Circuits and components of delta wave regulation

David S. Uygun*,
Radhika Basheer
VA Boston Healthcare System and Harvard Medical School, Dept. of Psychiatry, West Roxbury, MA 02132, USA

Abstract
Sleep is vital and the deepest stages of sleep occur within Non-rapid-eye-movement (NREM) sleep, defined by high electroencephalographic power in the delta (~0.5–4 Hz) wave frequency range. Delta waves are thought to facilitate a myriad of physical and mental health functions. This review aims to comprehensively cover the historical and recent advances in the understanding of the mechanisms orchestrating NREM delta waves. We discuss a complete neurocircuit – focusing on one leg of the circuit at a time – and delve deeply into the molecular mechanistic components that contribute to NREM delta wave regulation. We also discuss the relatively localized nature in which these mechanisms have been defined, and how likely they might generalize across distinct sensory and higher order modalities in the brain.

Keywords
Sleep; Non-rapid-eye-movement sleep; Electroencephalography; Brain waves; Delta waves; Neurocircuitry

1. Introduction
An important aspect of sleep is the deep stage of Non-rapid-eye-movement (NREM) sleep, which is characterized by high levels (i.e. high power) of electroencephalographic (EEG) signals called delta waves. Stark boundaries for the upper and lower limits of delta across the literature is elusive, but for the purposes of this review, delta will mean any band ≥0.5 ≤4 Hz since we will cover reports that define delta differently and focus on sleep, sedation or anesthesia (Fernandez et al., 2017; Adamantidis et al., 2019; Fultz et al., 2019; Nuñez et al., 1992; Wright et al., 1986). However, narrower definitions, such as 1.5–4 Hz, may be a better definition for future studies, as we will explore below (Hubbard et al., 2020). Delta waves are implicated in many processes that benefit health, including synaptic homeostasis (Tononi and Cirelli, 2006), cellular energy regulation (Dworak et al., 2010; Brown et al., 2012), clearance of toxic proteins (Xie et al., 2013; Morawska et al., 2016), cognitive performance, mood (Tononi and Cirelli, 2006; Dworak et al., 2010; Xie et al., 2013; Dolsen et al., 2017) and neuronal plasticity (Seibt and Frank, 2019; Timofeev and Chauvette, 2017; Tononi...
Disorders like insomnia, traumatic brain injury, obstructive sleep apnea, and other neuropsychiatric conditions that are associated with interrupted or fragmented sleep reduce deep NREM and delta wave power (Stepanski et al., 1984; Ellis et al., 2012; Sands et al., 2018; Luz et al., 2016; Garbarino et al., 2016). Patients with these disorders exhibit impaired cognitive performance and mood (Stepanski et al., 1984; Ellis et al., 2012; Sands et al., 2018; Luz et al., 2016; Garbarino et al., 2016). For instance, up to 80% of insomnia patients also have co-morbid psychiatric symptoms (Nutt and Stahl, 2010), and sleep disturbance overlaps so much with post-traumatic stress disorder that it is considered a ‘Hallmark feature’ (Kanady et al., 2018), typically preceding a diagnosis (Lipinska et al., 2016).

We know sleep in general is good for us, but are NREM delta waves important? Reduced delta waves coincide with fragmented or interrupted sleep (Lu et al., 2000). This leads to excessive day-time sleepiness, which in turn is a main factor in traffic collisions and deficient work performance (Banerjee et al., 2004). Suppression of NREM delta waves by sleep medicines (Wright et al., 1986; Alexandre et al., 2008; Uygun et al., 2016) impairs memory (Gunja, 2013). This indicates that sleep (presumably enriched with delta waves) is good for memory consolidation, (Stickgold, 2013) conversely sleep deprivation impairs memory (Raven et al., 2018). Sleep also is good for mental health - insomnia is a predictor of suicide (Miller et al., 2019; Woznica et al., 2015) and reduced delta power is associated with suicidal ideation (Dolsen et al., 2017). Yet, treating insomnia with delta suppressing drugs (Alexandre et al., 2008; Uygun et al., 2016) can exacerbate or even cause suicide risk (McCall et al., 2017). A rather new hypothesis suggests that a major function of NREM is to clear harmful proteins out of the brain which accumulate during wakefulness. These toxic products lead to dementia (Xie et al., 2013) and are increased after traumatic brain injury (Morawska et al., 2016). Importantly, only delta, and no other waveforms of NREM, seems to be involved in their clearance (Xie et al., 2013; Morawska et al., 2016), which involves large waves of cerebrospinal fluid (CSF) that couple to NREM delta waves – in this case defined as 0.2–4 Hz (Fultz et al., 2019).

Taken together, high delta wave power, as found in natural and healthy NREM sleep, seems to be critical for memory, cognition, sleep maintenance and mental health. This provides a powerful impetus to understand delta wave regulation in the sleeping brain. We have compiled a review describing potential brain regions, essential neuronal properties needed to regulate oscillatory activity rendered by specific neurons and how these neurons within these brain regions contribute to establishing a circuity for generating and maintaining high delta power during sleep. Previous reviews give excellent overviews of delta wave mechanisms – devoting a section within a broader sleep (Brown et al., 2012) and anesthesia (Franks, 2008) context. This review aims to provide a highly focused yet comprehensive perspective on NREM delta waves. One review (McCormick and Bal, 1997) from the 1990s covers the electrophysiology regulating thalamocortical oscillations, including delta waves, in detail. Cellular, molecular and circuit level research of channels and receptors mediating these functions advanced prolifically during the 21st century. This review aims to provide an update. The structure of the review is such that each section of the main text centers on one major leg of a complete circuit. Within these circuits of cells, many molecular components provide the necessary working parts that allow the cells to encode the delta waves. These
components are complex in their own right. Each major component is discussed in its own “box” which is signposted in the main body of the review where it is mentioned in context.

2. Neocortex → Thalamus

The neocortex is the site at which EEG signals are recorded, its oscillatory behavior is paramount to defining sleep by studying delta waves and for studying the delta waves themselves. At the least, the neocortex provides an important leg of circuitry that contributes to maintaining delta waves. However, it probably is not the delta wave generator. This, however, should not be confused with another ‘slow’ wave, typically described as below 1 Hz (Steriade et al., 1993a, 1993b; Amzica and Steriade, 1995a, 1995b) – similar to the issue with the definition of delta, the exact boundaries defining the speed of this slow wave varies between publications (Steriade et al., 1993a, 1993c). Classical evidence suggests the cortex can self-regulate these approximately < 1 Hz slow waves and is therefore the site of origin (Steriade et al., 1993a; Timofeev et al., 2000). Isolated cortical slab preparations have shown intact slow waves consisting of spike independent mini excitatory and inhibitory post synaptic currents (Bazhenov et al., 2002). These likely reflect a mechanism that also occurs in the intact brain in vivo. The thalamus does not generate the < 1 Hz slow wave (Timofeev and Steriade, 1996), although it is also involved in sustaining it (David et al., 2013; Sheroziya and Timofeev, 2014). Unfortunately, adding more confusion, a common term in the field is ‘slow wave activity’ which is a broader band that is essentially equivalent to one of the broadest definitions of delta (i.e. 0.5–4 Hz), overlapping with the < 1 Hz slow wave (Brown et al., 2012; Narikiyo et al., 2020). Meanwhile, the terms ‘delta’ and ‘slow waves’ have also been used interchangeably (Gent et al., 2018). Nevertheless, it is important to understand the concept of a cortically driven slow wave at the lower end of the spectrum verses a thalamically driven delta wave at the higher (faster) end of the spectrum. A recent study demonstrated that neurons in the claustrum can coordinate cortical slow-wave activity (i.e. equivalent to delta; 0.5–4 Hz) (Narikiyo et al., 2020). But, additional work would be needed to separate the contribution of the claustrum to coordinating the < 1 Hz slow-wave vs faster delta waves. Neocortical inhibitory interneurons, which express either parvalbumin (PV) or somatostatin, gate the transitions from up and down states which form the slow waves (Zucca et al., 2017). Up states begin in cortical layer 5 (Beltramo et al., 2013). Recent work demonstrated that fast delta (termed delta 2) is homeostatically regulated whereas slower delta 1 and slow waves are not, suggesting different mechanistic circuits (Hubbard et al., 2020). A comprehensive review of slow waves is not the focus of this article, however the sometimes-poor distinction between slow waves and delta is important to acknowledge. Below we will consider the possible cortical source of delta waves. However, the evidence that delta (not slow waves) is generated mostly by TC relay neurons in the thalamus is clearer and more convincing, which we discuss in the next section TC Relay Neurons → Neocortex.

Two cortical layers, L5 and L6 send projections to higher and first order subcortical regions. All the neocortex→thalamus (CT) neurons are glutamatergic pyramidal neurons. Only the deepest layers of the cortex, L5 & L6 project out. L5-CT innervates higher order thalamic nuclei, the striatum, brain stem and hypothalamus (Harris and Mrsic-Flogel, 2013;
Feldmeyer, 2012; Chou et al., 2002). Interestingly, prefrontal layer 5 projects to the sleep promoting ventrolateral preoptic hypothalamus (Chou et al., 2002).

