The thalamocortical network as a single slow wave-generating entity

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Abstract

During non-REM sleep the EEG is dominated by slow waves which result from synchronized UP and DOWN states in the component neurons of the thalamocortical network. This review focuses on four areas of recent progress in our understanding of these events. Thus, it has now been conclusively demonstrated that the full expression of slow waves, both of natural sleep and anesthesia, requires an essential contribution by the thalamus. Furthermore, the modulatory role of brainstem transmitters, the function of cortical inhibition and the relative contribution of single neocortical neurons to EEG slow waves have started to be carefully investigated. Together, these new data confirms the view that a full understanding of slow waves can only be achieved by considering the thalamocortical network as a single functional entity for the generation of this key EEG rhythm.

Introduction

Slow waves are one of the most ubiquitous features of the EEG in mammals and have been a focus of neuroscience research for many decades. Whilst slow waves are inextricably linked with sleep physiology, dominating the EEG during the deeper stages of non-NREM sleep (i.e. slow wave sleep), they are also present during certain types of anaesthesia and in response to sensory stimuli. As such, the relationship between slow waves and states of sleep and consciousness is a complex one that necessitates a detailed understanding at the cellular and network level of the mechanisms that lead to slow waves and their neuronal counterparts, i.e. UP and DOWN states. To this end, over the last 30 years an array of *in vivo* and *in vitro* electrophysiological studies has shed significant light on the intrinsic and synaptic events that lead to slow waves [1–3]. Such studies have naturally focused on thalamocortical interactions and have highlighted central roles for both the neocortex and thalamus in shaping the overall manifestation of slow waves. Indeed, the requirement for a particular architecture and connectivity capable of generating slow waves becomes evident if one considers non-neocortical areas such as the piriform cortex which has a laminar structure that is distinct from the neocortex, lacks thalamic input and neither generates typical slow waves [4,5] or exhibits coherence with neocortical territories in low frequency EEG bands in vivo [5]. However, in the intact brain, several other regions have their activity phase-locked to EEG slow waves, including the hippocampus [6], striatum [7] and cerebellum [8] as well as brainstem nuclei that are the main sources of modulatory transmitters such as the locus cœruleus for noradrenaline [9], the dorsal raphe for serotonin [10] and the pedonculopontine nucleus for acetylcholine [11].

Given that slow waves are also difficult to fully characterize due to their multiple forms of intrinsic expression (i.e. waveform, frequency and amplitude) and contextual manifestation (i.e. *in vivo* versus *in vitro* models and anesthesia versus natural sleep), the identification of the brain regions and mechanisms responsible for initiating, propagating, terminating and modulating slow waves remains challenging at the experimental, computational and theoretical levels. Notwithstanding this, important progress has been made in recent years, and this review will bring together recent advances in our understanding of the generation of slow wave and UP and DOWN states with the aim of developing a concise and coherent framework for explaining how this brain rhythm develops during natural sleep and anesthesia.

The thalamus is as essential as the cortex for the full expression of slow waves

One area that has often been proposed to play a key role in slow wave generation is the thalamus [12]. Four main lines of evidence support this assertion. **First**, the firing of thalamocortical (TC) neurons is tightly associated with EEG slow waves. Together with the strong afferent and efferent connections with neocortical layers [13–15] that are involved in slow waves [16–18], this suggests that thalamic nuclei can control UP and DOWN state dynamics in neocortical circuits. **Second**, TC neurons fire early in relation to the initiation of cortical UP states with the particular relative timing being dependent both on their affiliation to specific thalamic nuclei [19–21] and number of action potentials present in the high frequency bursts [19] that are the typical firing mode of these cells during sleep and anesthesia (Fig. 1A-C). **Third**, both TC neurons as well as neurons of the nucleus reticularis thalami (NRT) can exhibit robust, rhythmic UP and DOWN states

in isolated conditions *in vitro* that are fully intrinsic and not reliant on rhythmic synaptic input, be it from the neocortex or other brain regions [12,22,23]. These thalamic UP and DOWN states result from the particular array of ion channels present in both TC and NRT neurons and are sustained by a tonic activation of the metabotropic glutamate receptors (mGluRs) that are postsynaptic to cortical afferents in both thalamic cell types [12,22,23]. **Four**, selective optogenetic activation of TC neurons readily induces neocortical UP states in head-restrained mice [24] and powerfully controls EEG slow waves in rats [25], thus being as efficient as sensory stimuli [26,27] or optical [17,28], electrical [29], and magnetic [30] stimulation of the neocortex in eliciting UP and DOWN states and EEG slow waves.

