Role of GABA<sub>A</sub> receptors in the physiology and pharmacology of sleep

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Abstract

Most sedative-hypnotics used in insomnia treatment target the γ-aminobutyric acid (GABA)$_\text{A}$ receptors. A vast repertoire of GABA$_\text{A}$ receptor subtypes was identified and displays specific electrophysiological and functional properties. GABA$_\text{A}$-mediated inhibition traditionally refers to “phasic” inhibition, arising from synaptic GABA$_\text{A}$ receptors, which transiently inhibit neurons. However, there is growing evidence that peri/extra-synaptic GABA$_\text{A}$ receptors are continuously activated by low GABA concentrations and mediate a “tonic” conductance. This slower type of signaling appears to play a key role in controlling cell excitability. This review aims at summarizing recent knowledge on GABA transmission, including the emergence of tonic conductance, and highlighting the importance of GABA$_\text{A}$ receptor heterogeneity. The mechanism of action of sedative-hypnotic drugs and their effects on sleep and the electroencephalogram (EEG) will be reported. Furthermore, studies using genetically-engineered mice will be emphasized, providing insights into the role of GABA$_\text{A}$ receptors in mechanisms underlying physiological and pharmacological sleep. Finally, we will address the potential of GABA$_\text{A}$ receptors pharmacology for the treatment of insomnia.
1. Introduction

It is a common experience that sleep is critical to recover from tiredness and thereby has potent restorative virtues. Poor quality of sleep, typical for insomnia, has many adverse effects on daily life. Insomnia is highly prevalent. It is defined by complaints of disturbed sleep in the presence of adequate opportunity for sleep. The disturbances can consist of one or several features including difficulty in initiating sleep, maintaining sleep, waking up too early and, non-restorative or poor-quality sleep (Mendelson et al., 2004; Roth, 2007). It has been proposed that insomnia is a hyperarousal disorder which could reflect a deficit in sleep homeostasis (Borbély & Achermann, 2005; Vgontzas, 2005). Pharmacology plays an important role in insomnia therapy, targeting several transmitter and peptide systems, including γ-aminobutyric acid/GABAergic, serotonergic, as well as histaminergic and hypocretin/orexinergic systems (Wafford & Ebert, 2008). Past and current treatments have been dominated by sedative-hypnotic drugs acting at GABA\textsubscript{A} receptors, including the traditional benzodiazepines (BZ) and non-BZ drugs (i.e., “Z”-drugs: zolpidem, zaleplon, zopiclone, eszopiclone).

The classical view of neuronal activity relies on the balance between two modes of transmission, excitation mediated by glutamate and inhibition mediated by GABA. GABA, the predominant inhibitory transmitter in the brain, plays a key role in neuronal firing patterns and activity of neuronal networks. In the central nervous system, GABA exerts its effect via ionotropic GABA\textsubscript{A} and GABA\textsubscript{C} receptors, and metabotropic GABA\textsubscript{B} receptors (Barnard et al., 1998; Bowery, 2006). GABA\textsubscript{B} receptors are insensitive to drugs which target GABA\textsubscript{A} and GABA\textsubscript{C} receptors are primarily expressed in retina and optical layers of the superior colliculi. This review will thus focus on GABA\textsubscript{A} receptors. Since their first characterization as “benzodiazepine”-receptors in the late 70s (Braestrup et al., 1977; Möhler & Okada, 1977), GABA\textsubscript{A} receptors have been shown to be a major site for clinically-relevant drugs, including barbiturates, some general anesthetics, as well as ethanol (Mohler, 2006). A vast repertoire of GABA\textsubscript{A} receptor subtypes was identified, underlying the complexity of GABA\textsubscript{A}-mediated transmission. Recently, the emergence of tonic inhibition, mediated by GABA\textsubscript{A}
receptors located outside the synaptic junction, challenged the traditional view of GABA$_A$-mediated transmission. This novel type of conductance may provide further insights into the understanding of sleep mechanisms, as well as the effects of sedative-hypnotic drugs on sleep.

The following review will first recapitulate properties of GABA$_A$ receptors underlying the heterogeneity of GABA$_A$-mediated transmission. Then the knowledge on pharmacological profiles of sedative-hypnotic drugs and their effects on sleep will be summarized. Studies using genetically-engineered mice providing insights into the role of GABA$_A$ receptors in mechanisms underlying sleep will be highlighted.

2. Heterogeneity of GABA$_A$ receptors and GABA$_A$-mediated transmission

GABA is released from the presynaptic neuron into the synaptic cleft. It exerts its action by binding to two cognate sites on the extracellular surface of GABA$_A$ receptors. The activation of the ligand-gated ion channel results in a very rapid increase in permeability for chloride ions, leading to membrane hyperpolarization and inhibition of excitability (Maconochie et al., 1994).

In addition to the fast inhibitory transmission, there is increasing evidence that GABA$_A$ chloride conductance can depolarize postsynaptic neurons and may have excitatory actions. When combined with other depolarizing inputs within a specific time window, activation of GABA$_A$ receptors may facilitate action potential generation. Therefore, GABAergic inputs at the same synapse may result in different actions depending on intracellular chloride concentrations at the site of GABAergic input, as well as the spatio-temporal summation with other inputs (Gulledge & Stuart, 2003; Marty & Llano, 2005). Furthermore, but beyond the scope of this review, GABA$_A$ receptors not only mediate their action at the level of postsynaptic neurons but also presynaptically (Kullmann et al., 2005).
2.1. Heterogeneity of subunits forming a variety of GABA<sub>A</sub> receptors.

GABA<sub>A</sub> receptors are pentameric hetero-oligomers, the subunits’ assembly forming the central ion channel. Several subunit classes have been identified in mammalian genomes, each of the subunits displaying several isoforms (α1-6, β1-3, γ1-3, δ, ε, π, ρ1-3, and θ; (Barnard <i>et al.</i>, 1998; Whiting <i>et al.</i>, 1999). The potential permutations forming pentameric oligomers are considerable. However, not all subunits co-assemble to form GABA<sub>A</sub> subtypes. For instance, δ and γ subunits are mutually exclusive (Sieghart <i>et al.</i>, 1999). The δ, ε and π subunits are believed to substitute for the γ subunit, whereas the θ may replace the β subunit. The subunit composition and stochiometry of native GABA<sub>A</sub> receptors is not yet completely elucidated. The properties of native GABA<sub>A</sub> receptors could be recapitulated in vitro when α, β and γ subunits were co-expressed (Pritchett <i>et al.</i>, 1989). Further available evidence favors GABA<sub>A</sub> receptors containing α-, β-subunits, and in the vast majority of cases a γ subunit in a 2:2:1 stochiometry (Chang <i>et al.</i>, 1996; Tretter <i>et al.</i>, 1997). A 2:1:2 stochiometry has also been reported (Backus <i>et al.</i>, 1993). The majority of receptors appear to consist of a single α isoform. However, a minority of populations containing α1α2, α1α3, α1α5, α2α3 or α3α5 pairs have been detected (Barnard <i>et al.</i>, 1998).