The L6-CT on the other hand projects preferentially to first order nuclei and synapses collaterals in TRN (Harris and Mrsic-Flogel, 2013; Mease et al., 2014; Landisman and Connors, 2007). L6-CT also drives inhibition of other cortical layers, via interneurons (Bortone et al., 2014) except layer 5, which it potentiates (Kim et al., 2014). The firing frequency of L6-CT neurons plays a role in whether TC-relay neurons are stimulated or suppressed. Optogenetic stimulation of L6-CT terminals enhanced spiking in the thalamus when entrained to fire at 10 Hz. However, when the same terminals were entrained to fire at 0.1 Hz, spikes were suppressed in the TC-relay neurons because excitation in the TRN by CT collaterals was more dominant at this frequency due to intrinsic burst properties of the TRN neurons, leading to a net inhibitory effect in the TC-relay nucleus (Crandall et al., 2015). While this is not direct evidence that the cortex produces delta waves, it suggests how slower cortical rhythms might become stabilized in NREM as the action potentials leaving the cortex signal positive feedback to keep TC-relay neurons hyperpolarized and in burst firing mode. Experiments revealing whether L6-CT optogenetic stimulation at delta frequencies would lead to net excitation or inhibition in TC-relay neurons would be interesting and may suggest a cortical source of delta regulation (as opposed to slow waves). Consistent with this idea, entraining the cortex to low frequency rhythms, by electrical stimulation, induced sleep in cats (Peñaloza-Rojas et al., 1964). Therefore, in principle delta waves can travel “top-down”, which may be important for NREM sleep stability. Delta waves (Kim et al., 2020) and sleep spindles (Kim et al., 2015) localize in parts of the neocortex and propagate around the neocortex. Sleep spindles are regulated by many of the same circuits as NREM delta waves albeit involving different ionic/molecular mechanisms (McCormick and Bal, 1997). The definition of spindles has also been somewhat inconsistent (Uygun et al., 2019). We do not cover sleep spindles in depth in this review, but we will mention them throughout, because they are another key EEG hallmark of NREM sleep. Using such EEG hallmarks has brought about our contemporary understanding that sleep does not necessarily occur exclusively at the behavioral level, but rather it can be localized to discreet areas of the cortex (Vyazovskiy et al., 2011; Vantomme et al., 2019; Fernandez et al., 2018). Notably however, the delta wave does not seem to originate within the cortex (Baker et al., 2014).

3. TC Relay Neurons → Neocortex

The search for the origin of delta has led researchers to look deeper in the brain. Though delta waves are recorded cortically, seminal papers have shown they originate in the thalamus, by self-oscillating “pacemaker” cells which send axons upwards to entrain the cortex. Feedback returning from the cortex likely synchronizes the TC neurons to produce field potentials which contribute to the EEG signal (Steriade et al., 1991; Dossi et al., 1992; Timofeev et al., 2001). These pacemaker cells are called thalamocortical (TC) relay neurons (Fig. 1) because they constitute the relay station where sensory information from the environment arrives in the thalamus (thalamo) and is then relayed to the cerebral cortex (cortical). These relay neurons are also crucially important in sleep. TC neurons generate
delta waves when they are in “burst firing” mode, which occurs when they are within an optimal hyperpolarized voltage range (McCormick and Pape, 1990).

This pattern of activity has been shown in the primary visual (dorsal lateral geniculate nucleus; dLGN) and auditory (medial geniculate nucleus; MGN) (McCormick and Pape, 1990) thalamus in cats and guinea pigs and the primary visual and somatosensory (ventrobasal; VB) in cats and rats (Leresche et al., 1991) when the TC neurons membrane potentials were in the ranges of – 83–75 mV (McCormick and Pape, 1990), – 80 mV-55 (Leresche et al., 1990) or below – 60 mV (Soltesz et al., 1991). Intracellular recordings in cat thalamic nuclei revealed that membrane potentials of – 65 mV corresponded to the onset of delta oscillations in the cortex (Steriade et al., 1991). While these optimal voltage ranges vary across studies, the range in a given cell is reportedly quite narrow at around 15 mV (Leresche et al., 1991). In the dLGN of the cat thalamus, TC relay neurons produce low-threshold spikes which pulse at around 2 Hz (within the delta range). These spikes are mediated by calcium ions. Crowning these calcium spikes are rapid bursts of action potentials which get propagated up to the cortex (McCormick and Pape, 1990; Leresche et al., 1990; Jahnsen and Llinás, 1984). The 2 Hz rhythm is set by a period between the calcium spikes which is ruled by interplay between the so called h-current, conferred by HCN channels (Box 1.), and low-threshold voltage-sensitive calcium channels (Box 2.). Membrane depolarization caused by the h-current brings the membrane to the threshold for ‘t-type’ calcium channels to open (Box 2.). The TC relay neurons are enriched with a subtype called CaV3.1 channels (Choi et al., 2015; Talley et al., 1999) and devoid of CaV3.2 or CaV3.3 (Talley et al., 1999; Pellegrini et al., 2016). Calcium spikes mediated by these channels further depolarizes the cell, bringing it to the action potential threshold (McCormick and Bal, 1997). Activation of the calcium channels is rapid but inactivation is slower for a more gradual decent back to more hyperpolarized voltages. This keeps the membrane potential around the action potential threshold for a brief period enabling the burst of action potentials to occur. Fast-deactivating voltage sensitive potassium channels that give neurons very brief refractory periods (Box 3.) enabling a rapid succession of action potentials. This constitutes the ‘burst’. Once enough of the calcium channels have inactivated and the membrane potential is no longer at the action potential threshold, the membrane potential shifts back toward a hyperpolarized potential which activates the h-current again – resetting the cycle. Thus, this oscillatory activity arises from the cells by virtue of their voltage sensitive ion channel repertoire.

Delta frequency (2 Hz) burst firing occurs in in vitro slice preparations of cat thalamus without any current steps or other experimental intervention (McCormick and Pape, 1990) and in vivo in decorticated thalamus (Timofeev et al., 2001). This has led to a general view that burst-firing is therefore the default state in these cells (Franks, 2008). This concept posits that when depolarizing inputs from wake areas fall silent, the cells drift into the hyperpolarized range where the h-current initiates. Pharmacological studies, on the other hand, have shown that the TC cells switch to burst-firing mode (Cope et al., 2005) when hyperpolarized via extra-synaptic GABA_A receptors (Box 4.) composed of α-4β6 subunits to generate delta waves in vivo (Mesbah-Oskui et al., 2014). Steriade’s group (Dossi et al., 1992) had postulated early that the TRN might hyperpolarize TC relay neurons to trigger
intrinsic burst firing after finding the TC relay neurons are chief generators of delta waves, however no data was available to support this at the time.

It is tempting to generalize these well described phenomena to the whole thalamus: i.e. that delta frequency intrinsic burst-firing activity is the mechanism of the underlying generator of cortical delta waves. However, it is probably an oversimplification of the mechanism. As mentioned, most of the work describing delta frequency burst firing was performed in either dLGN, MGN or VB, primary sensory areas. However, the thalamus is highly complex and organized, comprised of many distinct nuclei, forming distinct circuits. Somewhat less clear is how the other relay nuclei of the thalamus might be involved in delta wave generation. In principle, each relay nucleus forms part of a thalamocortical loop, projecting to a cortical region corresponding to the sensory modality. Yet not all thalamic nuclei do correspond to a specific sensory modality and burst firing has not been recorded in all thalamic nuclei. It stands to reason that the lack of data on this matter is entirely due to the technical difficulty of performing exhaustive records. Nevertheless, a fair surrogate for whether a relay nucleus might generate delta waves is the expression patterns of HCN2&4 (Box 1.) and this has been carefully mapped exhaustively in the rodent brain (Notomi and Shigemoto, 2004). Based on this, and the cellular recordings that have been made, it does not seem that all TC relay neurons likely play a role in delta wave generation.

NREM delta wave generation within the complex and functionally diverse structure that constitutes the thalamus needed further investigation. Steriade et al., (1991) associated thalamic bursting with cortical delta waves, recording thalamic activity from ventroanterior, ventrolateral and ventroposterior thalamic regions, dLGN and the centrolateral-paracentral intralaminar wing. Primary nuclei in which most of the burst firing whole cell recordings have been published do not project to frontal regions. Even some of the higher order thalamic nuclei only project directly with associative cortical regions, typically within posterior regions, parietal and occipital. This may seem surprising given that delta waves appear with the most power in anterior cortical regions (Kim et al., 2020; Choi et al., 2015; Massimini et al., 2004). So, which thalamic regions do project to the frontal cortex to promote delta waves?

One can start by considering the anatomical data that demonstrates which thalamic nuclei form ‘frontal’ thalamocortical loops. i.e. TC nuclei projecting to the frontal cortex. Next, consider which of those neurons are known to have the h-current, or at least expression of HCN2 and HCN4 channels, indicating they are pacemaker cells. Retrograde tracing from frontal cortical injections (anterior lateral motor cortex) revealed ipsilateral labeling in the ventral-medial (VM), ventral anterior-lateral (VAL), medio-dorsal (MD), posterior and intralaminar thalamic nuclei, with VM and VAL showing the strongest connections (Guo et al.). MD, VM or VAL all have rich expression of HCN2 and HCN4 (Notomi and Shigemoto, 2004). The MD shares strong reciprocal innervation with the prefrontal cortex (PFC) (Kuroda et al., 1998). Burst-firing has been recorded from MD in vivo. However, it was not clear whether the bursts recorded in this study were within the delta range as inclusion criteria for burst activity covered up to nearly 10 Hz (Copeland et al., 2015), and the MD-PFC loop has been implicated in theta (and other) oscillations encoding aspects of wakefulness such as attention and working memory (Parnaudeau et al., 2013). The
strongest evidence that MD is a delta wave generator comes from whole-cell recordings from slice-preparations showing h-currents within the MD neurons (Lee et al., 2012) and its rich expression of HCN2 and HCN4 (Notomi and Shigemoto, 2004). Additionally, CaV3.1 (Box 2.) knock-out (KO) reduces delta waves in LFPs of MD and frontal cortex in drug (ketamine and ethanol) induced loss of consciousness (Choi et al., 2015) although this does not indicate the MD is generating the delta in these records per se. The VM has a prominent h-current between −65 and 75 mV in rats (Paz et al., 2007). Interestingly however, it does not seem to faithfully produce a barrage of action potentials on the calcium spike (Cain and Snutch, 2013). Perhaps the profile of KV3 (Box 3.) channels in this thalamic nucleus do not enable the fast-firing required for a burst. KV3.2 (Box 3.) are native to the relay nuclei in mice, but their expression is enriched in dorsal regions rather than ventral (Kaczmarek and Zhang, 2017; Rudy et al., 1999). Distribution patterns in the mouse brain have not been reported with sufficient thalamic resolution to know for certain (Su et al., 2007). Thus, it is possible the h-current in VM is involved in membrane potential regulation rather than oscillatory burst firing for delta generation.

