# Modulation of GABA$_A$ receptor function and sleep

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## Abstract

The intravenous general anaesthetics (propofol & etomidate), the barbiturates, steroids (*e.g.* alphaxalone, allopregnanalone), the benzodiazepines and the widely prescribed "sleeping pill", the imidazopyridine zolpidem, are all positive allosteric modulators (PAMs) of GABA$_A$ receptors. PAMs enhance ongoing GABAergic communication between neurons. For treating primary insomnia, zolpidem remains a gold-standard medication - it reduces the latency to NREM sleep with a rapid onset and short half-life, leading to relatively few hangover effects. In this review, we discuss the role of the different GABA$_A$ receptor subtypes in the action of sleep-promoting drugs. Certain neuronal hub areas exert disproportionate effects on the brain's vigilance states. For example, injecting GABA$_A$ agonists and PAMs into the mesopontine tegmental anaesthesia area (MPTA) induces an anaesthetic-like state. Similarly, by selectively increasing the GABA drive onto arousal-promoting nuclei, such as the histaminergic neurons in the tuberomammillary nucleus, a more natural NREM-like sleep emerges. Some patients suffering from idiopathic hypersomnia have an unidentified GABA$_A$ receptor PAM in their cerebral spinal fluid. Treating these patients with benzodiazepine PAM site antagonists improves their symptoms. More knowledge of endogenous GABA$_A$ receptor PAMs could provide insight into sleep physiology.

**Keywords**
GABA, histamine, propofol, zolpidem, sleep, anaesthesia

**Highlights:**
Binding sites on GABA$_A$ receptors have been located for steroids.

Zolpidem can induce sleep by inhibiting histamine neurons.

GABA$_A$ receptors in the MPTA induce anaesthesia.

GABA$_A$ receptor PAM site antagonists treat idiopathic hypersomnia.

**1. Introduction: positive allosteric modulators (PAMs) and GABA$_A$ receptors**

Billions of people suffer from poor sleep, placing them at greater risk of illness [1]. Although primary insomnia is ideally treated with cognitive behavioural therapy, insomniacs often resort to prescribed drugs ("sleeping pills"). Many of these drugs work on GABA$_A$ receptors [2-4]. The GABA$_A$ receptors, GABA-gated chloride channels, are the principal agents for fast inhibition in the brain, and have been extensively studied using positive allosteric modulators (PAMs) [5-9] - compounds that enhance GABA's ability to prolong Cl$^-$ influx. PAMs do not gate the receptor directly, but instead, by binding to different parts of the receptor complex distinct from the GABA binding site, enhance ongoing GABAergic transmission, often quite modestly. The subtlety and beauty of allosteric modulation is that these small increases in ongoing GABAergic tone triggered by PAMs can cause major shifts in operation of circuitry, tipping the brain from wakefulness to sleep/sedation, and even to general anaesthesia, depending on the particular drug.

The GABA$_A$ receptor belongs to the cys-loop superfamily [10,11]. Nineteen mammalian genes encode GABA$_A$ receptor subunits, but the ones to consider for drugs that might increase sleep propensity are five of the $\alpha$ subunits ($\alpha1 - \alpha5$), all three $\beta$ subunits ($\beta1 - \beta3$), two of the three $\gamma$ subunits ($\gamma1$ and $\gamma2$), the $\delta$, $\epsilon$ and the $\theta$ subunits [3]. The other subunits are either not expressed in the brain, or are expressed in areas unlikely to influence sleep propensity. GABA$_A$ receptor subunit genes are differentially expressed throughout the brain, and the subunits differentially assemble. Mechanisms governing the differential assembly for subunits within particular neurons are starting to be elucidated [12]. GABA$_A$ receptors are pentamers with an intrinsic ion channel [11], gated by the binding of two molecules of GABA at the $\alpha$ and $\beta$ subunit interfaces. The best studied GABA$_A$ receptor types contain two $\alpha$ subunits, two $\beta$ subunits and a single $\gamma2$ subunit. This $\alpha\beta\gamma2$ class makes up most GABA$_A$ receptors, and are the major PAM targets. These receptors are typically responsible for the inhibitory postsynaptic Cl$^-$ currents found throughout the nervous system *i.e.* fast/phasic (millisecond) inhibitory synaptic transmission. By binding the neuroligin2 and GARLH proteins, the $\gamma2$ subunit ensures that $\alpha\beta\gamma2$ GABA$_A$ receptors get enriched in the postsynaptic area opposite the neurotransmitter release sites [12,13]. Most