2.2. Specific anatomical expression of GABA<sub>A</sub> subtypes.

The assembly of subunits forming the GABA<sub>A</sub> receptors also depends on the regional distribution of each subunit in the brain (Wisden <i>et al.</i>, 1988; Fritschy & Mohler, 1995; Pirker <i>et al.</i>, 2000). Furthermore, the cellular (somato-dendritic versus axonal) and subcellular localization of GABA<sub>A</sub> receptors on neurons contributes to the complexity of GABA<sub>A</sub> receptor heterogeneity (Somogyi <i>et al.</i>, 1989; Kullmann <i>et al.</i>, 2005; Trigo <i>et al.</i>, 2008). While α1-, α2-, α3-containing subtypes are mainly localized synaptically, these receptor subtypes are also found outside the synapse (Table 1) (Somogyi <i>et al.</i>, 1989). The δ-containing receptors (i.e., α4βxδ, α1βxδ, and α6βxδ) appear to be predominantly or exclusively peri- and/or extra-synaptic, though morphological evidence for such extrasynaptic
localization is scarce (Nusser et al., 1998; Fritschy & Brunig, 2003). The α5-containing receptors are mainly extrasynaptic. Nevertheless, there is functional and morphological evidence that a pool of α5-containing receptors also concentrates at GABAergic synapses located on dendrites (Serwanski et al., 2006; Ali & Thomson, 2008). This segregation between synaptic and extrasynaptic GABA_A receptors is important since they are involved in mediating two distinct forms of GABA_A-transmission (Semyanov et al., 2004; Mody, 2005).

2.3. Two distinct modes of GABA_A-mediated transmission: phasic versus tonic inhibition

GABA_A-mediated transmission traditionally refers to the transient -“phasic”- inhibitory postsynaptic currents (IPSCs) following the activation of synaptic receptors by high concentration (mM range) of GABA released from presynaptic nerve terminals (Maconochie et al., 1994). However, there is growing evidence that extrasynaptic GABA_A receptors are continuously activated by low (<50 μM) GABA concentration and thereby mediate a persistent -“tonic”- inhibition (Fig. 1). Though the discovery of tonic inhibition is relatively recent, new insights emphasize the importance of this transmission (Semyanov et al., 2004; Cavelier et al., 2005; Farrant & Nusser, 2005; Mody, 2005; Walker & Semyanov, 2008).

Electrophysiological recordings in rodent brain slices demonstrated the presence of GABA_A-mediated tonic inhibition in several regions and specific cell-types, including cerebellar granule cells (Brickley et al., 1996), relay neurons of the ventrobasal thalamic nuclei (Porcello et al., 2003; Belelli et al., 2005; Jia et al., 2005), pyramidal cells of neocortical layers II-III and V (Drasbek & Jensen, 2006; Drasbek et al., 2007; Yamada et al., 2007; Vardya et al., 2008), as well as the hippocampal dentate gyrus granule cells and the hippocampal interneurons of the molecular layer (Glykys et al., 2008). The occurrence of tonic currents correlates with the regional and extrasynaptic distribution of specific GABA_A subtypes (Table 1, Fig.2). The majority of extrasynaptic receptors is believed to contain the δ-subunit. This subunit shows high expression levels in the cerebellum, numerous thalamic
relay nuclei, the outer layers of the cortex (I-III) and the dentate gyrus (Wisden et al., 1988; Fritschy & Mohler, 1995; Peng et al., 2002). The δ subunit is preferentially associated with the α4 subunit (Sur et al., 1999) and the α4 expression pattern parallels that of the δ subunit. Nonetheless, a fraction of γ2-containing receptors located extrasynaptically also contributes to tonic conductance when activated by ambient GABA.

2.4. GABA$_A$ subtypes display distinct electrophysiological properties.

The subcellular segregation of specific GABA$_A$ subtypes together with the biophysical properties of GABA$_A$ receptors (i.e., concentration of ligand to induce the half maximal response, rate and extent of activation, desensitization and deactivation of the current) define their respective contribution to phasic or tonic conductance (for review, Vicini & Ortinski, 2004; Farrant & Nusser, 2005). The subunit composition of the receptor has considerable impact on the biophysical properties of the chloride channel. The type of α-subunit determines the kinetics of receptor activation (Verdoorn et al., 1990). δ-containing receptors (i.e., α6β3δ and α4β3δ) require low concentrations of GABA to reach 50% activation of the maximal response (EC$_{50}$ = ~0.3-0.7 μM; (Brown et al., 2002; Farrant & Nusser, 2005). In contrast, γ2-containing receptors (e.g. α 1β3γ2 or α2β3γ2 combinations) have a lower affinity for GABA and thus higher EC$_{50}$ (~6-14 μM). δ containing receptors display lower rate of desensitization compared to γ-containing receptors, and the rate and extend of desensitization is also dependent on the type of α-subunit present.

Phasic and tonic conductance can be modulated by changes in the number and properties of receptors, as well as GABA release and uptake. Extrasynaptic receptors respond to low, persistent concentration of GABA in the extracellular space, potentially due to spillover from the synapse as well as release from astrocytes (Fig.1) (Walker & Semyanov, 2008). Other amino acids, such as taurine and β-alanine, may also be involved in the generation of tonic currents (Cavelier et al., 2005; Jia et al., 2008; Walker & Semyanov, 2008). Elevated ambient GABA (possibly drug-induced) or actions of
allosteric modulators, such as BZ and Z-drugs, may recruit other extrasynaptic and/or synaptic receptors in a given neuronal circuit which may contribute tonic conductance.

Overall, the affinity for GABA, the rate and extent of desensitization, together with the subcellular segregation of distinct GABA\textsubscript{A} subtypes are key parameters underlying their differential activation and their contribution to phasic or tonic conductance.

2.5. Physiological significance of phasic and tonic inhibition.

Phasic inhibition, mediated by the activation of synaptic GABA\textsubscript{A} receptors is characterized by IPSC currents that peak and decay within milliseconds (Mody \textit{et al.}, 1994). This transient activation is due to the rapid clearance of GABA from the synaptic cleft by active transporters and simple diffusion (Grabauskas, 2005).

In contrast, the persistent activation of extrasynaptic receptors may result in an increase of membrane conductance (Semyanov \textit{et al.}, 2004; Cavelier \textit{et al.}, 2005; Farrant & Nusser, 2005), thereby reducing the change in membrane voltage elicited by excitatory input currents. Tonic conductance was proposed to decrease spatial and temporal integration of excitatory signals (Pouille & Scanziani, 2001; Cavelier \textit{et al.}, 2005). In cerebellar granule cells, frequency of action potential firing induced by an excitatory input can be altered upon pharmacological blockade of tonic inhibition (Hamann \textit{et al.}, 2002). Tonic inhibition in this cell-type generated a mean inhibitory conductance 3-fold larger than currents produced by high frequency action potentials evoked by vesicular release of GABA. Moreover, it contributed to ~90% of the total inhibition (Hamann \textit{et al.}, 2002). The magnitude of tonic conductance varies among cell-types.

In summary, phasic conductance rapidly and transiently inhibits neuronal excitability. In contrast, the effects of the tonic “background” conductance, a slower type of signaling, reduce the capability of excitatory stimuli to generate action potentials. Thereby, tonic conductance modulates neuronal firing rates and adjusts the excitability of neuronal networks. This property of establishing a “baseline”
excitability level of neuronal networks raises the intriguing possibility that such a mechanism may be involved in sleep and cognition.

2.6 Tonic inhibition is present in brain regions and cell populations relevant for sleep.

The high expression levels of the extrasynaptic α4βxδ subtype, as well as the presence of GABA\textsubscript{A}-mediated tonic currents in the thalamus and cortex (see section 2.3) are of particular interest in the context of sleep physiology (Table 1, Fig. 2). Tonic currents represent 80% of the total GABA\textsubscript{A}-mediated transmission in relay cells of the thalamus, while no tonic currents were observed in the thalamic reticular nucleus (Belelli et al., 2005). The thalamus and cortex form a functional network via integrated recurrent loops, enabling the synchronized activity of large-scale ensembles of neurons which generate specific brain oscillations (Steriade et al., 1986; Jones, 2002; Steriade, 2003). The type of firing of thalamo-cortical neurons in vivo is behavioral-state dependent. Low-threshold bursting is associated with non-rapid eye movement (NREM) sleep, while tonic firing is typical for waking. Blocking tonic inhibition favors tonic firing in thalamic slices (Cope et al., 2005). On the other hand, pharmacological enhancement of tonic inhibition hyperpolarized thalamic relay neurons. Modulation of tonic conductance also alters the temporal precision of low-threshold burst firing (Bright et al., 2007). Interestingly, widespread changes in GABA release across the thalamus increased tonic inhibition in the thalamic relay neurons while a local increase in GABA had no effect (Bright & Brickley, 2008). By adjusting the excitability of neuronal circuitries, tonic inhibition may play an important role in modulating the magnitude and frequency of network oscillations characterizing vigilance states and their associated behaviors.
3. Pharmacological profiles of sedative-hypnotic drugs targeting GABA$_A$ receptors and their effects on sleep

The structural complexity of GABA$_A$ receptors, the multiple receptor subtypes with distinct electrophysiological properties (channel kinetics, affinity for GABA, rate of desensitization, phasic versus tonic conductance), and specific regional, cellular and subcellular distribution, underlie their functional properties and pharmacological profiles.

