit, are frequently associated with structural alterations in highly conserved regions, that are critical for receptor activation and signal transduction. These include changes in the GABA binding site, the linker region and the M1, M2 and M3 transmembrane domain. As with SLC6A1 variants, mutations in $GABA_A$ receptor subunits can generally be categorized into two distinct functional classes, one being the gain of function (GOF) and the other one being the loss of function (LOF) variant. Gain of function mutations are typically characterized by a reduction of GABA EC_{50}, implicating increased sensitivity to GABA. In Patients with developmental and epileptic encephalopathies, these variants are associated with a remarkably younger age of seizure onset, as well as a higher incidence of intellectual disability and focal seizures. On the contrary, loss of function mutations show an increased GABA EC_{50}, reflecting decreased receptor sensitivity. Patients carrying these variants typically present with later seizure onset, febrile seizures and milder cognitive impairment (Absalom et al., 2022; Bryson et al., 2023; Maillard et al., 2022).

Similarly, mutations in the GABRB2 gene, which encodes the β2 subunit, include several LOF and GOF variants, that partially share phenotypical outcomes with the GABRB3 mutations. As with GABRB3, distinct forms of disease manifestation can be associated with the functional profile of the mutation. LOF variants are generally linked to milder clinical symptoms, including limited cognitive impairment and febrile seizures. GOF mutations in contrast have been associated with more severe neurodevelopmental phenotypes and a higher incidence of movement disorders, such as dystonia and dyskinesia. This may suggest, that the β2 subunit

plays a particularly important role in GABAergic disinhibitory regulation within the basal ganglia, a brain region that is critically involved in motor control and commonly implicated in movement disorders (Mohammadi et al., 2024).

Regarding the β1 subunit, a recently characterized form of epilepsy, classified as developmental and epileptic encephalopathy 45 (DEE45), has been described in 4 patients. This condition has been linked to pathogenic variants in the β1 encoding GABRB1 gene. The identified mutations affect the transmembrane domain and are associated with symptoms including hypotonia, microcephaly and intellectual disability. Due to the clinical overlap with previously reported GOF phenotypes for GABRB2 and GABRB3 mutations, it is assumed, that the GABRB1 variants may also result in gain of function effects (Monfrini et al., 2023).

From a pharmacological perspective, GOF variants are of particular interest, since most conventional antiepileptic drugs, like benzodiazepines and valproic acid, are based on the assumption, that inhibitory GABAergic transmission is reduced in epilepsy (Maillard et al., 2022). These drugs are therefore designed to enhance GABAergic signaling, by increasing GABA availability or enhancing GABA-mediated chloride currents (Perucca et al., 2023). However, in patients with GOF variants, the resulting $GABA_A$ receptors are already enhanced in their function due to increased receptor sensitivity and prolonged channel opening. In this case, further enhancement of GABAergic inhibition may not only be ineffective but could even exacerbate tonic inhibition and worsen the clinical condition (Ahring et al., 2022). Clinical resistance to standard antiepileptic drugs has been observed in several patients carrying GABRB1, GABRB2 and GABRB3 GOF variants (Monfrini et al., 2023).

By contrast, LOF mutations are often responsive to traditional GABA-enhancing therapies, since these patients typically exhibit reduced inhibitory signaling (Richardson et al., 2024). However, not only distinct GOF and LOF variants but also mixed variants exist (Absalom et al., 2022; Kan et al., 2024). This further complicates adequate pharmacotherapy and highlights once more the importance of personalized therapeutic approach, tailored to the genetic profile of the patients (Bryson et al., 2023; Maillard et al., 2022; Richardson et al., 2024).

Beyond their role in the pathophysiology of epilepsy, the GABAergic system and mutations in $GABA_A$ receptor subunits have also been linked to various neurodevelopmental disorders, including autism spectrum disorder (ASD) and Angleman syndrome (AS). Although the pathophysiological mechanisms are not yet fully understood, it is assumed that ASD involves a disturbance in the excitatory/inhibitory balance, an idea which is further supported by the frequent co-concurrency of epilepsy and ASD (Zhao et al., 2022).

In ASD, the switch form excitatory to inhibitory GABAergic signaling during neuronal development (described in chapter 3.2) appears to be delayed, which not only affects the maturation of neurons and circuit formation but also increases the susceptibility to external insults, such as prenatal drug or alcohol exposure. These factors may lead to disruptions in synaptic plasticity and connectivity (Topchiy et al., 2024). This hypothesis is further supported in postmortem studies, where a general reduction in $GABA_A$ receptor density was demonstrated, suggesting weakened neurotransmission in brains of individuals with ASD (Cellot and Cherubini, 2014; Fatemi et al., 2009; Oblak et al., 2009).

Moreover, ASD is strongly associated with genetic alterations, particularly copy number variations within the chromosomal 15q11-q13 region, which contains genes encoding for several $GABA_A$ receptor subunits, including GABRB3, GABRA5 and GABRG3 (Delahanty et al., 2011; Guo et al., 2017; Zhao et al., 2022). Single-nucleotide polymorphisms in GABRB3, similar to mutations observed in epilepsy, have also been linked to deficits in social behavior, implying a relevance of this gene in ASD pathophysiology (Noroozi et al., 2018; Tanaka et al., 2012).

In Angelman syndrome, a closely related molecular mechanism is observed. Most commonly the disorder is caused by deletions within the 15q11-q13 chromosomal region. A full deletion typically results in a severe phenotype, characterized by intellectual disability, motor impairment and early onset epilepsy. It is hypothesized that the severity of the epilepsy in this case may be partly due to the loss of GABRB3, as similar symptoms have been observed in β3 deficient mouse models (Papandreou et al., 2016; Tanaka et al., 2012).

A smaller subset of patients with Angelman syndrome carry mutations in the maternally expressed UBE3A gene, which is also located within the 15q11-q13 region. Affected individuals generally exhibit a milder phenotype and less severe epilepsy (Tanaka et al., 2012).

4.2. Psychiatric disorders

Approximately 15% of the global population are affected by major depression disorder (MDD), which is characterized by persistent low mood lasting at least two weeks, accompanied by lacking interest in activities, fatigue and a disruption of neurovegetative functions such as sleep and appetite. Due to limited treatment effectiveness and accessibility, MDD remains the leading cause of disability worldwide, according to the world health organization (WHO) (Duman et al., 2019; Luscher et al., 2023). For decades it was assumed, that the main underlying cause of MDD is a deficit in monoamines, particularly serotonin, noradrenaline and dopamine, and pharmacological treatments were accordingly developed to target these

systems. However, this monoamine hypothesis has been increasingly questioned, giving rise to alternative models. It is assumed that a deficit in GABAergic inhibition significantly contributes to the pathophysiology of MDD, leading to the GABAergic deficit hypothesis (Luscher et al., 2023, 2011; Luscher and Fuchs, 2015). The involvement of the GABAergic system is not only implicated in MDD but also in other neuropsychiatric disorders such as bipolar disorder (BPD), anxiety and schizophrenia (SCZ) (Luscher et al., 2011). Especially anxiety and depression are closely related to each other and often occur simultaneously (Zhao et al., 2023).

One of the most relevant findings in this context are the reduced levels of GABA in plasma, cerebrospinal fluid and cortical tissue of MDD patients (Luscher et al., 2011). This was further supported by studies, which showed reduced GABA levels in various brain regions, especially in patients with melancholic depression subtypes (Duman et al., 2019; Lener et al., 2017; Luscher et al., 2011; Schür et al., 2016). After treatment with selective serotonin reuptake inhibitors, GABA levels were normalized which additionally implicates the involvement of GABA in the etiology (Luscher and Fuchs, 2015). However, these findings do not apply to patients with bipolar disorder, where GABA levels do not differ significantly from the normal levels (Kaufman et al., 2009; Schür et al., 2016). In case of schizophrenia GABA levels also appear to be mostly unchanged or even increased (Luscher and Fuchs, 2015). A lower level of GABA in brain and serum of anxiety patients has also been suggested in clinical studies (Zhao et al., 2023).

Another important factor is the interaction between the GABAergic system and the hypothalamic-pituitary-adrenal (HPA) axis. In MDD and anxiety patients, hyperactivity of the HPA axis and elevated glucocorticoid levels are commonly observed (Drevets et al., 2008; Luscher et al., 2011; Luscher and Fuchs, 2015; Zhao et al., 2023). Under normal conditions, $GABA_A$ receptors in the paraventricular nucleus (PVN) of the hypothalamus inhibit corticotropin-releasing hormone (CRH) neurons and therefore limit HPA axis activation during acute stress. However, under chronic or early life stress, the GABAergic inhibitory signaling is diminished, resulting in a loss of control over CRH-expressing neurons. One contributing mechanism is the downregulation of KCC2, which leads to a depolarizing shift in the Cl^- equilibrium potential. As a result, GABAergic transmission becomes less effective or even excitatory and further promotes HPA axis activation (Duman et al., 2019; Luscher et al., 2011; Luscher and Fuchs, 2015). Moreover, the compensatory upregulation of glutamatergic innervation in the PVN may reduce sensitivity of stress-regulating circuits to GABAergic inhibition (Gunn et al., 2013; Luscher and Fuchs, 2015).

Animal and human studies demonstrated stress-induced downregulation of GABA and $GABA_A$ receptor expression in the hippocampus and the frontal cortex, which further contributes to

HPA axis hyperactivity, due to the involvement of these regions in HPA axis feedback control (Luscher and Fuchs, 2015). Additionally, it is commonly known, that GABAergic inhibition is affected by stress induced release of endogenous neurosteroids, such as allopregnanolone, which acts as allosteric $GABA_A$ receptor modulator and increases tonic inhibition. Data from clinical and preclinical studies suggest, that plasma concentrations of neurosteroids are reduced in depressed patients, indicating that neurosteroids pose relevant pharmaceutical treatment options for affected patients (Luscher et al., 2023, 2011).

In general, $GABA_A$ receptor expression in patients with MDD seems to be unchanged in comparison to healthy subjects. It is evident though, that the expression of different $GABA_A$ receptor subunit transcripts is altered in abundance, which might act as a compensatory mechanism for low GABA levels. For example, upregulation of α5, β3, γ2 and δ subunit mRNA was observed in postmortem brain tissue from depressed patients (Luscher et al., 2011). There is also evidence indicating deficits in function of either $GABA_A$ and $GABA_B$ receptors in multiple neuropsychiatric disorders, including MDD, BPD, anxiety disorders and SCZ (Duman et al., 2019; Fee et al., 2017). In contrast to MDD, in anxiety, $GABA_A$ receptor binding sites seem to be generally reduced (Luscher et al., 2011).

Additionally, there is evidence, that mutations in $GABA_A$ receptor subunit genes are linked to several psychiatric diseases. Polymorphisms in GABRB1, GABRB2, GABRA4, GABRA5 and GABRR1 have been linked to BPD, while mutations in GABRA1, GABRA5, GABRA6 and GABRG2 are associated with MDD (Luscher et al., 2011). There is also evidence for the involvement of alternative splice variants of the β2 mRNA in BPD and SCZ, as their expression levels were increased in post mortem brain tissue (Barki and Xue, 2022; Zhao et al., 2009). GABRB2 polymorphisms have been implicated in schizophrenia, however, a recent meta-analysis suggested that there is no relation between GABRB2 polymorphisms and SCZ (Barki and Xue, 2022; Zhang et al., 2018). Altered N-glycosylation of β2 subunits on the other hand has been recently observed in schizophrenia brain tissue which further supports the notion of GABRB2 involvement in the pathology (Barki and Xue, 2022; Mueller et al., 2014). Apart from SCZ, GABRB2 mutations have also been associated with BPD (Zhao et al., 2012) and decreased expression levels in MDD patients have been observed (Barki and Xue, 2022).

4.3. Alcohol use disorder

Approximately 7% of the adult global population is affected by alcohol use disorder (AUD) according to WHO and the main active component of alcoholic beverages, ethanol, is the most widely consumed psychoactive substance worldwide (World Health Organization, 2024). What

makes alcohol so compelling, especially in social situations, is its anxiolytic and disinhibitory effects. However, when consumption becomes chronic, it is often associated with dysphoria and cognitive deficits and an increased risk of developing AUD. This is partly due to neuroadaptive changes and disruption of neurotransmitter homeostasis in the brain. While alcohol and its metabolites act on many pathways in the CNS, including dopaminergic and glutamatergic systems, one of its main effects is mediated through modulation of the GABAergic inhibitory pathway (Dharavath et al., 2023).

Even at small doses, acute ethanol exerts a profound effect on inhibitory neurotransmission in the CNS (Koulentaki and Kouroumalis, 2018; Roberto and Varodayan, 2017). It acts as a positive allosteric $GABA_A$ receptor modulator, therefore enhancing GABAergic inhibition, which contributes to a cascade of regulatory effects, leading to characteristic behavioral outcomes such as anxiolysis and relaxation (Dharavath et al., 2023; Ghit et al., 2021). Some studies suggest that acute ethanol exposure activates distinct extrasynaptic $GABA_A$ receptor subtypes within the central amygdala, which mediate tonic inhibition. However, these findings are inconsistent across laboratories (Dharavath et al., 2023; Herman and Roberto, 2016; Most et al., 2014; Roberto et al., 2021; Santhakumar et al., 2007). Additionally, direct, reversible effects on expression levels and subunit compositions of the $GABA_A$ receptors, like rapid downregulation of the extrasynaptic α4βδ subtype, were observed (Dharavath et al., 2023; Liang and Olsen, 2014; Olsen and Liang, 2017).

With rising ethanol levels, $GABA_A$ receptors in the ventral tegmental area (VTA), nucleus accumbens (NAc), hypothalamus and hippocampus are progressively activated, enhancing inhibitory neurotransmission (Dharavath et al., 2023; Liang and Olsen, 2014; Robinson et al., 2009). Furthermore, acute ethanol influences the mesolimbic reward system, by inhibiting GABAergic interneurons in the VTA. This results in the disinhibition of dopaminergic neurons, leading to an increased dopamine release in the NAc and may contribute to addictive properties of alcohol intake (Tateno and Robinson, 2011; Yorgason et al., 2022).

After chronic alcohol consumption, the CNS undergoes neuroadaptive changes in response to the constant presence of ethanol. These adaptions include the downregulation of certain $GABA_A$ receptor subtypes, particularly α1-containing receptors, and the upregulations of others, such as α4- and δ-containing receptors, across various brain regions (Koulentaki and Kouroumalis, 2018; Roberto and Varodayan, 2017). This alters both phasic and tonic inhibition as downregulation of the α1 subunit weakens fast synaptic inhibition, while postsynaptic α4 and δ-containing subtypes contribute to altered tonic inhibition. As a result, the excitatory/inhibitory balance becomes disrupted, leading to central hyperexcitability, indicating a compensatory mechanism to the potentiation of inhibitory transmission caused by ethanol (Koulentaki and Kouroumalis, 2018). Upon cessation of alcohol intake, these adaptions lead

to classic withdrawal symptoms, such as anxiety, dysphoria and seizures, and may promote alcohol seeking behavior via negative reinforcement mechanisms. (Dharavath et al., 2023; Liang and Olsen, 2014; Roberto and Varodayan, 2017).

Several genetic studies suggest that polymorphisms in $GABA_A$ receptor genes are associated with alcohol use disorder and may influence individual susceptibility. Among these, the most extensively researched gene is GABRA2, which encodes the α2 subunit. Animal studies indicate that this subunit plays a crucial role in mediating the anxiolytic effects of ethanol and thereby reinforces ethanol seeking behavior. Human genetic association studies have repeatedly demonstrated a strong link between SNPs in GABRA2 and increased risk for AUD (Koulentaki and Kouroumalis, 2018; Kumar et al., 2009; Li et al., 2014; Olsen and Liang, 2017; Stephens et al., 2017). Similarly, polymorphisms in the GABRB1 gene have been associated with AUD (Koulentaki and Kouroumalis, 2018), by impacting tonic and phasic inhibition and contributing to changes in $GABA_A$ receptor sensitivity (Anstee et al., 2013; Koulentaki and Kouroumalis, 2018).

The knowledge of the role of the GABAergic system in AUD leads to direct therapeutical implications. Since benzodiazepines, similar to ethanol, act as positive allosteric $GABA_A$ receptor modulators, they are commonly used during alcohol withdrawal to reduce symptoms like anxiety and seizures. However, benzodiazepines themselves carry a risk for unwanted side effects and have a high potential for dependence, which limits their use to short term treatment (Liang and Olsen, 2014). Recently, anticonvulsant medication such as gabapentin and pregabalin have gained attention as alternative treatment options for alcohol withdrawal. They present similar efficacy to benzodiazepines in alleviating withdrawal while exhibiting fewer and less severe adverse effects (Liang and Olsen, 2014; Mason et al., 2018). Additionally, neurosteroids such as allopregnanolone, which modulate extrasynaptic $GABA_A$ receptors, are being investigated for their potential in reducing alcohol seeking behavior. Preliminary evidence supports the idea that neurosteroid administration alleviates cravings, however, further research is needed to validate these findings (Peltier et al., 2024).

5. Binding sites of the GABAA receptor and their pharmacological significance

The $GABA_A$ receptor contains multiple binding sites, not only for GABA and endogenous neurosteroids, but also for a wide range of pharmacological agents. These are primarily positive allosteric modulators and include exogenous neurosteroids, barbiturates, general

anesthetics and benzodiazepines. The following chapters provide an overview of the most relevant binding sites and their pharmacological significance (Ghit et al., 2021; Olsen, 2018).

5.1. Benzodiazepines

For decades benzodiazepines have been widely used and are well known for their anxiolytic, sedative and muscle relaxant properties. Consequently, they are recommended for the treatment of epilepsy, anxiety and sleeping disorders and are also commonly applied in anesthesia (Olsen, 2018; Sigel and Ernst, 2018). Beyond these approved indications, benzodiazepines are frequently used off-label for conditions such as post-traumatic stress disorder and various mood disorders. The broad pharmacological effects are thought to arise from their relatively non-specific modulation of different $GABA_A$ receptor subtypes. As discussed in chapter 3.3, different α-subunits seem to mediate distinct effects. While the modulation of α1-containing subtypes is primarily associated with sedative properties, the anxiolytic effects are mainly mediated via α2-containing subtypes (Engin, 2023; Möhler, 2006). This lack of subtype selectivity results in a broad range of unwanted side effect, as, for instance, the sedative effects are often undesired when treating anxiety. Additionally, benzodiazepine carry a high risk of dependence, as a tolerance to their sedative effects develops rapidly. This is likely due to the downregulation of $GABA_A$ receptors after prolonged administration, as a compensatory response. It is estimated that the development of subtype-selective compounds may alleviate many of the unwanted side effects and reduce abuse and dependence potential. In particular, α1-sparing agents are suggested as promising candidates (Engin, 2023).

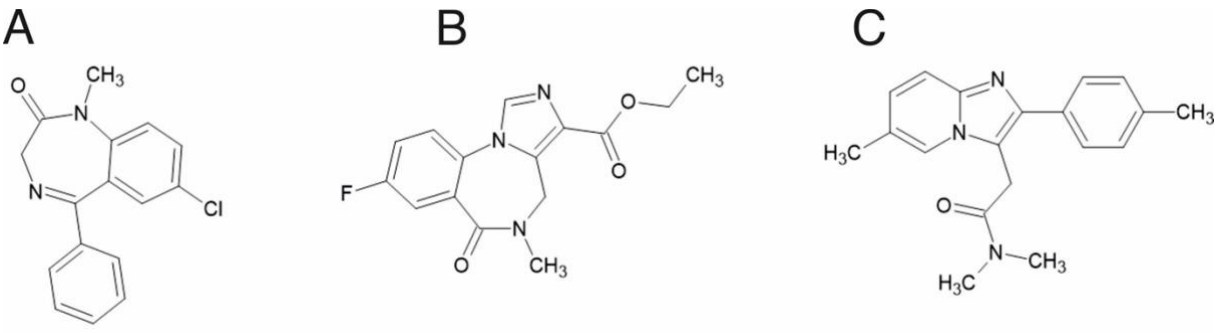

Figure 7: (A) Diazepam (**B**) Flumazenil (**C**) Zolpidem

Benzodiazepines generally act on high affinity bindings sites on $GABA_A$ receptors, where they function as either positive or negative allosteric modulators by inducing conformational changes. Positive allosteric modulators, such as diazepam (see **Figure 7A**), raise the opening probability of the Cl^- channel and thereby enhance GABA mediated inhibitory transmission. In contrast, negative allosteric modulators decrease the receptor response to GABA binding and consequently produce anxiogenic and pro-convulsant effects. Additionally, benzodiazepine site antagonists exist, most notably flumazenil (see **Figure 7B**), which are clinically used as benzodiazepine antidot. These antagonists bind to the benzodiazepine site and inhibit the action of allosteric modulators, without directly inducing conformational changes themselves. However, flumazenil exhibits weak negative allosteric modulation at low concentrations and weak positive allosteric modulation at higher concentrations. Apart from benzodiazepines, z-substances, such as zopiclone and zolpidem (see **Figure 7C**), also act as positive allosteric modulators at the same benzodiazepine binding site (Sigel and Ernst, 2018).

