

OSNABRÜCK UNIVERSITY

MASTER THESIS

**Optical Flow, Helmholtz Decomposition and
Autoencoders for the characterization of slow-waves**

A new approach for temporospatial pattern detection in neuroimaging

Author:
Michael GERSTENBERGER

Supervisors:
Dr. Dominik Jüstel
Prof. Peter König

*A thesis submitted in fulfillment of the requirements
for the degree of Master of Science
in the*

**Neurobiopsychology Group
Institute for Cognitive Science**

In cooperation with IBMI and ICI at Helmholtz Zentrum Munich
January 17, 2021

Declaration of Authorship

I, Michael GERSTENBERGER, declare that this thesis titled, “Optical Flow, Helmholtz Decomposition and Autoencoders for the characterization of slow-waves” and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed:

Date:

Abstract

Optical Flow, Helmholtz Decomposition and Autoencoders for the characterization of slow-waves

Neural signal transduction at different levels of alertness occurs in distinct shapes (Gemignani et al., 2015, p. 137f.). One may observe stimulus dependent activity during wakefulness but also spontaneous patterns of activation that dominate in stages of deep sleep (Dang-Vu et. al. 2011). Similarly, fast neuronal firing is replaced by slow, traveling waves of activation during anaesthesia. Cortical slow-waves can be captured by high-speed fluorescence microscopy of GCaMP channel activity in transgenic mice at up to 100Hz (Celotto et al. 2020).

Different types of slow-waves exist which can incorporate rather localized or more widespread regions of cortex. In principle, they are thought to occur spontaneously in various regions, however it was also noted that synchrony with somatosensory states and more importantly signals in subcortical structures exists during sleep. This synchrony is related to memory formation. Recently important differences between sleep slow-waves and their counterpart during anaesthesia were identified. This shows that slow-waves represent an important yet highly variable neural phenomenon and indicates that methods are required that allow to systematically distinguish slow-waves based on their properties.

Townsend and Gong (2018) suggest to characterize temporospatial properties of neural recordings by the detection of patterns in Dense Optical Flow. It shows, however, that the use is accompanied by two challenges: First it shows that focal brightness changes result in sources and sinks in the vector fields. Second successful estimation of Optical Flow is challenged by the quality of the contrast it is applied on. Here it is demonstrated how both challenges can be addressed and Dense Optical Flow used to characterize slow-waves. The presented procedure allows for a scale independent split of optogenetic recordings into several segments that relate to slow-waves. This allows for an event related analysis and the computation of a contrast that is independent from anatomical structures and meets the smoothness requirements of Dense Optical Flow. It is further demonstrated that Helmholtz-Decomposition can be used to distinguish between the effect of local sources of neural activation and global patterns of neural flow. Finally, it is shown how the measured high-level features can be analysed using variational autoencoders. The approach reveals a complex topology of the distribution of different features in latent slow-wave-shape space. These features include the direction of flow and the distribution of sources, phase and amplitude as well as the correlation with breathing. It is further suitable to investigate how individual waves and the combination of events differs between stages of anaesthesia.

In summary a new technique to characterize slow-waves is presented that can help to improve our understanding of neural processing in the brain under anaesthesia. The use of Optical Flow, Helmholtz-Decomposition and autoencoders to characterize and distinguish different types of slow-waves appears promising. It bears potentials to study the relationship between distributions of different slow-waves and other features including anaesthetics, experimental stimuli or animal behavior.

Acknowledgements

I would like to thank my supervisors for their support of my Master Thesis. Especially I want to thank Silviu Bodea who initiated the project, recorded all the data and made my contribution possible. Special thanks go to him and Prof. Gil Westermeyer of the Institute of Biological and Medical Imaging (IBMI) for the continuous feedback and advice regarding the results and further direction of my work. Besides I want to genuinely thank my first supervisor, Dr. Dominik Juestel for his input regarding the methodological questions and especially for pointing out the benefits of Helmholtz-Decomposition. I truly enjoyed taking part in the meetings of his group at the Institute for Computational Biology (ICI) and appreciate that I gained interesting insights in the work of the other members which gave variety to my work during the global pandemics. Finally I want to thank my second supervisor Prof. Peter König of Osnabrück University especially for his structured guidance regarding a cooperation with external partners during the Master Thesis.