pharmacological research has been done on this $\alpha\beta\gamma2$ class [10]. Swapping the $\gamma2$ subunit for a $\delta$ subunit, gives the $\alpha\beta\delta$ class [14], exclusively extrasynaptic receptors, often existing as $\alpha1\beta2\delta$ and $\alpha4\beta2\delta$ combinations, that produce tonic conductances [14,15]. Unlike the phasic IPSCs carried by the $\alpha\beta\gamma2$ class, tonic inhibitory conductances through $\alpha\beta\delta$ receptors do not convey precise inhibitory timing, but instead help set the gain of the system [14]. Extrasynaptic binary GABA$_A$ receptors, with only two subunit types in the pentamer, such as $(\alpha1)_3(\beta3)_2$, could also exist *in vivo* [16].

## 2. GABA$_A$ receptor PAMs are currently the best sleep-promoting and general anaesthesia-inducing drugs.

The intravenous general anaesthetics (propofol & etomidate), the barbiturates, certain steroids (*e.g.* pregnanalone, alphaxalone), the benzodiazepines, the pyrazolopyrimidine zaleplon, the cyclopyrrolone zopiclone and the imidazopyridine zolpidem are all PAMs of GABA$_A$ receptors and can produce sedation [3-5,9]. For most of their actions the benzodiazepines and the "z-drugs" (zalepon, zopiclone and zolpidem) bind between the $\alpha$ and $\gamma2$ subunit interface [17]. Zolpidem also binds at the $\alpha1$-$\alpha1$ subunit interface on binary $(\alpha1)_3(\beta3)_2$ receptors [16]. Propofol works on GABA$_A$ receptor types with $\beta$ subunits [4], which means it will likely work on all GABA$_A$ receptors [4]. It binds at the interface between the extracellular domain and the transmembrane domain [18-20]. The PAM steroids bind across the interfaces between the $\alpha$ and $\beta$ subunits [21]. Etomidate binds at the subunit interfaces at a site distinct from the steroids and propofol [21].

### 2.1. Zolpidem: a gold standard sleeping drug.

Zolpidem has been the gold standard "sleeping pill" for treating primary insomnia [22,23]. According to Forbes magazine (6 August 2015, www.forbes.com), the sales of zolpidem tartrate (Ambien) reached 2.8 billion US dollars in 2011, with 40 million prescriptions in the USA. Since then, with the introduction of generics, and competition from orexin receptor antagonists (Belsomra, also known as suvorexant), sales have declined but they still continue to be high (6 August 2015, www.forbes.com). Zolpidem induces a sleep-like state that resembles non-rapid eye-movement (NREM) sleep (EEG delta power elevated in the 0.5 to 4.5 Hz range of the EEG, reduced muscle