The high affinity binding site for benzodiazepines is located at the extracellular α+/γ- interface of $GABA_A$ receptors. Since benzodiazepines do not modulate α4- and α6-containing subtypes, they are labelled as diazepam-insensitive, while subtypes containing α1,2,3,5 and γ1-3 are considered diazepam sensitive. Among the γ subunits, benzodiazepines show the highest affinity for γ2-containing receptors (Olsen, 2018; Sigel and Ernst, 2018; Sigel and Steinmann, 2012). Since benzodiazepines do not bind to δ-containing subtypes, their action is restricted to synaptic $GABA_A$ receptors and they do not modulate tonic inhibition (Bryson et al., 2023; Sigel and Ernst, 2018; Sigel and Steinmann, 2012).

Structurally, the binding site at the α+/γ- interface is located beneath loop C of the extracellular receptor domain and is characterized by a high density of aromatic residues. A histidine residue at position 102 in the α1, α2, α3 and α5 subunits plays a critical role in ligand recognition by forming hydrogen bonds with functional groups of benzodiazepines. This histidine is absent in the α4 and α6 subunit, which likely accounts for their insensitivity to diazepam (Kim et al., 2020; Kim and Hibbs, 2021; Scott and Aricescu, 2019).

In addition to the high-affinity binding site, several low affinity binding sites have been identified within the transmembrane domain. This includes two sites at the β+/α- interface, which are structurally similar to the binding sites of general anesthetics (as discussed in chapter 5.3).

When diazepam binds to the α1β3γ2 subtype, it engages in hydrophobic interactions with methionine 286 (β3M286) and phenylalanine 289 (β3F289) of the β3 subunit. The phenyl-residue is oriented towards the proline at position 233 (α1P233) of the α1 subunit. (Masiulis et al., 2019; Zhu et al., 2022). These findings confirm former hypotheses suggesting the presence of additional transmembrane binding sites, which may contribute to the biphasic activity of

benzodiazepines. It is assumed, that this second binding site mediates benzodiazepines activity at nanomolar concentrations, independent of the γ subunit (Walters et al., 2000). A similar binding pattern has been observed for zolpidem at α1β2γ2 receptors, although the functional relevance of this secondary site in the case of zolpidem remains unclear (Zhu et al., 2022).

One additional diazepam binding site has been identified at the γ/β TMD interface of α1β2γ2 subtypes. In this configuration, the diazepam is positioned above serine 280 (γS280) at the γ subunit and interacts with phenylalanine in position 304 (γF304) at the γ subunit as well as with proline in position 228 (βP228) at the β subunit (Kim et al., 2020; Kim and Hibbs, 2021).

The mechanism of action of benzodiazepines has been described as a “lock-and-pull” mechanism. Upon GABA binding, a closure of loop C at the β subunits is triggered and initiates a rotation of their ECDs. This conformational change is passed on to the ECDs of adjacent subunits and subsequently to the transmembrane domains, where it ultimately results in channel opening. Benzodiazepines enhance this process by stabilizing the α+/γ- interface, thereby amplifying the rotational movement that promotes receptor activation (Masiulis et al., 2019; Scott and Aricescu, 2019).

5.2. Barbiturates

Barbiturates were first introduced in the early 20th century and are considered the first effective anticonvulsants as well as the first intravenous anesthetics. At the time, they were among the most widely used anesthetics, and were also prescribed for depression, anxiety and insomnia, despite their narrow therapeutic window (Cutler et al., 2023; López-Muñoz et al., 2005). Barbiturates not only modulate $GABA_A$ receptors, but also non-selectively inhibit other ligand-gated ion channels, including kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, thereby reducing glutamate release and excitatory neurotransmission (Cutler et al., 2023; Fourati et al., 2017). However, their actions on GABAergic signaling are considered the principal mechanism responsible for their antidepressant and sedative effect. This was shown by studies in transgenic mice with mutations in the $GABA_A$ β3 subunit, which exhibit reduced sensitivity to barbiturate-induced sedation and immobilization (Fourati et al., 2017; Löscher and Rogawski, 2012).

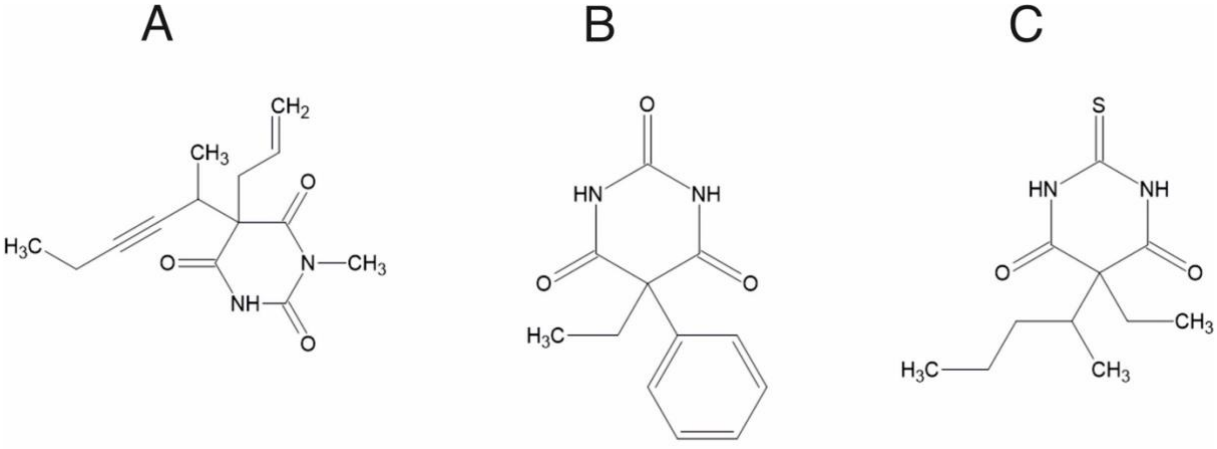

Figure 8: (**A**) Methohexital (**B**) Phenobarbital (**C**) Thiopental

Due to their serious side effects, including respiratory depression and a high potential for dependence, barbiturates have been mostly replaced by benzodiazepines. However, in some cases, including treatment resistant forms of epilepsy, severe convulsions and anesthesia induction, certain barbiturates, such as methohexital, phenobarbital and thiopental (**Figure 8**), are still in use (Cutler et al., 2023; Fourati et al., 2017; López-Muñoz et al., 2005).

Similar to benzodiazepines, barbiturates act as positive allosteric modulators of $GABA_A$ receptors and thereby potentiate the receptor´s response to GABA. They bind to both extrasynaptic and synaptic receptor subtypes, increasing the duration of Cl^- channel opening and thereby potentiate Cl^- conductance. Unlike benzodiazepines, sufficiently high concentrations of barbiturates can directly activate the receptor in the absence of GABA, which makes their mechanism of action partially GABA-independent (Cutler et al., 2023; Löscher and Rogawski, 2012).

Two distinct binding sites for barbiturates have been identified thus far. One is located at the α+/β- subunit interface, the other at the γ+/β- interface. In both cases, the binding pocket resides within the TMD of the β subunit, in a conserved motif below the M2-M3 loop. A key amino acid involved is βL223, which forms an electrostatic interaction with the nitrogen atom of the barbituric acid ring. Additionally the bound barbiturate is stabilized by surrounding van der Waals interactions (Cutler et al., 2023; Kim et al., 2020).

5.3. General anesthetics

A broad range of commonly used general anesthetics, similar to the previously discussed barbiturates, exert their effects by modulating $GABA_A$ receptors. Through positive allosteric modulation, relevant anesthetic functions, such as hypnosis, amnesia and suppression of spinal reflexes, are mediated. The most widely used agents include the intravenous anesthetics propofol and etomidate (**Figure 9**) as well as inhalational anesthetic agents, including isoflurane and enflurane, which all act on distinct binding sites (Philip et al., 2025). As with barbiturates, these agents not only act as positive allosteric modulators but can also directly activate $GABA_A$ receptors at higher concentrations (Ghit et al., 2021).

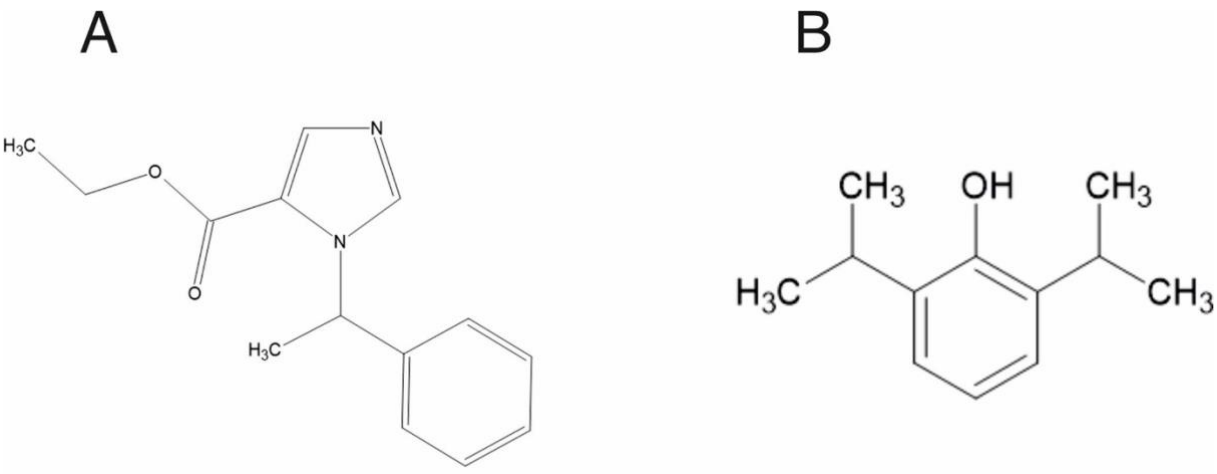

Figure 9: (**A**) Etomidat (**B**) Propofol

Propofol was introduced in 1977 and has since become the most widely used intravenous anesthetic agent. Its primary binding sites on the $GABA_A$ receptor have been identified at several subunit interfaces, including the αβ+/α-γ, γ+/β- and γβ+/α-β interfaces (Maldifassi and Sigel, 2017; Olsen, 2018) These findings suggest that α, β and γ subunits all contribute to propofol sensitivity (Philip et al., 2025). In addition, there is evidence that propofol exhibits greater affinity for β1-containing subtypes compared to those containing β2 and β3 (Eaton et al., 2016; Hao et al., 2020).

Etomidate was the first non-barbiturate intravenous anesthetic and is considered the most selective $GABA_A$ receptor modulator among currently used anesthetics. In contrast to propofol, there is strong evidence, that etomidate binds exclusively at the β+/α- interface (Kim et al., 2020; Olsen, 2018; Philip et al., 2025). Etomidate has also been shown to act on extrasynaptic

$GABA_A$ receptors, enhancing tonic inhibition, which may contribute more significantly to its anesthetic effects than phasic, synaptically mediated inhibition (Hao et al., 2020).

A recent cryo-electron microscopy study confirmed that the binding sites for both etomidate and propofol are located within the TMD of the β subunit. For etomidate the asparagine residue at position 265 (β2/3N265) in the M2 TMD and the methionine at position 286 (β2/3M286) in the M3 helix are particularly involved in forming the binding site (Hao et al., 2020; Kim et al., 2020; Olsen, 2018). The β2/3M286 residue also plays a critical role in propofol binding, which is further stabilized by a hydrogen bond with αI228 in the M1 domain (Kim et al., 2020).

Volatile anesthetics exhibit substance specific differences in their pharmacological profiles and pharmacokinetics. Nevertheless, they share common mechanisms of action, including the enhancement of extrasynaptic GABA-induced tonic currents and prolongation of synaptic inhibition (Hao et al., 2020; Kotani and Akaike, 2013; Philip et al., 2025). Their anesthetic effects are primarily mediated via α1-containing $GABA_A$ receptors. The main binding sites have been identified at the α+/β− and β+/α− interface within the TMD (Philip et al., 2025; Woll et al., 2018).

5.4. Neurosteroids

Endogenous neurosteroids are synthesized de novo in neurons and glial cells within the CNS and partake in the regulation of the excitatory/inhibitory balance. Generally, they can be classified into three subgroups: pregnane-derived (e.g. allopregnanolone (**Figure 10**)), androstane-derived (e.g. androstanediol) and sulfated (e.g. pregnenolone sulfate) (Cutler et al., 2023; Reddy, 2010). Neurosteroids modulate multiple ligand gated ion channels, including $GABA_A$, NMDA, and serotonin receptors. Consequently, they influence a wide range of brain functions such as mood regulation, cognition, memory and pain perception. This modulatory capacity underlies the therapeutic interest in exogenous neurosteroids, which exhibit antidepressant, anticonvulsant and anesthetic properties (Cutler et al., 2023).

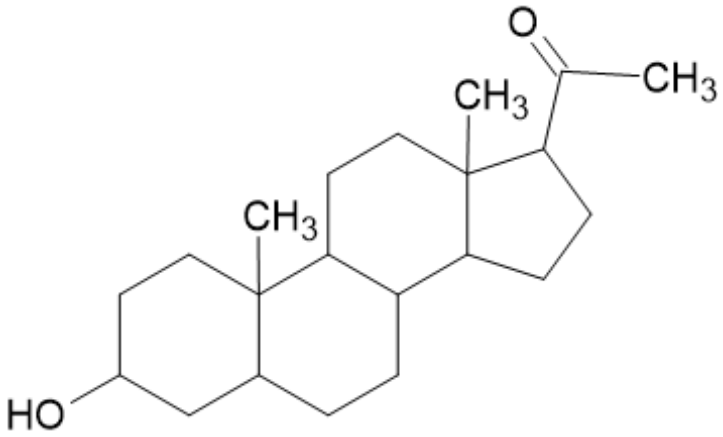

Figure 10: Allopreganolone

Certain neurosteroids, such as allopregnanolone and androstanediol, act as positive allosteric $GABA_A$ receptor modulators, whereas sulfated variants function as negative allosteric modulators and reduce receptor responsiveness to ligand binding (Chen et al., 2018; Zorumski et al., 2019). Positive modulation by neurosteroids increases the opening duration, resulting in enhanced Cl^- currents and decreased neuronal excitability. Similar to barbiturates and general anesthetics, high concentrations of neurosteroids can directly activate $GABA_A$ receptors even in the absence of GABA (Cutler et al., 2023; Reddy, 2010). Neurosteroids interact with various $GABA_A$ receptor subtypes, including extrasynaptic δ-subunit-containing receptors as well as synaptic γ-subunit-containing-receptors, thereby potentiating both tonic and phasic inhibition. (Cutler et al., 2023). However, it is generally assumed, that neurosteroids preferably target δ-containing subtypes at lower concentrations (Brickley and Mody, 2012).

Three distinct binding sites for neurosteroids on the $GABA_A$ receptor have been identified to date. One binding site is a so called “hydrophobic groove”, located at the β+/α- subunit interface. At this site, the A ring of the steroid forms a hydrogen bond with a glutamine at position 241 (αQ241) within the TMD1 of the α subunit. The functional relevance of this residue was confirmed by site-directed mutagenesis, where the substitution of glutamine with isoleucine resulted in a significant reduction of the receptor´s sensitivity to neurosteroid modulation. (Germann et al., 2021; Laverty et al., 2017; Miller et al., 2017).

The second binding site is located within the α subunit, specifically between the TM1 and TM4 domain at their extracellular ends. At this site, the steroid is positioned with its A ring facing the tyrosine at position 415 (αY415), while the D ring is positioned near an asparagine at position 407 (αN407) (Germann et al., 2021).

Finally, a third binding site has been identified within the β subunit, between TM 3 and TM4. Here, the A-ring points towards the extracellular end of TM4 near tyrosine 443 (βY443), while the D-ring is located near glycine 287 (βG287) within the TM3 (Chen et al., 2019; Germann et al., 2021).

As previously discussed in chapter 4.2 and 4.3, neurosteroids play an key role in psychiatric disorders such as MDD, as reduced levels of allopregnanolone are frequently reported in patients with mood disorders (Cutler et al., 2023; Zorumski et al., 2019). This reduction is associated with chronic stress, a major contributing factor in the development of both depressive and anxiety disorders, which negatively impacts the synthesis of endogenous neurosteroids (Cutler et al., 2023). This mechanism is particularly relevant in the context of postpartum depression (PPD), where an acute drop of endogenous neurosteroids, especially allopregnanolone, has been implicated, Simultaneously, a compensatory upregulation of $GABA_A$ receptor expression fails (Cutler et al., 2023; Gunduz-Bruce et al., 2022; Reddy et al., 2023). Several clinical trials investigating the administration of brexanolone, a synthetic formulation of allopregnanolone, for the treatment of PPD have shown significant improvement in depressive symptoms (Gunduz-Bruce et al., 2022; Reddy et al., 2023).

Exogenous neurosteroid analogs, such as ganaxolone, have shown promise in the treatment of certain forms of treatment-resistant epilepsy. Catamenial epilepsy, for instance, is a form of epilepsy, that shows variation in intensity and frequency, depending on the stage of the menstrual cycle. Clinical data suggest that progesterone may be an effective treatment option in this context, and the use of ganaxolone is currently under investigation (Brickley and Mody, 2012; Miziak et al., 2020). Similarly, allopregnanolone and its derivatives have been proposed for use in patients with intractable partial seizures and in children with refractory epilepsy, particularly when administered in combination with conventional antiepileptic drugs (Miziak et al., 2020).

Additionally, the progesterone analog alphaxolone has emerged as promising candidate for intravenous anesthesia. It exhibits rapid onset and offset kinetics and is associated with a reduced risk of cardiorespiratory depression compared to conventional general anesthetics. Of particular interest is its apparent lack of neurotoxicity, as repeated or prolonged exposure to traditional anesthetics agents during early development has been linked to neurodevelopmental impairments in children (Philip et al., 2025; Tesic et al., 2020).

6. Aims

$GABA_A$ receptors are the main inhibitory receptors in the mammalian brain and play a key role in the regulation of neuronal excitability. $GABA_A$ receptors are involved in various physiological processes such as sleep regulation, formation of memory, and motor control. Thus, any dysregulation of this intricate balance between GABAergic inhibitory currents and excitatory signaling is seen as a major contributor to the pathophysiology of several neurological and psychiatric disorders such as epilepsy, schizophrenia and anxiety (Sears and Hewett, 2021).

A key feature of the GABAergic system is the brain region-specific expression of distinct subtypes. Common ligands like benzodiazepines broadly modulate $GABA_A$ receptors, but their lack of subtype selectivity often causes sedation, dependence, and cognitive side effects (Engin, 2023; Möhler, 2006). Subtype-selective compounds may better target pathological circuits while preserving normal inhibition. While β2/β3-containing receptors have been well studied, due to the lack of β1-selective modulators, those receptors remain underexplored. However, given their low expression and reduced off-target potential, β1-selective modulators could be promising therapeutics and also serve as tools to better understand β1-subunit-containing $GABA_A$ receptors.

Thus, the primary aim of my thesis was to thoroughly characterize a focused compound library derived from a previously identified β1-selective PQ library (Simeone et al., 2017). For this purpose, I expressed $GABA_A$ receptors composed of either α1β1 or α1β3 in *Xenopus laevis* oocytes and measured the modulation of GABA-induced chloride currents (I_{GABA}) using the two-microelectrode voltage clamp technique. Active compounds were further studied on subunit compositions α1β2 and α1β2γ2 as well as on the recently identified β1 or β3 homopentamers. In parallel, I also studied potential I_{GABA} modulation at the major $GABA_A$ receptor subtype α1β2γ2 by 24 newly synthesized compounds (provided by the Schützenmeister Lab at the Department of Pharmaceutical Sciences) to provide for insights into potential unwanted side effects.

7. Materials and methods

The protocol for the methods used in this thesis were adopted from previous master´s theses, including those submitted by Mag. Marina Gehrmann, Mag. Katharina Neßmerak and Mag. Sabina Salkic, supervised by Ass. Prof. Dr. Sophia Khom-Steinkellner. I adapted these methods to suit my specific needs in the data collection process.

7.1. Expression of functional $GABA_A$ receptors by cRNA-injection into *Xenopus laevis* oocytes

Xenopus laevis oocytes were purchased weekly from Ecocyte Bioscience. Upon arrival, they were transferred under semi-sterile conditions to a petri dish, containing ND96-buffer. The ND96-buffer medium was prepared once a month as a 20-fold concentrated stock solution and diluted to a 1-fold concentration daily. The final concentration was 90 mM NaCl, 1 mM KCL, 1 mM MgCl2 · 6 H2O, 1 mM CaCl2, 5 mM HEPES. The pH of 7.4 was adjusted using 1 M NaOH-Solution. Milli-Q® water (highly purified water) was used as a diluent. 10 0000 Units Penicillin and 10 µg/ml Streptomycin were added to the storage solution.

To start the injection process, suitable oocytes were selected under a binocular at a 20-fold magnification based on the general criteria for oocyte health and quality: bicolored, round, no signs of damage, and placed on a mesh in a petri-dish containing ND96-buffer (see **Figure 11**).