The most dorsal part of the midline thalamus is the paraventricular nucleus (PVT). Bursting activity from single unit records shows PVT activity coincides instantaneously with the rising phase of the delta wave. In some reports, the PVT does not show intense staining of HNC2 and HCN4 (Notomi and Shigemoto, 2004), whereas others suggest HCN4 is the dominant subunit followed by HCN2 (Kolaj et al., 2012) and it is the only midline thalamic nucleus for which there is clear evidence of the h-current (Kolaj et al., 2014) and intrinsic burst firing (Kolaj et al., 2012). To use a strict definition of delta frequency pace making, based solely on the concept classically described in the primary sensory nuclei, PVT may be the only midline nucleus performing this role. Moreover, the dorsal thalamus is rich in CaV3.1 (Box 2.) and KV3.2 (Box 3.) expression encoding the other key ionic regulators governing delta waves. Paradoxically, thalamic KO of CaV3.1 raised delta waves in one study (Anderson et al., 2005). However developmental compensation is difficult to circumvent with constitutive KO studies, and if CaV3.3 channels compensated the loss, less hyperpolarized membrane potentials may become sufficient to initiate burst firing (Cain and Snutch, 2010) and may create cells that more readily enter burst mode, thus potentially elevating delta waves. Notwithstanding, another CaV3.1 KO study revealed diminished delta (Lee et al., 2004). Inducible KO studies that ablate CaV3.1 in adulthood might be interesting to definitively test the role of thalamic CaV3 channels in delta wave regulation. Recent evidence suggests that other midline nuclei typically, described as non-sensory or higher order, generate delta waves in vivo (Gent et al., 2018). But, can it be generalized that this is driven by the same intrinsic cell properties found in the primary areas dLGN, MGN or VB? How exactly these non-sensory nuclei might be generating delta is less clear at the cellular level. Just beneath the PVT, the intralaminar medio-dorsal nucleus (IMD; unlike its neighboring bilateral MD), is lacking in support of its role as a pacemaker. Below this is the central medial thalamic nucleus (CMT), rhomboideus and the nucleus reuniens. In these three nuclei, bursting activity recorded in vivo precedes cortical up-states in the cingulate nucleus of the frontal cortex, especially the ventral parts of CMT, the whole rhomboideus and the dorsal parts of the nucleus reuniens. All showed the earliest onset of firing before the delta wave. Moreover, burst-like optogenetic stimulation of the CMT generated delta in

Brain Res Bull. Author manuscript; available in PMC 2022 November 22.
the frontal cortex, whereas tonic optogenetic activation of the CMT, but not the VB, led to rapid wakefulness. Other experiments looking at changes in delta power in the CMT vs the cingulate cortex suggested the CMT precedes changes in the cortex (Baker et al., 2014). However, these studies (Gent et al., 2018; Baker et al., 2014) do not definitively preclude the possibility that the waves began first in other parts of the frontal cortex where recordings were not performed, such as in the prefrontal cortex which can produce early up-states – important in < 1 Hz waves (Sheroziya and Timofeev, 2014).

As described so far, dLGN neurons show bursts interspersed with quiet periods cycling at 2 Hz coinciding instantaneously with the delta waves in cortex. In the study by Gent et al. (2018), burst firing frequencies from single unit records in CMT were anything up to nearly 20 Hz (inter-burst intervals of 50 ms). If these frequencies were conferred faithfully to the cortex as with primary nuclei, this would constitute theta, sigma and beta as well as delta. It is important to make the distinction of these frequency bands because bursting in the sigma range would suggest a mechanism of reciprocal innervation with the TRN which generates sleep spindles (Steriade et al., 1985; Astori et al., 2011). This is a distinct mechanism from the h-current mediated intrinsic bursting that drives delta waves (McCormick and Pape, 1990). Additionally, other data from whole cell recordings demonstrate a lack of, or very small, h-currents in CMT (Jhangiani-Jashanmal et al., 2016) or Nucleus Reuniens (Walsh et al., 2017), suggesting these nuclei are not made up of pacemaking neurons, at least in the classical sense. Further evidence that these two midline nuclei are not pacemaker cells is that neither show strong expression of HCN2 or HCN4 channels (Notomi and Shigemoto, 2004) which is also the case for the rhomboid nucleus. This is evidence that the primary thalamic relay nuclei might be maintaining delta oscillations within their respective thalamocortical loops, whereas the CMT and perhaps other midline nuclei regulate delta more globally, by cellular mechanisms that are distinct from those described in primary relay nuclei, and are also poised to interrupt cortical delta waves globally, corresponding to behavioral wakefulness. An interesting finding from the Gent et al. study was that the CMT confers the entrainment of delta directly to the frontal cortex, but to posterior cortical regions (barrel and visual) via the antero-dorsal thalamus. This may explain why the anterior thalamus does not intrinsically generate delta (Dossi et al., 1992) if its main role is to relay information from the CMT. There are also at least two subsets of neuron, based on electrophysiologic properties of neuronal types in the CMT (Jhangiani-Jashanmal et al., 2016), and even more diversity in the Reuniens (Walsh et al., 2017), but there is no data on whether these subtypes may be organized into anatomically defined circuitries or other modalities. An interesting future study might involve retrograde tracers injected into the frontal cortex, followed by recording of intrinsic neuronal properties in the retrograde labeled cells in the CMT, rhomboideus or the nucleus reuniens to show if there is specific subpopulation of frontal cortex projecting neurons that do exhibit burst firing activity and identification of the relay nucleus.

Convergent wake-promoting, and generally excitatory, signals likely provide a concerted effort to keep TC relay neurons out of bursting mode. A comprehensive discussion of these arousal circuits is beyond the scope of this article because they are mechanisms of interrupting delta waves, not generating or promoting them. Moreover, they are described in detail elsewhere (Brown et al., 2012; Franks, 2008). Briefly though, the thalamus receives
ascending aminergic inputs: noradrenaline from the locus coeruleus, dopamine from the dorsal Raphé and histamine from the tuberomammillary nuclei. Additionally, the thalamus receives ascending orexinergic inputs from the lateral hypothalamus (Scammell et al., 2017) and acetylcholine from the brainstem (Brown et al., 2012), and descending glutamatergic inputs from the neocortex (Franks, 2008), acting on glutamate receptors mGluR1 (Hong et al., 2016) and NMDA (Wenzel et al., 1997; Dunah et al., 1998). However, other afferents synapsing on TC relay neurons do play a role in promoting delta waves, as described in the following section.

4. Thalamic Reticular Nucleus → TC Relay neurons

Until recently, the TRN has been described as a somewhat homogeneous nucleus, consisting entirely of GABAergic neurons (Fig. 1), the vast majority of which being also PV positive and richly expressing CaV3.2/CaV3.3 (Pellegrini et al., 2016) & KV3.1/KV3.3 channels (Espinosa et al., 2008). However, more recently, evidence has delineated the TRN by cell-subtype and anatomical connectivity. Interestingly, despite expression of HCN2&4 (Notomi and Shigemoto, 2004), voltage clamp recording of TRN neurons in mice initially showed no H-current (Santoro et al., 2000), although it was later shown to appear under conditions in which potassium leak currents were blocked (Rateau and Ropert, 2006). NMDA blockade in TRN hyperpolarized cell membranes and caused delta frequency burst firing in TRN (Zhang et al., 2009b). However, this effect was not repeatable when it was attempted in another lab (Liu et al., 2019). Notwithstanding, other work has shown the TRN has an important modulatory role in promoting delta waves (Lewis et al., 2015). It likely does this by promoting conditions that enable the TC neurons to remain in burst firing mode (Lewis et al., 2015). This is supported by recent work from our labs which revealed that attenuation of synaptic inhibition onto PV positive TRN neurons, thus enhancing the GABAergic inhibitory drive from TRN to TC relay neurons, boosted NREM delta waves (Uygun et al., 2022).

While brief dynamic pulsing optogenetic stimulation protocols (20 ms or 1 s waxing and waning) have caused TRN activity to promote spindles (Halassa et al., 2011; Thankachan et al., 2019), tonic optogenetic stimulation (30 s) of TRN neurons rapidly invokes high delta wave power in the neocortex (Lewis et al., 2015). This, effect occurs via the TC neurons of course, as the TRN neurons do not directly project to cortex. During optogenetic stimulation of TRN, unit records of TC neurons showed a shift from a firing rate of ~10 to ~4–5 Hz, and then a shift back to ~10 Hz when the TRN stimulation ended (Lewis et al., 2015). Optogenetic stimulation of the GABAergic afferent terminals that inhibit the TRN interrupts NREM and wakes mice up (Thankachan et al., 2019; Herrera et al., 2016). Opposite and complimentary to this, optogenetic inhibition of the terminals, attenuating inhibition onto TRN (net effect: TRN excitation), leads to more NREM sleep and delta wave power (Herrera et al., 2016). The voltage gated potassium channels Kv3.1 and Kv3.3, which have rapid kinetics enabling cells with fast-firing capabilities (Box 4.) are enriched within the TRN. KV3.1 knock-outs and Kv3.1/Kv3.3 double knock-outs have reduced delta wave power (Espinosa et al., 2008; Joho et al., 1999), likely reflecting the reduced neurotransmitter output from the TRN neurons. Similarly, rats with ibotenic acid lesions in
TRN have diminished delta waves following recovery from surgery (Marini et al., 2000). Taken together, a persistently active TRN seems to promote NREM delta wave power.