tone, and lower respiratory rate). Zolpidem's main effect in humans is to reduce the latency to NREM sleep [23]. It does not particularly effect the duration of NREM sleep (*i.e.* sleep maintenance) [23]. The zolpidem-evoked EEG is similar, but not identical, to the spectra observed during natural NREM sleep (**Figure 1A, B**). Compared with most benzodiazepines that were originally used as sleeping medications, zolpidem is a good sleeping pill because it has rapid onset kinetics, a short plasma half-life coupled with relatively short receptor occupancy, and it also works at a more limited receptor profile. Physiologically relevant $GABA_A$ receptor types modulated by zolpidem are the $\alpha1\beta\gamma2$, $\alpha2\beta\gamma2$, $\alpha3\beta\gamma2$ subunit-containing receptors [24]; zolpidem exhibits only 20-fold higher binding affinity at $\alpha1\beta\gamma2$-containing receptors [24], so in practice, zolpidem will work at all these receptor subtypes *in vivo*. Because the $\alpha1$, $\alpha2$ and $\alpha3\beta\gamma2$ target $GABA_A$ receptors for zolpidem are collectively widely expressed, many brain processes will be affected, but sleep is usually the first thing to happen after taking zolpidem. The short half-life means that if people do awaken on zolpidem they are less likely to experience hangover effects (*e.g.* ataxia, confusion) which cause accidents; however, for the elderly, zolpidem's use is discouraged because they seem likely to have more accidents when taking the drug.

## 2.2. Propofol-induced sedation probably arises from a broader range of $GABA_A$ receptor targets than those involved in zolpidem-induced sedation

Although propofol is the world's most used intravenous general anaesthetic, at lighter doses it induces a sedative state, induced clinically for investigative and therapeutic procedures. With sedative doses of propofol, the EEG is synchronised around 4 Hz, and there are also higher frequencies evoked in the $\beta$ and $\gamma$ range encompassing 20 - 40 Hz [25] (**Figure 1C**). The high frequency oscillations are synchronised between the neocortex and the thalamus [25,26]. By reducing responses to external stimuli, these oscillations may contribute to propofol-induced loss-of-consciousness [26]. As the propofol concentration is increased, general anaesthesia appears, with immobility and deep unconsciousness. Brain stem circuits become depressed, breathing and heart centres have to be artificially maintained, and the EEG takes on an isoelectric (flat) or burst suppression profile. Similar to zolpidem, propofol increases $GABA_A$ responses on arousal promoting histaminergic neurons [4,27]. But because the

number of GABA$_A$ receptor targets for propofol is so much greater than for zolpidem, propofol's sites of action to induce sedation is likely to be broader.

**3. Inducing sedation by inhibiting key nodal points in the circuitry**

Sedation and general anaesthesia emerge from both top-down and bottom-up mechanisms [25,28,29]. Increasing inhibition in the neocortex fragments intracortical signalling [28]. But inhibiting the wake-promoting areas in the hypothalamus and brainstem also play a role. Aminergic and peptidergic neuromodulator systems in the hypothalamus and brainstem promote and sustain wakefulness [30]. A feature of these neuromodulator cell types is that there are relatively few soma in the home nucleus, but they send axons extensively throughout the brain to release, by volume transmission, their particular transmitter. Examples are the histaminergic neurons whose cell bodies are located in the posterior hypothalamus, the tuberomammillary area, and the noradrenergic neurons whose cell bodies are in the brain stem locus coeruleus [30]. A GABA$_A$ receptor PAM that enhances inhibition onto *e.g.* histamine or noradrenergic neurons could thus have disproportionate influence on brain vigilance state [29,31]. Indeed, a new cluster of neurons in the rat brainstem – the mesopontine tegmental anaesthesia area (MPTA) - has been discovered which, when shut down, allows the emergence of anaesthesia with immobility (muscle atonia), analgesia and shift of the EEG to the $\delta$ range of frequencies [32,33]. Injection of GABA$_A$ receptor agonists and the PAMs pentobarbital and propofol into a volume containing as few as 1900 neurons in this MPTA area induced anaesthesia [32]. More work is needed to fully map out the connections of these MPTA neurons, but at the moment we know little of their circuitry.