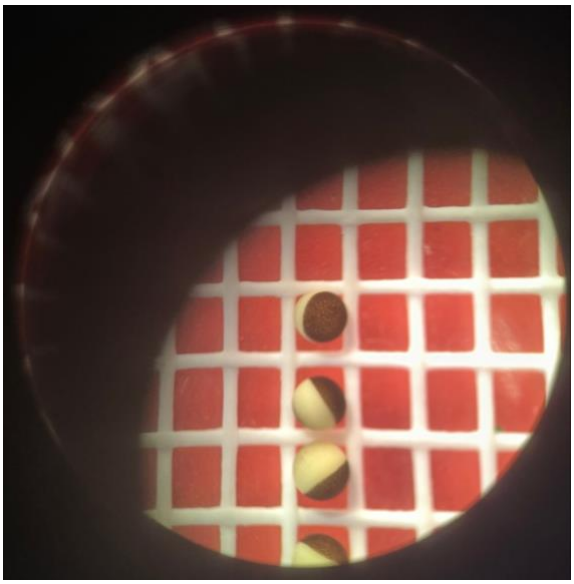

Figure 11: Xenopus laevis oocytes placed on a mesh

RNA encoding for the single GABAA receptors subunits was mixed under RNase free conditions, according to the optimal mixing ratio for the different subunits (α1β1 3:1, α1β2 and α1β3 1:1, α1β2γ2 1:1:10) (Boileau et al., 2002; Krishek et al., 1996). A needle was pulled out of a glass-capillary with the Narishige puller (npi Electronics). The needle was then filled with mineral oil (SIGMA-ALDRICH Co.), followed by the mixed RNA. The oocytes were then injected with 50 nl-100 nl RNA using a Nanoject III. After injection the cells were incubated for 24h at incubation temperature and the storage solution was replaced daily.

7.2. Preparation of the measurement solutions

Each day, a 1 mM GABA stock solution was freshly prepared by dissolving 5.15 mg GABA in 50 ml ND96 buffer. This solution was subsequently diluted using ND96 to obtain the required test concentrations which were individually determined for each oocyte.

The test substances were dissolved in pure dimethyl sulfoxide (DMSO) to a concentration of either 100 mM or, in cases of solubility limitations, 30 mM or even 10 mM. These stock solutions were kept at 4°C to ensure storage stability. Before application to the cells, the stock solutions were further diluted to their final concentration with ND96 together with the required amount of GABA stock solution.

7.3. Two-microelectrode voltage clamp technique and application of the substances

To measure the oocytes with the two-microelectrode voltage clamp technique, they were first placed in a modified perfusion chamber. The chamber is covered with a glass plate containing two access holes for the microelectrodes. On top of this platter sits a quartz funnel which serves as a reservoir for the measurement solutions. A pipetting robot applies the previously prepared solutions into the funnel. The solution is then drawn through the chamber outlet using a pump and perfuses the oocyte. Any remaining liquid is removed through outlets, which consist of two thin plastic tubes attached to the sides of the funnel. This is important to minimize the risk of cross-contamination between the different applied solutions (Baburin et al., 2006).

The microelectrodes were first pulled using a Narishige puller (npi Electronics) and were made of borosilicate glass. They were half-filled with 3M KCl-solution. A $AgCl_2$-wire was inserted into the filled microelectrodes to collect the measured signals, which are transmitted through the KCl-solution. It is important, that the electrodes are filled without any air bubbles, as these would interfere with current conductance. After placing the oocyte in the funnel, the microelectrodes were inserted through the access holes using a binocular.

The two microelectrodes each serve a distinct function. One is referred to as “potential electrode” and measures the actual membrane potential of the oocyte. The difference between the measured potential and the desired holding potential, which is set to -70 mV, is calculated by the amplifier (TurboTec 05X, npi Electronics). Simultaneously the “current electrode” injects the necessary current to maintain the membrane potential at -70 mV. This feedback mechanism enables clamping of the oocyte membrane at the desired potential and allows for the measurement of ion currents (Bianchi, 2006).

At the beginning of each experiment GABA was applied at various concentrations to determine the effective concentration that induces currents corresponding to 5-10% of the maximal GABA-evoked response. This concentration (GABA $EC_{5\text{-}10}$) was then used for co-application with the test compounds. The compounds were applied either at a concentration of 10 or 30 µM for screening purposes, or at increasing concentrations to generate concentration-response curves.

The pipetting robot was controlled using the Robosoft software. For the recording and analysis of GABA-induced currents and modulated currents, Clampex 10.7 (Molecular Devices, San Jose) software was used.

7.4. Evaluation and statistical analysis

The analysis of the experiments was performed using Clampfit 10.7 by measuring the amplitudes of GABA-induced currents and the currents modulated by the test compounds relative to the baseline.

The resulting data were then standardized in Origin 7.0 using the formula $\frac{[(I_{GABA+compound})-(I_{GABA})]}{I_{GABA}} \times 100$ for the test compounds and $\frac{I_{GABA}}{I_{GABAmax}} \times 100$ for the GABA concentration-response curves. To generate concentration-response curves, the normalized data were plotted in a semi-logarithmic coordinate system, and a sigmoidal curve was fitted using the Hill equation. This allowed the determination of the parameters for the maximum

effect (E_{max}) and the effective concentrations, that causes 50% of the maximal effect (EC_{50}). Additional descriptive statistics, such as the number of experiments (n), the mean and the standard error of the mean (SEM) were also calculated.

The remaining statistical analysis and plotting of the screening data were performed with GraphPad Prism version 10.5. *One-way ANOVA* followed by a *Post hoc Tukey test* was used to compare the EC_{50} and E_{max} values across different $GABA_A$ receptor subtypes when more than two subtypes were analyzed. For comparison between two distinct subtypes, an *unpaired t-test* was used. Screening data were evaluated using a *one-sample t-test*, to assess differences from zero. A *P*-value of $P < 0.05$ was the criteria for statistical significance.

To create the figures, representative current traces were first filtered in Clampfit 10.7 using a low-pass, Bessel filter (8-Pole). The traces were then exported and arranged in CorelDRAW 2025. Similarly, the concentration-response curves were exported from Origin 7.0 and finalized in CorelDRAW.

8. Results

8.1. GABA dose-response curves at different receptor subtypes

The aim of my studies was to characterize I_{GABA} modulation at submaximal GABA concentrations by two distinct compound libraries provided by Dr. Dominik Schnalzer and Assoz. Prof. Nina Schützenmeister. Therefore, I first established concentration-response relationships for all $GABA_A$ receptor subtypes that were studied in this thesis. $GABA_A$ receptors composed of α1β2γ2, α1β2, α1β1 and α1β3 were expressed in *Xenopus laevis* oocytes and the amplitudes in response to increasing GABA concentrations were measured. The concentration-response curves were then fitted with the Hill-equation (see **Figure 12** and **Table 1**).

As shown in **Table 2**, a *one-way ANOVA* identified significant differences in EC_{50} values (F (3, 16) = 19.58, $P < 0,0001$) between the studied subtypes. The *Post hoc Tukey test* revealed that the EC_{50} values at α1β2γ2 receptors (15.5 ± 2.5) were significantly higher than at α1β2 (3.7 ± 0.4, $P < 0.0001$), α1β1 (5.1 ± 0.7, $P = 0.0002$) and α1β3 (3 ± 0.3, $P < 0.0001$), while comparable EC_{50} values were determined for all binary receptors ($P > 0.5$ for all comparisons, *Post hoc Tukey test*).

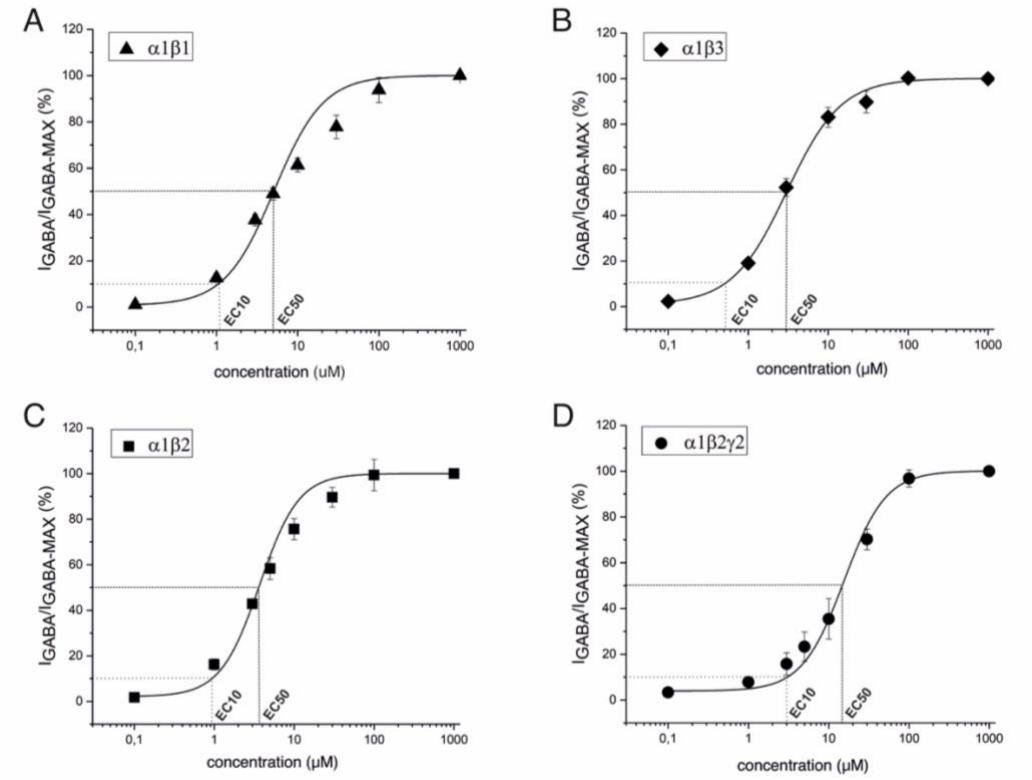

Figure 12: GABA concentration-response curves on (**A**) α1β1, (**B**) α1β3, (**C**) α1β2 and (**D**) α1β2γ2 receptors. The figure shows GABA-concentrations in µM on the X-axis and $I_{GABA}/I_{GABA\text{-}max}$ (%) on the Y-axis. The EC_{10} and EC_{50} values are represented by dotted lines. Symbols represent the mean-values ± SEM (standard error of the mean). Note that the data for the GABA concentration response were generated in joint effort with Susanne Kasper.

	EC_{50} (µM)	**nH**	**n**
α1β1	5.1 ± 0.7	1.4 ± 0.3	5
α1β2	3.7 ± 0.4	1.8 ± 0.4	5
α1β3	3 ± 0.3	1.3 ± 0.1	5-7
α1β2γ2	15.5 ± 2.5	1.7 ± 0.3	5

Table 1: Representation of the mean effective concentration (EC_{50}) in µM, the hill coefficient (nH) and the range in number of experiments (n) for the different $GABA_A$ receptor subtypes.

	EC_{50}
α1β2γ2 vs. α1β2	**** ($P < 0.0001$)

α1β2γ2 vs. α1β1	*** (*P* = 0.0002)
α1β2γ2 vs. α1β3	**** (*P* < 0.0001)
α1β2 vs. α1β1	ns (*P* = 0.8758)
α1β2 vs. α1β3	ns (*P* = 0.9815)
α1β1 vs. α1β3	ns (*P* = 0.6810)

Table 2: Comparison of EC_{50} values of the different $GABA_A$ receptor subtypes α1β2γ2, α1β2, α1β1 and α1β3. The statistical evaluation was made using *one-way ANOVA* followed by *Post hoc Tukey test* (ns = not significant, *P* = *P*-value, **** = *P* < 0.0001, *** = *P* < 0.001).

8.2. DCBS substances

First, I tested the effects of all compounds from the PQ library (provided by Dr. Dominik Schnalzer) on GABA-induced currents. The compounds were applied at a concentration of 10 µM at both α1β1 and α1β3 receptors. For each oocyte the EC_{3-7} was determined individually by application of increasing GABA concentrations. This was followed by co-application of the compounds together with the GABA concentration corresponding to a GABA EC_3-EC_7.

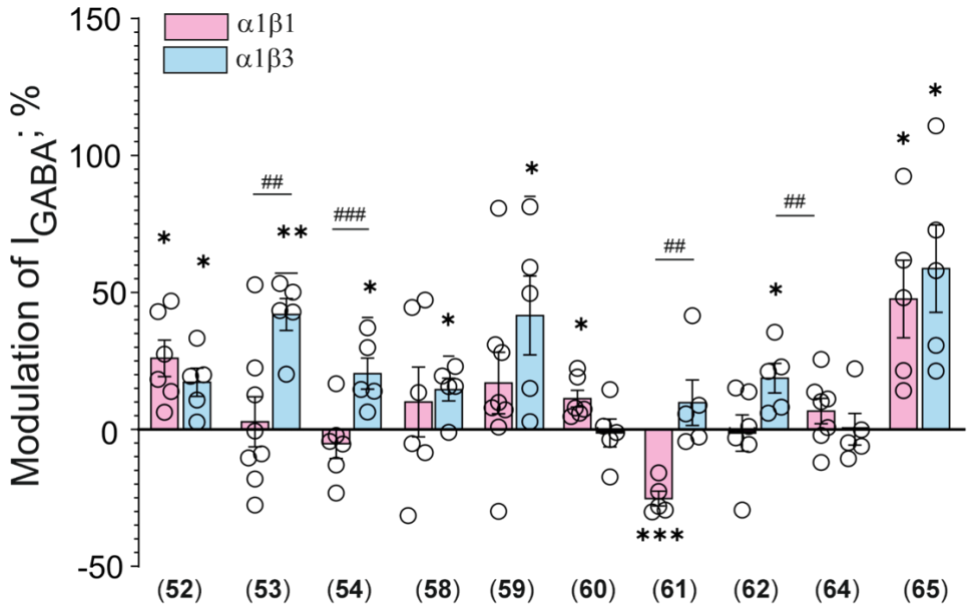

Figure 13: Modulation of I_{GABA} (%) by compounds of the PQ library. The bars show the mean value ± SEM on either α1β1 (pink) or α1β3 receptors (blue). Data was derived from at least five different oocytes and at least two different frogs. A *one sample t-test* revealed significant differences from zero (* = *P*

<0.05, ** = $P < 0.01$, *** = $P < 0.001$), whereas hashtags represent results from the *unpaired t-test* which shows significant differences in I_{GABA} modulation at the two different subtypes (## = $P < 0.01$, ### = $P < 0.001$, #### = $P < 0.0001$)

As shown in **Figure 13** and **Table 3,** a *one-sample t-test* revealed that compounds 48, 49, 50, 51 and 65 modulate I_{GABA} through α1β1 ($P < 0.05$) and compounds 48, 49, 50, 53, 59 and 65 enhanced I_{GABA} at α1β3 ($P < 0.05$) by more than 30%. The effects of the remaining compounds were either below this threshold, inactive or even inhibited I_{GABA}. Furthermore, using *unpaired t-test*, I found that the effect of active compounds was significantly higher on the α1β3 subtype compared to the α1β1 for compound 53 (t = 3.117, df = 11, $P = 0.0098$).

Compound	Subtype	I_{GABA} enhancement (%)	n	one-sample t-test	Unpaired t test	selectivity
52	α1β1	26 ± 7	6	*t*=3.905, *df*=5 *P* = 0.0114	*t*=1.006, *df*=9 *P* = 0.3405	no
	α1β3	17 ± 5	5	*t*=3.358, *df*=4 *P* = 0.0284		
53	α1β1	3 ± 9	8	*t*=0.3034, *df*=7 *P* = 0.7704	***t*=3.117, *df*=11 *P* = 0.0098**	**β3**
	α1β3	**42 ± 6**	**5**	***t*=7.224, *df*=4 *P* = 0.0019**		
54	α1β1	-5 ± 5	6	*t*=0.9748, *df*= 5 *P* = 0.3744	*t*=3.260, *df*=9 *P* = 0.0098	β3
	α1β3	20 ± 6	5	*t*=3.595, *df*=4 *P* = 0.0229		
58	α1β1	10 ± 13	6	*t*=0.7840, *df*=5 *P* = 0.4685	*t*=0.3105, *df*=9 *P* = 0.7632	no
	α1β3	15 ± 4	5	*t*=3.512, *df*=4 *P* = 0.0246		
59	**α1β1**	**17 ± 11**	**8**	***t*=1.507, *df*=7 *P* = 0.1756**	*t*=1.353, *df*=11 *P* = 0.2032	no
	α1β3	**42 ± 14**	**5**	***t*=2.883, *df*=4 *P* = 0.0449**		
60	α1β1	11 ± 3	6	*t*=3.660, *df*=5 *P* = 0.0146	*t*=2.193, *df*=9 *P* = 0.0560	no

	α1β3	-1 ± 5	5	*t*=0.2587, *df*=4 *P* = 0.8086		
61	α1β1	-25 ± 3	5	*t*=9.403, *df*=4 *P* = 0.0007	*t*=4.004, *df*=8 *P* = 0.0039	no
	α1β3	10 ± 8	5	*t*=1.171, *df*=4 *P* = 0.3067		
62	α1β1	-1 ± 7	6	*t*=0.2043, *df*=5 *P* = 0.8462	*t*=2.282, *df*=9 *P* = 0.0484	β3
	α1β3	19 ± 5	5	*t*=3.472, *df*=4 *P* = 0.0255		
64	α1β1	7 ± 5	7	*t*=1.443, *df*=6 *P* = 0.1992	*t*=0.907, *df*=10 *P* = 0.3859	no
	α1β3	0 ± 6	5	*t*=0.00653, *df*=4 *P* = 0.9951		
65	**α1β1**	**48 ± 14**	**5**	***t*=3.361, *df*=4 *P* = 0.0283**	*t*=0.5202, *df*=8 *P* = 0.6170	no
	α1β3	**59 ± 16**	**5**	***t*=3.677, *df*=4 *P* = 0.0213**		

Table 3: Results from screening for modulatory effects of substances at the α1β1 and the α1β3 subtype. Data was derived from at least five oocytes and at least two frogs. A *one-sample t-test* was used to detect differences from zero. *Unpaired t-test* was applied to show significant differences between the two distinct subtypes.

Next, as it is a known phenomenon in the *Xenopus* expression system that upon injection of β1 or β3 subunits homomeric $GABA_A$ receptors can be formed, I studied the effect of all compounds at the β1 and β3 homomers. Four compounds (48, 49, 50, 51), that were identified as highly selective α1β1 modulators in a previous screening by Mag. Angelika Doppler, were tested as well.

Importantly, these receptors are not activated by GABA as they are lacking the classical GABA binding sites. However, they can be activated by changes in extracellular pH (Garifulina et al., 2022). In addition, these receptors are usually spontaneously active which can be seen by relatively high holding currents (average of -0.5 µA for β1 homomers as opposed to oocytes expressing for instance α1β2γ2, where holding currents range from 0 to -0.1 µA).

Therefore, I applied acidified ND96 buffer (pH=6) to verify the expression of functional homomers, followed by application of all compounds in non-acidic ND96 in a concentration of 30 µM. As shown in **Table 4** and **Figure 14B**, a *one-sample t-test* revealed, that all compounds

except for 48, 49, 50, 51, 62 and 64 changed the holding currents with similar efficacy (P < 0.05), ranging from 0.0104 µA (compound 59) to 0.4173 µA (compound 61). This suggests that these compounds are active at β1 homomers, however they exhibit inhibitory effects. For selected representative current traces see **Figure 14A.**

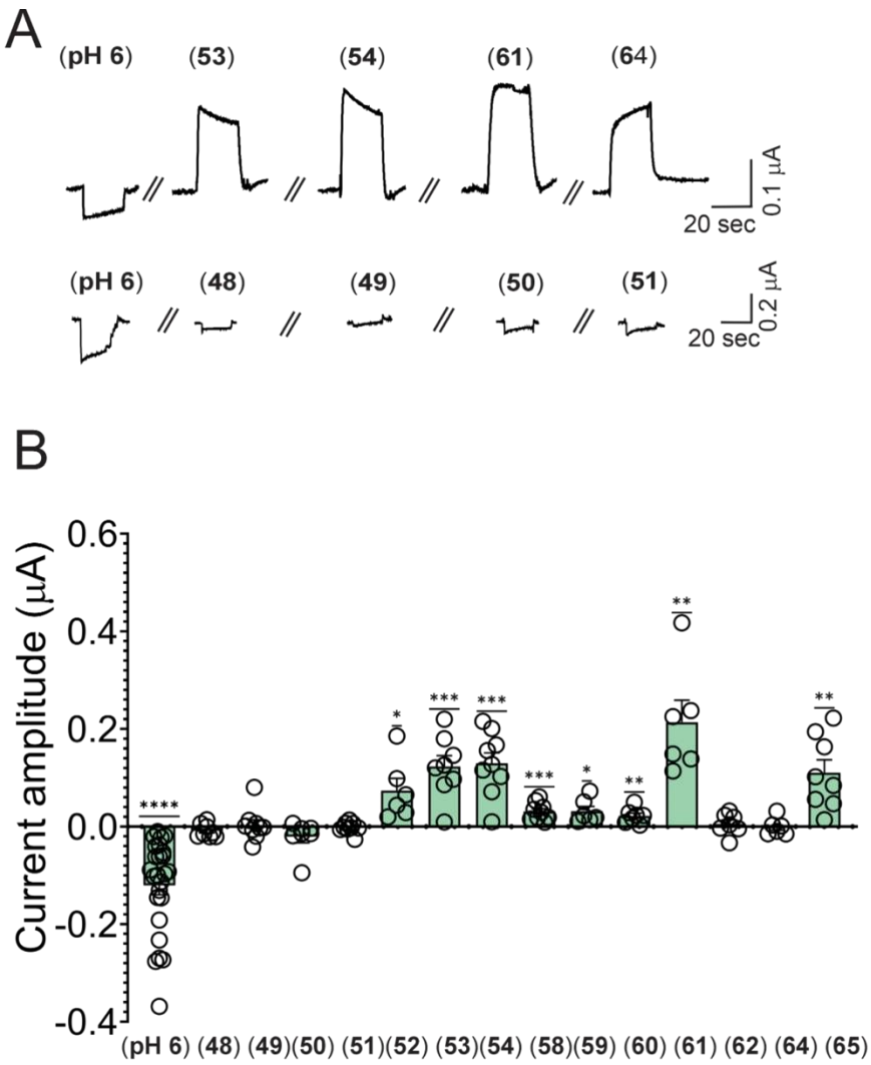

Figure 14: (**A**) Representative traces of the significant inhibitory effect of selected compounds (53, 54, 61 and 64) at spontaneously active β1- homomers (shown in the upper panel) and the lack of this effect for other compounds shown in the lower panel (48, 49, 50, 51). (**B**) The bars represent mean changes in current amplitudes ± SEM after application of the compounds (30 µM). A *one-sample t-test* revealed significant differences from zero (* = P < 0.05, ** = P < 0.01, *** = P < 0.001, **** = P < 0.0001)

Next, I studied the effect of the compounds on β3 homomers in the same manner (**Figure 15A** for selected representative traces). Likewise, the *one-sample t-test* revealed, that the holding current changed, ranging from 0.0028 µA (compound 65) to 0.0173 µA (compound 52) after application of compounds 52, 58, 59, and 65 (P < 0.05) (**Table 4, Figure 15B**) The effect,

indicating closure of the receptor induced by the compounds, was therefore observed for both β1 and β3 homomers.