Contents

Declaration of Authorship	i
Abstract	ii
Acknowledgements	iii
1 Introduction	1
2 Slow-waves	4
2.1 Overview of proposed types and functions	4
2.2 Effects of anaesthesia on single cells and neural networks	6
2.3 Slow-waves in anaesthesia and deep sleep	9
2.3.1 Three systems for sleep regulation	9
2.3.2 Neural oscillators	12
2.3.3 Neocortical slow waves during sleep and anaesthesia	13
2.4 Sleep slow-waves and memory consolidation	14
2.5 A working definition	15
3 Methods	17
3.1 Dense Optical Flow	17
3.2 Helmholtz-Decomposition	17
3.3 Helmholtz-Decomposition of Dense Optical Flow	17
4 Results	18
4.1 Main Section 1	18
4.1.1 Subsection 1	18
4.1.2 Subsection 2	18
4.2 Main Section 2	19
5 Discussion	20
5.1 Main Section 1	20
5.1.1 Subsection 1	20
5.1.2 Subsection 2	20
5.2 Main Section 2	21
A Frequently Asked Questions	22

A.1 How do I change the colors of links?	22
Bibliography	23

List of Figures

2.1 Oscillatory centres and the ascending reticular activation system	16
---	----

List of Abbreviations

LAH List Abbreviations Here
WSF What (it) Stands For

Chapter 1

Introduction

Anaesthesia inhibits neural activity and alters the signals in the brain. Depending on the dosage of the anaesthetic in use different patterns can be observed (Eger, 1981). At intermediate concentrations many agents cause propagating low-frequency waves that occur in close succession and manifest as slow oscillations in EEG (Brown et al., 2012; Eger, 1981). Similar patterns can be observed during deep sleep. Neocortical slow waves differ in shape and size and with respect to specific temporo-spatial features: Most importantly the source location of recurrent activation, the direction and speed of spread and different sets of functionally connected regions can be observed (Brown et al., 2012). These features can be measured using modern imaging techniques that capture the fluorescence of genetically modified GCaMP channels.

The development of new experimental technologies to capture GCaMP fluorescence with an increasing temporal resolution of up to 100Hz offers new opportunities for the study of the neural circuitry and dynamic state changes of the brain under anaesthesia. At the same time, they also impose new challenges that result from the complexity of the recorded data. These challenges must be addressed by a well-controlled experiment or suitable approaches for data analysis.

Strict experimental control simplifies data processing. Under certain experimental conditions, high amplitude slow waves with a good signal to noise ratio and contrast to the hemodynamic autofluorescence occur in a regular pattern. For ketamine induced anaesthesia (100mg/kg), regular patterns of neocortical slow waves with highly similar shapes, durations and amplitudes occur. This simplifies the separation of events and the subsequent analysis (Celotto et al. 2020). However, such strict control means that the variance of the captured slow waves is highly reduced.

More general findings regarding the structure of neocortical slow waves are only possible in less controlled settings. This means, however, that suitable methods for data processing and analysis must be established that are both

robust and suitable to capture the dynamics of neocortical slow waves of different shapes and signal strengths.

Hence a new approach was developed that allows to distinguish and characterize neocortical slow waves of various shapes and forms. It reveals a complex topology of events and shows how different levels of isoflurane change the way signals travel in the brain. The development was accompanied by two major questions: (1) What are slow-waves and (2) how can their temporospatial properties be measured and characterized. To answer the earlier question and provide a working-definition for neural slow-waves that allows to distinguish different events, relevant slow wave literature was systematically reviewed. To tackle the latter question it was investigated how Dense Optical Flow can be used to measure the strengths and direction of neural flow and how Helmholtz-Decomposition can help to distinguish it from neural sources and areas of extended recurrent activity. As not only features but characteristic patterns are of interest it was further assessed how autoencoders can be used for dimensionality reduction of the measured properties. In the following the results of named literature review are presented first (section 2). It shows that anaesthetics change important properties of neurons most prominently their excitability which explains why bistable states of network activity that can be observed in neocortex empirically (see section 2.2). While important differences exist between anaesthesia and sleep, slow waves can be observed in both states. One may distinguish corticothalamic slow waves in the delta range and neocortical slow waves of a duration above one second (see section 2.3). The latter kind of slow waves is of main interest here. They are understood as extended periods of recurrent neural activation that manifests as above baseline activity (see section 2.5).