Most sedative-hypnotics used in the treatment of insomnia target the GABA$_A$ receptors. It is commonly accepted that anxiolytic, anticonvulsant, myorelaxant, as well as amnestic, sedative and hypnotic actions of these drugs are due to their “general” inhibitory effect on neuronal activity (Rudolph & Mohler, 2004). Understanding how these various sedative-hypnotic drugs enhance phasic and/or tonic GABA$_A$-mediated transmission is of particular importance.

3.1 Binding sites on the GABA$_A$ receptors & pharmacological profiles of sedative-hypnotic drugs.

GABA binds at the interface between $\alpha$ and $\beta$ subunits. BZs and Z-drugs act via the so-called BZ binding site located at the interface between $\alpha$ and $\gamma$ subunits (Sigel & Buhr, 1997), and thereby specifically bind to $\gamma$-containing receptors. In contrast, GABA$_A$ agonists bind to the same binding sites as GABA, between the $\alpha$ and $\beta$ subunits (Amin & Weiss, 1993; Smith & Olsen, 1994). The $\delta$ subunit was recently reported to confer high agonist sensitivity (You & Dunn, 2007).

The requirement of the $\gamma$ subunit for the binding inferers that BZs and Z-drugs will act on the majority of GABA$_A$ receptors (Table 1). BZ and Z-drugs are positive allosteric modulators, i.e., they enhance the GABA-induced chloride current. They have little intrinsic activity in the absence of GABA. The binding of BZs and Z-drugs alters the duration of IPSCs, primarily determined by the $\alpha$ subunit present in the GABA$_A$ receptor subtypes they bind to (for review, Möhler et al., 2002).
The specificity of sedative-hypnotic drugs for distinct GABA<sub>A</sub> receptor subtypes is usually characterized \textit{in vitro}, using \textit{Xenopus} oocytes or cell systems expressing recombinant GABA<sub>A</sub> receptors. Animal models have proved to be a powerful tool to assess \textit{in vivo} the drugs’ specificity for the various GABA<sub>A</sub> receptor subtypes (discussed in section 4).

3.2. \textit{Effects of sedative-hypnotics on sleep physiology.}

Vigilance states (i.e., waking, sleep) are characterized by complex patterns of brain activity reflected by the electroencephalogram (EEG). Sleep is divided into two distinct stages: NREM sleep and REM sleep. In humans, NREM sleep is further subdivided into 4 stages 1-4 according to the predominance of slow waves. In stage 2, the frequency of brain waves is reduced and EEG hallmarks called spindles occur. Responsiveness to stimuli decreases as EEG slow waves become more predominant. Thus, stages 3 and 4, also referred to as “deep” sleep or “slow-wave” sleep, are characterized by high amplitude slow waves.

Each vigilance state has its EEG signature. Quantitative EEG analysis allows the extraction of important functional parameters such as slow-wave-activity (SWA, or “delta” activity) in NREM sleep, encompassing components of the EEG signal in the frequency range of approximately 0.5-4.5 Hz. SWA is considered as an indicator of sleep “depth” or “intensity”. The amplitude of slow waves occurring after sleep onset, and reflected by SWA, depends on the amount of time previously spent awake (Borbély \textit{et al.}, 1981). After a night’s sleep, slow waves show markedly decreased amplitude in the morning. Interestingly, individuals showing a lower proportion of deep sleep are more likely to self-medicate (Roth, 2007). NREM sleep stage 2 is characterized by spindle activity, defined as power in the frequency range between \textasciitilde12-15 Hz. The functional significance of slow waves and spindles is still unknown. REM sleep and waking are characterized in animals (i.e., rodents) by a pronounced activity in the theta (6-9 Hz) frequency range.
Ideally, a hypnotic drug should reduce the latency to sleep onset, reduce waking after sleep onset, enhance aspects of sleep such as NREM sleep duration and SWA, without affecting sleep architecture (i.e., the alternation of NREM-REM sleep cycles). The mechanism of action of drugs traditionally used to treat insomnia, and their effects on sleep, are summarized below. Drugs which recently received considerable attention for their potential to treat insomnia (THIP/gaboxadol, Tiagabine), and other GABA<sub>A</sub> modulators studied for their effects on sleep (e.g., alcohol, neurosteroids) are also discussed.

3.2.1. Positive allosteric modulators mainly target synaptic γ2-containing GABA<sub>A</sub> receptors mediating phasic inhibition.

Historically, barbiturates were the first widely prescribed sedatives in the early 1900s. At low concentrations, they act as positive allosteric modulators of GABA<sub>A</sub> receptors, at a binding site distinct from that of BZs. At high doses, they directly act at the GABA binding site. Due to their toxicity and development of tolerance and dependency, their prescription and use as hypnotics has been discontinued. Barbiturates markedly alter the EEG in NREM sleep, especially reducing activity <10 Hz. In contrast, frequency components above 10 Hz, encompassing the spindle frequency range are increased. Barbiturates also increase NREM sleep duration and suppress REM sleep (Lancel, 1999).

Classical BZs (e.g., diazepam (valium), flunitrazepam (Rohypnol®), flurazepam (Dalmadorm®), triazolam (Halcion®), midazolam (Dormicum®)) were introduced in the 60s. They were widely prescribed due to their potent anxiolytic and sedative properties. Importantly, BZs lacked the toxicity of barbiturates, as well as the liability of overdose due to their pharmacokinetics (Visser et al., 2003; Mendelson, 2005). BZs act at α1, α2, α3 and α5 subtypes containing the γ2 subunit, while α4 and α6 subtypes are insensitive to BZs (Table 1) (Möhler et al., 2002). Thus, BZs target the majority of GABA<sub>A</sub> receptors, producing sedative, anxiolytic, muscle relaxant, anticonvulsant and
cognition-impairing effects. The slow-elimination of various compounds (e.g., flunitrazepam, flurazepam) was linked to residual effects such as daytime sleepiness and impaired performance. Compounds with a shorter half-life were then developed (e.g. triazolam, midazolam) and seemed to lack residual side-effects (Borbély et al., 1983a). BZs reliably shorten sleep onset latency and increase sleep continuity. REM sleep is typically reduced and REM sleep latency prolonged (Borbély et al., 1991; Mendelson, 2005; Lemmer, 2007). All compounds induce dramatic reduction of the EEG power in the low frequencies (< 10 Hz), encompassing SWA (~0.5-4.5 Hz), while spindle activity is increased (Fig. 3 and 4). These significant changes persisted in the subsequent “drug-free” night (Borbély et al., 1983b; Borbély et al., 1991). The clinical and functional significance of pharmacologically suppressing vigilance states and altering the EEG is uncertain. Interestingly, the typical declining trend of SWA over consecutive NREM sleep episodes is not altered by BZs, suggesting that the homeostatic process of sleep regulation is not disrupted (Borbély & Achermann, 1991). Usage of BZs is curtailed by their side-effects, such as next-day sedation, cognitive impairment and amnesic effects (Fig. 4; for review, Korpi et al., 1997). Their long-term use can also lead to development of tolerance and dependence, as well as rebound symptoms at withdrawal (Roehrs et al., 1990; O'Brien C, 2005; Stewart, 2005). These side effects, together with the need for a more restricted activity profile, have led to the screening of novel compounds also acting at GABA\textsubscript{A} BZ-binding site.