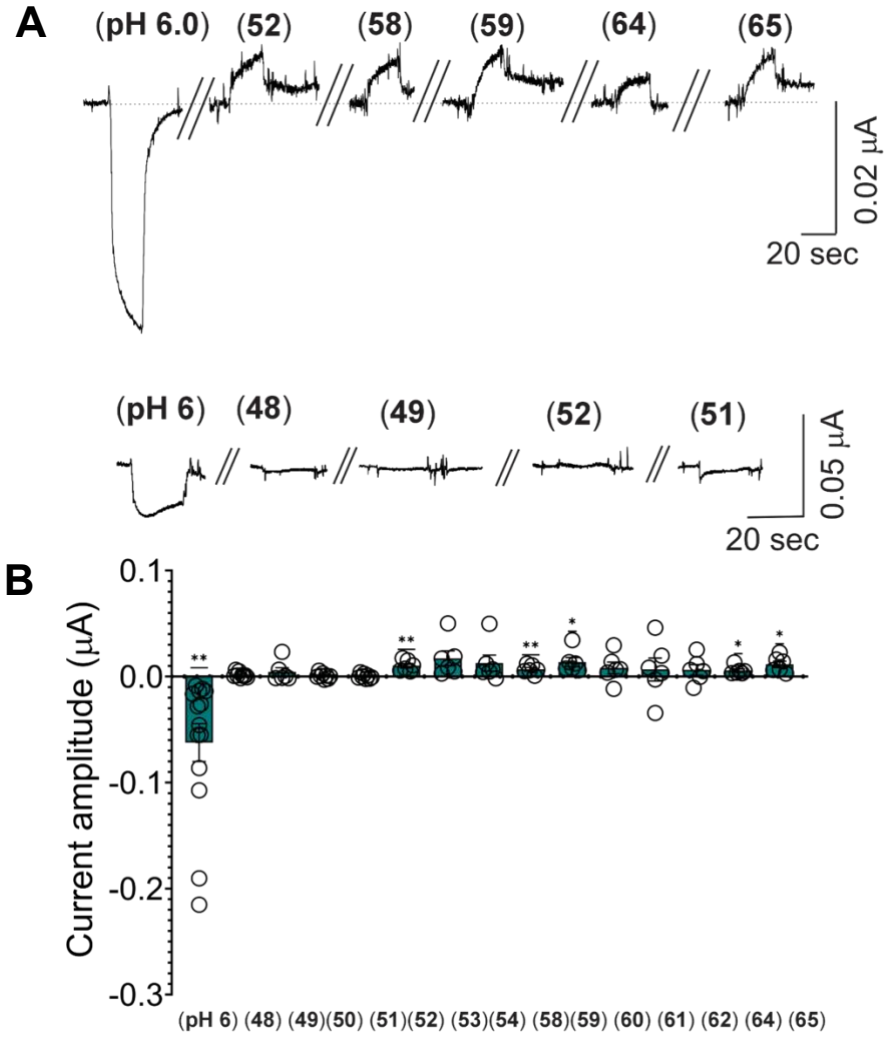

Figure 15: (**A**) Current traces that are representative for the inhibitory effects of selected compounds on β3 homomers (upper panel). In the lower panel a representation of currents after the application of compounds which did not exhibit this effect. (**B**) The mean change in current amplitude ± SEM is represented through bars. The compounds were applied at a concentration of 30 µM. At least five oocytes of two different frogs were evaluated. A *one-sample t-test* showed significant differences from zero (* = $P < 0.05$, and ** = $P < 0.01$).

compound	subtype	mean change of current amplitude (µA)	n	one-sample t-test

48	β1	-0.009 ± 0.005	8	t = 1.828, df = 7 *P* = 0.1102
	β3	0.001 ± 0.001	9	t = 1.716, df = 8 *P* = 0.1244
49	β1	0.003 ± 0.011	9	t = 0.2672, df = 8 *P* = 0.7961
	β3	0.004 ± 0.004	6	t = 1.080, df = 5 *P* = 0.3296
50	β1	-0.021 ± 0.013	7	t = 1.616, df = 6 *P* = 0.1573
	β3	0.001 ± 0.001	8	t = 0.6300, df = 7 *P* = 0.5487
51	β1	-0.002 ± 0.004	9	t = 0.5472, df = 8 *P* = 0.5992
	β3	0.000 ± 0.001	8	t = 0.2218, df = 7 *P* = 0.8308
52	**β1**	**0.073 ± 0.025**	**6**	**t = 2.916, df = 5** ***P* = 0.0332**
	β3	**0.010 ± 0.002**	**6**	**t = 4.819, df = 5** ***P* = 0.0048**
53	**β1**	**0.123 ± 0.022**	**8**	**t = 5.485, df = 7** ***P* = 0.0009**
	β3	0.017 ± 0.008	6	t = 2.396, df = 5 *P* = 0.0619
54	**β1**	**0.129 ± 0.021**	**9**	**t = 6.022, df = 8** ***P* = 0.0003**
	β3	0.013 ± 0.008	6	t = 1.653, df = 5 *P* = 0.1593
58	**β1**	**0.031 ± 0.006**	**9**	**t = 5.246, df = 8** ***P* = 0.0008**
	β3	**0.007 ± 0.002**	**6**	**t = 4.054, df = 5** ***P* = 0.0098**
59	**β1**	**0.031 ± 0.010**	**6**	**t = 3.091, df = 5** ***P* = 0.0271**
	β3	**0.014 ± 0.004**	**6**	**t = 3.132, df = 5** ***P* = 0.0259**
60	**β1**	**0.022 ± 0.006**	**7**	**t = 3.777, df = 6** ***P* = 0.0092**

	β3	0.008 ± 0.005	6	t = 1.476, df = 5 *P* = 0.2000
61	**β1**	**0.213 ± 0.045**	**6**	**t = 4.692, df = 5** ***P* = 0.0054**
	β3	0.007 ± 0.011	6	t = 0.6168, df = 5 *P* = 0.5644
62	β1	0.005 ± 0.008	7	t = 0.6172, df = 6 *P* = 0.5598
	β3	0.006 ± 0.005	6	t = 1.268, df = 5 *P* = 0.2607
64	β1	-0.001 ± 0.007	6	t = 0.08357, df = 5 *P* = 0.9366
	β3	**0.006 ± 0.002**	**6**	**t = 3.514, df = 5** ***P* = 0.0170**
65	**β1**	**0.110 ± 0.027**	**8**	**t = 4.153, df = 7** ***P* = 0.0043**
	β3	**0.011 ± 0.003**	**6**	**t = 3.753, df = 5** ***P* = 0.0133**

Table 4: Summary of the effects of the compounds on β1 and β3 homomers, displayed as mean change in current amplitude ± SEM. At least five experiments were evaluated for every compound. The *one-sample t-test* revealed significant differences from zero for certain compounds.

8.3. Concentration-response curves for compounds 58, 59, 64 and 65

Concentration-response curves for the 4 most selective compounds, which were identified in a previous screening, were generated by Mag. Angelika Doppler and can therefore be found in her master´s thesis as well as in the manuscript (Schnalzer, Haller, Doppler et al., submitted).

I generated concentration response curves for compounds 58, 59, 64 and 65 by applying increasing compound concentrations along with the corresponding GABA EC_{3-7} concentration at both α1β1 and α1β3 receptors (representative traces shown in **Figure 17** and **Figure 18**, concentration-response curves shown in **Figure 16**).

As shown in

Table 5, compounds 58, 59 and 65 significantly enhanced I_{GABA} through α1β3 receptors in a concentration-dependent manner. A *one-sample t-test* revealed that the first significant effects

for compound 59 and 65 occurred at a concentration of 3 µM (59: *P* = 0.0386, 65: *P* = 0.031). Compound 58 exhibited its first significant effects at a concentration of 10 µM (*P* = 0.0246, one-sample t-test). At the α1β1 subtype no concentration-response relationship could be established for compound 58, 59 and 65. Compound 64 was not active on either subtype. Since no concentration-response curve for the α1β1 subtype could be established for neither of the tested compounds, a comparison between the EC_{50} and E_{max} with the corresponding curves on α1β3 could not be made.

compound	subtype	EC_{50} (µM)	E_{max} (%)	nH	n
58	α1β1	-	-	-	6-11
	α1β3	27.6 ± 1.3	105 ± 5	3.3 ± 0.7	5-6
59	α1β1	-	-	-	7-9
	α1β3	35.7 ± 9.1	318 ± 47	1.4 ± 0.3	5-7
64	α1β1	-	-	-	5-7
	α1β3	-	-	-	5-7
65	α1β1	-	-	-	6-9
	α1β3	15.4 ± 4.5	210 ± 24	1.7 ± 0.4	5

Table 5: Display of the mean effective concentration (EC_{50}) in µM, the maximal potentiation (E_{max}) in %, the hill-coefficient (nH) and the range of experiments (n) for the compounds 58, 59, 64 and 65 at the α1β1and α1β3 subtype.

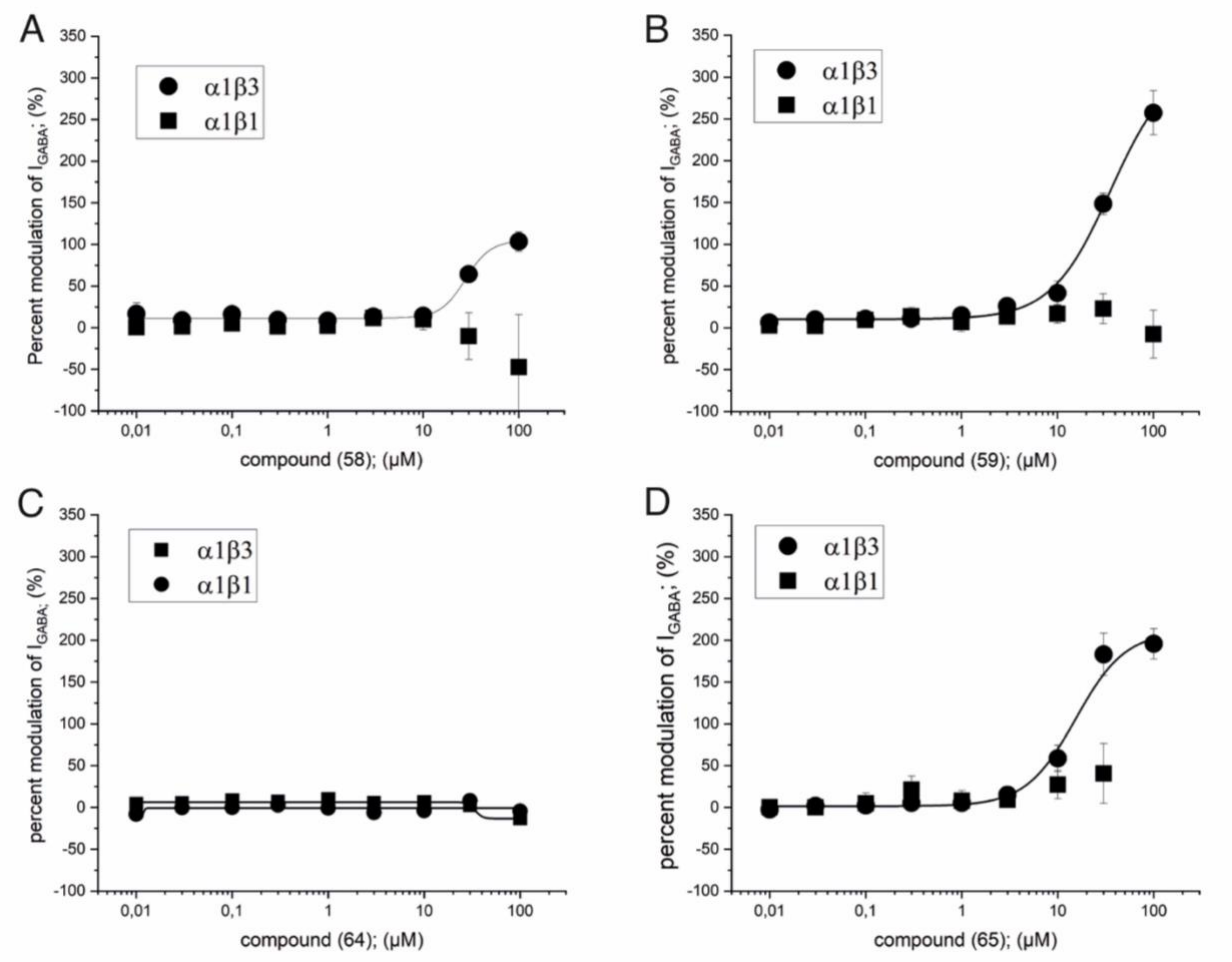

Figure 16: Generated concentration-response curves for the compounds 58, 59, 64 and 65 at α1β1 and α1β3 receptors in comparison. X-axis represents the concentration of the applied compound, which was applied in combination with the corresponding GABA $EC_{3\text{-}7}$ concentration. The Y-axis shows the modulation of I_{GABA} in (%). The symbols represent the mean ± SEM of the experiments carried out on at least five oocytes in two different batches. Comparison of concentration response-curve for compound 58 (**A**), 59 (**B**), 64 (**C**) and 65 (**D**) at α1β1 and α1β3 receptors, respectively.

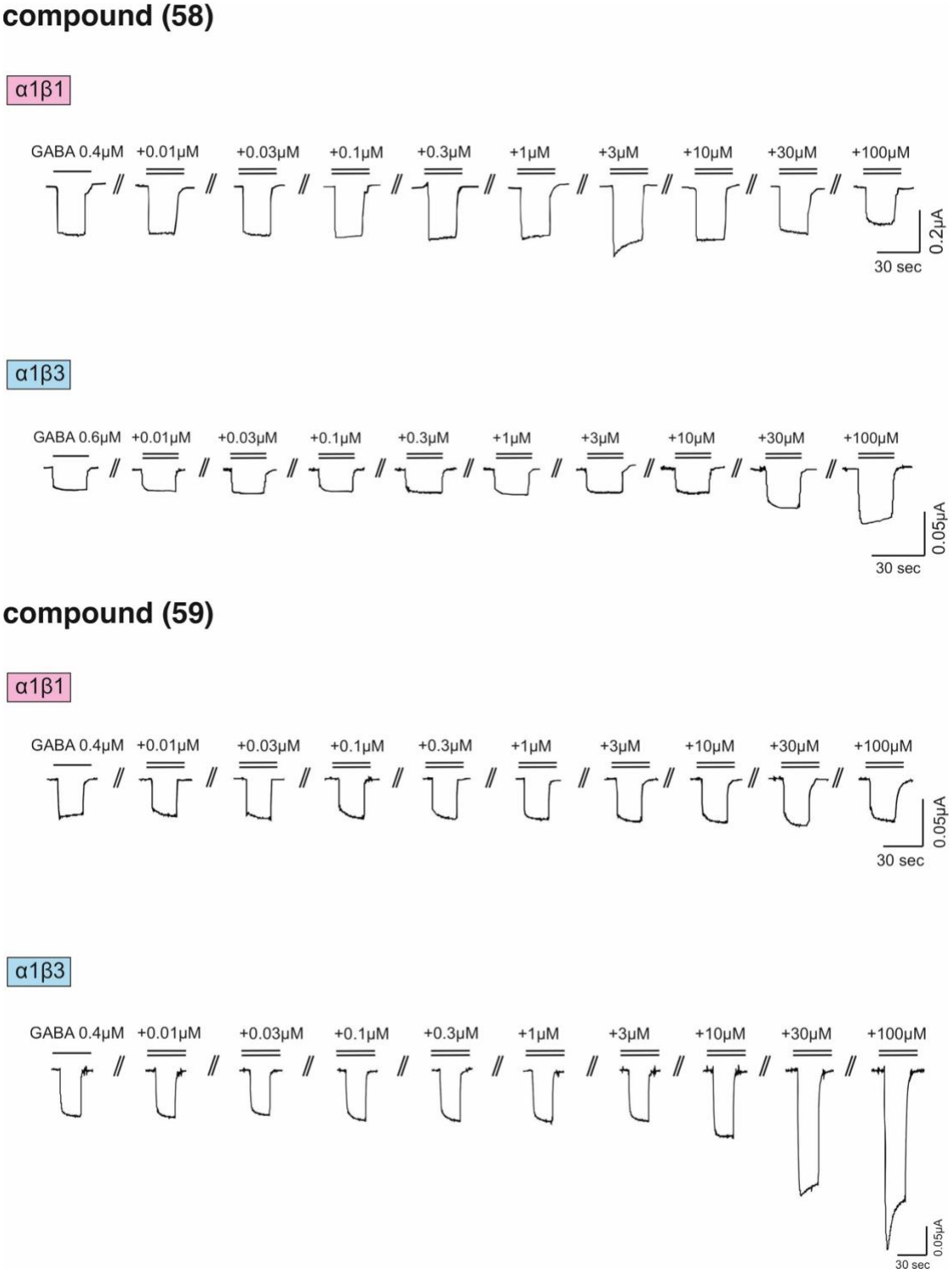

Figure 17: Representative current traces for the modulation of I_{GABA} by compound 58 (upper two panels) and compound 59 (lower two panels) at the $GABA_A$ receptor subtypes α1β1 and α1β3. The single bars above the first traces indicate the application of GABA at a concentration corresponding to the $EC_{3\text{-}7}$, which was evaluated individually for each oocyte. The double bars indicate the application of GABA in combination with the compound in increasing concentrations.