Despite all the above experimental results and human evidence showing altered slow waves following thalamic disruption in a patient with fatal familial insomnia [31], until very recently, only *in vitro* experiments had shown that the probability of generating cortical UP and DOWN states is conditioned by intact thalamocortical connections (Fig. 2E) [32–34]. Indeed, since the original experiments by Steriade's group in 1993 [3], it had always been assumed that slow waves of natural sleep and anesthesia are cortically generated since they could still be recorded after thalamic deafferentation *in vivo*. However, studies in rats and cats have now conclusively demonstrated that both under anesthetic and natural sleep conditions [25], EEG and cortical slow waves are significantly disrupted following thalamic deafferentation, be it achieved through either physical [35] or pharmacological interventions [25,35] (Fig. 2A,B). In particular, using a combination of EEG, thalamic multiple single unit recordings with a silicone probe and optogenetics coupled to reverse microdialysis [36], it was shown that slow wave frequency is reduced

by more than 50% following intrathalamic application of tetrodotoxin both in anesthetized and naturally-sleeping rats (Fig. 2C,D) [25]. Interestingly, under ketamine-xylazine anesthesia neocortical slow wave activity is regained 30 hours after thalamic deafferentation [35] (Fig. 2A,B), possibly explaining the minimal effects of thalamic deafferentation that had been observed in the original studies [3] where animals were recorded 2 days after the lesion. Although homeostatic plasticity is one candidate mechanism for explaining the recovery of slow waves *in vivo*, e.g. by an atypical up-scaling of synapses [37], other complex mechanisms might be involved. Importantly, the reduction/block of slow waves after thalamic deafferentation *in vivo* during natural sleep and anesthesia also explains why this rhythm with all its characteristic features cannot be fully reproduced in an isolated cortex *in vitro* unless either the ionic composition of the extracellular medium is modified [16,18,32,38] or neuromodulators are added to the *in vitro* neocortical preparation [12,34] (see below). In summary, thalamic inputs are intimately involved in the generation of slow waves, and as we recently proposed [12], the thalamocortical circuit should be viewed as a single functional and dynamic entity when considering the generation of slow waves of natural sleep and anesthesia (Fig. 2F).

Intrinsic and network mechanisms of UP and DOWN states in the neocortex

Within this framework of the thalamocortical circuit being a unified slow wave-generating entity, it still remains of great significance to fully understand the intrinsic and/or synaptic origin of UP and DOWN states in the different thalamic and cortical neuronal populations. While the ability of NRT and TC neurons in both sensory and intralaminar thalamic nuclei to generate intrinsic rhythmic UP and DOWN states was established a few years ago

[22,39] (and extensively reviewed in [12]), some recent key investigations have shed additional light on the nature of the UP and DOWN state dynamics in neocortical neurons. In particular, a study in a thalamocortical network preserved *in vitro* [38] and an *in vivo* investigation using selective optogenetic stimulation [17] have now confirmed the original observation [16] that *within the neocortical network*, layer 5 neurons play the most central role in triggering UP states and bringing about their propagation. In contrast, layers 2/3 may assist in UP state generation but are not necessary for their propagation. Moreover, selectively inhibiting layer 5 neurons with either tetrodotoxin [38] or halorhodopsin/archaerhodopsin combined with optical stimulation [17] impairs the generation and propagation of slow waves whereas the same procedure applied to layer 2/3 neurons fails to prevent the generation of UP states. However, the above *in vivo* study was conducted under anesthesia, and species- and paradigm-specific differences also need to be taken into account. For example, the contribution of supragranular layers to slow waves in humans during natural sleep appears to be more prominent than in anesthetized rodents [40]. In addition, as we now know that afferents from different thalamic nuclei are not restricted to cortical layer 4 [15,41,42] the potential diverse weight of direct thalamic inputs carrying activity at slow wave frequency to different cortical layers should also be carefully investigated, ideally *in vivo* during slow waves of natural sleep.