The methods to analyse slow waves are explained in the second part. First a new approach is presented that allows to track hemoglobin rich blood in vessels of various size. It is shown that high amplitude oscillations result from intracranial bloodflow and band-stop filtering can be used to reduce this effect. Second an overview of the processing steps is provided. The procedure of splitting events is reported, then Dense Optical Flow is introduced and it is demonstrated how Helmholtz Decomposition can be used to distinguish between the effect of local brightness changes and global flow. Subsequently the use of autoencoders for unsupervised characterization of slow-waves is discussed and the hyperparameters of the models used are presented.

The results of the approach are shown in the third part. First simple statistics regarding the dataset, selected features and the experimental conditions are reported. Then the relationship between properties of waves is summarized for representative events. Finally the results of the autoencoders are reported.

In the last part the findings regarding the methods and the achieved results are discussed. Optical-flow, Helmholtz decomposition and autoencoders are a suitable tool to study slow waves. Feature engineering helps to select relevant and robust features. Optical flow can be used in combination with Helmholtz-Decomposition to distinguish between local sources and global pathways of neural flow. Autoencoders represent an easy to use tool for the analysis of high level features. The results confirms the existence of fundamentally different types of slow waves. The approach allows for a fine grained distinctions and reveals a complex topology of static and dynamic properties in latent slow wave space.

Chapter 2

Slow-waves

2.1 Overview of proposed types and functions

There is a large body of literature that addresses slow oscillations in the brain. Slow waves in the delta range (0.5-4 Hz) can be identified in electroencephalograms (EEG) under sleep and anaesthesia. Neurons that switch between up and down states in the respective frequency are present in thalamus indicating that this kind of slow waves is of thalamic origin (Brown et al., 2012, p. 1110). More recently neocortical slow oscillations that occur with a frequency below 1Hz have been studied as well. As they do not necessarily occur in regular time intervals they are referred to as neocortical slow waves here (see also section 2.5). Both electrophysiological recordings as well as two-photon and wide field fluorescence microscopy have been used to investigate neocortical slow waves (Niethard et al., 2018; Celotto et al., 2020). Neocortical slow waves with a typical duration of more than one second are the main subject of the empirical analysis presented here.

On one side these low frequency neocortical slow waves and EEG delta waves are recognized as distinct phenomena (Brown et al., 2012, p. 1110). On the other hand simultaneous imaging and electrophysiological recordings indicate that both signals relate to each other (Niethard et al., 2018). One interpretation is that neocortical slow oscillations bind together spindles and delta waves (Brown et al., 2012, p. 1110). Thereafter one could consider them to be the signature of the same distributed slow wave process. To better understand how different oscillatory patterns in the brain relate to neocortical slow waves is crucial to summarize our knowledge about them. A clear understanding is not only necessary to discern events and measure slow wave properties but it is also important for the cross-validation and interpretation of the results achieved. Hence selected literature on slow waves, sleep and the effect of anaesthetics is briefly reviewed in this chapter ¹.

¹For an outstanding review with a focus on sleep that includes a discussion about the origins of different kinds of rhythms in the brain see Brown et al. (2012).

The effects of anaesthesia on the properties and the spiking behavior of individual cells are reported first. The modes of action of anaesthetics such as isoflourane have been studied extensively (Qazzaz and Winlow, 2017; Moghadam et al., 2019; Eger, 1981; Jenkins, Franks, and Lieb, 1999). It shows that anaesthetics change several properties of neurons which modify the temporal and spatial integration of signals and cause bistable states in distributed neural networks. This can be demonstrated using spiking network models which provide an explanation for the occurrence of neocortical slow waves under anaesthesia that is coherent with named changes of neural properties (Nghiem et al., 2018). Stronger inhibition of neural activity also exists during sleep. However important characteristics of the dynamics of neocortical slow waves differ between NREM sleep and anaesthesia. Hence it was argued that two different types of slow waves occur during sleep and anaesthesia (see section 2.2).