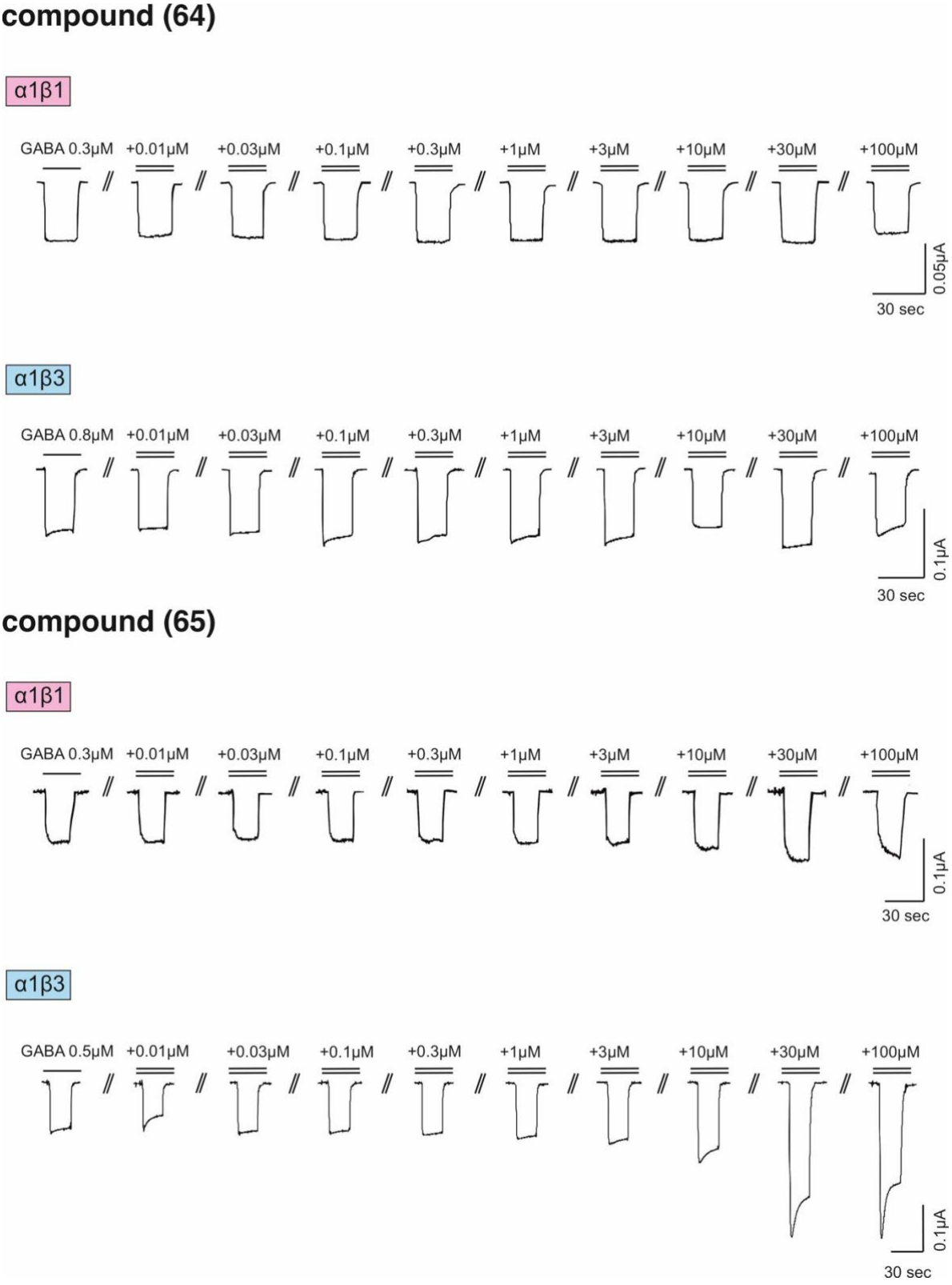

Figure 18: Representative current traces for the modulation of I_{GABA} by compound 64 (upper two panels) and compound 65 (lower to panels) at the $GABA_A$ receptor subtypes α1β1 and α1β3. The single bars above the first traces indicate the application of GABA at a concentration corresponding to the $EC_{3\text{-}5}$, which was evaluated individually for each oocyte. The double bars indicate the application of GABA in combination with the compound in increasing concentrations.

8.4. Compound 48

Based on the results from my studies and Mag. Angelika Doppler´s studies we found that from this series the most promising α1β1 selective compound was identified as compound 48. This compound showed a maximal I_{GABA} potentiation of 427 ± 20% and an EC_{50} value of 0.8 ± 0.1 µM at α1β1 versus E_{max} = 91 ± 28% and EC_{50} = 6.2 ± 5.3 µM at α1β3 (data kindly provided by Angelika) (**Table 6**). Therefore, I lastly studied the effect of this compound on the most abundant $GABA_A$ receptor subtype α1β2γ2. I also studied the beta subunit´s influence on the modulatory effect by replacing the β3 with the β2 subunit (for concentration-response curves see **Figure 19**, representative traces see **Figure 20**).

A *one-way ANOVA* revealed no significant differences in the EC_{50} values of the different subunit combinations (F (3, 17) = 1.145, P = 0.3593). It did, however, detect a significant difference in maximal GABA modulation (E_{max}, %) between the different subtypes (F (3, 17) = 129.5, P < 0.0001). The following *Post hoc Tukey test* revealed, that the E_{max} at the α1β1 subtype (427± 20) was significantly higher than at α1β3 (91 ± 28, P < 0.0001), α1β2 (50 ± 8, P < 0.0001) and α1β2γ2 (-14.9 ± 1.9, P < 0.0001). Additionally, the E_{max} at α1β3 (91 ± 28) is significantly higher than at α1β2γ2 (-14.5 ± 1.9, P = 0.004214). No difference in E_{max} was detected for the remaining subunit combinations (P > 0.05) (**Table 7**).

	EC_{50} (µM)	**E_{max} (%)**	**nH**	**n**
α1β1	0.8 ± 0.1	427 ± 20	1.4 ± 0.1	6-7
α1β3	6.2 ± 5.3	91 ± 28	1.2 ± 0.6	5-6
α1β2	3.1 ± 1.1	50 ± 8	1.8 ± 1.3	5
α1β2γ2	0.01 ± 0.1	-14.9 ± 1.9	1.5 ± 5	5-6

Table 6: Display of the mean effective concentration (EC_{50}) in µM, the maximal potentiation (E_{max}) in %, the hill-coefficient (nH) and the range of experiments (n) for compound 48 at the α1β1, α1β3, α1β2 and α1β2γ2 subtype.

	EC_{50}	**E_{max}**
α1β1 vs. α1β3	ns (P = 0.4489)	**** (P < 0.0001)

α1β1 vs. α1β2	ns (*P* = 0.9153)	**** (*P* < 0.0001)
α1β1 vs. α1β2γ2	ns (*P* = 0.9960)	**** (*P* < 0.0001)
α1β2 vs. α1β3	ns (*P* = 0.8374)	ns (*P* = 0.4214)
α1β3 vs. α1β2γ2	ns (*P* = 0.3706)	** (*P* = 0.0042)
α1β2γ2 vs. α1β2	ns (*P* = 0.8387)	ns (*P* = 0.0988)

Table 7: Comparison of EC_{50} values of compound 48 between receptor subtypes α1β2γ2, α1β2, α1β1 and α1β3. The statistical evaluation was made using *one-way ANOVA* followed by *Post hoc Tukey test* (ns = not significant, P = *P*-value, **** = *P* < 0.0001, ** = *P* = 0.01).

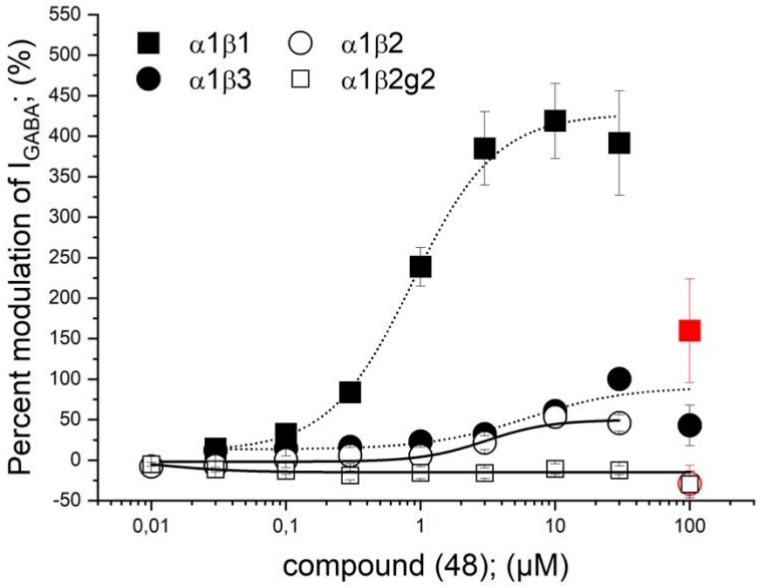

Figure 19: Comparison of the generated concentration-response curves of compound 48 at α1β2γ2, α1β2, α1β1 and α1β3. X-axis represents the concentration of the applied compound, which was applied in combination with the corresponding GABA EC_{3-7}. The Y-axis shows the modulation of I_{GABA} in (%). The symbols represent the mean ± SEM of the experiments carried out on at least five oocytes. Red data points were excluded from the fit.

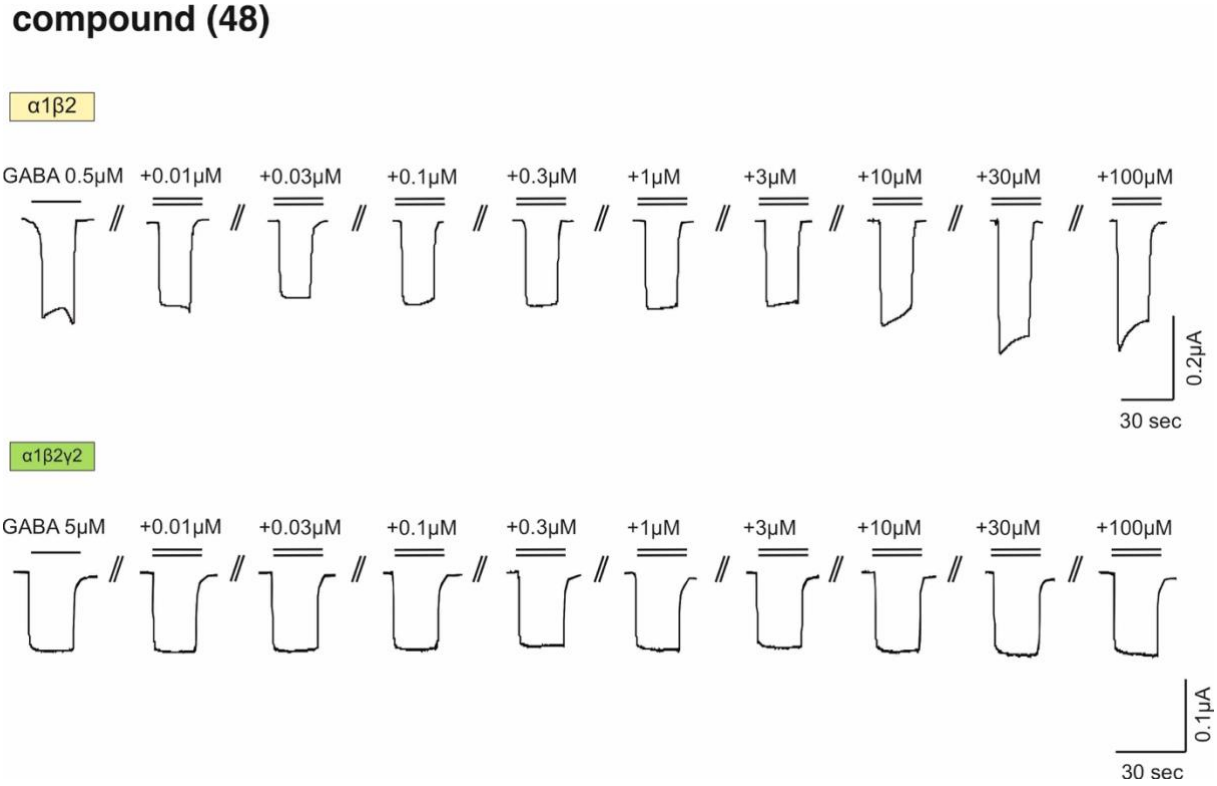

Figure 20: Representative current traces for the concentration-dependent modulation of I_{GABA} by compound 48 at the $GABA_A$ receptor subtypes α1β2 (upper panel) and α1β2γ2 (lower panel). The single bars above the first traces indicate the application of GABA at a concentration corresponding to the EC_{3-5}, which was evaluated individually for each oocyte. The double bars indicate the application of GABA in combination with the compound in increasing concentrations.

8.5. CS6

In a separate study, I determined potential adverse effects on $GABA_A$ receptors by a series of natural compound derivates in collaboration with the group of Assoz. Prof. Nina Schützenmeister.

For this purpose, I expressed the most abundant $GABA_A$ receptor subtype α1β2γ2 in *Xenopus laevis* oocytes and studied potential modulatory effects on I_{GABA} by those compounds at a concentration of 30 μM. As shown in **Figure 21** and **Table 8**, from this series, only compound CS6 potentiated I_{GABA} by more than 30% (*one-sample t-test*, $P < 0.05$). I_{GABA} enhancement by the other compounds was either around or below the threshold for biological activity (< 30%) or did not show any significant effect at all.

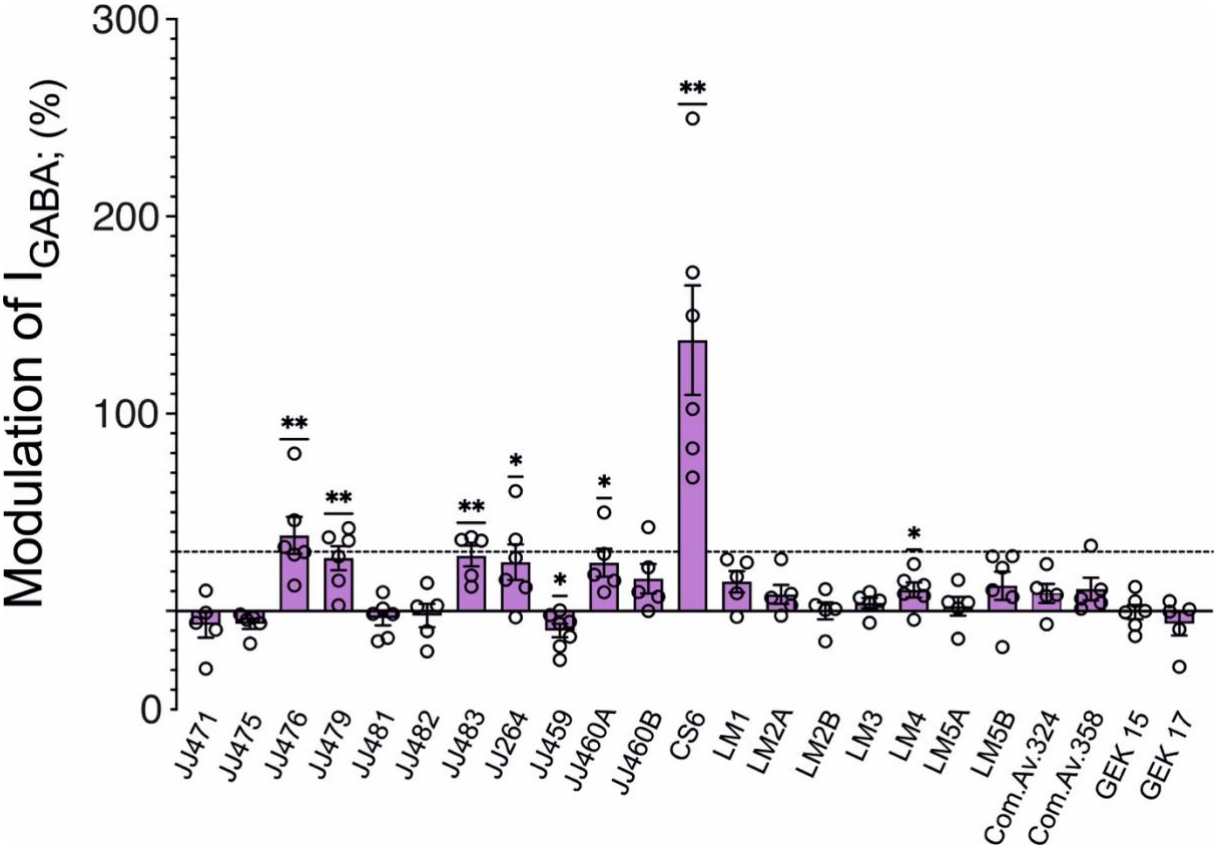

Figure 21: Modulation of I_{GABA} (%) by compounds provided by Assoz. Prof. Nina Schützenmeister. The bars show the mean value ± SEM on α1β2γ2 receptors. The substances were applied at a concentration of 30 µM. Data was derived from at least five different oocytes and at least two different frogs. A *one sample t*-test revealed significant differences from zero (* = P <0.05, ** = P < 0.01).

Compound	I_{GABA} enhancement (%)	n	one-sample t-test
JJ471	-7 ± 7	5	t=1.097, df=4 P= 0.3342
JJ475	-7 ± 3	5	t=2.492, df=4 P= 0.0674
JJ476	38 ± 9	6	t=4.090, df=5 P= 0.0094
JJ479	27 ± 6	6	t=4.380, df=5 P= 0.0072
JJ481	-4 ± 4	6	t=0.9371, df=5 P= 0.3917
JJ482	-2 ± 6	5	t=0.3928, df=4 P= 0.7145

JJ483	28 ± 5	5	t=5.262, df=4 *P*= 0.0062
JJ264	25 ± 9	6	t=2.731, df=5 P= 0.0412
JJ459	-10 ± 3	7	t=2.913, df=6 P= 0.0269
JJ460A	24 ± 7	5	t=3.433, df=4 P= 0.0265
JJ460B	16 ± 7	5	t=2.182, df=4 P= 0.0945
CS6	**137 ± 28**	**6**	**t=4.953, df=5 P= 0.0043**
LM1	15 ± 5	5	t=2.742, df=4 P= 0.0518
LM2A	8 ± 5	5	t=1.733, df=4 P= 0.1581
LM2B	0 ± 4	5	t=0.008056, df=4 P= 0.9940
LM3	4 ± 3	5	t=1.486, df=4 P= 0.2115
LM4	11 ± 4	6	t=2.778, df=5 P= 0.0390
LM5A	2 ± 5	5	t=0.4892, df=4 P= 0.6503
LM5B	13 ± 7	6	t=1.780, df=5 P= 0.1352
Com. Av. 324	9 ± 5	5	t=1.819, df=4 P= 0.1430
Com. Av. 358	11 ± 6	5	t=1.937, df=4 P= 0.1248
GEK 15	-1 ± 4	6	t=0.1643, df=5 P= 0.8759
GEK 17	-6 ± 6	5	t=1.088, df=4 P= 0.3376

Table 8: Results from screening for modulatory effects of substances at the α1β2γ2 subtype. Data was derived from at least five oocytes and at least two frogs. A *one-sample t-test* was used to detect differences from zero.

For the only active compound, CS6, I next studied the concentration dependent effect on the α1β2γ2 subtype and found that the first significant drug effect can be observed at a concentration of 1 µM (*P* = 0.0039, *one-sample t-test*) and maximal potentiation reached 272 ± 52% with an EC_{50} value of 4.1 ± 2.3 µM (**Table 9**).

Next, to gain insight into a potential subunit-selectivity, I investigated the impact of the γ2 subunit on I_{GABA} enhancement, by generating concentration-response curves on the α1β2 subtype thus omitting incorporation of a γ2 subunit. As shown in **Table 10**, the unpaired t-test revealed similar maximal I_{GABA} enhancement (*unpaired t-test*, t = 0.3636, df = 8, *P* > 0.05) and EC_{50} values (*unpaired t-test*, t = 1.934, df = 8, *P* > 0.05) at both the α1β2 and the α1β2γ2 subtype. Lastly, I studied whether replacing the β2 for the β1 subunit would affect I_{GABA} enhancement. I found that the modulatory activity of CS6 at the α1β1 subtype was 5 times more pronounced compared to its effects at the α1β2 receptor (α1β1: E_{max}= 1302 ± 306 % vs. α1β2: E_{max}= 245 ± 53 %, *P* = 0.0129, t = 0.02139, df = 8, *unpaired t-test*). EC_{50} values for α1β1 and α1β2, however, did not differ (*unpaired t-test*, t = 0.8836, df = 9, *P* > 0.05). For representative current traces and concentration-response curves, see **Figure 22**, **Figure 23** and **Figure 24**. Lastly, I examined potential agonistic effects by CS6 at the α1β2γ2 subtype and indeed, in the absence of GABA, CS6 at a concentration of 30 µM induced a current corresponding to approximately 3% of I_{max} induced by application of 1 mM GABA.

Collectively, my experiments provided functional evidence not only for the lack of significant neurotoxicity of 23 out of 24 compounds. But in addition, I identified a novel scaffold interacting with β1-perferrring $GABA_A$ receptors with functional selectivity and which does not interact with the benzodiazepine binding site.

	EC_{50} (µM)	**E_{max} (%)**	**nH**	**n**
α1β1	14.9 ± 7.6	1302 ± 306	1.7 ± 0.5	6
α1β2	26.7 ± 11.5	245 ± 53	1.1 ± 0.2	5-6
α1β2γ2	4.1 ± 2.3	272 ± 52	1.1 ± 0.5	5-11

Table 9: Display of the mean effective concentration (EC_{50}) in µM, the maximal potentiation (E_{max}) in %, the hill-coefficient (nH) and the range of experiments (n) for the compounds CS6 at the α1β2, α1β1 and α1β2γ2 subtype.

	EC_{50}	E_{max}
α1β2γ2 vs. α1β2	t=1.934, df=8 *P* = 0.0892	t=0.3636, df=8 *P* = 0,7256
α1β1 vs. α1β2	t=0.8836, df=9 *P* = 0.3999	**t=3.094, df=9** ***P* = 0.0129**

Table 10: Comparison of EC_{50} and E_{max} values of compound CS6 between receptor subtypes α1β2γ2, α1β2, using an *unpaired t-test*. Likewise, the *unpaired t-test* was used to detect differences between EC_{50} and E_{max} between α1β1 and α1β2.