The complete ablation of slow waves by ionotropic glutamate receptor antagonists in neocortical slices [16] strongly support the view that the UP and DOWN state dynamic within this brain regios is generated mainly by excitatory and inhibitory synaptic barrages. This, however, does not rule out the putative contribution of intrinsic neuronal activity by some sparse neocortical neuron population(s). Indeed, using the reduced Ca^{2+}/Mg^{2+}

model of UP and DOWN states in cortical slices two groups of neurons were found to elicit low frequency rhythmic firing at slow wave frequency in the absence of synaptic transmission, namely a subset of pyramidal neurons in layers 2/3 and 5 and a group of Martinotti cells in layer 5 [43]. Recently, using an alternative *in vitro* model of slow waves, whereby the cholinergic drive is reinstated by application of the cholinergic agonist carbachol, slow waves could be recorded at both the network level, as evident in the local field potential, and at the level of individual neurons (Fig. 3A) [12]. In this model too, while the majority of neurons cease firing altogether following the block of glutamate and GABA receptors, a small subset continue to exhibit rhythmic firing at slow wave frequency similar to that observed in control conditions (Fig. 3B). Again, notwithstanding the importance of these findings, the existence of intrinsic activity at slow wave frequency in a subset of neocortical neurons still needs to be confirmed as being relevant to natural sleep.

Importantly, whereas most reports of single cell contributions to slow waves have been of a correlative nature, a recent *in vivo* study has provided convincing evidence for causal interactions between single neuron activity and slow waves. Switching on single layer 5 bursting neurons in anesthetized rats can lead to a state change from slow waves to continuous UP states, highlighting the power of individual neurons in the control of network oscillations and global brain states [44]. Moreover, this single neuron contribution to network activity might be underestimated when interpreting the data obtained in rodents, since single action potentials are able to trigger robust polysynaptic events lasting for tens of milliseconds in human neocortical slices, arguing for an unexpectedly strong spike-to-spike coupling [45]. Nevertheless, the global synchrony of UP states throughout the neocortex [46] makes it unlikely that individual neurons can substantially

contribute to the propagation of UP states. Intriguingly, although UP states are generally considered as global phenomena in some instances small populations of neocortical neurons can undergo local UP and DOWN state transitions during wakefulness [47], a phenomenon which is also known to occur in human subjects [48].

Neocortical inhibitory mechanisms involved in slow waves

Although slow waves in the neocortex are widely accepted to be mainly due to a finely tuned dynamic balance between excitation and inhibition, it is somewhat surprising that, relatively fewer studies have investigated in detail the inhibitory mechanisms involved in this brain rhythm. Nevertheless, a recent study in ferret slices has shown that GABA-A receptor-mediated fast inhibition is crucial for maintaining the appropriate balance of persistent excitatory and inhibitory synaptic activity during slow waves, i.e. bicuculline shortens Up states and prolongs DOWN states ([49], see also [50]). GABA-B receptors on the other hand appear to selectively contribute to the termination of UP states in layer 2/3 pyramidal neurons in enthorhinal cortex slices [51], because GABA-B antagonists drastically prolong the UP state duration and impair the ability of layer 1 stimulation to terminate UP states [52]. Interestingly, the main source of GABA-B receptor mediated IPSPs in the neocortex are neurogliaform neurons [53], a neuron type known to release GABA in the extracellular space leading to promiscuous inhibition of a large population of neighbouring neurons [54]. This cortical cell type, therefore, may be a potentially important contributor to local and global neocortical slow waves, particularly in the termination of UP states which, differently from the start of UP states, has been reported to occur almost simultaneously even in relatively distant cortical regions (see Fig. 1C)

[21,46]. Undoubtedly, a major challenge of future research will be the much needed characterization of the contribution of individual neocortical interneneurons to the dynamics of UP and DOWN state generation during slow waves of natural sleep and anesthesia.