More recently, a better understanding of the heterogeneity of the TRN is coming to light. Expression of genetic markers secreted phosphoprotein 1 (Spp1) and endothelin converting enzyme like-1 (Ecel1) seem to be mutually exclusive in the TRN, delineating two major subpopulations. The Spp1 + population approximates a TRN ‘core’ and corresponds to first order, primary sensory, thalamocortical circuits, while the Ecel1 + population approximates a TRN ‘shell’ which corresponds to higher order, non-sensory, nuclei (Li et al., 2020). Disrupting the trafficking of T-type channels (Box 2) in the TRN revealed a reduction of both delta and spindles, but only in the Spp1 +, first order, parts of the TRN (Li et al., 2020). Moreover, as tested in somatosensory loops vs MD↔PFC loop (Fernandez et al., 2018), TRN neurons that form part of primary sensory circuits play more of a role in spindle generation than non-sensory areas. CaV3.3 (Box 2) KO mice lost this separation of specialized circuit roles, suggesting CaV3.3 channels may by enriched in TRN neurons of primary sensory modalities (Fernandez et al., 2018). Therefore, it may be interesting to quantify the colocalization of CaV3.3 channels with Spp1 and Ecel1 (Li et al., 2020) in the TRN to support this. These data fit the notion that first-order thalamocortical loops are delta regulators and higher order loops are less so, as discussed in the section above on the TC relay nuclei. Thus, the TRN is an important regulator of localized sleep signatures, including delta waves, at the cortical level. However, Li et al., (2020) focused on disrupting T-type calcium channels. Though T-type calcium channels are important for delta in TC-relay neurons, and spindles in TRN, they may not be the sufficient mechanism of delta modulation by TRN, given the role for TRN in modulating delta seems to involve tonic excitatory tone. It may be that if the appropriate molecular target were perturbed, or if optogenetics or chemogenetics were used, both TRN subpopulations may affect delta. Interestingly, optogenetic stimulation of a single unilateral TRN location induced delta power in the cortex either locally or globally depending on how strongly the TRN neurons were stimulated (Lewis et al., 2015). The mechanism of this remains speculative, but it may occur via the strong connectivity throughout the TRN by electrical synapses, also called gap junctions, which are formed of connexin36 (Landisman et al., 2002; Zolnik and Connors, 2016). Perhaps the TRN is the lynchpin between local and global sleep regulation. Several other recent papers have described subpopulations of the TRN often based on anatomical circuitry and sometimes including information about genetic expression profiles (Fernandez et al., 2018; Li et al., 2020; Clemente-Perez et al., 2017; Halassa et al., 2014). These studies are building evidence for niche circuits traversing a highly organized TRN that regulate NREM sleep and sleep oscillations vs non-sleep related functions. Due in part by the historically well-known role for the TRN in spindles, papers often largely focus on spindles and overlook or do not report findings on delta. One such study dissected two TRN subpopulations in which neuronal spiking of sensory (visual modality) projecting neurons were positively correlated to spindle activity whereas anterior (non-sensory specific) were negatively correlated (Halassa et al., 2014). However, despite a new appreciation for the heterogeneity of the TRN, its cells seem to play a role in both spindles and delta wave generation depending on their membrane potentials (Fernandez et al., 2018).
Similar to the section on TC relay neurons, we will not comprehensively cover how delta is interrupted to promote wakefulness via the TRN, but briefly, evidence suggests it occurs by receiving GABAergic inputs from wake active areas: BF, LH (Thankachan et al., 2019; Herrera et al., 2016). As for afferents that might promote delta waves, the neocortex may contribute to stabilizing TRN activity by descending glutamatergic projection neurons acting upon GluN2C-NMDA receptors to bolster delta waves (Fernandez et al., 2017).

5. Conclusion

In this review, we covered what has become a well studied and characterized neurocircuit mechanism regulating NREM delta waves. We expanded on earlier reviews, to bring in more recent molecular findings, and to delve more deeply into defined nuclei (TRN, dLGN, MGN, VB, VM, VAL, MD, PVT, IMD, CMT, rhomboideus and nucleus reuniens of the thalamus.) within gross structures. We also explored some of the remaining open questions within this niche area of sleep research.

Acknowledgments

This work was supported by a VA Biomedical Laboratory Research and Development Service Merit Award I01 BX001404 (R.B.) & VA CDA IK2 BX004905 (D.S.U.); and NIH support from R01 NS119227 (R.B.). D. S.U. & R.B. are Research Health Scientists at VA Boston Healthcare System, West Roxbury, MA. The contents of this work do not represent the views of the US Department of Veterans Affairs or the United States Government.

Abbreviations:

- CMT: central medial thalamic nucleus
- CSF: cerebrospinal fluid
- CT: Corticothalamic
- dLGN: dorsal lateral geniculate nucleus
- EEG: Electroencephalogram/Electroencephalography/Electroencephalograhic
- HCN: Hyperpolarization-activated cyclic nucleotide-gated
- KO: knock-out
- MD: medio-dorsal
- MGN: medial geniculate nucleus
- NREM: non-rapid-eye-movement-sleep
- PFC: prefrontal cortex
- PV: Parvalbumin
- PVT: paraventricular nucleus of the thalamus
- TC: thalamocortical
TRN  Thalamic reticular nucleus
VAL  ventral anterior-lateral
VB   ventrobasal
VM   ventral-medial

References

Achermann P, Borbély AA, 1997. Low-frequency (< 1 hz) oscillations in the human sleep electroencephalogram. Neuroscience 81, 213–222. [PubMed: 9300413]
Adamantidis AR, Gutierrez Herrera C, Gent TC, 2019. Oscillating circuitries in the sleeping brain. Nat. Rev. Neurosci 20, 746–762. [PubMed: 31616106]
Alexandre C, et al. , 2008. Sleep-stabilizing effects of E-6199, compared to zopiclone, zolpidem and THIP in mice. Sleep 31, 259–270. [PubMed: 18274274]
Alldred MJ, 2005. Distinct 2 subunit domains mediate clustering and synaptic function of postsynaptic GABAA receptors and gephyrin. J. Neurosci 25, 594–603. [PubMed: 15659595]
Amzica F, Steriade M, 1995a. Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. J. Neurosci 15, 4658–4677. [PubMed: 7790931]
Amzica F, Steriade M, 1995b. Short- and long-range neuronal synchronization of the slow (<1 Hz) cortical oscillation. J. Neurophysiol 73, 20–38. [PubMed: 7714565]
Anderson MP, et al. , 2005. Thalamic Cav3.1 T-type Ca2+ channel plays a crucial role in stabilizing sleep. Proc. Natl. Acad. Sci. USA 102, 1743–1748. [PubMed: 15677322]
Astori S, et al. , 2011a. The Ca v3.3 calcium channel is the major sleep spindle pacemaker in thalamus. Proc. Natl. Acad. Sci. USA 108, 13823–13828. [PubMed: 21808016]
Baker R, et al. , 2014. Altered activity in the central medial thalamus precedes changes in the neocortex during transitions into both sleep and propofol anesthesia. J. Neurosci 34, 13326–13335. [PubMed: 25274812]
Banerjee D, Vitiello MV , Grunstein RR, 2004. Pharmacotherapy for excessive daytime sleepiness. Sleep Med. Rev 8, 339–354. [PubMed: 15336235]
Bazhenov M, Timofeev I, Steriade M, Sejnowski TJ, 2002. Model of thalamocortical slow-wave sleep oscillations and transitions to activated states. J. Neurosci 22, 8691–8704. [PubMed: 12351744]
Beltramo R, et al. , 2013. Layer-specific excitatory circuits differentially control recurrent network dynamics in the neocortex. Nat. Neurosci 16, 227–234. [PubMed: 23313909]
Biel M, Wahl-Schott C, Michalakis S, Zong X, 2009. Hyperpolarization-activated cation channels: From genes to function. Physiol. Rev 89.
Bortone DS, Olsen SR, Scanziani M, 2014. Translaminar inhibitory cells recruited by layer 6 corticothalamic neurons suppress visual cortex. Neuron 82, 474–485. [PubMed: 24656931]
Bright DP, Smart TG, 2013. Methods for recording and measuring tonic GABAA receptor-mediated inhibition. Front. Neural Circuits 7.
Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW, 2012. Control of sleep and wakefulness. Physiol. Rev 92, 1087–1187. [PubMed: 22811426]
Cain SM, Snutch TP, 2010. Contributions of T-type calcium channel isoforms to neuronal firing. Channels 4.
Cain SM, Snutch TP, 2013. T-type calcium channels in burst-firing, network synchrony, and epilepsy. Biochim. Biophys. Acta - Biomembr 1828, 1572–1578.
Catterall WA, 2011. Voltage-gated calcium channels. Cold Spring Harb. Perspect. Biol 3, 1–23.
Chemin J, et al. , 2002. Specific contribution of human T-type calcium channel isoforms (α1G, α1H and α1I) to neuronal excitability. J. Physiol 540 (3).
Choi S, Yu E, Lee S, Llinás RR, 2015. Altered thalamocortical rhythmicity and connectivity in mice lacking CaV3.1 T-type Ca2+ channels in unconsciousness. Proc. Natl. Acad. Sci. USA 112, 7839–7844. [PubMed: 26056284]
Chou TC, et al., 2002. Afferents to the ventrolateral preoptic nucleus. J. Neurosci 22, 977–990. [PubMed: 11826126]

Chow A, et al., 1999. K+ channel expression distinguishes subpopulations of parvalbumin- and somatostatin-containing neocortical interneurons. J. Neurosci 19, 9332–9345. [PubMed: 10531438]

Clemente-Perez A, et al., 2017. Distinct thalamic reticular cell types differentially modulate normal and pathological cortical rhythms. Cell Rep. 19, 2130–2142. [PubMed: 28591583]

Cope DW, Hughes SW, Crunelli V, 2005. GABAA receptor-mediated tonic inhibition in thalamic neurons. J. Neurosci 25, 11553. [PubMed: 16354913]

Copeland CS, Neale SA, Salt TE, 2015. Neuronal activity patterns in the mediadorsal thalamus and related cognitive circuits are modulated by metabotropic glutamate receptors. Neuropharmacology 92, 16. [PubMed: 25576798]

Crandall SR, Cruikshank SJ, Connors BW, 2015. A corticothalamic switch: controlling the thalamus with dynamic synapses. Neuron 86, 768–782. [PubMed: 25913856]

David F, et al., 2013. Essential thalamic contribution to slow waves of natural sleep. J. Neurosci 33, 19599–19610. [PubMed: 24336724]

Dolphin AC, 2016. Voltage-gated calcium channels and their auxiliary subunits: physiology and pathophysiology and pharmacology. J. Physiol. Neurosci 594, 19.