The generation following the BZs, known as “Z”-drugs, was launched in the 80s. This new class of drugs includes imidazopyridines (zolpidem, Stilnox®, Ambien®, generics available), cyclopyrrolones (zopiclone; eszopiclone, S-enantiomer of zopiclone, Lunesta®), pirazolopyrimidines (zaleplon, Sonata®). Indiplon (a derivative of zaleplon) and eszopiclone are currently under review by the US Food and Drug administration for their potential in treating insomnia. Z-drugs are structurally different from the BZs. However, they act on the same binding site. They display high potency at \(\alpha_1\)-containing receptors, medium potency at \(\alpha_2\) and \(\alpha_3\)-containing receptors and lack of interaction at the \(\alpha_5\)-subtype (Dämgen & Lüddens, 1999). Overall, Z-drugs show similar actions on sleep as BZs, including the shortening of latency to sleep and a dose-dependent reduction of REM sleep (Fig. 4)
(Brunner et al., 1991; Lader, 1992). Strikingly, Z-drugs share the “spectral EEG signature” of BZs, i.e., consistent reduction of low EEG components (<10 Hz) comprising the SWA range, and an increase in EEG power in the spindle frequency range during NREM sleep (Trachsel et al., 1990; Brunner et al., 1991; Aeschbach et al., 1994). The dynamic pattern of SWA and spindle frequency activity within NREM sleep episodes were also shown to be preserved after administration of Z-drugs (Aeschbach et al., 1994).

These findings suggest that drugs acting at the BZ’s binding site do not interfere with the homeostatic aspect of sleep regulation, but rather with EEG generating mechanisms (Borbély et al., 1991). Overall, while Z-drug hypnotics represent effective sleeping aids, concerns remain regarding their side-effects. In particular, amnesia and risk of tolerance and dependence occur when these compounds are used over an extended period of time (George, 2001).

3.2.2. Effects of drugs modulating tonic inhibition.

Recent studies emphasize the potential role of extrasynaptic receptors in the modulation of sleep. A selective GABA\(_A\) agonist, THIP (4, 5, 6, 7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol; gaboxadol) was recently investigated for its potential to treat insomnia. Moreover, several compounds studied for their sedative-hypnotic actions have been characterized as endogenous (e.g., neuroactive steroids) or exogenous (e.g tiagabine, alcohol) modulators of GABA\(_A\)-tonic conductance.

THIP (gaboxadol) is a relatively old compound. It is a derivative of muscimol sharing a similar structure with GABA (Krogsgaard-Larsen et al., 1977). In a Xenopus oocyte recombinant expressing system, THIP acts as a low-potency agonist at \(\alpha 1\beta 3\gamma 2\) subtype and is a partial agonist at \(\alpha 4\beta 3\gamma 2\) (Brown et al., 2002; Krogsgaard-Larsen et al., 2004). However, it is a full agonist at \(\alpha 4\beta 3\delta\) receptors and generates currents larger than those produced by GABA (Brown et al., 2002; Storustovu & Ebert, 2006). The presence of \(\delta\) subunit was shown to be a strong determinant of the increased pharmacological activity of agonist compounds, while the presence of \(\gamma\) subunit precludes the response
to an agonist (Storustovu & Ebert, 2006). The exquisite selectivity of THIP is used as a tool to identify tonic currents in electrophysiological studies. THIP effects on sleep differ substantially from those induced by allosteric modulators (Fig. 3 and 4). Several studies in human subjects reported a decrease of wakefulness after sleep onset and/or an increase in total sleep time, while REM sleep was not altered. THIP also induced an increase in EEG power encompassing SWA, and a selective decrease in the spindle frequencies during NREM sleep (Faulhaber et al., 1997; Lancel et al., 2001; Mathias et al., 2001a; Mathias et al., 2005; Walsh et al., 2007; Lankford et al., 2008). This compound was discontinued in March 2007 while it was in Phase III clinical trial. Nonetheless, THIP is a powerful tool to investigate the role of the unique GABA\(_A\) receptor populations composed of \(\delta\)-containing receptors (discussed in Section 4).

Another compound, tiagabine, has been recently investigated for its potential as a hypnotic. Tiagabine is a GABA uptake inhibitor launched initially as an anticonvulsant in treatment of epilepsy. It specifically inhibits the GABA transporter GAT-1 (Fig. 1). Such pharmacological manipulation may sustain synaptically-released GABA levels in the synaptic cleft, thereby increasing GABA\(_A\)-mediated inhibition as well as activation of GABA\(_B\) receptors. The effects of tiagabine on sleep are similar to those evoked by selective GABA\(_A\) agonists. Indeed, tiagabine elevates EEG power density in frequencies <10 Hz during NREM sleep, including the SWA range (Mathias et al., 2001b), and increases sleep continuity and time spent in NREM sleep stage 3-4 (Mathias et al., 2001b; Walsh et al., 2005; Roth et al., 2006; Walsh et al., 2006a; Walsh et al., 2006b).

Neuroactive steroids represent a major group of endogenous modulators of GABA\(_A\) (Belelli & Lambert, 2005). Extrasynaptic \(\alpha_4\beta_3\delta\) receptors are sensitive to positive allosteric modulation by neurosteroids of the pregnane class (Mihalek et al., 1999; Adkins et al., 2001; Brown et al., 2002; Wohlfarth et al., 2002). Hence, endogenous neurosteroids can selectively enhance GABA\(_A\)-mediated tonic conductance (Stell et al., 2003; Herd et al., 2007). The scarce studies addressing the effects of
neuroactive steroids on sleep-wake behavior suggest that different compounds exert distinct effects on sleep (for review, Lancel, 1999; Steiger, 2007).

Alcohol is also a potential allosteric modulator of extrasynaptic GABA<sub>A</sub> receptors. Its use to counteract difficulties to sleep is fairly common (Johnson et al., 1998). δ-GABA<sub>A</sub> receptors may be sensitive to low ethanol concentrations (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003; Wei et al., 2004). However, the demonstration of the selective action of ethanol on the δ subtype awaits further confirmation since recent studies found no effects of alcohol on these receptors (Borghese et al., 2006; Korpi et al., 2007). Ethanol increases EEG power in the low 0.25-1 Hz frequency range in NREM sleep, while EEG power in the spindle frequency range is decreased (Dijk et al., 1992). The increase in frequencies below 1 Hz is interesting since they comprise slow (<1 Hz) oscillations, an EEG hallmark of sleep. Slow oscillations in thalamo-cortical neurons correspond to the slow membrane potential oscillation consisting of up and down states (Steriade et al., 1993a; Steriade et al., 1993b; 1993c). The effects of ethanol differ from those of BZs, Z-drugs and selective GABA<sub>A</sub> agonists.

Overall, allosteric modulators acting at γ2-containing GABA<sub>A</sub> receptors reduce sleep latency and sleep fragmentation. They alter sleep architecture, promoting NREM sleep while suppressing REM sleep. Furthermore, nearly all BZs and Z-drug compounds reduce SWA and increase spindle occurrence. In contrast, drugs modulating extrasynaptic δ-containing receptors do not alter sleep latency, may increase sleep continuity depending on the dose administered, and increase EEG power in the SWA range (Fig.4). These diverse effects on sleep are due to the pharmacokinetics and the neuronal networks targeted by sedative-hypnotic drugs. This raises the interesting possibility that activation of synaptic GABA<sub>A</sub> receptors by allosteric modulators plays a role in mechanisms underlying induction/consolidation of NREM sleep and spindles, while suppressing mechanisms
triggering REM sleep and processes underlying slow wave generation. On the other hand, extrasynaptic receptors mediating tonic inhibition may be involved in EEG synchronization.