Neuromodulation of slow waves

As mentioned earlier, rhythmic firing that is phase-locked to EEG slow waves and UP and DOWN state dynamics occur not only in cortex and thalamus but also in many other brain regions, which interestingly include those brainstem nuclei that are the main source of modulatory transmitters such as noradrenaline [9], serotonin [10] and acetylcholine [11]. In view of the known role that these transmitters play in regulation of brain states [55–57] and their effects on slow waves as described in the original studies by Steriade's group [55], it is surprising that an increased interest in investigating the precise contribution to, and/or modulation of, slow waves by these transmitters has only occurred relatively recently.

An elegant study that used local field potential, intracellular and multiunit extracellular recordings combined with voltage-sensitive dye imaging in thalamocortical slices has shown that the effect of bath-applied acetylcholine on cortical slow waves depends on its concentration and the presence of intact thalamo-cortical connections [34]. In particular, the local cortical circuit shows an increased number of UP states in response to both low and high doses of ACh if the thalamo-cortical connections are intact, whereas only low doses of ACh can increases UP state transitions when thalamocortical connections are cut (Fig. 3C) [34] (as is the case in the cortical slow wave model described in Fig. 3A,B).

A recent study has also shown that the desynchronization of the EEG by selective cholinergic optogenetic stimulation has both muscarinic and nicotinic contributions [58]. However, another key investigation in anesthetized rats has demonstrated that the cortical block of acetylcholine receptors has little effect on cortical UP and DOWN state transitions [59]. These apparently contrasting results can be reconciled by considering that the effects observed by Wester and Contreras (2013) [34] were mediated by activation of cholinergic receptors on both thalamic and cortical neurons while the contribution of only cortical receptors was assessed in the other study [59]. On the other hand, unilateral locus coeruleus lesioning and noradrenergic blockers applied to the cortex *in vivo* abolish local UP and DOWN states [59]. This is in contrast with the findings obtained in thalamocortical slices where noradrenaline markedly reduces excitatory conductances driven by intracortical afferents [60]. In summary, although important data are starting to emerge on slow wave modulation by brainstem transmitters, further work needs to be carried out to fully unravel the interplay of these different neuromodulators as well as the multiple pre- and post-synaptic receptors subtypes through which they signal during natural sleep

Conclusions

Over the last few years a number of important and conclusive studies has significantly furthered our understanding of slow wave generation. Thus, the widely accepted view that the slow waves observed in natural sleep and during anesthesia are generated entirely within neocortical territories is no longer tenable: neither the isolated cortex (nor the isolated thalamus) can express identical slow waves to those observed *in vivo* but

their full expression requires an intact thalamocortical network as a unified functional slow wave-generating entity. The precise contribution of different cortical neuronal populations to slow wave generation, termination and propagation as well as of inhibition, both within neocortical territories and in the thalamus, still needs to be fully deciphered. As we have previously argued [12], these future studies, and those needed to fully characterize the actions of neuromodulators, will of course need to take into account the requirement of an intact and fully functional thalamocortical network to be able to provide novel and meaningful information. This will be clearly helped by the advent of novel molecular and optogenetic tools which allow the selective targeting of distinct neuronal populations on the basis of their specific molecular markers, location, connectivity or a combination of these features [61].

Figure 1. Cell type-specific features of firing phase preference in the thalamocortical network relative to slow waves.