Dolsen EA, et al., 2017. Neurophysiological correlates of suicidal ideation in major depressive disorder: hyperarousal during sleep. J. Affect. Disord 212, 160–166. [PubMed: 28192765]

Dossi RC, Nuñez A, Steriade M, 1992. Electrophysiology of a slow (0.5–4 Hz) intrinsic oscillation of cat thalamocortical neurones in vivo. J. Physiol 447, 215–234. [PubMed: 1593448]

Dunah AW, Luo J, Wang YH, Yasuda RP, Wolfe BB, 1998. Subunit composition of N-methyl-D-aspartate receptors in the central nervous system that contain the NR2D subunit. J. Physiol 519, 16. [PubMed: 9495808]

Dworak M, McCarley RW, Kim T, Kalinychuk AV, Basheer R, 2010. Sleep and brain energy levels: ATP changes during sleep. J. Neurosci 30, 9007–9016. [PubMed: 20592221]

Ellis JG, Perlis ML, Neale LF, Espie CA, Bastien CH, 2012. The natural history of insomnia: focus on prevalence and incidence of acute insomnia. J. Psychiatr. Res 46, 1278–1285. [PubMed: 22800714]

Espinosa F, Marks G, Heintz N, Joho RH, 2004. Increased motor drive and sleep loss in mice lacking Kv3-type potassium channels. Genes Brain Behav. 3, 90–100. [PubMed: 15005717]

Espinosa F, Torres-Vega MA, Marks GA, Joho RH, 2008. Ablation of Kv3.1 and Kv3.3 potassium channels disrupts thalamocortical oscillations in vitro and in vivo. J. Neurosci 28, 5570–5581. [PubMed: 18495891]

Essrich C, Lorez M, Benson JA, Fritschy J-M, Lüscher B, 1998. Postsynaptic clustering of major GABA A receptor subtypes requires the γ2 subunit and gephyrin. Nat. Neurosci 17 (1), 563–571.

Farrant M, Nusser Z, 2005. Variations on an inhibitory theme: phasic and tonic activation of GABA A receptors. Nat. Rev. Neurosci 63 (6), 215–229.

Feldmeyer D, 2012. Excitatory neuronal connectivity in the barrel cortex. Front. Neuroanat 6.

Fernandez LM, et al., 2018. Thalamic reticular control of local sleep in mouse sensory cortex. Elife 7.

Fernandez LMJ, et al., 2017. Cortical afferents onto the nucleus Reticularis thalami promote plasticity of low-threshold excitability through GluN2C-NMDARs. Sci. Rep 7, 12271. [PubMed: 28947779]

Franks NP, 2008. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat. Rev. Neurosci 9, 370–386. [PubMed: 18425091]

Fultz NE, et al., 2019. Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. Science 366, 628–631. [PubMed: 31672896]

Garbarino S, Guglielmi O, Sanna A, Mancardi GL, Magnavita N, 2016. Risk of occupational accidents in workers with obstructive sleep apnea: systematic review and meta-analysis. Sleep 39, 1211–1218. [PubMed: 26951401]

Gent TC, Bandarabadi M, Herrera CG, Adamantidis AR, 2018. Thalamic dual control of sleep and wakefulness. Nat. Neurosci 21, 974–984. [PubMed: 29892048]
Ghoshal A, et al., 2020. Effects of a patient-derived de novo coding alteration of CACNA1I in mice connect a schizophrenia risk gene with sleep spindle deficits. Transl. Psychiatry 10, 1–12. [PubMed: 32066695]

Gunja N, 2013. In the Zzz zone: the effects of z-drugs on human performance and driving. J. Med. Toxicol 9, 163–171. [PubMed: 23456542]

Guo ZV et al. Maintenance of persistent activity in a frontal thalamocortical loop. Halassa MM, et al., 2011. Selective optical drive of thalamic reticular nucleus generates thalamic bursts & cortical spindles. Nat. Neurosci 14, 1118. [PubMed: 21785436]

Halassa MM, et al., 2014. State-dependent architecture of thalamic reticular subnetworks. Cell 158, 808–821. [PubMed: 25126786]

Harris KD, Mrsic-Flogel TD, 2013. Cortical connectivity and sensory coding. Nature 503, 51–58. [PubMed: 24201278]

Hering S, et al., 2018. Calcium channel gating. Pflug. Arch 470, 1291.

Herrera CG, et al., 2016. Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. Nat. Neurosci 19, 290–298. [PubMed: 26691833]

Hong J, et al., 2016. The thalamic mGluR1-PLCβ4 pathway is critical in sleep architecture. Mol. Brain 9, 1–12. [PubMed: 26739966]

Hörttagl H, et al., 2013. Patterns of mRNA and protein expression for 12 GABAA receptor subunits in the mouse brain. Neuroscience 236, 345–372. [PubMed: 23337532]

Hubbard J, et al., 2020. Rapid fast-delta decay following prolonged wakefulness marks a phase of wake-inertia in NREM sleep. Nat. Commun 11 (11), 1–16.

Jahnsen H, Llinás R, 1984. Voltage-dependent burst-to-tonic switching of thalamic cell activity: an in vitro study. Arch. Ital. Biol 122, 73–82. [PubMed: 6087765]

Jhangiani-Jashanmal IT, Yamamoto R, Gungor NZ, Paré D, 2016. Electroresponsive properties of rat central medial thalamic neurons. J. Neurophysiol 115, 1533–1541. [PubMed: 26763778]

Joho RH, Ho CS, Marks GA, 1999. Increased gamma- and decreased delta-oscillations in a mouse deficient for a potassium channel expressed in fast-spiking interneurons. J. Neurophysiol 82, 1855–1864. [PubMed: 10515974]

Joho RH, Marks GA, Espinosa F, 2006. Kv3 potassium channels control the duration of different arousal states by distinct stochastic and clock-like mechanisms. Eur. J. Neurosci 23, 1567–1574. [PubMed: 16553620]

Kaczmarek LK, Zhang Y, 2017. Kv3 channels: enablers of rapid firing, neurotransmitter release, and neuronal endurance. Physiol. Rev 97, 1431–1468. [PubMed: 28904001]

Kanady JC, et al., 2018. Cognitive behavioral therapy for insomnia reduces fear of sleep in individuals with posttraumatic stress disorder. J. Clin. Sleep Med 14, 1193–1203. [PubMed: 29991428]

Kim B, Hwang E, Strecker RE, Choi JH, Kim Y, 2020. Differential modulation of NREM sleep regulation and EEG topography by chronic sleep restriction in mice. Sci. Rep 101 (10), 1–13.

Kim D, Hwang E, Lee M, Sung H, Choi JH, 2015. Characterization of topographically specific sleep spindles in mice. Sleep 38, 85–96. [PubMed: 25325451]

Kim J, Matney CJ, Blankenship A, Hestrin S, Brown SP, 2014. Layer 6 corticothalamic neurons activate a cortical output layer, layer 5a. J. Neurosci 34, 9656–9664. [PubMed: 25031405]

Kolaj M, Zhang L, Rønnekleiv OK, Renaud LP, 2012. Midline thalamic paraventricular nucleus neurons display diurnal variation in resting membrane potentials, conductances, and firing patterns in vitro. J. Neurophysiol 107, 1835. [PubMed: 22219029]

Kolaj M, Zhang L, Hermes MLHJ, Renaud LP, 2014. Intrinsic properties and neuropharmacology of midline paraventricular thalamic nucleus neurons. Front. Behav. Neurosci 8.

Kopp C, Rudolph U, Tobler I, 2004. Sleep EEG changes after zolpidem in mice. Neuroreport 15, 2299–2302. [PubMed: 15371753]

Kovács K, Sik A, Ricketts C, Timofeev I, 2010. Subcellular distribution of low-voltage activated T-Type Ca2+ channel subunits (Cav3.1 and Cav3.3) in reticular thalamic neurons of the cat. J. Neurosci. Res 88, 448–460. [PubMed: 19774668]
Kuroda M, Yokofujita J, Murakami K. 1998. An ultrastructural study of the neural circuit between the prefrontal cortex and the mediodorsal nucleus of the thalamus. Prog. Neurobiol 54, 417–458. [PubMed: 9522395]

Landisman CE, et al. 2002. Electrical synapses in the thalamic reticular nucleus. J. Neurosci 22, 1002–1009. [PubMed: 11862128]

Landisman CE, Connors BW. 2007. VPM and PoM nuclei of the rat somatosensory thalamus: intrinsic neuronal properties and corticothalamic feedback. Cereb. Cortex 17, 2853–2865. [PubMed: 17389627]

Lau D, et al. 2000. Impaired fast-spiking, suppressed cortical inhibition, and increased susceptibility to seizures in mice lacking Kv3.2 K+ channel proteins. J. Neurosci 20, 9071. [PubMed: 11124984]

Lee J, et al. 2013. Sleep spindles are generated in the absence of T-type calcium channel-mediated low-threshold burst firing of thalamocortical neurons. Proc. Natl. Acad. Sci. USA 110, 20266–20271. [PubMed: 24282303]

Lee J, Kim D, Shin H-S. 2004. Lack of delta waves and sleep disturbances during non-rapid eye movement sleep in mice lacking alpha1G-subunit of T-type calcium channels. Proc. Natl. Acad. Sci. USA 101, 18195–18199. [PubMed: 15601764]

Lee S, et al. 2012. Bidirectional modulation of fear extinction by mediodorsal thalamic firing in mice. Nat. Neurosci 15, 308–314.

Leresche N, Jassik-Gerschenfeld D, Haby M, Soltesz I, Crunelli V. 1990. Pacemaker-like and other types of spontaneous membrane potential oscillations of thalamocortical cells. Neurosci. Lett 113, 72–77. [PubMed: 1973275]

Leresche N, Lightowler S, Soltesz I, Jassik-Gerschenfeld D, Crunelli V. 1991. Low-frequency oscillatory activities intrinsic to rat and cat thalamocortical cells. J. Physiol 441, 155–174. [PubMed: 1840071]

Lewis LD, et al. 2015. Thalamic reticular nucleus induces fast and local modulation of arousal state. Elife 4, e08760. [PubMed: 26460547]

Li Y, et al. 2020. Distinct subnetworks of the thalamic reticular nucleus. Nature 583, 819–824. [PubMed: 32699411]

Lipinska G, Baldwin DS, Thomas KGF. 2016. Pharmacology for sleep disturbance in PTSD. Hum. Psychopharmacol 31, 156–163. [PubMed: 26856810]

Liu J, Shelkar GP, Zhao F, Clausen RP, Dravid SM. 2019. Modulation of burst firing of neurons in nucleus reticularis of the thalamus by GluN2C-containing NMDA receptors. Mol. Pharmacol 96, 193–203.