Certain GABAergic neurons, when active, increase the probability to enter NREM sleep. The most well studied are the GABAergic projection neurons in the preoptic hypothalamus, which become active just before the entry into NREM sleep and release GABA onto wake-promoting histamine neurons and other ascending arousal pathways, such as the noradrenergic locus coeruleus neurons [30,34,35] (**Figure 2**). Rather than zolpidem working over the whole brain to produce sedation, we tested if it was sufficient for zolpidem to cause sedation by increasing inhibitory drive at the histamine neurons (**Figure 2**), thus mimicking the situation occurring at natural NREM

sleep onset. A custom pharmacogenetic experiment was designed. Mice harbouring a global knock-in F77I mutation of the GABA$_A$ receptor $\gamma2$ subunit have abolished zolpidem binding to their GABA$_A$ receptors [29]. The GABA$_A$ receptors still work normally, but zolpidem cannot induce NREM sleep in these mice [29]. Reintroduction of the wild-type and zolpidem-sensitive $\gamma2$ subunit selectively into the histamine neurons partially rescued zolpidem's ability to induce NREM sleep [29]. Consistently, optogenetic inhibition of histamine neurons also induces NREM sleep [36]. Therefore, zolpidem could in part induce sleep by enhancing GABA's actions on histamine neurons, so mimicking the natural mechanism for entry into NREM sleep (**Figure 2**). Enhancing inhibition onto histamine neurons is not the whole story. In addition to the preoptic to aminergic hub neurons, various other GABA pathways also induce NREM sleep or behavioural arrest when their activity is artificially increased optogenetically or pharmaco-genetically [37,38]. Zolpidem will be working on all these pathways simultaneously if they terminate with $\alpha1\beta\gamma2$, $\alpha2\beta\gamma2$ or $\alpha3\beta\gamma2$ GABA$_A$ receptors.

## 4. Orthosteric activation of GABA$_A$ receptors can induce sleep

Ten years ago there was excitement about a promising sleep-promoting drug, THIP (4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol, also known as Gaboxadol). THIP promoted NREM-like sleep in humans. This compound is not a GABA$_A$ receptor PAM, but an orthosteric agonist, muscimol is another example, docking at the GABA$_A$ receptor binding site and mimicking GABA's agonist actions [17]. In the forebrain, THIP activates extrasynaptic $\alpha1\beta2\delta$ or $\alpha4\beta2\delta$ GABA$_A$ receptors to increase the tonic conductance on *e.g.* thalamic relay neurons [2,39,40]; this membrane hyperpolarisation of thalamic relay neurons helps generate the delta oscillations in NREM sleep [41]. Prominent $\delta$ power in the EEG, however, does not necessarily mean sleep. In mice THIP and muscimol cause catalepsy, with high EEG $\delta$ power even though the mice are awake [2,40]; the drugs actually substantially delay NREM sleep onset [42]. Gaboxadol failed Phase III clinical trials – in humans it did not cause catalepsy, so there is a clear species difference with mice, but Gaboxadol had variable efficacy in inducing and maintaining sleep [43,44]. It is not clear why the efficacy was variable. An account by the journalist Hamilton Morris, who took Gaboxadol, gives the interesting background on the history of this drug's development (see *Harper's Magazine*, August 2013, available online). Some people given Gaboxadol reported

dizziness and nausea [44], possibly because the drug enhances tonic inhibition on cerebellar granule cells [45]. Gaboxadol has now been renamed OV101, and is being evaluated for treating Angleman and Fragile X syndromes. In Angleman syndrome tonic inhibition is reduced on granule cells, and elevating the tonic inhibition with Gaboxadol/OV101 could reduce the motor discoordination [46].