Evidence from several decades of research highlights the role thalamic circuits play for the generation of rhythmical activity, the awake state and slow wave sleep (Brown et al., 2012). While slow wave sleep is arguably promoted by several mechanisms including the cardiac cycle and the release of melatonin that alters neural excitability, sleep spindles of thalamic origin are assumed to be the trigger for a transition to deep sleep (Montagna, 2005, p. 347). Besides specific ion channels have been identified in thalamus that presence of which correlates with slow waves in the delta range (Brown et al., 2012, p. 1112). Sleep spindles also precede many of the neocortical slow waves observed on the level of single cells using two-photon imaging (Niethard et al., 2018). This contrasts with the assumption that neocortical slow waves form in a spontaneous and fully independent manner in different regions of cortex if the excitability is reduced. Not all mechanisms of sleep are fully understood. It must be mentioned, however, that sleep is a vegetative state and thalamic nuclei play a crucial role in the generation of neural oscillations including spindles and slow-waves in the theta range (see section 2.3).

Sleep slow-waves are known to be involved in processes of memory consolidation. The assumed modes of action have been described in the hippocampal neocortical dialog model (Buzsáki, 1996). Many results support the hypothesis that memory replay occurs during sleep and that it includes both hippocampus and neocortex. One may hypothesize that neocortical slow waves represent the neural signature of an important part of this process. Memory consolidation is arguably the most prominent function of slow waves (see section 2.4).

As explained above slow-waves differ with respect to several features. This

holds both for the duration and frequency at which they occur and the assumed sites of origin. Neocortical slow waves can emerge from various regions. EEG studies in humans indicate that prefrontal-orbitofrontal regions act as the preferred location of early above average activity (Brown et al., 2012, p. 1110). First steps to distinguish slow-waves in a data driven approach were carried out by Bernardi et al. (2018). Two types of sleep slow-waves may be distinguished in EEG. This distinction is based on a metric that includes a shape parameter and a term that indicates whether slow-waves are rather local or widespread. The distribution of these types of slow-waves differs during different stages of deep sleep. It highlights the importance of means to discern slow-waves based on their shape and spatial pattern of activation. A working definition that allows to detect and separate slow-waves is derived from the developed understanding of slow-waves (see section 2.5).

2.2 Effects of anaesthesia on single cells and neural networks

Anaesthetics alter the spiking behaviour of cortical neurons. Under deep anaesthesia the activity of cells is highly reduced and quiescence can be observed for most units. However, the modes of action that cause the respective changes of neural properties differ between anaesthetic agents. In addition, the effects can depend on the exact concentration of the drug in use and low dosages may, in some cases, even have opposing effects (Moghadam et al., 2019). As isoflourane was used to acquire the dataset at hand its effects on individual cells is shortly summarized highlighting important differences to other anaesthetics when necessary. It is known that alterations of the properties of individual cells change the dynamics of neural interaction on a population level. By adjusting the properties of cells in simple models of neural networks bistable states can be reproduced that resemble slow-waves under anaesthesia (Jercog et al., 2017).

The exact pharmacological mechanisms of action of inhalant anaesthetics such as isoflourane remain uncertain (Miller, Theodore, and Widrich, 2020). Effects on several ion channels have been reported including both chemically gated CL⁻ channels (GABA receptors and glycine receptors) and K⁺ channels (Glutamate receptors). For example isoflourane is known to reduce the hyperpotentiation that results from CL⁻ influx as a consequence of in vitro GABA administration (Jenkins, Franks, and Lieb, 1999). Hence it can be assumed to have excitatory effects in the nervous system as it decreases the inhibition due to GABA. However isoflourane also has inhibitory effects as it suppresses K⁺ channel currents resulting in smaller electrically triggered peak

amplitudes of action potentials (Buljubasic et al., 1992). Besides a potentiation of glycine receptors is assumed alongside other neurochemical mechanisms that alter the excitability of neurons (National Center for Biotechnology 2021). In vivo studies indicate that the net-effect of isoflourane appears to be inhibitory for all relevant dosages. In this respect isoflourane contrasts with other anaesthetics (including e.g. halothane and ketamine) that show concentration dependence (Moghadam 2019 comparative). It shall be noted however that differences in the density of different types of receptors exist in different areas of the brain. While isoflourane can be assumed to decrease the excitability of neurons and inhibit neural signaling at all concentrations this effect could differ between brain areas.