Löw K, et al. 2000. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 290, 131–134. [PubMed: 11021797]

Lu J, Greco MA, Shiromani P, Saper CB. 2000. Effect of lesions of the ventrolateral preoptic nucleus on NREM and REM sleep. J. Neurosci 20, 3830–3842. [PubMed: 10804223]

Luz GP, et al. 2016. Impaired sustained attention and lapses are present in patients with mild obstructive sleep apnea. Sleep Breath. 20, 681–687. [PubMed: 26564169]

Marini G, Ceccarelli P, Mancia M. 2000. Effects of bilateral microinjections of ibotenic acid in the thalamic reticular nucleus on delta oscillations and sleep in freely-moving rats. J. Sleep Res 9, 359–366. [PubMed: 11132522]

Massimini M, Huber R, Ferrarelli F, Hill S, Tononi G. 2004. The sleep slow oscillation as a traveling wave. J. Neurosci 24, 6862–6870. [PubMed: 15295020]

McCall WV, et al. 2017. Hypnotic medications and suicide: risk, mechanisms, mitigation, and the FDA. Am. J. Psychiatry 174, 18–25. [PubMed: 27609243]

McCormick DA, Bal T. 1997. Sleep and arousal: thalamocortical mechanisms. Annu. Rev. Neurosci 20, 185–215. [PubMed: 9056712]

McCormick DA, Pape HC. 1990. Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. J. Physiol 431, 291–318. [PubMed: 1712843]

McKernan RM, et al. 2000. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABAA receptor α1 subtype. Nat. Neurosci 3, 587–592. [PubMed: 10816315]
Mease RA, Krieger P, Groh A, 2014. Cortical control of adaptation and sensory relay mode in the thalamus. Proc. Natl. Acad. Sci. USA 111, 6798–6803. [PubMed: 24748112]

Mesbah-Oskui L, Horner RL, Orser BA, Horner RL, 2014. Thalamic δ-subunit containing GABA A receptors promote electrocortical signatures of deep non-REM sleep but do not mediate the effects of etomidate at the thalamus in vivo. J. Neurosci 34, 12253–12266. [PubMed: 25209268]

Miller BJ, Parker CB, Rapaport MH, Buckley PF, McCall WV, 2019. Insomnia and suicidal ideation in nonaffective psychosis. Sleep 42.

Moosmang S, Biel M, Hofmann F, Ludwig A, 1999. Differential distribution of four hyperpolarization-activated cation channels in mouse brain. Biol. Chem 380.

Moraw ska MM, et al., 2016. Sleep modulation alleviates axonal damage and cognitive decline after rodent traumatic brain injury. J. Neurosci 36, 3422–3429. [PubMed: 27013672]

Moreno H, et al., 1995. Thalamocortical projections have a K+ channel that is phosphorylated and modulated by cAMP-dependent protein kinase. J. Neurosci 15, 5486–5501. [PubMed: 7643197]

Narikiyo K, et al., 2020. The claustrum coordinates cortical slow-wave activity. Nat. Neurosci 23, 741–753. [PubMed: 32393895]

Notomi T, Shigemoto R, 2004. Immunohistochemical localization of Ih channel subunits, HCN1–4, in the rat brain. J. Comp. Neurol 471.

Nuñez A, Amzica F, Steriade M, 1992. Intrinsic and synaptically generated delta (1–4 Hz) rhythms in dorsal lateral geniculate neurons and their modulation by light-induced fast (30–70 Hz) events. Neuroscience 51, 269–284. [PubMed: 1465192]

Nusser Z, Sieghart W, Somogyi P, 1998. Segregation of different GABA A receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J. Neurosci 18, 1693–1703. [PubMed: 9464994]

Nutt DJ, Stahl SM, 2010. Searching for perfect sleep: the continuing evolution of GABA A receptor modulators as hypnotics. J. Psychopharmacol 24, 1601–1612. [PubMed: 19942638]

Olsen RW, Sieghart W, 2009. GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56, 141–148. [PubMed: 18760291]

Pape HC, 1996. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. Annu. Rev. Physiol 58.

Parnaudeau S et al. Inhibition of medio-dorsal thalamus disrupts thalamo-frontal connectivity and cognition. (2013) doi:10.1016/j.neuron.2013.01.038.

Pauls TL, Cox JA, Berchtold MW, 1996. The Ca2+–binding proteins parvalbumin and oncomodulin and their genes: new structural and functional. Biochim. Biophys. Acta - Gene Struct. Expr 1306, 39–54.

Paz JT, Chavez M, Saïllet S, Deniau J-M, Charpier S, 2007. Activity of ventral medial thalamic neurons during absence seizures and modulation of cortical paroxysms by the nigrothalamic pathway. J. Neurosci 27, 929–941. [PubMed: 17251435]

Pellegrini C, Lecci S, Lüthi A, Astori S, 2016. Suppression of sleep spindle rhythmogenesis in mice with deletion of CaV3.2 and CaV3.3 T-type Ca2+ channels. Sleep 39, 875–885. [PubMed: 26612388]

Peñaloza-Rojas JH, Elterman M, Olmos N, 1964. Sleep induced by cortical stimulation. Exp. Neurol 10, 140–147. [PubMed: 14206370]

Porcello DM, Ho CS, Joho RH, Huguenard JR, 2002. Resilient RTN fast spiking in Kv3.1 Null mice suggests redundancy in the action potential repolarization mechanism. J. Neurophysiol 87, 1303–1310. [PubMed: 11877504]

Ralvenius WT, Benke D, Acuña MA, Rudolph U, Zeilhofer HU, 2015. Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABA A receptor subtype. Nat. Commun 6, 6803. [PubMed: 25865415]

Rateau Y, Ropert N, 2006. Expression of a functional hyperpolarization-activated current (Ih) in the mouse nucleus reticularis thalami. J. Neurophysiol 95, 3073–3085. [PubMed: 16617177]

Raven F, Van der Zee EA, Meerlo P, Havekes R, 2018. The role of sleep in regulating structural plasticity and synaptic strength: implications for memory and cognitive function. Sleep Med. Rev 39, 3–11. [PubMed: 28641933]
Rudolph U, Knoflach F, 2011. Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. Nat. Rev. Drug Discov 10, 685–697. [PubMed: 21799515]

Rudy B, et al. , 1999. Contributions of Kv3 channels to neuronal excitability. Ann. N. Y. Acad. Sci 868, 304–343. [PubMed: 10414303]

Rudy B, McBain CJ, 2001. Kv3 channels: voltage-gated K+ channels designed for high-frequency repetitive firing. Trends Neurosci. 24, 517–526. [PubMed: 11506885]

Sands SA, et al. , 2018. Quantifying the arousal threshold using polysomnography in obstructive sleep apnea. Sleep 41, 1–9.

Santoro B, et al. , 2000. Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. J. Neurosci 20.

Santoro B, Tibbs GR, 1999. The HCN gene family: molecular basis of the hyperpolarization-activated pacemaker channels. Ann. N. Y. Acad. Sci 868.

Scammell TE, Arrigoni E, Lipton JO, 2017. Neural circuitry of wakefulness and sleep. Neuron 93, 747–765. [PubMed: 28231463]

Schweizer C, et al. , 2003. The γ2 subunit of GABAA receptors is required for maintenance of receptors at mature synapses. Mol. Cell. Neurosci 24, 442–450. [PubMed: 14572465]

Seibt J, Frank MG, 2019. Primed to sleep: the dynamics of synaptic plasticity across brain states. Front. Syst. Neurosci 13, 2. [PubMed: 30774586]

Sheroziya M, Timofeev I, 2014. Global intracellular slow-wave dynamics of the thalamocortical system. J. Neurosci 34, 8875–8893. [PubMed: 24966387]

Sigel E, Steinmann ME, 2012. Structure, function, and modulation of GABAA receptors. J. Biol. Chem 287, 40224–40231. [PubMed: 23038269]

Soltesz I, et al. , 1991. Two inward currents and the transformation of low-frequency oscillations of rat and cat thalamocortical cells. J. Physiol 441, 175–197. [PubMed: 1667974]

Sperk G, et al. , 2020. Immunohistochemical distribution of 10 GABAA receptor subunits in the forebrain of the rhesus monkey Macaca mulatta. J. Comp. Neurol 528, 2551–2568. [PubMed: 32220012]

Stamenic TT, et al. , 2021. The T-type calcium channel isoform Cav3.1 is a target for the hypnotic effect of the anaesthetic neurosteroid (3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile. Br. J. Anaesth 126, 245. [PubMed: 32859366]

Stepanski E, Lamphere J, Badia P, Zorick F, Roth T, 1984. Sleep fragmentation and daytime sleepiness. Sleep 7, 18–26. [PubMed: 6718922]

Steriade M, Deschenes M, Domic L, Mulle C, 1985. Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. J. Neurophysiol 54, 1473–1497. [PubMed: 4087044]

Steriade M, Dossi RC, Nunez A, 1991. Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortically induced synchronziation and brainstem cholinergic suppression. J. Neurosci 11, 3200–3217. [PubMed: 1941080]

Steriade M, Nunez A, Amzica F, 1993. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. Soc. Neurosci 73, 3252–3285.