## 4.1 Tonic GABA co-released from histamine neurons acts as a break on wakefulness

Ironically, in spite of the clinical failure of THIP/Gaboxadol, $GABA_A$ receptors contributing to the tonic conductance are important for reducing the duration of wakefulness in mice [47]. Wake-promoting histamine neurons co-release GABA in the neocortex [47] (**Figure 2**). This GABA, broadcast non-synaptically from histaminergic axons, contributes to tonic extrasynaptic inhibition [47] (**Figure 2**). Reduction of GABA release from histamine axons (by knocking down the vesicular GABA transporter gene from histamine neurons) caused the mice to be more active in the "lights off"/night period. This could mean that the GABA is acting through the extrasynaptic $GABA_A$ receptors as a break on the intensity of wakefulness. Too much wakefulness can be part of mood disorders, such as bipolar disorder.

## 5. Waking up with PAMs: Can zolpidem promote arousal from altered states of consciousness?

When the brain is physically damaged, the balance between excitatory and inhibitory pathways can become maladjusted. $GABA_A$ receptor PAMs may provide a way to partially correct this. Based on case studies, zolpidem can improve the motor symptoms in Parkinson's disease, possibly by enhancing GABAergic transmission in basal ganglia pathways [48]. There are also some remarkable case studies reporting that zolpidem becomes wake-promoting in certain types of coma (chronic disorders of consciousness or minimally conscious states) [49,50]. This is not a common occurrence – most patients with chronic disorders of consciousness do not respond to zolpidem, and if they do, the effect lasts between one to four hours [50]. How could this work? Some GABAergic pathways are actually wake-promoting [34,51-53]. Selectively enhancing the GABAergic drive through these pathways with $GABA_A$ receptor PAMs could actually promote wakefulness. For example, a subset of lateral hypothalamic GABAergic neurons project to the GABAergic reticular thalamus

neurons [51]. Selectively activating these lateral hypothalamic GABAergic neurons optogentically produces wakefulness, and can even cause emergence from general anaesthesia [51]. Thus, depending on the type of damage associated with the coma, zolpidem might be able to selectively stimulate these pathways.

## 6. Waking up and sleeping with endozepines: Idiopathic hypersomnia.

Certain endogenous steroids are physiological GABA$_A$ receptor PAMs that induce sedation [9]. But there are other endogenous PAM molecules, termed endozepines. One of these is the peptide diazepam binding inhibitor (DBI), which is released from astrocytes to enhance GABA$_A$ receptor currents through $\alpha 3\beta\gamma 2$ type-GABA$_A$ receptors on reticular thalamic neurons [41,54,55]. By reducing GABA input onto thalamic relay neurons from the reticular neurons, DBI could hinder NREM sleep induction and promote arousal.

A rare number of people suffer from excessive daytime sleepiness of unknown cause (idiopathic hypersomnia). The cerebrospinal fluid (CSF) of some these patients contains an unknown GABA$_A$ receptor PAM, between 300 to 500 Da, that is inactivated by trypsin, suggesting it is a peptide [56]. The molecule enhances the action of GABA particularly at $\alpha 2\beta\gamma 2$ recombinant receptors [56]. Those hypersomnia patients who have the sleep-inducing CSF-inducing biomarker regain more normal wakefulness when treated with antagonists (flumazenil) of the benzodiazepine site [56,57], or with negative allosteric modulators of the GABA$_A$ receptor [58]. Identifying this endogenous GABA$_A$ receptor PAM may give insight into endogenous mechanisms controlling sleep.

## 8. Perspective

Sleep is universally craved. The market for medications remains huge [22]. But despite the success of zolpidem, and also the new orexin receptor antagonists, we still need better sleep medications. Most researchers working on new GABA$_A$ receptor drugs are indeed actively trying to remove the sedative properties of these drugs and concentrate on developing the analgesic, anxiogenic or cognition enhancing facets [6,59,60]. Nevertheless, it would certainly be ideal to have a drug that induces a

completely natural NREM sleep. Identifying novel endozepine-type molecules could be one strategy.