4. Dissection of the role of GABA<sub>A</sub> receptors in sleep and sleep regulation: insights from animal models and pharmacology

Deciphering the sedative-hypnotic properties of drugs modulating either synaptic or extrasynaptic GABA<sub>A</sub> receptors relates to the functional analysis of the multiple GABA<sub>A</sub> subtypes. The combination of pharmacological approaches with genetically-engineered mice (knock-in and knock-out (KO) mice) proved to be a powerful approach to investigate the function of distinct GABA<sub>A</sub> subtypes in mediating the effects of drugs. It certainly emphasized the importance of GABA<sub>A</sub> receptor heterogeneity.

4.1. Which GABA<sub>A</sub> subtypes mediate the sedative-hypnotic effects of allosteric modulators acting at mainly synaptic GABA<sub>A</sub> receptors?

Great progress was achieved in identifying the GABA<sub>A</sub> receptor subtypes involved in the effects of BZs and Z-drugs using genetically engineered mice. A point mutation (“knock-in”) in the α1, α2, α3, or α5 subunits was introduced to prevent the binding of diazepam at the BZ binding site (α4 and α6 subtypes being insensitive to BZs). Such point mutation preserves the physiological functions of the receptor, and consists of replacing the conserved histidine residue, prerequisite to BZ binding, by an arginine residue (α1(H101R), α2(H101R), α3(H126R) and α5(H105R)) (Rudolph & Mohler, 2004). Studies using these knock-in mice showed that the α1 subtype mediates both sedative (Rudolph et al., 1999; Crestani et al., 2000b; McKernan et al., 2000) and amnesic actions of the BZs (Rudolph et al., 1999). On the other hand, muscle relaxant effects are mediated by α2 subtype and at high dose by α3 receptors (Crestani et al., 2001).

In contrast, characterizing which subtype(s) underlie(s) the hypnotic properties of BZs proved to be complex. The α1 subtype was expected to fulfill this role since it mediates the sedative actions of
diazepam. However, α1-point mutated (H101R) mice displayed a similar response to diazepam (3 mg/kg) compared to control mice (Tobler et al., 2001). In particular, action of diazepam on sleep latency, amount of sleep and suppression of REM sleep does not require the α1 subtype. Strikingly, the reduction of SWA induced by diazepam was even more pronounced in α1(H101R) mice. Sleep continuity was enhanced by diazepam only in α1(H101R) mice, suggesting the involvement of other GABA₆ subtypes. The α3-subtype does not appear to mediate the effects of diazepam on sleep architecture, as shown by studies on α3(H126R) mice and α1(H101R)/α3(H126R) double mutant mice (Kopp et al., 2003; Kopp et al., 2004b). In α2(H101R) mice, the diazepam-induced decrease of SWA activity in NREM sleep was reduced compared to control mice, as well as the enhancement of theta activity in REM sleep (Kopp et al., 2004a). These findings suggest that α2-containing receptors, in particular those expressed in the hypothalamic and pontine ascending pathways, are involved in the generation of NREM sleep. The α2-subtype is also localized in the brainstem-septo-hippocampal systems involved in the generation of theta oscillations (Table 1 & Fig. 2) (Wisden et al., 1992; Fritschy & Mohler, 1995; Vertes & Kocsis, 1997; Jones, 2005; Luppi et al., 2006). Overall, these data indicate that the sedative and hypnotic actions of diazepam are dissociated and mediated by different neuronal circuits.

Whereas diazepam and other BZs have a high affinity for α1, α2, α3 and α5-subtypes, zolpidem has a 10-fold higher affinity for α1- than for α2- and α3-subtypes and does not recognize the α5-subtype (Dämgen & Lüddens, 1999). Knock-in mice confirmed in vivo its preferential affinity for the α1-subtype. Sedative effects of zolpidem are mediated through the α1-subtype (Crestani et al., 2000a). The in vivo contribution of the γ2 subunit to the actions of zolpidem was also confirmed in point mutated γ2(F77I) mice (Cope et al., 2004). Regarding the hypnotic actions of zolpidem, α1(H101R) mice did not show the marked reduction in SWA observed in control mice (Kopp et al., 2004b). In contrast, the drug induced an increase in NREM sleep and suppression of REM sleep in both
genotypes. These data suggest that zolpidem-promoting effects on sleep are mediated by α2 and/or α3 subtypes, whereas its effects on the sleep EEG are mediated by the α1 subtype.

4.2. Further characterization of drugs acting at extrasynaptic receptors on sleep and the sleep EEG

The full GABA<sub>A</sub> agonist THIP is a powerful compound to assess the involvement of tonic conductance in sleep and further investigate its effects on the sleep EEG. We used a knock-out mice model (δ-subunit KO) to assess whether the effects of THIP on sleep and the sleep EEG are mediated in vivo by the extrasynaptic δ-subtype. After THIP administration, δ-KO mice lacked the massive alterations of the EEG and vigilance states observed in wild-type control mice, demonstrating that the effects of THIP on vigilance states are mediated in vivo by the extrasynaptic δ-containing receptors (Winsky-Sommerer et al., 2007). In control mice, THIP did not shorten sleep latency but rather reduced the amount of NREM sleep. At higher doses, REM sleep was suppressed (Vyazovskiy et al., 2005; Winsky-Sommerer et al., 2007; Alexandre et al., 2008). Strikingly, THIP consistently induced an abnormal EEG pattern (i.e., recurrent spike wave events, Fig. 3), resulting in a massive increase of EEG power in both waking and NREM sleep in the low frequencies encompassing the SWA range (Vyazovskiy et al., 2005; Winsky-Sommerer et al., 2007; Alexandre et al., 2008). This increase in power may be misleading. One should be aware that the recurrence of spike-wave events, at a frequency of approximately 0.5-1 Hz, as well as their asymmetric shape and high amplitude of the waves, largely contributed to the increase in SWA (Vyazovskiy et al., 2005). An abnormal EEG pattern was observed in the rat, also there contributing to the increase in SWA (Lancel & Faulhaber, 1996; Lancel, 1997).

High amplitude EEG spike-waves seem to be a common feature of drugs enhancing GABA<sub>A</sub>-mediated tonic conductance (Fig. 3). Muscimol, another selective GABA<sub>A</sub> agonist showing very high affinity for δ-containing receptors (Quirk et al., 1995; Huh et al., 1996; Mihailek et al., 1999), induced such EEG patterns in several species (Pedley et al., 1979; Fariello et al., 1981; Peeters et al., 1989;
Lancel et al., 1996; Lancel et al., 1997; Vyazovskiy et al., 2007). Tiagabine was reported to elicit similar alterations of the EEG (Walton et al., 1994; Coenen et al., 1995; Lancel et al., 1998). It is tempting to draw parallels with spike-wave events induced by the anesthetic etomidate (Reynolds et al., 2003) or the neuromodulator $\gamma$-hydroxybutyrate (Kaupmann et al., 2003; Meerlo et al., 2004; Vienne & Tafti, 2008). Etomidate is a potent modulator of extrasynaptic GABA$_\text{A}$ receptors (Belelli et al., 2005). In contrast, the effects of exogenous $\gamma$-hydroxybutyrate on sleep result from activation of the GABA$_\text{B}$ receptors which also mediate a slower type of transmission (Bowery, 2006; Vienne & Tafti, 2008).