A) Nuclear specificity of firing phase preference in the thalamus. Population phase histograms of firing for neurons in the ventrobasal (VB, blue histogram) and posterior (Po, red histogram) nucleus. Note how the vast majority of units in the Po fire before the transition from DOWN to UP state (marked by 0 on the abscissa). In the Po, bursts with a higher number of action potentials occur progressively earlier during an UP state (right plot). **B**) Nuclear specificity of firing phase preference in various thalamic nuclei (the start of the UP state is indicated by the dotted vertical line at time 0, and the red lines represent the median for each nucleus. (Cth: corticothalamic; VL: ventrolateral thalamic nucleus; VA/VM: ventral anterior/ventromedial thalamic nuclei; Rt: reticular thalamic nucleus). **C**) Averaged membrane potentials of neurons recorded at various cortical (top) and thalamic (bottom) sites plotted against the phase of the slow waves. (M1: primary motor cortex; S1: primary somatosensory cortex; V1; primary visual cortex; PF: parafascicular nucleus; PO: posterior nucleus; AV: anterior thalamic nucleus). (A, B and C: reproduced and modified with permission from ref. [19], [20] and [21], respectively).

Figure 2. Thalamic contribution to sleep slow waves.

A) EEG recordings before, 1h hour after and 30h after pharmacological inactivation of the thalamus by local application of lidocaine show a drastic reduction of slow waves. **B)** Corresponding intracellular recordings in affected neurons (Cell 2) compared to non-affected neurons (Cell 1 and 3) before (top traces) and after the slow wave recovery

process (bottom traces). **C)** Effect of intrathalamic reverse microdialysis application of the T-type Ca^{2+} channel blocker TTA-P2 and the sodium channel blocker tetrodotoxin (TTX) on EEG slow waves of naturally sleeping rats. **D)** Reduction of slow wave frequency under ketamine-xylazine anesthesia (left) and natural sleep conditions (right) (black: control; red: TTA-P2; blue: TTX). **E**) In thalamocortical slices, there is a reduction in the frequency of UP states (top) but not in their duration (bottom) when the thalamic afferents are removed ("Non-connected"), a result similar to that observed with pharmacological de-afferentation of the thalamus (cf. **C** and **D**). **F**) Schematic representation of the thalamocortical network and key elements of the slow wave generating mechanism. See Ref [12] for further details. (A-B, C-D, E and F: reproduced and modified with permission from [35], [25], [34], [12] respectively).

Figure 3. The cholinergic system affects the dynamics of slow waves in the neocortex *in vitro*.

A) Reinstating the cholinergic drive to slices of mouse neocortex maintained *in vitro* using the non-specific agonist carbachol (CCH) results in the apperance of prominent and rhythmic UP states-linked firing as indicated by the multi-unit activity (MUA) and the local field potential (LFP) (red trace). **B)** Some neocortical L5 neurons can generate slow rhythmic firing in the absence of synaptic transmission. Recording of local filed oscillations (LFO) and two single units during CCH-induced slow waves (left) shows that neurons fire synchronously during UP states. During block of glutamate and GABA receptors with the illustrated drugs (right) the majority of neurons seizes rhythmic action potential output

(unit 1) as also evident from the lack of oscillations in the LFP, but a minority of neurons, however, continues to generate low frequency rhythmic activity. **C**) Bath application of low (right, upper traces) but not high (right, bottom traces) doses of acetylcholine increases the frequency and rhythmicity of the ongoing slow waves in the neocortex of thalamocortical slices which lack the thalamic input. (C: reproduced and modified with permission from [34]).