Single cell recordings reveal decisive effects of Isoflourane on the properties and the spontaneous behavior of neurons. Moghadam et. al (2019) performed a comparative study of systemic and volatile general anesthetics in single cell cultures and the isolated brain of *lymnaea stagnalis*. The substances tested include the volatile agents sodium pentobarbital, sodium thiopentone, ketamine on one side as well as halothane, enflurane and isoflourane on the other. It was found that isoflourane causes a gradual decline of both amplitude and frequency of spontaneous action potentials. Differences between the six types of neurons studied were found to be marginal. Upon stimulation neurons remained silent at all examined concentrations of isoflourane. In contrast a gradual decline of evoked action potentials showed for increasing levels of enflourane. Using either halothane or barbiturates the authors were also able to produce bistable states in vitro during which periods of rapid spiking and quiescence alternate spontaneously.

Besides named alternations in spiking, effects on subthreshold properties of neurons have been identified. Increasing levels of isofourane can cause a decrease in the membrane time constant i.e. the duration between stimulus onset and 63% potential change of the cell membrane. Under normal conditions it takes longer for the neuron to reach maximal voltage as compared to anaesthesia with isoflourane. This is reflected in the estimated membrane capacitance that analogously decreases. The membrane capacitance is especially interesting because of the role it plays in the integration of electrical inputs (Golowasch & Nadim 2014). The most likely explanation for the apparent reduction is however an increase in the leakage current. This is because it is (1) rather unplausible for the capacitance of the constant size membrane to change significantly and (2) because of the abovementioned interaction between membrane proteins and anaesthetics (Quazzaz & Winlow 2017). Neurons act as temporal integrators and fire if the combined voltage of input spikes that occur simultaneously or in short succession exceeds threshold. If

the leakage current is stronger the timing of input spikes becomes more critical as the membrane potential goes back to baseline more quickly which may prohibit charge accumulation. Hence it can be hypothesized that anaesthetics affect temporal integration.²

Arguably the abovementioned effects explain important features of population level dynamics. Eger (1981) systematically studied the EEG patterns in the awake state and during anaesthesia with isoflourane for five different dosages at up to 2.9% in humans. For very light anaesthesia (iso = .56%) low voltage fast activity can be observed. At a light surgical level (isp = .96% and 1.78%) slow oscillations are present that change from more regular to irregular patterns and alternating patterns with high amplitude oscillations at a moderate surgical level (iso = 2.2%). For deep anaesthesia only occasional low voltage activity shows (iso = 2.9%). Isoflourane administration leads to a gradual shift from a stable awake state to bistable states and finally deep anaesthesia where quiescence dominates.

The effects of anesthesia presented here provide the background for a mechanistic interpretation of slow-wave activity. The overall dynamics that arise from changes of neural properties can be modeled using a simple model that employs Adaptive Exponential Integrate And Fire cells as shown by Nghiem et al. (2018): Simulations of a network that consists of 80% excitatory cells and 20% fast spiking inhibitory neurons produce sequences of continuous activity (up-states) and widespread absence of action potentials (down-states). A shift from down-states to and up state can be triggered by background noise while "spike-frequency adaptation on excitatory cells produces a self-inhibition that, destabilizing the up state, causes a reset to the down state" (Nghiem et. al 2018, p. 2). Spiking network models can reproduce alternating sequences of up states and down states, an important characteristic of population level activity under anaesthesia.