This drug-induced spike-wave activity was reported to be accompanied by a sedated behavioral state (Walton et al., 1994; Meerlo et al., 2004; Vyazovskiy et al., 2005; Vyazovskiy et al., 2007; Winsky-Sommerer et al., 2007; Alexandre et al., 2008; Vienne & Tafti, 2008). During the catalepsy-like state induced by muscimol, spike-waves occur asynchronously in the frontal and parietal EEGs (Vyazovskiy et al., 2007). This synchronous occurrence of high amplitude waves across cortical areas induced by muscimol is consistent with recent data suggesting an important role of inhibitory neurons in the large-scale synchronization of the slow <1 Hz oscillation in membrane potential undergone by cortical neurons during sleep (Volgushev et al., 2006).

Overall, drug-induced increase of GABA$_\text{A}$ tonic conductance resulted in dramatic EEG alterations. This “excessive” conductance is inherent to the high sensitivity of GABA$_\text{A}$ agonists compared to GABA (Storustovu & Ebert, 2006). It was shown that relatively minor changes in chloride concentrations within the postsynaptic neurons can lead to a switch from hyperpolarizing to depolarizing activity. Thus, the effects of drugs acting at extrasynaptic receptors (THIP, muscimol, tiagabine, etomidate), especially in the thalamo-cortical networks, may result in quite profound alterations in properties of the networks. Furthermore, these findings suggest that tonic inhibition can easily be shifted outside the homeostatic range. Thereby, the dose-window of drugs acting directly at extrasynaptic GABA$_\text{A}$ receptors will be inevitably narrow. In the same line, GABA is a high affinity but low-efficacy agonist at extrasynaptic $\delta$-containing receptors emphasizing the impact of
endogenous modulators of tonic inhibition. For instance, neuroactive steroids may have profound effects by enhancing the efficacy of GABA (Stell et al., 2003; Belelli & Lambert, 2005). In the opposite situation, loss of tonic conductance in thalamo-cortical networks might induce seizure activity if such conductance is critical to limit increased excitability. δ-KO mice showed some seizures episodes (Olsen et al., 1997), suggesting an increased excitability or impaired inhibitory timing within the thalamus (Porcello et al., 2003).

General anesthesia and sleep depress consciousness. They may share common neurophysiological elements (Tung & Mendelson, 2004; Franks, 2008). Although anesthetics are beyond the scope of this review, it is noteworthy that a number of them (e.g, etomidate, propofol, isoflurane) have been recently described as potent modulators of extrasynaptic GABA_δ-containing receptors (Bonin & Orser, 2008). The use of genetically-engineered mice has also played a critical role in distinguishing the role of specific GABA_δ subtypes in mediating their sedative, anesthetic and amnesic actions (Rudolph & Mohler, 2004).

4.3. Evaluate the role of GABA_δ receptors expressed in specific neuronal networks

Knock-out mice lacking distinct GABA_δ subunits exhibit compensatory changes. For instance, α1-KO mice show major alterations in the expression of several GABA_δ subtypes to compensate for the loss of expression of the α1 gene (Sur et al., 2001; Kralic et al., 2006). These compensatory changes may thereby preclude the dissection of each subtype’s contribution to an observed sleep phenotype.

However, such potential caveats uncovered adaptative mechanisms. For instance, studies in α6-KO mice showed that the loss of tonic inhibition due to the lack of extrasynaptic α6βδ receptors was compensated for, underlining the importance of tonic inhibition in the cerebellar granule cells. The compensatory mechanism was identified as an increased expression of the TASK-1 potassium channel.
which also inhibits cellular excitability in a continuous “tonic” manner (Brickley et al., 2001). The opposite studies were recently performed, analyzing the consequences of the loss of TASK-1 potassium conductance. TASK-1 KO mice showed an increased impact of the GABA<sub>A</sub> receptor activation. Moreover, TASK-1 female KO mice were more affected by THIP compared to controls, while males showed similar responses in both genotypes. These findings suggest that a sex-dependent regulation of extrasynaptic GABA<sub>A</sub> receptors is involved in the changes induced by TASK-1 deletion (Linden et al., 2008).

The role of β3 and α3 subtypes in sleep regulation was particularly interesting to address due to their expression in the reticular nucleus of the thalamus (nRT; Fig. 2). As previously mentioned, typical oscillations in the sleep EEG are closely associated with specific patterns of neuronal activity arising from the thalamo-cortical networks. Hence, the progressive hyperpolarization of thalamocortical neurons occurring during the progression from waking to deep sleep results in fluctuations in the membrane potential, which are initially in the frequency range of sleep spindles and then of slow waves (Steriade, 2003). The nRT plays a pivotal role in the thalamo-cortical circuitry by providing the main inhibitory input onto the thalamic relay nuclei (Huguenard & Prince, 1994; Cox et al., 1996). Only 10% of mice with a disruption of the β3-subunit survive (Homanics et al., 1997). They display symptoms reminiscent of the human Angelman syndrome, including hyperactivity, poor motor coordination, repetitive stereotypical behavior, seizures and EEG abnormalities (DeLorey et al., 1998). In thalamic slices from β3-KO mice, GABA<sub>A</sub> inhibition was disrupted in the nRT and oscillatory synchrony dramatically intensified (Huntsman et al., 1999). A sleep study showed that consistent with the disrupted inhibitory transmission in the nRT, the usual increase of EEG power in the spindle frequency range at the transition of NREM sleep to REM sleep was reduced in β3-KO mice. Moreover, SWA during NREM sleep was significantly higher in the β3-KO mice (Wisor et al., 2002). These findings are however difficult to interpret since the study is performed on a “survivor” population where compensatory mechanisms are likely to occur.
The α3 subtype displays a restricted distribution and is predominantly expressed in several neuronal populations crucial for sleep (Fig. 2, Table 1). It is strategically located in the nRT, the cortical layer VI, as well as in the monoaminergic and serotonergic neurons of the brainstem and the cholinergic neurons of the basal forebrain. In α3-KO mice, no compensation of the α3-GABA<sub>Λ</sub> receptors by other α subtypes seemed to occur in the nRT (Studer et al., 2006). Baseline sleep recordings did not reveal any major differences between α3-KO mice and wild-type controls (Winsky-Sommerer et al., 2008). Consistent with the results in β3-KO mice, we observed in α3-KO mice a reduced increase in power in the spindle frequency band (10–15 Hz) at the transition between NREM–REM sleep. Furthermore, sleep pressure did not uncover differences in sleep regulation between the genotypes. The puzzling lack of sleep phenotype suggested compensatory mechanisms. Synaptic GABA<sub>Λ</sub> clustering plays is important for the function of inhibitory synapses. Strikingly, despite the disruption of postsynaptic GABA<sub>Λ</sub> receptor clusters in α3-KO mice, GABA<sub>Λ</sub>-mediated inhibition is retained in the thalamic reticular nucleus (Winsky-Sommerer et al., 2008). These findings indicate that other GABA<sub>Λ</sub> receptor subtypes are present. Since the ε subunit is coexpressed with α3, the compensatory mechanism could be due to this subtype (Moragues et al., 2000). The structure (subunit partner) and function of native GABA<sub>Λ</sub> receptors containing the ε subunit are unknown. This subtype shows a very discrete distribution and in particular in regions involved in sleep (Fig. 2, Table 1). It is predominantly expressed in the locus coeruleus and the hypothalamus, where 70 % of orexin neurons and 25 % of histaminergic neurons express the ε subunit mRNA (Sinkkonen et al., 2000; Sergeeva et al., 2005). It is also localized in the cholinergic and monoaminergic neurons, as well as the hippocampus (Whiting et al., 1997). Interestingly, the ε gene is clustered on the same chromosome (Xq28) as the α3 and θ genes (Whiting et al., 1999). The recombinant ε subtype has peculiar electrophysiological properties: it shows a high sensitivity to GABA, and seems to be insensitive to BZs, which is expected if it substitutes the γ subunit (Ranna et al., 2006). Presence of the ε subunit also reduced neurosteroid and anesthetic modulation (Davies et al., 1997; Belelli et al., 2002). Moreover, some findings are suggestive of spontaneous channel opening (Neelands et al., 1999;
Maksay et al., 2003; Wagner et al., 2005), which would produce a tonic inhibition comparable to the one mediated by δ-containing receptors. Due to its expression in neuronal populations crucial for sleep and peculiar electrophysiological characteristics, it will be interesting to investigate the role of the ε-subtype in sleep. These findings show that the adaptative changes taking place are sophisticated enough to sustain thalamo-cortical function underlying complex behaviors and related brain activity in α3-KO mice. The recent description of the imidazopyridine TP003 compound, which appears to be selective at the α3-containing receptors, may provide an alternative approach to investigate the role of the α3 subtype in sleep (Dias et al., 2005).