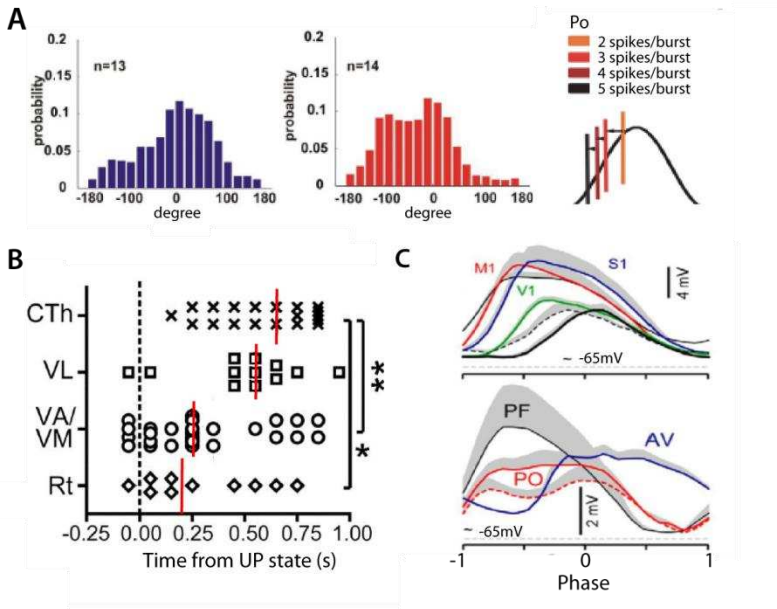
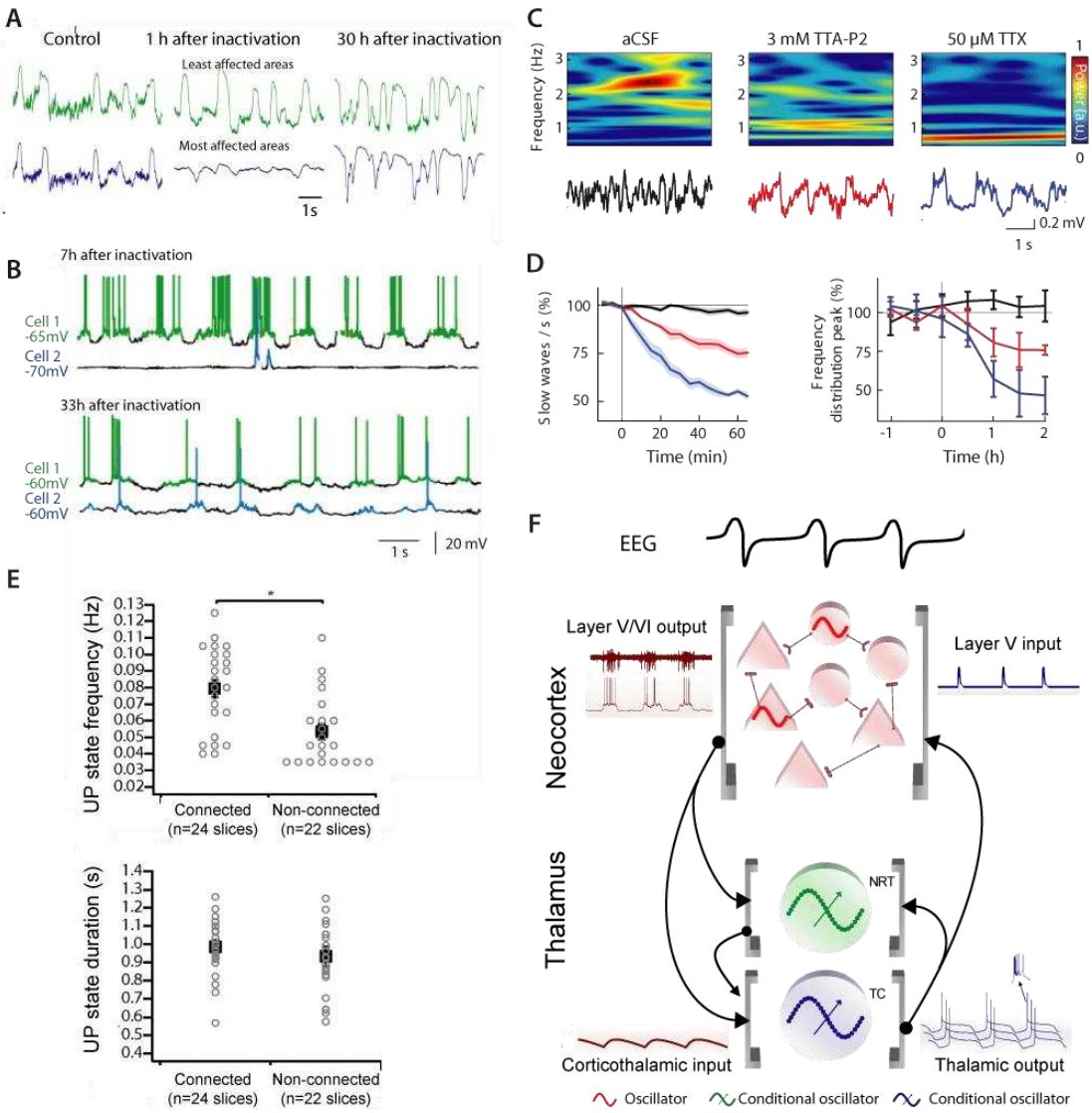