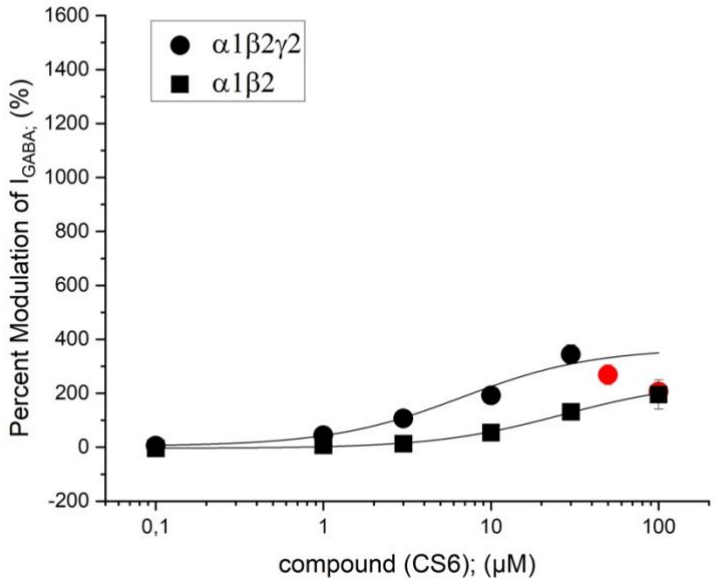

Figure 22: Comparison of the concentration-response curves of compound CS6 at the α1β2γ2 and the α1β2 subtype. The X-axis represents the concentration of the applied compound, which was applied in combination with the corresponding GABA EC $_{3\text{-}7}$. The Y-axis shows the modulation of I_{GABA} in (%). The symbols represent the mean ± SEM of the experiments carried out on at least five oocytes.

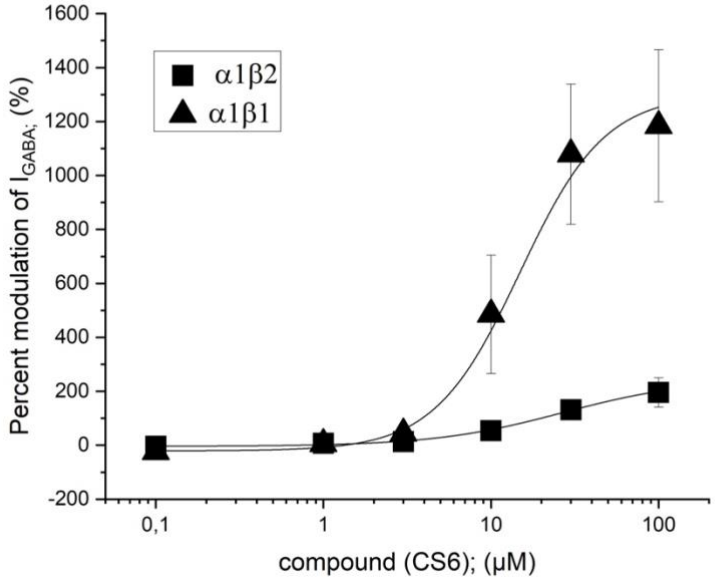

Figure 23: Comparison of the generated concentration-response curves of compound CS6 at the α1β2 and the α1β1 subtype. The X-axis represents the concentration of the applied compound, which was applied in combination with the corresponding GABA EC $_{3\text{-}7}$. The Y-axis shows the modulation of I_{GABA} in (%). The symbols represent the mean ± SEM of the experiments carried out on at least five oocytes.

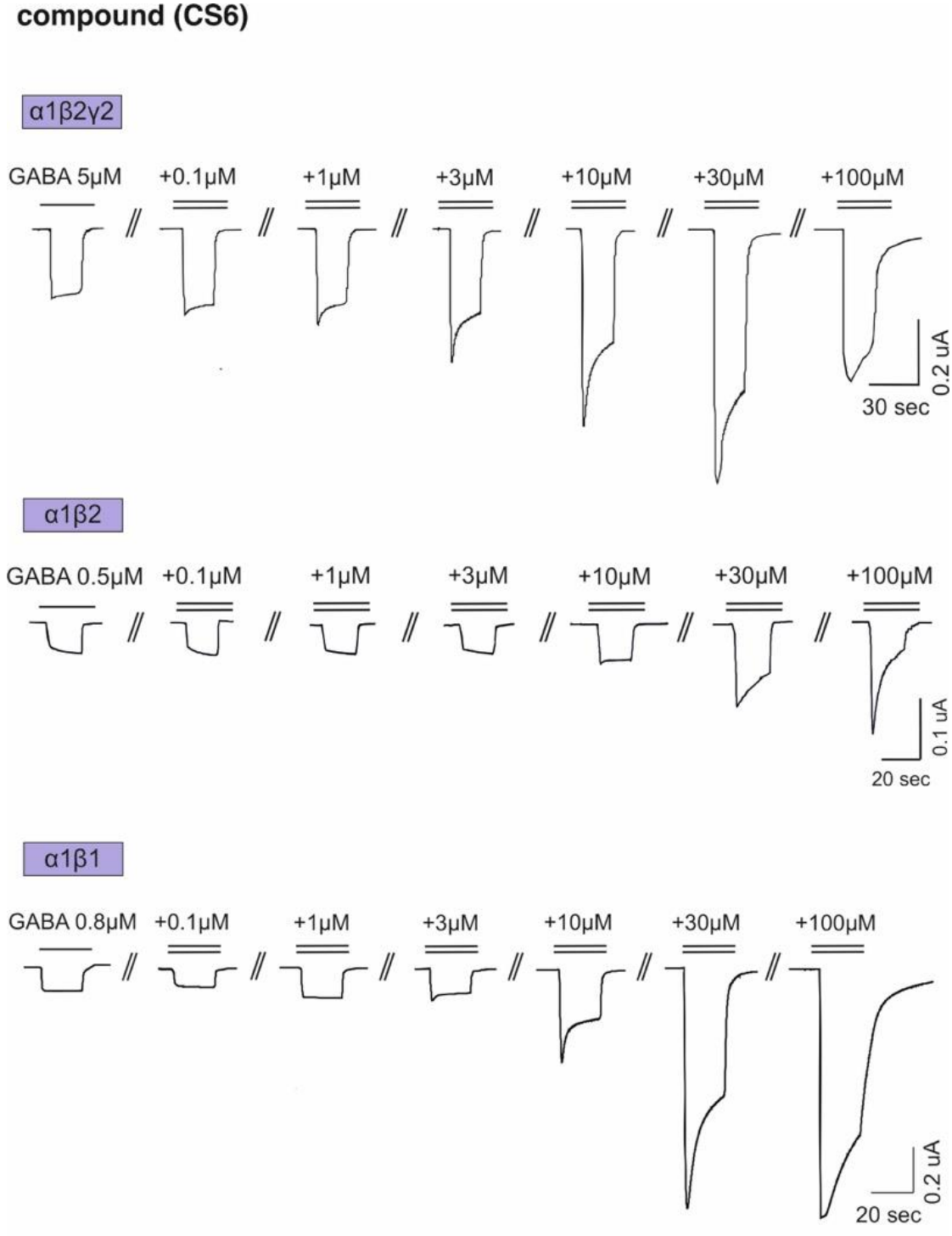

Figure 24: Representative current traces for compound CS6 at different $GABA_A$ receptor subtypes. The upper panel shows concentration dependent effects on the α1β2γ2 subtype, mid-panel on the α1β2 subtype and lower panel on the α1β1 subtype. The single bar indicates the application of GABA in a concentration corresponding to GABA EC_{5-10}, which was determined individually for each oocyte. The double bars represent the application of GABA in combination with rising compound concentrations.

9. Discussion

GABA is the primary inhibitory neurotransmitter in the CNS and thus plays a key role in keeping the balance between excitatory/inhibitory signalling. This regulatory effect is mainly exerted through its interaction with ionotropic $GABA_A$ receptors, which are critically involved in regulating an abundance of physiological processes and neuronal development (Knoflach et al., 2016). This not only includes effects in the central nervous system, but also in other organs, as $GABA_A$ receptors are expressed in peripheral tissue as well (Gajić Bojić et al., 2025). In the CNS, GABA and $GABA_A$ receptors are amongst other effects important for the formation of memory and the regulation of mood and sleep. Hence, in many neurological and neuropsychiatric diseases such as epilepsy and mood disorders, such as major depression disorder and anxiety, a disruption of the GABAergic system contributes to the dysregulation of the excitatory/inhibitory balance (Ghit et al., 2021).

As this knowledge is broadly established, $GABA_A$ receptors still represent important targets for pharmacological agents. A range of different $GABA_A$ receptor modulators have been in clinical use for decades, including benzodiazepines, general anaesthetics, barbiturates and, since very recently, also neurosteroids. What most of these agents have in common, is their rather unselective activity and broad modulation of many $GABA_A$ receptor subtypes. This is especially relevant concerning the most frequently prescribed $GABA_A$ receptor modulators, benzodiazepines, as they exert a range of different effects which can be ascribed to distinct $GABA_A$ receptor subtypes. Negative, unwanted effects include sedative properties as well as cognitive side effects and a high potential for dependence (Olsen, 2018). These adverse effects may be diminished, by developing agents that selectively act at $GABA_A$ receptors of a defined subunit composition (Engin, 2023).

The brain region specific expression of different subtypes is an important feature of the GABAergic system. Some subtypes, including the most abundant subtype α1β2γ2, are expressed in nearly all brain regions and have been extensively studied in the past. However selected subtypes, especially with regards to the different β subunits, have been less well explored. This includes receptors containing the β1 subunit. Isoforms containing this subunit have lower expression levels in the CNS and their regional distribution is thought to be less broad. As there is a lack of β1-selective modulators, these receptors have not been well studied thus far (Olsen and Sieghart, 2008). The development of β1-selective agents would not only aid in further characterizing and mapping the receptors distribution, but they may also present novel therapeutic options.

The primary goal of my thesis was to identify and further characterize β1-selective compounds from a pyrazoloquinolinone library, provided by Dr. Dominik Schnalzer. My colleague Angelika Doppler previously studied four compounds from the same library and demonstrated that those compounds display significant functional selectivity on α1β1 over α1β3 receptors. I aimed to find additional selective modulators by testing the remaining twelve compounds from this series at α1β1 and the α1β3 receptors. Yet, none of those compounds displayed selectivity for α1β1 receptors while displaying I_{GABA} enhancement at α1β3 receptors. These compounds where thus further characterized by generating concentration response curves.

During the process of generating the concentration-response curves at the α1β1 subtype, I detected that some compounds, despite their lack of positive modulatory effect, exerted a strong inhibitory effect on GABA-induced currents. In some cases, the inhibitory effect was so strong, that the effect of the co-applied GABA was completely suppressed. To further investigate this phenomenon, I first examined, whether the substances would also exhibit this effect at the α1β1 subtype in the absence of GABA. Indeed, while the application of GABA as a control substance led to the expected effect of channel opening (e.g. measured currents of -0.07μA corresponding for GABA EC_{3-7}), the application of the compounds alone apparently resulted in the closure of the $GABA_A$ receptor, shown by a decrease in the holding currents. Surprisingly, those effects were not observed at each oocyte, suggesting that this inhibitory effect may not be mediated by an interaction of the compounds with α1β1 receptors but rather by an interaction with spontaneously active channels formed by exclusive assembly of β1 subunits which is known to happen upon heterologous receptor expression. Those receptors are characterized by relatively high holding currents (e.g. average of approximately - 0.5 μA for β1 experiments) compared to heteropentameric receptors (e.g. holding currents ranging from 0 to 0.1 μA for α1β2γ2 subtypes), which may be explained by their spontaneous activity (Garifulina et al., 2022; Krishek et al., 1996). As I frequently observed high holding currents for my experiments (e.g. - 0.4 μA), I speculated that the effect of the substances may be due to this said interaction with homopentamers in the background. To confirm this hypothesis, I initiated an additional screening of all compounds at β1 homopentamers. For this purpose, I injected only β1 subunits into the oocytes. As these homopentamers lack a GABA binding site but are pH sensitive, the expression of the pentamers was verified with the application of acidified ND96 buffer at the beginning of each experiment. The following application of the substances, each at a concentration of 30 μM, once again led to the supposed closure of the channel.

Interestingly, this inhibitory effect at the α1β1 subtype at concentrations ≥ 30µM was either significantly less pronounced or even absent for the β1-selective compounds characterized by my colleague Angelika Doppler. Nonetheless, I included these compounds (48, 49, 50, 51) in the homopentamer screening. Interestingly, 9 compounds from the series closed the spontaneously active β1-receptors, while only 3 compounds had some minor effects on β3-receptors. Thus, I conclude that some of the compounds (e.g. 53, 54, 60) indeed show β1 subtype selectivity, however their effects seem to be mediated through β1 homopentamers instead of binary α1β1 receptors. In contrast, the previously characterized pyrazoloquinolinones (48, 49, 50, 51) showed no effect at β1 and β3 homopentamers.

To finalize the research for this compound library I lastly generated two additional concentration-response curves for the most promising β1-selective compound, compound 48. First, I examined whether the compound was active at the most abundant $GABA_A$ receptor subtype α1β2γ2, which would be not desired. Indeed, 48 did not show any significant effect at this subunit combination at any concentration. This confirmed that the modulatory effect of the compound is not dependent on the benzodiazepine binding site. In addition, I generated a concentration-response curve at the α1β2 subtype, to further determine the influence of the different β subunits on the compound´s modulatory effects. The compound´s effect at this subtype did not differ significantly from the α1β3 subtype, while its effect was significantly lower than at the α1β1 subtype. This further confirms the subtype selectivity of the examined compound. These data will soon be published.

Additionally, I screened a library of natural compound precursors and derivatives, provided by the laboratory of Assoz. Prof. Nina Schützenmeister. In this case, the aim was to investigate whether these compounds display adverse effects at the $GABA_A$ receptor subtype α1β2γ2. One compound (CS6) showed significant enhancement of I_{GABA} and was therefore further characterized. First, I established a concentration-response curve for this compound at the α1β2γ2 subtype. Next, I generated an additional concentration-response curve at the binary α1β2 receptor, to investigate whether the γ subunit has an influence on the compound´s modulatory effects. As the EC_{50} and E_{max} values for these two subtypes did not differ significantly, I concluded that the benzodiazepine binding site does not influence the compound´s effects. To gain insights into a possible subtype selectivity of CS6 I created an additional concentration-response curve at the α1β1 subtype. The compound´s E_{max} was significantly higher at this subtype than at any of the others. This led to the assumption that this substance exhibits β1 selectivity as well.

There is an abundance of subtype-selective compounds, especially regarding selectivity for the different α subunits. An example for this are Z-drugs, which exert their sedative effects through α1-containing receptors in contrast to benzodiazepines, which act on a variety of receptor subtypes. Concerning the β subunit selectivity, the general anesthetic etomidate has been identified as β2/β3 selective. In contrast, compounds that exclusively modulate β1-containing subtypes are rare. Only, salicylidene salicylhydrazide (Olsen and Sieghart, 2008; Thompson et al., 2004) and compounds with a pyrazoloquinolinone scaffold, which are also studied in this thesis, have been identified as β1 selective modulators (Simeone et al., 2017).

Yet not only the compound´s selectivity for different $GABA_A$ receptor subtypes needs to be considered for future studies, but also potential off-target effects. Specifically, as $GABA_A$ receptors are part of the Cys-loop receptor family, some binding sites are highly conserved among these receptors (Kim and Hibbs, 2021), a modulatory effect of the substances at other ionotropic receptors cannot be excluded with certainty at this point. This may lead to additional, unintended pharmacological effects.

Another important factor to consider for future studies is the compound´s ability to cross the blood brain barrier (BBB) and therefore act in the central nervous system. This is dependent on the molecule's lipid solubility, its molecular weight and its capability to form hydrogen bonds (Pardridge, 2012). As the BBB´s permeability for a substance substantially determines its in vivo effects, this aspect is especially important to consider during substance development and when further studying the compounds effects.

The final objective surely will be to map the binding site(s) of the compounds at the β1-containing subtype. Previous studies suggest that the PQ binding site is located at the extracellular α+/β- interface. An arginine residue at position 41 at the β1 subunit might be critically involved in mediating the subtype selectivity (Simeone et al., 2017). Additional docking studies to further investigate the involvement of different amino acids will be included in the paper (Schnalzer, Haller, Doppler, et al., submitted).

For the compound, provided by the Schützenmeister lab, no binding site could be identified thus far. In this case future mutational or docking studies might provide more insight.

Taken together, the findings of my master thesis led to the identification of novel β1 selective ligands. These represent promising candidates for further characterization in future studies.

Bibliography

Absalom, N.L., Liao, V.W.Y., Johannesen, K.M.H., Gardella, E., Jacobs, J., Lesca, G., Gokce-Samar, Z., Arzimanoglou, A., Zeidler, S., Striano, P., Meyer, P., Benkel-Herrenbrueck, I., Mero, I.-L., Rummel, J., Chebib, M., Møller, R.S., Ahring, P.K., 2022. Gain-of-function and loss-of-function GABRB3 variants lead to distinct clinical phenotypes in patients with developmental and epileptic encephalopathies. Nat Commun 13, 1822. https://doi.org/10.1038/s41467-022-29280-x

Ahring, P.K., Liao, V.W.Y., Gardella, E., Johannesen, K.M., Krey, I., Selmer, K.K., Stadheim, B.F., Davis, H., Peinhardt, C., Koko, M., Coorg, R.K., Syrbe, S., Bertsche, A., Santiago-Sim, T., Diemer, T., Fenger, C.D., Platzer, K., Eichler, E.E., Lerche, H., Lemke, J.R., Chebib, M., Møller, R.S., 2022. Gain-of-function variants in GABRD reveal a novel pathway for neurodevelopmental disorders and epilepsy. Brain 145, 1299–1309. https://doi.org/10.1093/brain/awab391

Andersen, J.V., 2025. The Glutamate/GABA-Glutamine Cycle: Insights, Updates, and Advances. Journal of Neurochemistry 169, e70029. https://doi.org/10.1111/jnc.70029

Andersen, J.V., Marian, O.C., Qvist, F.L., Westi, E.W., Aldana, B.I., Schousboe, A., Don, A.S., Skotte, N.H., Wellendorph, P., 2024. Deficient brain GABA metabolism leads to widespread impairments of astrocyte and oligodendrocyte function. Glia 72, 1821–1839. https://doi.org/10.1002/glia.24585

Andersen, J.V., Schousboe, A., 2023. Milestone Review: Metabolic dynamics of glutamate and GABA mediated neurotransmission — The essential roles of astrocytes. Journal of Neurochemistry 166, 109–137. https://doi.org/10.1111/jnc.15811

Anstee, Q.M., Knapp, S., Maguire, E.P., Hosie, A.M., Thomas, P., Mortensen, M., Bhome, R., Martinez, A., Walker, S.E., Dixon, C.I., Ruparelia, K., Montagnese, S., Kuo, Y.-T., Herlihy, A., Bell, J.D., Robinson, I., Guerrini, I., McQuillin, A., Fisher, E.M.C., Ungless, M.A., Gurling, H.M.D., Morgan, M.Y., Brown, S.D.M., Stephens, D.N., Belelli, D., Lambert, J.J., Smart, T.G., Thomas, H.C., 2013. Mutations in the Gabrb1 gene promote alcohol consumption through increased tonic inhibition. Nat Commun 4, 2816. https://doi.org/10.1038/ncomms3816

Avoli, M., de Curtis, M., Lévesque, M., Librizzi, L., Uva, L., Wang, S., 2022. GABAA signaling, focal epileptiform synchronization and epileptogenesis. Front Neural Circuits 16, 984802. https://doi.org/10.3389/fncir.2022.984802

Baburin, I., Beyl, S., Hering, S., 2006. Automated fast perfusion of Xenopus oocytes for drug screening. Pflugers Arch 453, 117–123. https://doi.org/10.1007/s00424-006-0125-y

Bak, L.K., Schousboe, A., Waagepetersen, H.S., 2006. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. Journal of Neurochemistry 98, 641–653. https://doi.org/10.1111/j.1471-4159.2006.03913.x

Barki, M., Xue, H., 2022. *GABRB2*, a key player in neuropsychiatric disorders and beyond. Gene 809, 146021. https://doi.org/10.1016/j.gene.2021.146021

Bhat, R., Axtell, R., Mitra, A., Miranda, M., Lock, C., Tsien, R.W., Steinman, L., 2010. Inhibitory role for GABA in autoimmune inflammation. Proc Natl Acad Sci U S A 107, 2580–2585. https://doi.org/10.1073/pnas.0915139107

Bianchi, L., 2006. Heterologous expression of C. elegans ion channels in Xenopus oocytes. WormBook. https://doi.org/10.1895/wormbook.1.117.1

Biermann, B., Ivankova-Susankova, K., Bradaia, A., Abdel Aziz, S., Besseyrias, V., Kapfhammer, J.P., Missler, M., Gassmann, M., Bettler, B., 2010. The Sushi Domains of $GABA_B$ Receptors Function as Axonal Targeting Signals. J. Neurosci. 30, 1385–1394. https://doi.org/10.1523/JNEUROSCI.3172-09.2010

Boileau, A.J., Baur, R., Sharkey, L.M., Sigel, E., Czajkowski, C., 2002. The relative amount of cRNA coding for γ2 subunits affects stimulation by benzodiazepines in GABAA receptors expressed in Xenopus oocytes. Neuropharmacology 43, 695–700. https://doi.org/10.1016/s0028-3908(02)00036-9

Brickley, S.G., Mody, I., 2012. Extrasynaptic GABAA receptors: Their function in the CNS and implications for disease. Neuron 73, 23–34. https://doi.org/10.1016/j.neuron.2011.12.012

Bryson, A., Reid, C., Petrou, S., 2023. FUNDAMENTAL NEUROCHEMISTRY REVIEW: $GABA_A$ receptor neurotransmission and epilepsy: Principles, disease mechanisms and pharmacotherapy. Journal of Neurochemistry 165, 6–28. https://doi.org/10.1111/jnc.15769