## Acknowledgements

**Figure legends**

**Figure 1. Comparing natural sleep with zolpidem-induced sleep and propofol-induced sedation.** Experiments illustrating the changes in muscle (EMG) and brain activity (EEG) that occur during natural sleep compared with the changes observed following injection of zolpidem or propofol into wild-type mice or rats. The plot with propofol illustrates recordings made at higher bandwidths directly from the cortex of rats using the local field potential (LFP), at the point when the animal lost its righting reflex (LORR). The oscillatory behaviour observed during propofol sedation generally occurs across the higher frequency domains. These data were re-drawn from Baker *et al* (2014)[25]. The data in the top panels were re-drawn from Uygen *et al* (2016) [29]. The heat maps illustrate the increase in power at the various frequency domains. The low frequency oscillations observed during NMREM sleep are also observed with zolpidem sedation in wild-type mice.
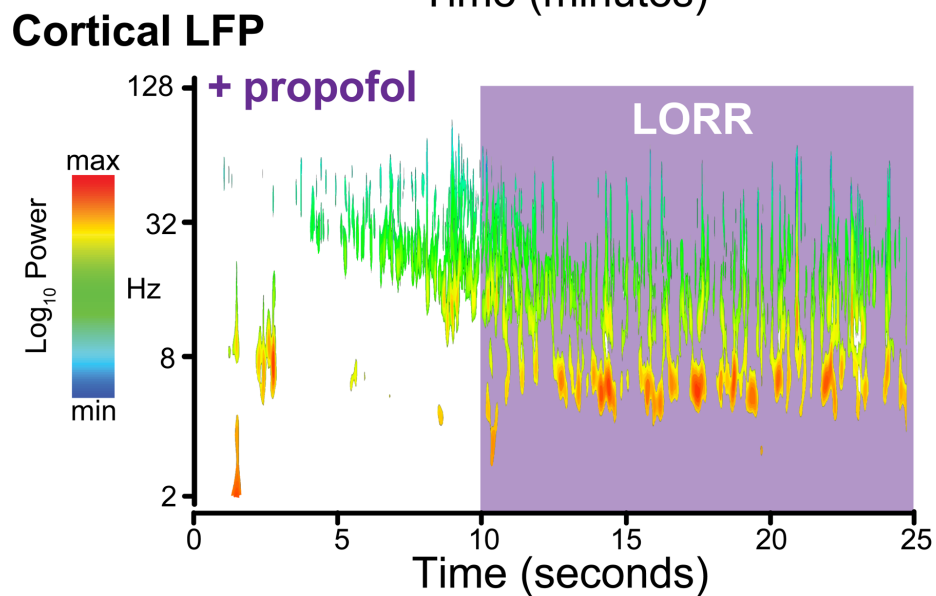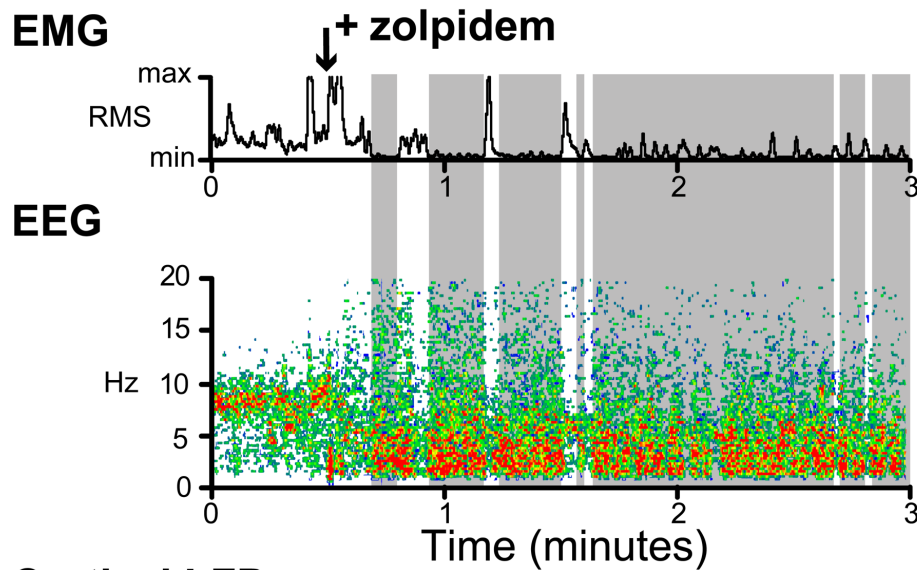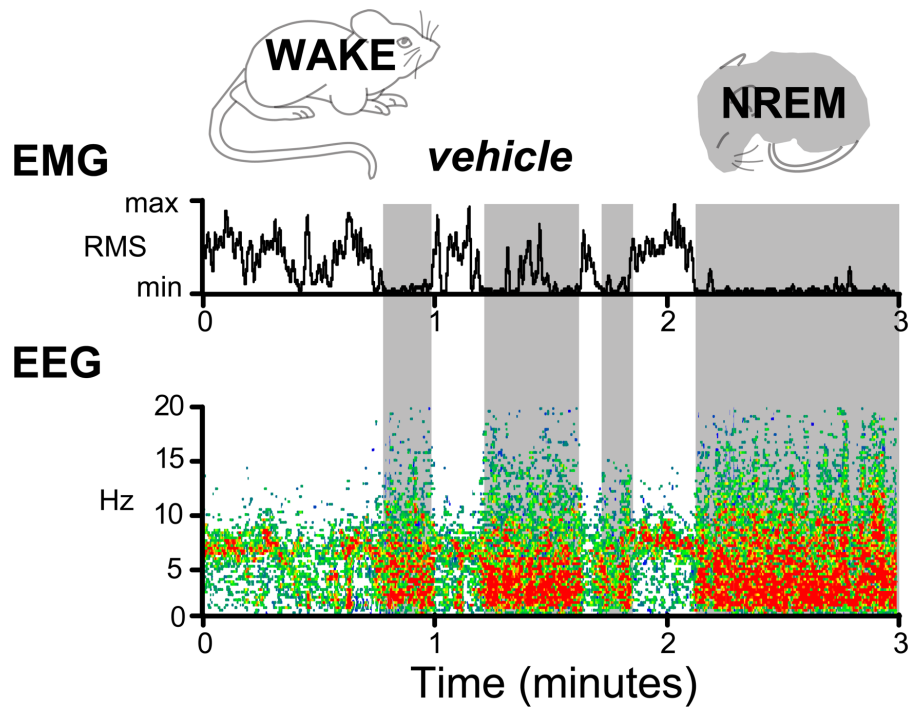