Figure 1


A
Control
1 h after inactivation
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Least affected areas
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B
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Cell 1
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Cell 2
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Cell 1
-60mV
Cell 2
-60mV
1 s
20 mV
C
aCSF
3 mM TTA-P2
50 µM TTX
Frequency (Hz)
Power (a.u)
0.2 mV
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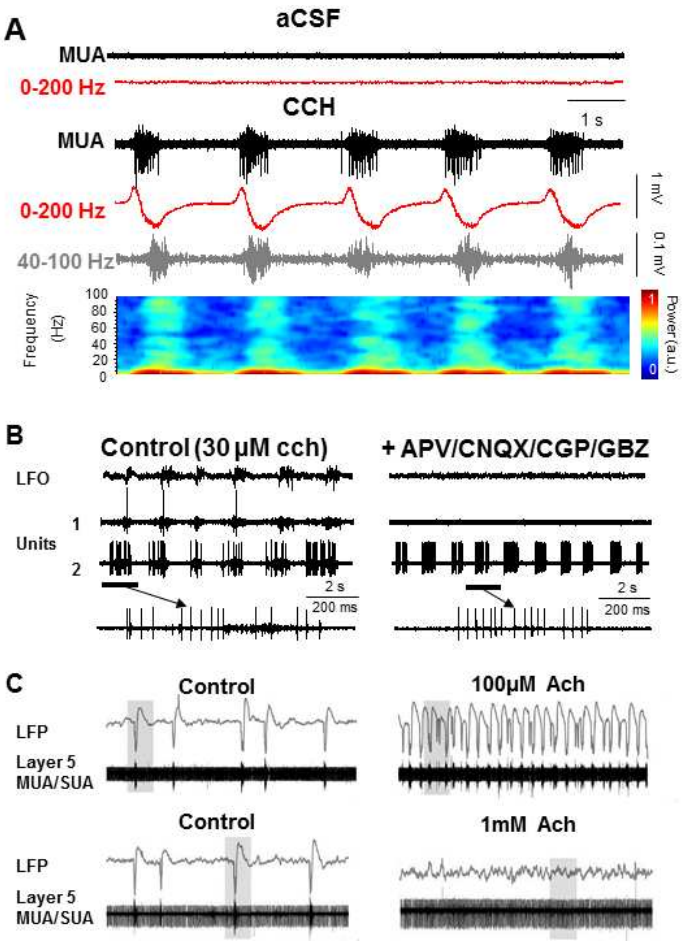
D
Slow waves / s (%)
Time (min)
Frequency distribution peak (%)
Time (h)
E
UP state frequency (Hz)
Connected (n=24 slices)
Non-connected (n=22 slices)
UP state duration (s)
Connected (n=24 slices)
Non-connected (n=22 slices)
F
EEG
Layer V/VI output
Layer V input
Neocortex
Thalamus
NRT
TC
Corticothalamic input
Thalamic output
Oscillator
Conditional oscillator
Conditional oscillator

Figure 2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Power (a.u.)
B
Control (30 µM cch)
+ APV/CNQX/CGP/GBZ
LFO
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1
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2 s
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200 ms
C
Control
100µM Ach
LFP
Layer 5 MUA/SUA
Control
1mM Ach
LFP
Layer 5 MUA/SUA

Annotated References

* of special interest

** of outstanding interest

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