Cellot, G., Cherubini, E., 2014. GABAergic Signaling as Therapeutic Target for Autism Spectrum Disorders. Front Pediatr 2, 70. https://doi.org/10.3389/fped.2014.00070

Chen, Q., Wells, M.M., Arjunan, P., Tillman, T.S., Cohen, A.E., Xu, Y., Tang, P., 2018. Structural basis of neurosteroid anesthetic action on GABAA receptors. Nat Commun 9, 3972. https://doi.org/10.1038/s41467-018-06361-4

Chen, Z.-W., Bracamontes, J.R., Budelier, M.M., Germann, A.L., Shin, D.J., Kathiresan, K., Qian, M.-X., Manion, B., Cheng, W.W.L., Reichert, D.E., Akk, G., Covey, D.F., Evers, A.S., 2019. Multiple functional neurosteroid binding sites on GABAA receptors. PLoS Biol 17, e3000157. https://doi.org/10.1371/journal.pbio.3000157

Chuang, S.-H., Reddy, D.S., 2018. Genetic and Molecular Regulation of Extrasynaptic GABA-A Receptors in the Brain: Therapeutic Insights for Epilepsy. J Pharmacol Exp Ther 364, 180–197. https://doi.org/10.1124/jpet.117.244673

Colombi, I., Rastogi, M., Parrini, M., Alberti, M., Potenzieri, A., Chellali, M.M., Rosati, S., Chiappalone, M., Nanni, M., Contestabile, A., Cancedda, L., 2024. Heterogeneous subpopulations of GABAAR-responding neurons coexist across neuronal network scales and developmental stages in health and disease. iScience 27, 109438. https://doi.org/10.1016/j.isci.2024.109438

Cutler, A.J., Mattingly, G.W., Maletic, V., 2023. Understanding the mechanism of action and clinical effects of neuroactive steroids and GABAergic compounds in major depressive disorder. Transl Psychiatry 13, 228. https://doi.org/10.1038/s41398-023-02514-2

Delahanty, R.J., Kang, J., Brune, C.W., Kistner, E.O., Courchesne, E., Cox, N.J., Cook, E.H., Macdonald, R.L., Sutcliffe, J.S., 2011. Maternal transmission of a rare GABRB3 signal peptide variant is associated with autism. Mol Psychiatry 16, 86–96. https://doi.org/10.1038/mp.2009.118

Delaney, A.J., Crane, J.W., 2016. Presynaptic GABAB receptors reduce transmission at parabrachial synapses in the lateral central amygdala by inhibiting N-type calcium channels. Sci Rep 6, 19255. https://doi.org/10.1038/srep19255

Dharavath, R.N., Pina-Leblanc, C., Tang, V.M., Sloan, M.E., Nikolova, Y.S., Pangarov, P., Ruocco, A.C., Shield, K., Voineskos, D., Blumberger, D.M., Boileau, I., Bozinoff, N., Gerretsen, P., Vieira, E., Melamed, O.C., Sibille, E., Quilty, L.C., Prevot, T.D., 2023. GABAergic signaling in alcohol use disorder and withdrawal: pathological involvement and therapeutic potential. Front. Neural Circuits 17, 1218737. https://doi.org/10.3389/fncir.2023.1218737

Domi, E., Domi, A., Adermark, L., Heilig, M., Augier, E., 2021. Neurobiology of alcohol seeking behavior. Journal of Neurochemistry 157, 1585–1614. https://doi.org/10.1111/jnc.15343

Drevets, W.C., Price, J.L., Furey, M.L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct Funct 213, 93–118. https://doi.org/10.1007/s00429-008-0189-x

Duman, R.S., Sanacora, G., Krystal, J.H., 2019. Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversal by novel treatments. Neuron 102, 75–90. https://doi.org/10.1016/j.neuron.2019.03.013

Eaton, M.M., Germann, A.L., Arora, R., Cao, L.Q., Gao, X., Shin, D.J., Wu, A., Chiara, D.C., Cohen, J.B., Steinbach, J.H., Evers, A.S., Akk, G., 2016. Multiple Non-Equivalent Interfaces Mediate Direct Activation of GABAA Receptors by Propofol. Curr Neuropharmacol 14, 772–780. https://doi.org/10.2174/1570159x14666160202121319

Engin, E., 2023. GABAA receptor subtypes and benzodiazepine use, misuse, and abuse. Front Psychiatry 13, 1060949. https://doi.org/10.3389/fpsyt.2022.1060949

Enz, R., Cutting, G.R., 1998. Molecular composition of GABAC receptors. Vision Research 38, 1431–1441. https://doi.org/10.1016/S0042-6989(97)00277-0

Ernst, M., Brauchart, D., Boresch, S., Sieghart, W., 2003. Comparative modeling of GABAA receptors: limits, insights, future developments. Neuroscience 119, 933–943. https://doi.org/10.1016/S0306-4522(03)00288-4

Farrant, M., Nusser, Z., 2005. Variations on an inhibitory theme: phasic and tonic activation of GABAA receptors. Nat Rev Neurosci 6, 215–229. https://doi.org/10.1038/nrn1625

Fatemi, S.H., Reutiman, T.J., Folsom, T.D., Thuras, P.D., 2009. GABAA receptor downregulation in brains of subjects with autism. J Autism Dev Disord 39, 223–230. https://doi.org/10.1007/s10803-008-0646-7

Fee, C., Banasr, M., Sibille, E., 2017. Somatostatin-Positive Gamma-Aminobutyric Acid Interneuron Deficits in Depression: Cortical Microcircuit and Therapeutic Perspectives. Biological Psychiatry, Information Processing Alterations in Depression 82, 549–559. https://doi.org/10.1016/j.biopsych.2017.05.024

Forkuo, G.S., Guthrie, M.L., Yuan, N.Y., Nieman, A.N., Kodali, R., Jahan, R., Stephen, M.R., Yocum, G.T., Treven, M., Poe, M.M., Li, G., Yu, O.B., Hartzler, B.D., Zahn, N.M., Ernst, M., Emala, C.W., Stafford, D.C., Cook, J.M., Arnold, L.A., 2016. Development of GABAA Receptor Subtype-Selective Imidazobenzodiazepines as Novel Asthma Treatments. Mol Pharm 13, 2026–2038. https://doi.org/10.1021/acs.molpharmaceut.6b00159

Fourati, Z., Ruza, R.R., Laverty, D., Drège, E., Delarue-Cochin, S., Joseph, D., Koehl, P., Smart, T., Delarue, M., 2017. Barbiturates Bind in the GLIC Ion Channel Pore and Cause Inhibition by Stabilizing a Closed State♦. J Biol Chem 292, 1550–1558. https://doi.org/10.1074/jbc.M116.766964

Frangaj, A., Fan, Q.R., 2018. Structural biology of GABAB receptor. Neuropharmacology 136, 68–79. https://doi.org/10.1016/j.neuropharm.2017.10.011

Gajić Bojić, M., Aranđelović, J., Škrbić, R., Savić, M.M., 2025. Peripheral GABAA receptors - Physiological relevance and therapeutic implications. Pharmacology & Therapeutics 266, 108759. https://doi.org/10.1016/j.pharmthera.2024.108759

Garifulina, A., Friesacher, T., Stadler, M., Zangerl-Plessl, E.-M., Ernst, M., Stary-Weinzinger, A., Willam, A., Hering, S., 2022. β subunits of GABAA receptors form proton-gated chloride channels: Insights into the molecular basis. Commun Biol 5, 784. https://doi.org/10.1038/s42003-022-03720-2

Gassmann, M., Bettler, B., 2012. Regulation of neuronal GABAB receptor functions by subunit composition. Nat Rev Neurosci 13, 380–394. https://doi.org/10.1038/nrn3249

Germann, A.L., Pierce, S.R., Tateiwa, H., Sugasawa, Y., Reichert, D.E., Evers, A.S., Steinbach, J.H., Akk, G., 2021. Intrasubunit and Intersubunit Steroid Binding Sites Independently and Additively Mediate α1β2γ2L GABAA Receptor Potentiation by the Endogenous Neurosteroid Allopregnanolone. Mol Pharmacol 100, 19–31. https://doi.org/10.1124/molpharm.121.000268

Ghit, A., Assal, D., Al-Shami, A.S., Hussein, D.E.E., 2021. GABAA receptors: structure, function, pharmacology, and related disorders. J Genet Eng Biotechnol 19, 123. https://doi.org/10.1186/s43141-021-00224-0

Gunduz-Bruce, H., Takahashi, K., Huang, M., 2022. Development of neuroactive steroids for the treatment of postpartum depression. J Neuroendocrinol 34, e13019. https://doi.org/10.1111/jne.13019

Gunn, B.G., Cunningham, L., Cooper, M.A., Corteen, N.L., Seifi, M., Swinny, J.D., Lambert, J.J., Belelli, D., 2013. Dysfunctional Astrocytic and Synaptic Regulation of Hypothalamic Glutamatergic Transmission in a Mouse Model of Early-Life Adversity: Relevance to Neurosteroids and Programming of the Stress Response. J Neurosci 33, 19534–19554. https://doi.org/10.1523/JNEUROSCI.1337-13.2013

Guo, H., Peng, Y., Hu, Z., Li, Y., Xun, G., Ou, J., Sun, L., Xiong, Z., Liu, Y., Wang, T., Chen, J., Xia, L., Bai, T., Shen, Y., Tian, Q., Hu, Y., Shen, L., Zhao, R., Zhang, X., Zhang, F., Zhao, J., Zou, X., Xia, K., 2017. Genome-wide copy number variation analysis in a Chinese autism spectrum disorder cohort. Sci Rep 7, 44155. https://doi.org/10.1038/srep44155

Hao, X., Ou, M., Zhang, D., Zhao, W., Yang, Y., Liu, J., Yang, H., Zhu, T., Li, Y., Zhou, C., 2020. The Effects of General Anesthetics on Synaptic Transmission. Curr Neuropharmacol 18, 936–965. https://doi.org/10.2174/1570159X18666200227125854

Herman, M.A., Roberto, M., 2016. Cell-type-specific tonic GABA signaling in the rat central amygdala is selectively altered by acute and chronic ethanol. Addiction Biology 21, 72–86. https://doi.org/10.1111/adb.12181

Jin, Z., Mendu, S.K., Birnir, B., 2013. GABA is an effective immunomodulatory molecule. Amino Acids 45, 87–94. https://doi.org/10.1007/s00726-011-1193-7

Johnston, G.A., 2013. Advantages of an antagonist: bicuculline and other GABA antagonists. Br J Pharmacol 169, 328–336. https://doi.org/10.1111/bph.12127

Jurd, R., Arrasa, M., Lambert, S., Drexler, B., Siegwart, R., Crestani, F., Zaugg, M., Vogt, K.E., Ledermann, B., Antkowiak, B., Rudolph, U., 2003. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABAA receptor β3 subunit. The FASEB Journal 17, 250–252. https://doi.org/10.1096/fj.02-0611fje

Kaila, K., Price, T.J., Payne, J.A., Puskarjov, M., Voipio, J., 2014. Cation-chloride cotransporters in neuronal development, plasticity and disease. Nat Rev Neurosci 15, 637–654. https://doi.org/10.1038/nrn3819

Kan, A.S.H., Kusay, A.S., Mohammadi, N.A., Lin, S.X.N., Liao, V.W.Y., Lesca, G., Souci, S., Milh, M., Christophersen, P., Chebib, M., Møller, R.S., Absalom, N.L., Jensen, A.A., Ahring, P.K., 2024. Understanding paralogous epilepsy-associated $GABA_A$ receptor variants: Clinical implications, mechanisms, and potential pitfalls. Proc Natl Acad Sci U S A 121, e2413011121. https://doi.org/10.1073/pnas.2413011121

Kasaragod, V.B., Mortensen, M., Hardwick, S.W., Wahid, A.A., Dorovykh, V., Chirgadze, D.Y., Smart, T.G., Miller, P.S., 2022. Mechanisms of inhibition and activation of extrasynaptic αβ GABAA receptors. Nature 602, 529–533. https://doi.org/10.1038/s41586-022-04402-z

Kaufman, R.E., Ostacher, M.J., Marks, E.H., Simon, N.M., Sachs, G.S., Jensen, J.E., Renshaw, P.F., Pollack, M.H., 2009. Brain GABA levels in patients with bipolar disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry 33, 427–434. https://doi.org/10.1016/j.pnpbp.2008.12.025

Khazipov, R., Valeeva, G., Khalilov, I., 2014. Depolarizing GABA and Developmental Epilepsies. CNS Neurosci Ther 21, 83–91. https://doi.org/10.1111/cns.12353

Kilb, W., 2021. When Are Depolarizing GABAergic Responses Excitatory? Front. Mol. Neurosci. 14, 747835. https://doi.org/10.3389/fnmol.2021.747835

Kim, J.J., Gharpure, A., Teng, J., Zhuang, Y., Howard, R.J., Zhu, S., Noviello, C.M., Walsh, R.M., Lindahl, E., Hibbs, R.E., 2020. Shared structural mechanisms of general anaesthetics and benzodiazepines. Nature 585, 303–308. https://doi.org/10.1038/s41586-020-2654-5

Kim, J.J., Hibbs, R.E., 2021. Direct Structural Insights into GABAA Receptor Pharmacology. Trends in Biochemical Sciences 46, 502–517. https://doi.org/10.1016/j.tibs.2021.01.011

Knoflach, F., Hernandez, M.-C., Bertrand, D., 2016. GABAA receptor-mediated neurotransmission: Not so simple after all. Biochemical Pharmacology 115, 10–17. https://doi.org/10.1016/j.bcp.2016.03.014

Kotani, N., Akaike, N., 2013. The effects of volatile anesthetics on synaptic and extrasynaptic GABA-induced neurotransmission. Brain Research Bulletin, Extrasynaptic ionotropic receptors 93, 69–79. https://doi.org/10.1016/j.brainresbull.2012.08.001

Koulentaki, M., Kouroumalis, E., 2018. GABAA receptor polymorphisms in alcohol use disorder in the GWAS era. Psychopharmacology 235, 1845–1865. https://doi.org/10.1007/s00213-018-4918-4

Krishek, B.J., Moss, S.J., Smart, T.G., 1996. Homomeric beta 1 gamma-aminobutyric acid A receptor-ion channels: evaluation of pharmacological and physiological properties. Mol Pharmacol 49, 494–504.

Kumar, S., Porcu, P., Werner, D.F., Matthews, D.B., Diaz-Granados, J.L., Helfand, R.S., Morrow, A.L., 2009. The role of GABAA receptors in the acute and chronic effects of ethanol: a decade of progress. Psychopharmacology (Berl) 205, 529. https://doi.org/10.1007/s00213-009-1562-z

Laverty, D., Desai, R., Uchański, T., Masiulis, S., Stec, W.J., Malinauskas, T., Zivanov, J., Pardon, E., Steyaert, J., Miller, K.W., Aricescu, A.R., 2019. Cryo-EM structure of the human α1β3γ2 GABAA receptor in a lipid bilayer. Nature 565, 516–520. https://doi.org/10.1038/s41586-018-0833-4

Laverty, D., Thomas, P., Field, M., Andersen, O.J., Gold, M.G., Biggin, P.C., Gielen, M., Smart, T.G., 2017. Crystal structures of a GABAA-receptor chimera reveal new

endogenous neurosteroid-binding sites. Nat Struct Mol Biol 24, 977–985. https://doi.org/10.1038/nsmb.3477

Lener, M.S., Niciu, M.J., Ballard, E.D., Park, M., Park, L.T., Nugent, A., Zarate, C.A., 2017. Glutamate and GABA Systems in the Pathophysiology of Major Depression and Antidepressant Response to Ketamine. Biol Psychiatry 81, 886–897. https://doi.org/10.1016/j.biopsych.2016.05.005

Li, D., Sulovari, A., Cheng, C., Zhao, H., Kranzler, H.R., Gelernter, J., 2014. Association of Gamma-Aminobutyric Acid A Receptor α2 Gene (GABRA2) with Alcohol Use Disorder. Neuropsychopharmacol 39, 907–918. https://doi.org/10.1038/npp.2013.291

Liang, J., Olsen, R.W., 2014. Alcohol use disorders and current pharmacological therapies: the role of GABAA receptors. Acta Pharmacol Sin 35, 981–993. https://doi.org/10.1038/aps.2014.50

López-Muñoz, F., Ucha-Udabe, R., Alamo, C., 2005. The history of barbiturates a century after their clinical introduction. Neuropsychiatric Disease and Treatment 1, 329.

Löscher, W., Rogawski, M.A., 2012. How theories evolved concerning the mechanism of action of barbiturates. Epilepsia 53, 12–25. https://doi.org/10.1111/epi.12025

Luscher, B., Fuchs, T., 2015. GABAergic control of depression-related brain states. Adv Pharmacol 73, 97–144. https://doi.org/10.1016/bs.apha.2014.11.003

Luscher, B., Maguire, J.L., Rudolph, U., Sibille, E., 2023. GABAA receptors as targets for treating affective and cognitive symptoms of depression. Trends in Pharmacological Sciences 44, 586–600. https://doi.org/10.1016/j.tips.2023.06.009

Luscher, B., Shen, Q., Sahir, N., 2011. The GABAergic Deficit Hypothesis of Major Depressive Disorder. Mol Psychiatry 16, 383–406. https://doi.org/10.1038/mp.2010.120

Magnin, E., Francavilla, R., Amalyan, S., Gervais, E., David, L.S., Luo, X., Topolnik, L., 2019. Input-Specific Synaptic Location and Function of the α5 $GABA_A$ Receptor

Subunit in the Mouse CA1 Hippocampal Neurons. J. Neurosci. 39, 788–801. https://doi.org/10.1523/JNEUROSCI.0567-18.2018

Maillard, P.-Y., Baer, S., Schaefer, É., Desnous, B., Villeneuve, N., Lépine, A., Fabre, A., Lacoste, C., El Chehadeh, S., Piton, A., Porter, L.F., Perriard, C., Wardé, M.-T.A., Spitz, M.-A., Laugel, V., Lesca, G., Putoux, A., Ville, D., Mignot, C., Héron, D., Nabbout, R., Barcia, G., Rio, M., Roubertie, A., Meyer, P., Paquis-Flucklinger, V., Patat, O., Lefranc, J., Gerard, M., Epigen Consortium, de Bellescize, J., Villard, L., De Saint Martin, A., Milh, M., 2022. Molecular and clinical descriptions of patients with GABAA receptor gene variants (GABRA1, GABRB2, GABRB3, GABRG2): A cohort study, review of literature, and genotype-phenotype correlation. Epilepsia 63, 2519–2533. https://doi.org/10.1111/epi.17336

Maldifassi, M.C., Sigel, E., 2017. Functional sites for anesthetics in GABAA receptors. Oncotarget 8, 10779–10780. https://doi.org/10.18632/oncotarget.14616

Mao, C., Shen, C., Li, C., Shen, D.-D., Xu, C., Zhang, S., Zhou, R., Shen, Q., Chen, L.-N., Jiang, Z., Liu, J., Zhang, Y., 2020. Cryo-EM structures of inactive and active GABAB receptor. Cell Res 30, 564–573. https://doi.org/10.1038/s41422-020-0350-5

Masiulis, S., Desai, R., Uchański, T., Serna Martin, I., Laverty, D., Karia, D., Malinauskas, T., Zivanov, J., Pardon, E., Kotecha, A., Steyaert, J., Miller, K.W., Aricescu, A.R., 2019. GABAA receptor signalling mechanisms revealed by structural pharmacology. Nature 565, 454–459. https://doi.org/10.1038/s41586-018-0832-5

Mason, B.J., Quello, S., Shadan, F., 2018. Gabapentin for the treatment of alcohol use disorder. Expert Opin Investig Drugs 27, 113–124. https://doi.org/10.1080/13543784.2018.1417383

Mermer, F., Poliquin, S., Rigsby, K., Rastogi, A., Shen, W., Romero-Morales, A., Nwosu, G., McGrath, P., Demerast, S., Aoto, J., Bilousova, G., Lal, D., Gama, V., Kang, J.-Q., 2021. Common molecular mechanisms of *SLC6A1* variant-mediated neurodevelopmental disorders in astrocytes and neurons. Brain 144, 2499–2512. https://doi.org/10.1093/brain/awab207

Miller, P.S., Aricescu, A.R., 2014. Crystal structure of a human GABAA receptor. Nature 512, 270–275. https://doi.org/10.1038/nature13293

Miller, P.S., Scott, S., Masiulis, S., De Colibus, L., Pardon, E., Steyaert, J., Aricescu, A.R., 2017. Structural basis for GABAA receptor potentiation by neurosteroids. Nat Struct Mol Biol 24, 986–992. https://doi.org/10.1038/nsmb.3484

Miziak, B., Chrościńska-Krawczyk, M., Czuczwar, S.J., 2020. Neurosteroids and Seizure Activity. Front Endocrinol (Lausanne) 11, 541802. https://doi.org/10.3389/fendo.2020.541802

Mohammadi, N.A., Ahring, P.K., Liao, V.W.Y., Chua, H.C., Rosa, S.O. de la, Johannesen, K.M., Michaeli-Yossef, Y., Vincent-Devulder, A., Meridda, C., Bruel, A.-L., Rossi, A., Patel, C., Klepper, J., Bonanni, P., Minghetti, S., Trivisano, M., Specchio, N., Amor, D., Auvin, S., Baer, S., Meyer, P., Milh, M., Salpietro, V., Maroofian, R., Lemke, J.R., Weckhuysen, S., Christophersen, P., Rubboli, G., Chebib, M., Jensen, A.A., Absalom, N.L., Møller, R.S., 2024. Distinct neurodevelopmental and epileptic phenotypes associated with gain- and loss-of-function GABRB2 variants. eBioMedicine 106. https://doi.org/10.1016/j.ebiom.2024.105236

Möhler, H., 2006. GABAA receptor diversity and pharmacology. Cell Tissue Res 326, 505–516. https://doi.org/10.1007/s00441-006-0284-3

Möhler, H., Benke, D., 2001. GABAB-receptor isoforms Molecular architecture and distribution. Life Sciences.