Steriade M, Nunez A, Amzica F, 1993a. Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J. Neurosci 13, 3266–3283. [PubMed: 8340807]

Steriade M, Contreras D, Dossi RC, Nunez A, 1993b. The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: Scenario of sleep rhythm generation in interacting thalamic and neocortical networks. J. Neurosci 13, 3284–3299. [PubMed: 8340808]

Stickgold R, 2013. Parsing the role of sleep in memory processing. Curr. Opin. Neurobiol 23, 847–853. [PubMed: 23618558]

Su YC, et al. , 2007. Distribution of Kv3.3 potassium channel subunits in distinct neuronal populations of mouse brain. J. Comp. Neurol 502, 953–972. [PubMed: 17444489]

Talley EM, et al. , 1999. Differential distribution of three members of a gene family encoding low voltage-activated (t-type) calcium channels. J. Neurosci 19, 1895–1911. [PubMed: 10066243]
Thankachan S, et al., 2019. Thalamic reticular nucleus parvalbumin neurons regulate sleep spindles and electrophysiological aspects of schizophrenia in mice. Sci. Rep 9, 1–16. [PubMed: 30626917]

Timofeev I, Chauvette S, 2017. Sleep slow oscillation and plasticity. Curr. Opin. Neurobiol 44, 116–126. [PubMed: 28453998]

Timofeev I, Steriade M, 1996. Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. J. Neurophysiol 76, 4152–4168. [PubMed: 8985908]

Timofeev I, Grenier F, Bazhenov M, Sejnowski TJ, Steriade M, 2000a. Origin of slow cortical oscillations in deafferented cortical slabs. Cereb. Cortex 10, 1185–1199. [PubMed: 11073868]

Timofeev I, Bazhenov M, Sejnowski TJ, Steriade M, 2001. Contribution of intrinsic and synaptic factors in the desynchronization of thalamic oscillatory activity. Thalamus Relat. Syst 1, 53–69.

Tononi G, Cirelli C, 2006. Sleep function and synaptic homeostasis. Sleep Med. Rev 10, 49–62. [PubMed: 16376591]

Tononi G, Cirelli C, 2014. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. Neuron 81, 12–34. [PubMed: 24417297]

Uygun DS, et al., 2016. Bottom-up versus top-down induction of sleep by zolpidem acting on histaminergic and neocortex neurons. J. Neurosci 36, 11171–11184. [PubMed: 27807161]

Uygun DS, et al., 2019. Validation of an automated sleep spindle detection method for mouse electroencephalography. Sleep 42, 1–13.

Uygun DS, et al., 2022. Knockdown of GABAA alpha3 subunits on thalamic reticular neurons enhances deep sleep in mice. Nat. Commun 13, 2246. [PubMed: 35473906]

Vantomme G, Osorio-Forero A, Lüthi A, Fernandez LMJ, 2019a. Regulation of local sleep by the thalamic reticular nucleus. Front. Neurosci 13, 576. [PubMed: 31231186]

Vyazovskiy VV, et al., 2002. Sleep EEG in mice that are deficient in the potassium channel subunit K.v.3.2. Brain Res. 947, 204–211. [PubMed: 12176162]

Vyazovskiy VV, et al., 2011. Local sleep in awake rats. Nature 472, 443–447. [PubMed: 21525926]

Walsh DA, Brown JT, Randall AD, 2017. In vitro characterization of cell-level neurophysiological diversity in the rostral nucleus reuniens of adult mice. J. Physiol 595, 3549. [PubMed: 28295330]

Wenzel A, Fritschy JM, Mohler H, Benke D, 1997. NMDA receptor heterogeneity during postnatal development of the rat brain: differential expression of the NR2A, NR2B, and NR2C subunit proteins. J. Neurochem 68, 469–478. [PubMed: 9003031]

Winsky-Sommerer R, et al., 2008. Normal sleep homeostasis and lack of epilepsy phenotype in GABAA receptor alpha3 subunit-knockout mice. Neuroscience. 10.1016/j.neuroscience.2008.03.081.

Woznica AA, Carney CE, Kuo JR, Moss TG, 2015. The insomnia and suicide link: toward an enhanced understanding of this relationship. Sleep Med. Rev 22, 37–46. [PubMed: 25454672]

Wright NA, Belyavin A, Borland RG, Nicholson AN, 1986a. Modulation of delta activity by hypnotics in middle-aged subjects: studies with a benzodiazepine (flurazepam) and a cyclopyrrolone (zopiclone). Sleep 9, 348–352. [PubMed: 3507374]

Xie L, et al., 2013. Sleep drives metabolite clearance from the adult brain. Science 342, 373–377. [PubMed: 24136970]

Zhang Q, Huang A, Lin YC, Yu HG, 2009a. Associated changes in HCN2 and HCN4 transcripts and If pacemaker current in myocytes. Biochim. Biophys. Acta - Biomembr 1788.

Zhang Y, Linas RR, Lisman JE, 2009b. Inhibition of NMDARs in the nucleus reticularis of the thalamus produces delta frequency bursting. Front. Neural Circuits 3.

Zolnik TA, Connors BW, 2016. Electrical synapses and the development of inhibitory circuits in the thalamus. J. Physiol 594, 2579–2592. [PubMed: 26864476]

Zucca S, et al., 2017. An inhibitory gate for state transition in cortex. Elife 6.
Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are key molecular mediators of delta waves. The expression of select HCN channel types in a neuronal population provides the neurons with a crucial component of the mechanism to exhibit a pattern of action potential firing termed ‘burst firing’. Unlike tonic firing, in which a neuron will fire action potentials non-rhythmically, burst-firing is characterized by a rapid succession of action potentials followed by a temporally precise period of quiescence – before releasing another rapid succession of action-potentials (McCormick and Pape, 1990). HCN channels enable cells to autonomously exhibit this pattern of rhythmic firing without requiring synaptic inputs to maintain the rhythm. The kinetics of the channel dictates the frequency of the rhythm. This works as follows. Aptly named HCN channels are activated (meaning opened, enabling the membrane to become permeable to certain ions) when the cell is more negatively charged than its resting membrane potential. Hyperpolarization immediately following an action potential activates the HCN channels, this hyperpolarized voltage opens the channels, ion flow provides an electrical current which depolarizes the cell – counteracting the hyperpolarized voltage which started the process. Eventually, this leads to new action potentials which cause another repetition of the cycle. Importantly, the duration from HCN channel activation to the action potential is precise – dictated by the HCN channel kinetics – setting the frequency (i.e. cycles per second; Hz) of burst firing. This HCN channel mediated depolarizing current is commonly annotated as \( I_h \) for ‘hyperpolarization activated current (I)’ and is commonly termed the H-current. HCN channels are localized throughout the nervous system and in the heart. Although certain subtypes are virtually ubiquitous, others express selectively within certain cellular species to perform specialized roles. Four types of HCN channels exist and are named HCN1–4 (Santoro and Tibbs, 1999). Different isoforms have different kinetics (Santoro et al., 2000), distributions (Moosmang et al., 1999) and functions (Pape, 1996). Select types provide their native cells with intrinsic burst firing properties. HCN2 & HCN4 seem to the key subtypes involved in delta frequency burst firing. Although HCN2 is expressed ubiquitously in the brain, one of the regions with the most enrichment is in the thalamus. Meanwhile HCN4 is restricted to few areas including the thalamus (Notomi and Shigemoto, 2004). Remaining unclear is whether delta oscillatory networks (or other systems) co-express HCN2 homomers and HCN4 homomers or express HCN2/4 heteromers (Biel et al., 2009). Some indirect evidence comparing electrophysiological properties in expression systems with HCN2 and HCN4 (Xenopus oocytes) to TC relay neurons support the idea that the two subtypes form homomers which are co-expressed (Santoro et al., 2000). However, better clarification is needed in order to search for druggable targets on these molecules, to pharmacologically promote delta oscillations. One potential way in which these could be targeted is by development of a drug that would modulate the voltage sensitivity of the channel to more depolarized membrane potentials, which may theoretically induce or better maintain deep NREM sleep by enabling burst-firing in pacemaker cells even when wake promoting inputs are still relatively active. However, this would have to be a highly...
selective compound to be safe since the channels are expressed in many peripheral tissues including the heart.

Recently, the different frequency bands have further characterized ‘subtypes’ of delta waves suggesting faster delta is homeostatically regulated in mice, and is therefore for sensitive to sleep debt, whereas slower delta is not (Hubbard et al., 2020). In humans, brain waves < 1 Hz do not attenuate over the sleep period, as sleep pressure presumably dissipates, whereas faster delta does (Achermann and Borbély, 1997). Some evidence from oocytes and rat myocytes suggests that the ratio of HCN2:HCN4 plays an important role in kinetics (Zhang et al., 2009a). Different ratios within distinct TC relay nuclei may underlie these newly detailed ‘subtypes’ of delta waves.
## Box 2

### CaV3 channels.

Voltage gated calcium channels are widespread in mammalian tissues performing many functions from muscle contraction to hormone secretion to electrophysiologic rhythmicity in the heart and brain (Catterall et al., 2011 & Cain & Snutch, 2013). CaV channels are made of multiple subunits – the α1 subunit forms the pore whose structure selectively permits calcium permeability. 10 known α subunit isoforms provide specific voltage sensitivities and kinetics, and thus distinct functions. The α subunit is made of four pore forming domains, each with six transmembrane segments and intracellular N and C termini. On each of the four domains, the fourth transmembrane segment constitutes the voltage sensor – shifting out and rotating in response to changing voltage and initiating a conformational change to open the channel pore (Catterall, 2011; Hering et al., 2018). β, α2 − δ and γ subunits also make up the full channel protein complex – these also modulate kinetics and help localize the channels (Dolphin, 2016). So called T-type CaV channels are named because their spikes are transient. T-Type calcium channels are termed CaV3 and three subtypes are known: CaV3.1, CaV3.2 and CaV3.3 (Cain and Snutch, 2013). A defining aspect of T-type CaV channels is that their thresholds – the voltage at which the channel opens – are low, more negative/hyperpolarized, compared with the action potential threshold (Unlike L-type whose thresholds are less negative, closer to the action potential). T-type calcium channels are important for two key brainwaves of NREM sleep, spindles and delta waves. The ‘transient’ calcium spike mediated by T-type CaV channels follows hyperpolarization, because hyperpolarization “de-inactivates” (readies) the CaV channels, once de-inactivated, they are poised to react to membrane depolarization where they open and further depolarize the cell. This small calcium spike brings the cell to the action potential, but only briefly, enabling a transient opportunity for action potentials to fire. Then the channel inactivates and the membrane hyperpolarizes again. CaV channel isoforms are diverse with highly organized stoichiometry, delineating anatomical regions. CaV3 channels are especially implicated in rhythmicity of NREM brainwaves (Cain and Snutch, 2010, 2013). Based on in vivo KO studies, CaV3.1 channels are important in delta wave regulation in both physiological and drug-induced NREM sleep (Choi et al., 2015; Lee et al., 2004; Stamenic et al., 2021) and they also play a role in sleep stability (Anderson et al., 2005) which is likely one of the functions of delta waves. CaV3.1&3.2 have roughly 10 mV lower thresholds than CaV3.3 in vitro (Chemin et al., 2002) which is necessary for generating sleep spindles rather than delta (Thankachan et al., 2019; Ghoshal et al., 2020). In the thalamus, CaV3.1 channels localize in the soma and dendrites and CaV3.3 localize in soma of cat TRN neurons (Kovács et al., 2010). Whereas, CaV3.2 and CaV3.3 express in the TRN of mice (Talley et al., 1999; Pellegrini et al., 2016). CaV3.1 are found in the TC relay neurons in mice (Talley et al., 1999; Lee et al., 2013).
Box 3