Mice lacking the γ2 subunit die within the first 2 postnatal weeks. The number of BZ sites is reduced by 90% in these mice, confirming that γ2 is necessary for BZ binding (Gunther et al., 1995). A sleep study in γ2-heterozygous mice did not reveal any difference in vigilance states or EEG hallmarks in these mice compared to their wild-type controls, suggesting the occurrence of compensatory mechanisms (I. Tobler, unpublished results).

The use of knock-in and knock-out mice has provided considerable insights into the physiological, pharmacological and behavioral role of individual GABA_A receptor subtypes, as well as the neurophysiological mechanisms underlying sleep. These studies emphasized the necessity to consider GABA_A receptor-mediated transmission as a highly flexible process. This also revealed potent homeostatic mechanisms maintaining balance between excitation and inhibition in the CNS (e.g. α1-KO, α3-KO, α6-KO). New models allowing temporally and/or spatially controlled deletion of genes in mice will help refine the precision of analysis. Notably, a novel approach restoring drug sensitivity in chosen cell types was recently developed and may prove to be invaluable to investigate the role of specific GABA_A subtypes and neuronal circuits (Wulff et al., 2007). Moreover, drugs that specifically target defined GABA_A receptors are expected to provide new prospects for insomnia therapy and be used as tools to study sleep.
5. GABA<sub>A</sub> receptors pharmacology: future prospects for the treatment of insomnia

BZs and Z-drugs are the predominant hypnotics used in the treatment of insomnia. Meanwhile, multiple modes of GABA-mediated transmission have been characterized, i.e., the tonic conductance mediated by the extrasynaptic GABA<sub>A</sub> receptors, as well as GABA<sub>A</sub> subtype-dependent properties of phasic IPSCs. These distinct modes are localized in defined neuronal networks, in a cell-specific manner, often operating in parallel in a given structure. These findings suggest the value to develop drugs based on the specificity for GABA<sub>A</sub> subtypes in distinct neuronal circuits. Moreover, since the tonic conductance may have profound effects on neuronal excitability and thereby network activity, further characterizing its physiological role in sleep is of importance.

Three GABA<sub>A</sub> allosteric modulators are currently in clinical development: Indiplon-IR and -MR (Immediate/Modified Release; developed by Neurocrine Biosciences) are awaiting approval from the US Food and Drug Administration. Indiplon is a derivative of zaleplon and shows a similar pharmacological profile, including selectivity for the GABA<sub>A</sub> α1 subtype (Neubauer, 2005; Marrs, 2008). The EVT-201 compound (developed by Evotec) and Adipiplon (NG2-73; developed by Neurogen) are in phase II clinical trials. They both have the characteristic to be partial agonists at the BZ binding site. Interestingly, NG2-73 may modulate a more restricted set of neuronal networks involved in sleep, if its selectivity for the α3 subtype is confirmed. Only brief descriptions of their effects are available, and to date, no results have been published.

The GABA<sub>A</sub> δ subtype has emerged as an important pharmacological target for insomnia. Novel compounds selectively acting as allosteric modulators of the δ subtype have been described very recently (Wafford <i>et al.</i>, 2008). These compounds potentiate tonic currents mediated by the δ subtype. They may provide useful tools to assess in vivo the impact of tonic conductance in sleep and the benefits of its increase in a manner which, in contrast to selective agonists such as THIP or muscimol, may prove to stay within a homeostatic range. Further characterization of their actions on neurophysiology will provide important insights to predict the potential therapeutic effects of this class of drugs. Drugs effective in insomnia treatment should not impair daytime performance. Thus, in
addition to sleep, these novel compounds will also be useful to assess the impact of tonic conductance on cognition.

In the context of drugs targeting the GABA δ subtype, the interactions between neurosteroids and extrasynaptic receptors should be taken into consideration. They may predict a gender-specific drug responsiveness to the drug (Linden et al., 2008). Several findings indicate that neurosteroids exert a subtle regulation of extrasynaptic GABA_A receptors (Herd et al., 2007). Fluctuations of circulating neurosteroids accompanying the oestrous cycle, gestation, or stress, induce profound and rapid alterations of the GABA_A-mediated tonic conductance (Maguire et al., 2005; Maguire & Mody, 2007; , 2008).

GABA_A receptors are subjected to a highly dynamic regulation, including multiple mechanisms such as phosphorylation, post-translational modifications, and regulation of receptor trafficking (Leidenheimer et al., 1991; Fritschy & Brunig, 2003; Luscher & Keller, 2004). In this line, agonist activation of GABA_A receptors as well as chronic BZ treatment have been shown to increase GABA_A receptor endocytosis (Tehrani & Barnes, 1991; Calkin & Barnes, 1994). Epigenetic mechanisms also modulate GABA_A receptor expression in response to stressful stimuli. These emerging regulatory mechanisms could be relevant in the pathophysiology of insomnia.

6. Conclusions.

The heterogeneity of GABA_A receptors, the emergence of tonic conductance, as well as the potent adaptive mechanisms taking place in genetically-engineered mice, provide attractive avenues to further investigate the role of GABA_A receptors in the homeostasis of sleep. Understanding the mechanisms underlying physiological sleep may provide an optimal basis to develop novel GABA_A subtype-selective compounds to treat sleep disorders, as well as to characterize their effects on the EEG and performance in human subjects. Because of the central role of GABAergic transmission on
regulation of neuronal networks and complex behaviors, it would be of interest to investigate whether GABA_A receptors function is altered in insomnia and/or undergo dynamic changes to maintain homeostasis in this sleep disorder.
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**Abbreviations**

ACh: acetylcholine; ADO: adenosine; BZ: benzodiazepine; DA: dopamine; DpMe: deep mesencephalic reticular nucleus; DR: dorsal raphe nucleus; EEG: electroencephalogram; GABA: γ-aminobutyric acid; Glu: glutamate; Hcrt: hypocretins/orexins; His: histamine; IPSCs: inhibitory postsynaptic currents; KO: knockout; LC: locus coeruleus; LDTg: laterodorsal tegmental nucleus; MCH: melanin concentrating hormone; NA: noradrenaline; n.d: non determined; NREM sleep: non rapid-eye movement sleep; nRT: reticular nucleus of the thalamus; PeF: perifornical nucleus. PH: posterior hypothalamus area; PPTg: pedunculopontine tegmental nucleus; SubC: subcoeruleus nucleus; SWA: slow-wave activity; TM: tuberomammillary nuclei; VLPAG: ventrolateral periacqueductal gray; VLPO: ventrolateral preoptic area; 5-HT: serotonin.
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**Table 1. Characteristics of the main GABA<sub>A</sub> receptor subtypes.**