Monfrini, E., Borellini, L., Zirone, E., Yahya, V., Mauri, E., Molisso, M.T., Mameli, F., Ruggiero, F., Comi, G.P., Barbieri, S., Di Fonzo, A., Dilena, R., 2023. *GABRB1* -related early onset developmental and epileptic encephalopathy: Clinical trajectory and novel de novo mutation. Epileptic Disorders 25, 867–873. https://doi.org/10.1002/epd2.20132

Mortensen, M., Patel, B., Smart, T.G., 2012. GABA Potency at GABAA Receptors Found in Synaptic and Extrasynaptic Zones. Front Cell Neurosci 6, 1. https://doi.org/10.3389/fncel.2012.00001

Mortensen, M., Smart, T.G., 2006. Extrasynaptic αβ subunit GABAA receptors on rat hippocampal pyramidal neurons. J Physiol 577, 841–856. https://doi.org/10.1113/jphysiol.2006.117952

Most, D., Ferguson, L., Harris, R.A., 2014. Molecular basis of alcoholism. Handb Clin Neurol 125, 89–111. https://doi.org/10.1016/B978-0-444-62619-6.00006-9

Mueller, T.M., Haroutunian, V., Meador-Woodruff, J.H., 2014. N-Glycosylation of GABAA Receptor Subunits is Altered in Schizophrenia. Neuropsychopharmacol 39, 528–537. https://doi.org/10.1038/npp.2013.190

Naffaa, M.M., Hung, S., Chebib, M., Johnston, G.A.R., Hanrahan, J.R., 2017. GABA-ρ receptors: distinctive functions and molecular pharmacology. Br J Pharmacol 174, 1881–1894. https://doi.org/10.1111/bph.13768

Noroozi, R., Taheri, M., Ghafouri-Fard, S., Bidel, Z., Omrani, M.D., Moghaddam, A.S., Sarabi, P., Jarahi, A.M., 2018. Meta-analysis of GABRB3 Gene Polymorphisms and Susceptibility to Autism Spectrum Disorder. J Mol Neurosci 65, 432–437. https://doi.org/10.1007/s12031-018-1114-2

Oblak, A., Gibbs, T. t., Blatt, G. j., 2009. Decreased GABAA receptors and benzodiazepine binding sites in the anterior cingulate cortex in autism. Autism Research 2, 205–219. https://doi.org/10.1002/aur.88

Olsen, R.W., 2018. GABAA Receptor: Positive and Negative Allosteric Modulators. Neuropharmacology 136, 10–22. https://doi.org/10.1016/j.neuropharm.2018.01.036

Olsen, R.W., Liang, J., 2017. Role of GABAA receptors in alcohol use disorders suggested by chronic intermittent ethanol (CIE) rodent model. Mol Brain 10, 45. https://doi.org/10.1186/s13041-017-0325-8

Olsen, R.W., Sieghart, W., 2009. GABAA receptors: Subtypes provide diversity of function and pharmacology. Neuropharmacology 56, 141–148. https://doi.org/10.1016/j.neuropharm.2008.07.045

Olsen, R.W., Sieghart, W., 2008. International Union of Pharmacology. LXX. Subtypes of γ-Aminobutyric AcidA Receptors: Classification on the Basis of Subunit Composition, Pharmacology, and Function. Update. Pharmacol Rev 60, 243–260. https://doi.org/10.1124/pr.108.00505

Papandreou, A., McTague, A., Trump, N., Ambegaonkar, G., Ngoh, A., Meyer, E., Scott, R.H., Kurian, M.A., 2016. GABRB3 mutations: a new and emerging cause of

early infantile epileptic encephalopathy. Dev Med Child Neurol 58, 416–420. https://doi.org/10.1111/dmcn.12976

Pardridge, W.M., 2012. Drug transport across the blood–brain barrier. J Cereb Blood Flow Metab 32, 1959–1972. https://doi.org/10.1038/jcbfm.2012.126

Peltier, M.R., Verplaetse, T.L., Altemus, M., Zakiniaeiz, Y., Ralevski, E.A., Mineur, Y.S., Gueorguieva, R., Picciotto, M.R., Cosgrove, K.P., Petrakis, I., McKee, S.A., 2024. The role of neurosteroids in posttraumatic stress disorder and alcohol use disorder: A review of 10 years of clinical literature and treatment implications. Front Neuroendocrinol 73, 101119. https://doi.org/10.1016/j.yfrne.2023.101119

Perucca, E., Bialer, M., White, H.S., 2023. New GABA-Targeting Therapies for the Treatment of Seizures and Epilepsy: I. Role of GABA as a Modulator of Seizure Activity and Recently Approved Medications Acting on the GABA System. CNS Drugs 37, 755–779. https://doi.org/10.1007/s40263-023-01027-2

Philip, A.B., Brohan, J., Goudra, B., 2025. The Role of GABA Receptors in Anesthesia and Sedation: An Updated Review. CNS Drugs 39, 39–54. https://doi.org/10.1007/s40263-024-01128-6

Pirker, S., Schwarzer, C., Wieselthaler, A., Sieghart, W., Sperk, G., 2000. GABAA receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101, 815–850. https://doi.org/10.1016/S0306-4522(00)00442-5

Purwana, I., Zheng, J., Li, X., Deurloo, M., Son, D.O., Zhang, Z., Liang, C., Shen, E., Tadkase, A., Feng, Z.-P., Li, Y., Hasilo, C., Paraskevas, S., Bortell, R., Greiner, D.L., Atkinson, M., Prud'homme, G.J., Wang, Q., 2014. GABA Promotes Human b-Cell Proliferation and Modulates Glucose Homeostasis.

Reddy, D.S., 2010. Neurosteroids: Endogenous Role in the Human Brian and Therapeutic Potentials. Prog Brain Res 186, 113–137. https://doi.org/10.1016/B978-0-444-53630-3.00008-7

Reddy, D.S., Mbilinyi, R.H., Estes, E., 2023. Preclinical and clinical pharmacology of brexanolone (allopregnanolone) for postpartum depression: a landmark journey from concept to clinic in neurosteroid replacement therapy. Psychopharmacology (Berl) 240, 1841–1863. https://doi.org/10.1007/s00213-023-06427-2

Richardson, R.J., Petrou, S., Bryson, A., 2024. Established and emerging GABAA receptor pharmacotherapy for epilepsy. Front Pharmacol 15, 1341472. https://doi.org/10.3389/fphar.2024.1341472

Roberto, M., Kirson, D., Khom, S., 2021. The Role of the Central Amygdala in Alcohol Dependence. Cold Spring Harb Perspect Med 11, a039339. https://doi.org/10.1101/cshperspect.a039339

Roberto, M., Varodayan, F.P., 2017. Synaptic targets: Chronic alcohol actions. Neuropharmacology, Alcoholism 122, 85–99. https://doi.org/10.1016/j.neuropharm.2017.01.013

Robinson, D.L., Howard, E.C., McConnell, S., Gonzales, R.A., Wightman, R.M., 2009. Disparity between tonic and phasic ethanol-induced dopamine increases in the nucleus accumbens of rats. Alcohol Clin Exp Res 33, 1187–1196. https://doi.org/10.1111/j.1530-0277.2009.00942.x

Sallard, E., Letourneur, D., Legendre, P., 2021. Electrophysiology of ionotropic GABA receptors. Cell Mol Life Sci 78, 5341–5370. https://doi.org/10.1007/s00018-021-03846-2

Santhakumar, V., Wallner, M., Otis, T.S., 2007. Ethanol acts directly on extrasynaptic subtypes of GABAA receptors to increase tonic inhibition. Alcohol 41, 211–221. https://doi.org/10.1016/j.alcohol.2007.04.011

Sarasa, S.B., Mahendran, R., Muthusamy, G., Thankappan, B., Selta, D.R.F., Angayarkanni, J., 2020. A Brief Review on the Non-protein Amino Acid, Gamma-amino Butyric Acid (GABA): Its Production and Role in Microbes. Curr Microbiol 77, 534–544. https://doi.org/10.1007/s00284-019-01839-w

Sarlo, G.L., Holton, K.F., 2021. Brain concentrations of glutamate and GABA in human epilepsy: A review. Seizure - European Journal of Epilepsy 91, 213–227. https://doi.org/10.1016/j.seizure.2021.06.028

Schulte, J.T., Wierenga, C.J., Bruining, H., 2018. Chloride transporters and GABA polarity in developmental, neurological and psychiatric conditions. Neuroscience & Biobehavioral Reviews 90, 260–271. https://doi.org/10.1016/j.neubiorev.2018.05.001

Schür, R.R., Draisma, L.W.R., Wijnen, J.P., Boks, M.P., Koevoets, M.G.J.C., Joëls, M., Klomp, D.W., Kahn, R.S., Vinkers, C.H., 2016. Brain GABA levels across psychiatric disorders: A systematic literature review and meta-analysis of 1H-MRS studies. Hum Brain Mapp 37, 3337–3352. https://doi.org/10.1002/hbm.23244

Scott, S., Aricescu, A.R., 2019. A structural perspective on GABAA receptor pharmacology. Current Opinion in Structural Biology 54, 189–197. https://doi.org/10.1016/j.sbi.2019.03.023

Sears, S.M., Hewett, S.J., 2021. Influence of glutamate and GABA transport on brain excitatory/inhibitory balance. Exp Biol Med (Maywood) 246, 1069–1083. https://doi.org/10.1177/1535370221989263

Shaye, H., Stauch, B., Gati, C., Cherezov, V., 2021. Molecular mechanisms of metabotropic GABAB receptor function. Sci. Adv. 7, eabg3362. https://doi.org/10.1126/sciadv.abg3362

Sieghart, W., Chiou, L.-C., Ernst, M., Fabjan, J., Savić, M.M., Lee, M.T., France, C., 2022. α6-Containing GABAA Receptors: Functional Roles and Therapeutic Potentials. Pharmacological Reviews 74, 238–270. https://doi.org/10.1124/pharmrev.121.000293

Sigel, E., Ernst, M., 2018. The Benzodiazepine Binding Sites of GABAA Receptors. Trends in Pharmacological Sciences 39, 659–671. https://doi.org/10.1016/j.tips.2018.03.006

Sigel, E., Mamalaki, C., Eric A, B., 1982. Isolation of a GABA receptor from bovine brain using a benzodiazepine affinity column. FEBS Letters 147, 45–48. https://doi.org/10.1016/0014-5793(82)81008-9

Sigel, E., Steinmann, M.E., 2012. Structure, Function, and Modulation of GABAA Receptors. Journal of Biological Chemistry 287, 40224–40231. https://doi.org/10.1074/jbc.R112.386664

Simeone, X., Siebert, D.C.B., Bampali, K., Varagic, Z., Treven, M., Rehman, S., Pyszkowski, J., Holzinger, R., Steudle, F., Scholze, P., Mihovilovic, M.D., Schnürch, M., Ernst, M., 2017. Molecular tools for GABAA receptors: High affinity ligands for β1-containing subtypes. Sci Rep 7, 5674. https://doi.org/10.1038/s41598-017-05757-4

Smart, T.G., Stephenson, F.A., 2019. A half century of γ-aminobutyric acid. Brain Neurosci Adv 3, 2398212819858249. https://doi.org/10.1177/2398212819858249

Soltani, N., Qiu, H., Aleksic, M., Glinka, Y., Zhao, F., Liu, R., Li, Y., Zhang, N., Chakrabarti, R., Ng, T., Jin, T., Zhang, H., Lu, W.-Y., Feng, Z.-P., Prud'homme, G.J., Wang, Q., 2011. GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. Proceedings of the National Academy of Sciences 108, 11692–11697. https://doi.org/10.1073/pnas.1102715108

Staley, K., 2015. Molecular mechanisms of epilepsy. Nat Neurosci 18, 367–372. https://doi.org/10.1038/nn.3947

Stephens, D.N., King, S.L., Lambert, J.J., Belelli, D., Duka, T., 2017. $GABA_A$ receptor subtype involvement in addictive behaviour. Genes Brain and Behavior 16, 149–184. https://doi.org/10.1111/gbb.12321

Sun, C., Zhu, H., Clark, S., Gouaux, E., 2023. Cryo-EM structures reveal native GABAA receptor assemblies and pharmacology. Nature 622, 195–201. https://doi.org/10.1038/s41586-023-06556-w

Tanaka, M., DeLorey, T.M., Delgado-Escueta, A., Olsen, W., 2012. GABRB3, Epilepsy, and Neurodevelopment.

Tateno, T., Robinson, H.P.C., 2011. The mechanism of ethanol action on midbrain dopaminergic neuron firing: a dynamic-clamp study of the role of Ih and GABAergic synaptic integration. Journal of Neurophysiology 106, 1901–1922. https://doi.org/10.1152/jn.00162.2011

Tesic, V., Joksimovic, S.M., Quillinan, N., Krishnan, K., Covey, D.F., Todorovic, S.M., Jevtovic-Todorovic, V., 2020. Neuroactive steroids alphaxalone and CDNC24 are effective hypnotics and potentiators of GABAA currents, but are not neurotoxic to the developing rat brain. Br J Anaesth 124, 603–613. https://doi.org/10.1016/j.bja.2020.01.013

Thompson, S.A., Wheat, L., Brown, N.A., Wingrove, P.B., Pillai, G.V., Whiting, P.J., Adkins, C., Woodward, C.H., Smith, A.J., Simpson, P.B., Collins, I., Wafford, K.A., 2004. Salicylidene salicylhydrazide, a selective inhibitor of β1-containing GABAA receptors. Br J Pharmacol 142, 97–106. https://doi.org/10.1038/sj.bjp.0705689

Tian, J., Dang, H., Middleton, B., Kaufman, D.L., 2017. Clinically applicable GABA receptor positive allosteric modulators promote ß-cell replication. Sci Rep 7, 374. https://doi.org/10.1038/s41598-017-00515-y

Topchiy, I., Mohbat, J., Folorunso, O.O., Wang, Z. (Zephyr), Lazcano-Etchebarne, C., Engin, E., 2024. GABA System as the Cause and Effect in Early Development. Neurosci Biobehav Rev 161, 105651. https://doi.org/10.1016/j.neubiorev.2024.105651

Tretter, V., Moss, S.J., 2008. GABAA Receptor Dynamics and Constructing GABAergic Synapses. Front Mol Neurosci 1, 7. https://doi.org/10.3389/neuro.02.007.2008

Walters, R.J., Hadley, S.H., Morris, K.D.W., Amin, J., 2000. Benzodiazepines act on GABAA receptors via two distinct and separable mechanisms. Nat Neurosci 3, 1274–1281. https://doi.org/10.1038/81800

Winsky-Sommerer, R., 2009. Role of GABAA receptors in the physiology and pharmacology of sleep. European Journal of Neuroscience 29, 1779–1794. https://doi.org/10.1111/j.1460-9568.2009.06716.x

Woll, K.A., Zhou, X., Bhanu, N.V., Garcia, B.A., Covarrubias, M., Miller, K.W., Eckenhoff, R.G., 2018. Identification of binding sites contributing to volatile anesthetic effects on GABA type A receptors. The FASEB Journal 32, 4172–4189. https://doi.org/10.1096/fj.201701347R

World Health Organization, 2024. Global status report on alcohol and health and treatment of substance use disorders. World Health Organization.

Xu, C., Zhang, W., Rondard, P., Pin, J.-P., Liu, J., 2014. Complex GABAB receptor complexes: how to generate multiple functionally distinct units from a single receptor. Front Pharmacol 5, 12. https://doi.org/10.3389/fphar.2014.00012

Yee, B.K., Keist, R., von Boehmer, L., Studer, R., Benke, D., Hagenbuch, N., Dong, Y., Malenka, R.C., Fritschy, J.-M., Bluethmann, H., Feldon, J., Möhler, H., Rudolph, U., 2005. A schizophrenia-related sensorimotor deficit links α3-containing GABAA receptors to a dopamine hyperfunction. Proc Natl Acad Sci U S A 102, 17154–17159. https://doi.org/10.1073/pnas.0508752102

Yip, G.M.S., Chen, Z.-W., Edge, C.J., Smith, E.H., Dickinson, R., Hohenester, E., Townsend, R.R., Fuchs, K., Sieghart, W., Evers, A.S., Franks, N.P., 2013. A propofol binding site on mammalian GABAA receptors identified by photolabeling. Nat Chem Biol 9, 715–720. https://doi.org/10.1038/nchembio.1340

Yorgason, J.T., Wadsworth, H.A., Anderson, E.J., Williams, B.M., Brundage, J.N., Hedges, D.M., Stockard, A.L., Jones, S.T., Arthur, S.B., Hansen, D.M., Schilaty, N.D., Jang, E.Y., Lee, A.M., Wallner, M., Steffensen, S.C., 2022. Modulation of dopamine release by ethanol is mediated by atypical GABAA receptors on cholinergic interneurons in the nucleus accumbens. Addict Biol 27, e13108. https://doi.org/10.1111/adb.13108

Zhang, T., Li, J., Yu, H., Shi, Y., Li, Z., Wang, Linyan, Wang, Z., Lu, T., Wang, Lifang, Yue, W., Zhang, D., 2018. Meta-analysis of GABRB2 polymorphisms and the risk of schizophrenia combined with GWAS data of the Han Chinese population and psychiatric genomics consortium. PLoS One 13, e0198690. https://doi.org/10.1371/journal.pone.0198690

Zhao, C., Wang, F., Pun, F.W., Mei, L., Ren, L., Yu, Z., Ng, S.-K., Chen, J., Tsang, S.-Y., Xue, H., 2012. Epigenetic regulation on *GABRB2* isoforms expression: Developmental variations and disruptions in psychotic disorders. Schizophrenia Research 134, 260–266. https://doi.org/10.1016/j.schres.2011.11.029

Zhao, C., Xu, Z., Wang, F., Chen, J., Ng, S.-K., Wong, P.-W., Yu, Z., Pun, F.W., Ren, L., Lo, W.-S., Tsang, S.-Y., Xue, H., 2009. Alternative-Splicing in the Exon-10 Region of GABAA Receptor β2 Subunit Gene: Relationships between Novel Isoforms and Psychotic Disorders. PLoS One 4, e6977. https://doi.org/10.1371/journal.pone.0006977

Zhao, H., Mao, X., Zhu, C., Zou, X., Peng, F., Yang, W., Li, B., Li, G., Ge, T., Cui, R., 2022. GABAergic System Dysfunction in Autism Spectrum Disorders. Front. Cell Dev. Biol. 9. https://doi.org/10.3389/fcell.2021.781327

Zhao, H., Zhou, M., Liu, Y., Jiang, J., Wang, Y., 2023. Recent advances in anxiety disorders: Focus on animal models and pathological mechanisms. Animal Model Exp Med 6, 559–572. https://doi.org/10.1002/ame2.12360

Zhou, J., Noviello, C.M., Teng, J., Moore, H., Lega, B., Hibbs, R.E., 2025. Resolving native GABAA receptor structures from the human brain. Nature 638, 562–568. https://doi.org/10.1038/s41586-024-08454-1

Zhou, Y., Danbolt, N.C., 2013. GABA and Glutamate Transporters in Brain. Front. Endocrinol. 4. https://doi.org/10.3389/fendo.2013.00165

Zhu, S., Noviello, C.M., Teng, J., Walsh, R.M., Kim, J.J., Hibbs, R.E., 2018. Structure of a human synaptic GABAA receptor. Nature 559, 67–72. https://doi.org/10.1038/s41586-018-0255-3

Zhu, S., Sridhar, A., Teng, J., Howard, R.J., Lindahl, E., Hibbs, R.E., 2022. Structural and dynamic mechanisms of GABAA receptor modulators with opposing activities. Nat Commun 13, 4582. https://doi.org/10.1038/s41467-022-32212-4

Zizzo, M.G., Cicio, A., Raimondo, S., Alessandro, R., Serio, R., 2022. Age-related differences of γ-aminobutyric acid (GABA)ergic transmission in human colonic smooth muscle. Neurogastroenterology & Motility 34, e14248. https://doi.org/10.1111/nmo.14248

Zorumski, C.F., Paul, S.M., Covey, D.F., Mennerick, S., 2019. Neurosteroids as novel antidepressants and anxiolytics: GABA-A receptors and beyond. Neurobiol Stress 11, 100196. https://doi.org/10.1016/j.ynstr.2019.100196