**Figure 2. Action of zolpidem on the histamine arousal pathway and GABA-histamine co-release in the neocrtex.** Histaminergic axons from the hypothalamic tuberomammillary nucleus (TMN) is responsible for GABA (red) and histamine (blue) co-release in the neocortex during wakefulness [47]. A drawing of a small region of the neocortex (Ctx) illustrates the location of synaptic (green) and extra-synaptic (red, $\delta$ subunit containing) GABA$_A$ receptor populations in the neocortex, the GABAergic terminals (orange) from local interneurons that release GABA onto the soma (axo-somatic synapses) and dendrites (axo-dendritic synapses) of glutamatergic pyramidal cells, the glutamatergic synapses (olive) excite local dendrites, the histamine axons arriving from the TMN (blue), co-releasing histamine and GABA. The blue oval illustrates histamine receptors that will respond to the histamine release (blue) from these terminals. Histamine and GABA co-release from histamine axons is not associated with synapses but involves volume transmission. The GABA released from these axons generates a tonic conductance by activating high-affinity extrasynaptic ($\delta$ subunit-containing) GABA$_A$ receptors [47]. During NREM sleep, histamine-GABA release from the TMN is absent because the histamine soma receive strong synaptic inhibition from GABAergic neurons originating from the preoptic hypothalamic area. The GABA works synaptically at $\alpha\beta\gamma2$-type GABA$_A$ receptors on the histamine neurons. A phasic inhibitory post-synaptic conductance change (IPSC) is shown (black trace) Zolpidem induces a NREM-like sleep, in part, by acting at these abg2 receptors to prolong IPSCs (purple) on these histamine neurons, thus mimicking the effect of what happens during natural NREM sleep when GABAergic drive onto histamine neurons increases [29].
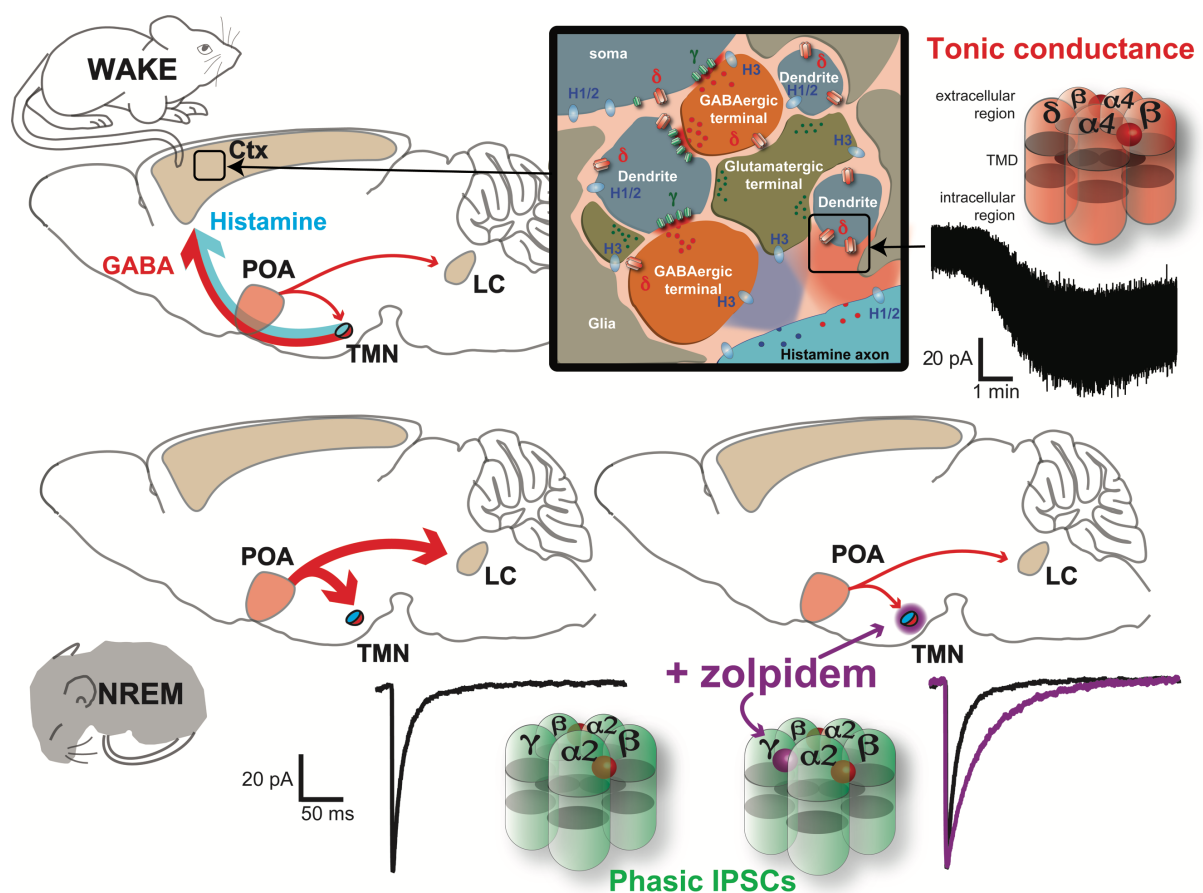
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