<table>
<thead>
<tr>
<th>Subunit composition</th>
<th>Expression in specific cell types</th>
<th>Subcellular distribution</th>
<th>GABA&lt;sub&gt;A&lt;/sub&gt;-mediated transmission</th>
<th>Ligands relevant in sleep</th>
</tr>
</thead>
</table>
| α1β2γ2              | -Cortex, hippocampus: principal cells & interneurons  
- Thalamus: relay neurons  
- Pallidum, substantia nigra: GABAergic neurons  
- Cerebellum: Purkinje cells, stellate, basket cells & granule cells | synaptic, extrasynaptic | phasic / tonic | BZ, Z-drugs |
| α2β3γ2              | -Hippocampus: principal cells  
- Striatum: spiny stellate neurons  
- Inferior olivary neurons  
- Motoneurons | synaptic, extrasynaptic | Phasic / tonic | BZ, Z-drugs |
| α3βγ2               | -Cortex: principal cells in layers V-VI  
- Basal forebrain cholinergic neurons  
- Thalamus: reticular thalamic neurons  
- Brainstem: serotonergic (dorsal raphe) & noradrenergic (locus coeruleus) neurons  
- Cerebellum: Golgi cells  
- Inferior olivary neurons | synaptic, extrasynaptic | phasic / tonic | BZ, Z-drugs |
| α4β2γ2              | -Hippocampus: dentate gyrus granule cells  
- Thalamus: relay neurons  
- Hippocampus: dentate gyrus granule cells  
- Thalamus: relay neurons | synaptic*, extrasynaptic* | phasic / tonic | Insensitive: BZ- & Z-drugs |
| α4βδ                | -Hippocampus: granule cells  
- Thalamus: relay neurons  
- Hippocampus: dentate gyrus granule cells  
- Thalamus: relay neurons | extrasynaptic* | tonic | Insensitive: BZ- & Z-drugs |
| α5βγ2               | -Hippocampus: granule cells  
- Spinal trigeminal neurons | synaptic, extrasynaptic | phasic / tonic | BZ |
| α6βγ2               | Cerebellum: granule cells  
| α6βδ                | Cerebellum: granule cells | synaptic | phasic | Insensitive: BZ- & Z-drugs |
| ε-subtype           | -Septum: GnRH neurons  
- Basal forebrain: cholinergic cells  
- Hypothalamus: hypocretinergic, histaminergic, oxytocinergic, dopaminergic neurons  
- Brainstem: dopaminergic (substantia nigra), serotonergic (raphe nuclei) & noradrenergic (locus coeruleus) neurons | n.d | tonic | Insensitive: BZ |
| θ-subtype           | -Hippocampus: dentate gyrus granule cells  
- Basal forebrain: cholinergic cells  
- Hypothalamus: dopaminergic neurons  
GnRH: gonadotropin-releasing hormone; n.d: not determined. x: subunit isoform undetermined. ?: matter of debate. *: scarce neuromorphological evidence. For the regional and cellular expression of GABA\textsubscript{A} subtypes, see (Bonnert \textit{et al.}, 1999; Moragues \textit{et al.}, 2000; Möhler \textit{et al.}, 2002; Moragues \textit{et al.}, 2002; Fritschy & Brunig, 2003).
Figure legends

Figure 1. Scheme of a GABAergic synapse and diversity of GABA-mediated transmission. GABA released in the synaptic cleft exerts its action via presynaptic and postsynaptic GABA_A as well as GABA_B receptors. Binding of GABA at synaptic GABA_A receptors results in inhibitory postsynaptic currents (IPSCs) which transiently inhibit neurons for 10-100 ms. In contrast, activation of extrasynaptic GABA_A receptors mediates tonic currents, playing a key role in adjusting membrane conductance. GABA transporters (GAT-1, GAT-3) can operate in a bidirectional way (i.e., remove GABA from the extracellular space or provide a source of GABA), thereby potentially modulating tonic inhibition. Synaptic and extrasynaptic receptors, which underlie these two forms of transmission, differ in their structural composition, subcellular localization, as well as electrophysiological characteristics, resulting in distinct pharmacological and functional properties.

Figure 2. Expression of GABA_A subtypes in major structures and neurotransmitter systems involved in the regulation of vigilance states. In blue, predominant GABA_A subtype; In black, other subtype(s) present. Red stars indicate regions where tonic currents were identified by electrophysiology. The activity of the major neuronal networks involved in waking, NREM and REM sleep is reviewed elsewhere (Jones, 2005; Luppi et al., 2006). ACh, acetylcholine. ADO, adenosine. DA, dopamine. DpMe, deep mesencephalic reticular nucleus. DR, dorsal raphe nucleus. Glu, glutamate. Hcrt, hypocretins/orexins. His, histamine. LC, locus coeruleus. LDTg, laterodorsal tegmental nucleus. MCH, melanin concentrating hormone. NA, noradrenaline. n.d: non determined. nRt, reticular nucleus of the thalamus. PeF, perifornical nucleus. PH, posterior hypothalamus area. PPTg, pedunculopontine tegmental nucleus. SubC, subcoeruleus nucleus. TM, tuberomammillary nuclei. VLPAG, ventrolateral periacqueductal gray. VLPO, ventrolateral preoptic area. 5-HT, serotonin.
Figure 3. Representative examples of 12-sec electroencephalogram traces (frontal EEG) and EEG power spectra, during waking and NREM sleep, following administration of vehicle or sedative-hypnotic drugs in mice.

Benzodiazepines and Z-drugs (e.g., diazepam 3 mg/kg and zolpidem 5 mg/kg, respectively; in blue) do not induce visually observable changes at the level of the raw EEG compared to the placebo condition (i.e., vehicle; in black). In contrast, after administration of a low dose of two GABA_A agonists (muscimol (2 mg/kg) or THIP (4 mg/kg); in red), the EEG pattern is characterized, both during waking and NREM sleep, by recurring spike-wave events. Muscimol and THIP both act as superagonists at the δ-subtype. By enhancing tonic conductance, both drugs dramatically altered the brain electrical activity. Spectral analysis reveals profound alterations induced by sedative-hypnotic drugs in the EEG activity. GABA_A allosteric modulators (e.g. diazepam) and GABA_A agonists (e.g., THIP) induce opposite changes on the NREM sleep EEG spectra, illustrated for the first 90 minutes after drug administration. The slow-wave activity increase induced by THIP, both in the waking and NREM sleep spectra, is largely due to the recurring spike-events observed at the raw EEG level. Gray bars indicate the slow-wave activity frequency range (~0.5-4. Hz) and the spindle frequency range (~10-15 Hz).

Figure 4. Overview of the effects induced by sedative-hypnotic drugs on sleep and other parameters, depending which GABA_A subtypes the drugs target.

Blue and red indicate γ2- and δ-subtype, respectively. Freq: frequency.
### Effects of drugs on sleep and other parameters:

**GABA<sub>A</sub> subtype modulated by sedative-hypnotics:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>γ&lt;sub&gt;2&lt;/sub&gt;-subtype</th>
<th>δ-subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep onset latency</td>
<td>↓</td>
<td>not altered</td>
</tr>
<tr>
<td>Sleep continuity</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Total sleep time</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>REM sleep</td>
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<td>not altered</td>
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<tr>
<td>REM sleep latency</td>
<td>↑</td>
<td>not altered</td>
</tr>
<tr>
<td>EEG power</td>
<td>↓ in freq. &lt;10 Hz</td>
<td>↑ in freq. &lt;10 Hz</td>
</tr>
<tr>
<td></td>
<td>↑ in spindle freq.</td>
<td>↓ in spindle freq.</td>
</tr>
<tr>
<td>Residual effects</td>
<td>sedation</td>
<td>none reported</td>
</tr>
<tr>
<td>Effects on cognitive performance</td>
<td>cognitive impairment &amp; amnesic effects</td>
<td>not altered &amp; sustain attention during sleep restriction</td>
</tr>
<tr>
<td>Development of tolerance and/or dependance</td>
<td>√</td>
<td>?</td>
</tr>
<tr>
<td>Rebound insomnia</td>
<td>√</td>
<td>?</td>
</tr>
</tbody>
</table>