**KV3 channels.**

In an action potential, the refractory period is determined by how rapidly voltage sensitive potassium channels deactivate (close). It is the length of the refractory period that dictates how long the interval between subsequent action potentials can be. Voltage sensitive potassium channel subtypes are diverse, and some are selectively native to cell-types. Of all potassium channels in mammals, KV3 have the fastest kinetics, providing the cells on which they are expressed with fast-firing capabilities (Kaczmarek and Zhang, 2017; Rudy and McBain, 2001). KV3 channels are tetramers – four subunits bundle to form a central pore. Each subunit is made of six transmembrane domains with internal N and C termini. KV3.3 and KV3.4 also have N-terminal ball structures to enable rapid inactivation (channel self-blocking) in response to sustained depolarization. The ability to repeatedly fire action potentials in rapid succession a key mechanism in burst-firing. The orchestrated brief rapid bursts cresting upon small transient calcium spikes, which in turn are rhythmically timed by the h-current, are the key molecular components regulating delta rhythmicity within a cell. Although fast firing neurons express PV, a small calcium binding protein, parvalbumin itself is not the mechanism that gives rise to fast firing (Rudy and McBain, 2001) – instead it has been suggested to be a protective buffer to the cell (Pauls et al., 1996). KV3.1 and KV3.3 is typically co-expressed with PV (Espinosa et al., 2008) and KV3.2 is also expressed in fast-firing neurons (Lau et al., 2000). Global KV3.1/KV3.3 double KO mice had sleep loss (Espinosa et al., 2004) via many short or unstable NREM bouts. i.e. sleep fragmentation (Joho et al., 2006) and an approximate 70% reduction in 1–4 Hz delta power in NREM sleep (Espinosa et al., 2008). Moreover, rebound sleep after sleep deprivation, which normally presents with heightened delta power, did not show increased delta in the KO mice. KV3.1 single KOs also had reduced delta power (Joho et al., 1999). Milder effects in single KOs of each channel have indicated that these two channels are functionally interchangeable, leading some researchers to describe the occurrence of KV3.1 & KV3.3 to be evolutionarily redundant (Porcello et al., 2002). However, at the molecular level, clear distinctions exist in their mechanisms (Kaczmarek and Zhang, 2017). It is likely that they are simply similar enough to compensate for one another if one is lost. Kv3.2 channels are widespread including TC relay nuclei (Moreno et al., 1995), however the TRN is devoid of them (Chow et al., 1999). KV3.2 KOs do not survive to adulthood, so these channels are vital. EEG analysis in young surviving KV3.2 KO mice showed a reduction in power in faster delta frequencies overlapping with theta (3.25–6 Hz) and this was in both NREM and REM. Moreover, rebound sleep did not have an altered EEG profile (Vyazovskiy et al., 2002).
Box 4

**GABA<sub>A</sub> receptors.**

Given that these specialized voltage sensitive channels (HCN2/4, CaV3, KV3) orchestrate within intrinsically oscillating pace-maker cells the next level to consider is the modulatory efforts by other receptors to promote or attenuate the generation of burst-firing. Ih requires a hyperpolarized membrane potential of an optimal range (see section on TC relay nuclei). Only when pacemaker neurons are hyperpolarized are they in “burst-firing mode”. Therefore, hyperpolarizing inhibitory inputs are important for initiating and or promoting delta, whereas excitatory inputs attenuate or block them.

GABA is the main inhibitory neurotransmitter in the forebrain. GABA<sub>A</sub> receptors are widely distributed throughout the brain. However, many distinct GABA<sub>A</sub> receptor isoforms exist, and the expression patterns of these isoforms are selective, defining nuclei and circuits (Hörtnagl et al., 2013). Different isoforms have distinct receptor kinetics, and this provides different brain regions with distinct GABAergic physiology (Farrant and Nusser, 2005). GABA<sub>A</sub> receptors are pentameric ligand gated chloride channels, meaning they are made up of five subunits of various related types, which come together to form a central pore that is permeable to chloride. Gating of the channel is controlled by the binding of GABA (Rudolph and Knoflach, 2011). These pentamers are formed from 19 known subunits: α<sub>1</sub>−6, β<sub>1</sub>−3, γ<sub>1</sub>−3, δ, ε, θ, π which form heteromers & ρ<sub>1</sub>−3 which form homomers. However, the composition of any one GABA<sub>A</sub> receptor is highly organized and specific to cell-types and brain regions. The archetypal GABA<sub>A</sub> receptor is a heteromer formed from 2α<sub>1</sub>−3, 2β<sub>1</sub>−3 & 1 γ<sub>2</sub> subunit. This composition, including a γ<sub>2</sub> subunit, localizes in the synapse and has rapid activation (channel opening) and deactivation (channel closing) to provide fast phasic inhibition (Schweizer et al., 2003; Alldred, 2005; Essrich et al., 1998) and can found almost everywhere in the brain with only a few exceptions. Notably, the less common variants also play crucial roles in physiology. Absolute expression patterns or levels are not necessarily the main indicator of physiologic relevance, because the kinetics and affinity to GABA can vary greatly, giving rise to highly specialized roles. In other words, one GABA<sub>A</sub> receptor type might require a lot of GABA to be around and then be open only briefly. Whereas another type may need very low concentrations but keep hyperpolarizing a cell for quite a while. For instance, γ and δ subunits seem to be mutually exclusive, never forming a channel together. δ containing receptors are not as widespread as γ<sub>2</sub> containing receptors (Olsen and Sieghart, 2009) expressing only in select brain regions (Hörtnagl et al., 2013). However, because the δ containing subunits are extrasynaptic (Nusser et al., 1998), they have much higher affinity for GABA, enabling sensitivity to concentrations of GABA much lower than presumed in the synaptic cleft. δ subunit containing GABA<sub>A</sub> receptors mediate a persistent, so called ‘tonic’, inhibition (Bright and Smart, 2013). GABA has two binding pockets at the interfaces between the two α and the two β subunits (Sigel and Steinmann, 2012). However, the GABA<sub>A</sub> receptor is a highly druggable target with many important allosteric binding pockets, sometimes at the other subunit interfaces. An import allosteric binding pocket is the benzodiazepine (BZ) binding pocket which is located at the interface between α<sub>1</sub>−3 or 5 & γ<sub>2</sub> subunits (Sigel and Steinmann,
2012). Both BZs and so-called z-drugs rely on the BZ site. Point mutations that removed drug sensitivity in a selected \(\alpha\) subunit led researchers to dissect the component effects of hypnotic drugs, eg sedative vs anxiolytic vs myorelaxant. The distinction between these effects were likely a function of the distribution of the receptor types in behavioral circuits. For example, anxiolytic effects were linked to \(\alpha_2\) subunits (Löw et al., 2000; Ralvenius et al., 2015), which are expressed in fear and anxiety circuits. Conversely, the \(\alpha_1\) subunit has been associate with sedation (McKernan et al., 2000) and the suppression of NREM delta oscillations by one of the z-drugs, zolpidem (Kopp et al., 2004) which reflects the dense expression of \(\alpha_1\) subunits in wake active regions and oscillation networks where is exhibits highly organized expression patterns even within the constituent parts of these specialized circuits (Hörtnagl et al., 2013; Sperk et al., 2020). The \(\alpha_3\) subunit is also expressed in oscillatory circuits but was paradoxically not been linked to sleep-wake regulation (Winsky-Sommerer et al., 2008). However methodological limitations, such as compensatory mechanisms in the constitutive \(\alpha_3\)KO, may explain this lack of association. Recent work in our lab demonstrated \(\alpha_3\) knock down in PV+ TRN neurons enhanced deep sleep by boosting NREM delta waves. To conduct this, we used in vivo genetic abscission by CRISPR-Cas9 in the adult brain, which may have been necessary to circumvent genetic compensation (Uygun et al., 2022). The GABA\(_A\) receptors that contain either \(\alpha_1, \alpha_3, \gamma_2\) and/or \(\delta\) subunits are perhaps the most important in the context of regulating delta oscillations.
Fig. 1.
Model diagram of the thalamocortical circuitry driving delta waves during NREM sleep. Glutamatergic thalamocortical (TC) relay neurons (orange) project to the cortex and receive glutamatergic inputs returning from the neocortex. The thalamic reticular nucleus (TRN) does not project to the cortex. Instead, the TRN modulates cortical activity via the TC relay neurons, by providing them with their primary source of inhibitory drive. Both major types of thalamic population shown here are defined by their expression of native specialized subtypes of channels and receptors (expanded notes), providing the cells with electrophysiologic mechanisms to generate (TC relay) and promote (TRN) NREM delta waves.

EEG signal - where delta waves are recorded

“Pacemaker” cells generate delta waves
HCN2/4 channels mediate hyperpolarization-activated depolarizing h
CaV3.1 channels mediate a depolarizing but transient calcium spike
KV3.2 channels mediate high-frequency barrage of action potentials
GABA\textsubscript{\alpha} receptors containing either \(\alpha1\) or \(\alpha4\) mediate hyperpolarization
Intrinsically burst-fire at delta frequency when hyperpolarized

Inhibitory cells promote delta waves
CaV3.2 & CaV3.3 channels mediate a depolarizing but transient calcium spike
KV3.1 & KV3.2 channels mediate high-frequency barrage of action potentials
GABA\textsubscript{\alpha} receptors containing \(\alpha3\) mediate hyperpolarization
Provides source of hyperpolarizing drive to TC-relay “pacemaker” cells