While simple mechanistic models explain some of the features of anaesthesia on a population level they represent a coarse generalization. It was highlighted that the exact mechanisms of action of anaesthetics remain uncertain. Generalizing over different agents and dosages is not necessarily justified and the dynamic of neural signalling might be affected by differences in the distribution of receptors for different neurotransmitters. Besides the complex anatomy of the brain including the various pathways that connect different regions uni- or bidirectionally is typically not reflected in simple mechanistic models. More precise measurements of the pathways and dynamics of neural signal transduction during slow-wave anaesthesia are necessary to understand bistable states of the brain. This is especially important also because it was recently shown that slow-wave sleep differs significantly from

²Note that other anaesthetics were found to increase the membrane capacitance

anaesthesia.

2.3 Slow-waves in anaesthesia and deep sleep

Recently it was argued that the dynamics of slow-wave sleep and slow-wave anaesthesia differ substantially (Nghiem et al., 2018). Jercog et al. (2017) found that the length of up states and the subsequent down state is correlated during urethane induced anaesthesia in rats for clearly synchronized periods where high-amplitude, slow fluctuations are present in local field potentials. Coefficients indicate a very weak relationship ($r = .2$). However, the correlation was found to be consistently positive across experiments whereas the correlation with later down-state periods (time-lag > 2) is close to zero (Jercog et al., 2017). This pattern in the dynamic of neural firing can be reproduced using simple mechanistic models (see section ??). During human non-REM sleep, however, no such relationship holds. As a tendency, long down states are followed by short up states instead as indicated by a very weak but significant negative correlation ($r = -.04$). A negative peak in the temporal cross correlation exists around zero (Nghiem et al., 2018). This gave rise to the argument that sleep-slow waves are fundamentally different from their counterpart during anaesthesia (Nghiem et al., 2018). Hence the mechanisms that enable slow wave sleep are more closely examined in this section.

2.3.1 Three systems for sleep regulation

It is long known that the anatomical substrate of sleep is a distributed system rather than a single region in the brain (Akert, 1965). Mammalian sleep represents a physiological process that is arguably regulated by the interaction between various control mechanisms. In general, one may categorize these processes into three classes each of which affects the activity of cortical neurons directly or via intermediate events. First there are processes that decrease neural excitability rather unpacificaly by a release or an accumulation of disperse chemical messengers. Second processes exist that incorporate electrical signalling and include the neurotransmitter systems that correspond to the ascending reticular activating system. Third there are processes that lead to oscillations in the firing rate of distinct neural populations. The latter can include spike rates that follow the circadian rhythm and alter the both the release of neurohormones and the excitation of cortex via spiking activity. However oscillatory activity also occurs in the range of delta slow waves. As it is not possible to discuss all features of sleep here, important

examples are presented that fall under named categories. The aim is to characterize the circumstances under which sleep slow waves occur.

Melatonin is the messenger of a system for sleep regulation that falls under the first category. In healthy subjects, melatonin concentrations alternate according to the cardiac cycle (Montagna, 2005). Melatonin can pass the blood brain barrier and can hence diffuse to the central nervous system where it accumulates (Aulinas, 2019). It acts as a neurohormone and has an inhibitory effect on the excitability of cortical neurons via different modes of action. Melatonin receptors in the neural membrane have been identified. Besides it was found that melatonin interacts with voltage-sensitive Ca^{2+} channel and inhibits the release of neurotransmitters as well as synaptic transmission (Choi et al., 2014). As it decreases the neural excitability of cortical neurons it shares some of the effects of mild anaesthesia. Melatonin reduces the excitability of disperse populations of neurons in the central nervous system promoting drowsiness and sleep.

The suprachiasmatic nuclei (SCN) represent an example for the third of the abovementioned categories. It interacts with the melatonin system. Melatonin is produced by pinealocytes that compose 95% of the cells in the pineal gland (Aulinas, 2019). The pineal gland resides outside the blood brain barrier and extends hypothalamus ventrally and represents an interface between neural signalling and the endocrine system. It is innervated by centres in the brain stem. Most importantly it is known to receive signals which are related to retinal activity from the SCN. The SCN is capable not only of creating a light-dependant circadian rhythm, but also of maintaining an entrained rhythm (Koella, 1984). The latter could be demonstrated in electrophysiological recordings in vitro. Isolated SCN show firing rates that alternate in a 24 hour pattern (De Berardis et al., 2011). Arguably this explains changes of melatonin release that follows the circadian cycle.

While melatonin is evidently part of sleep regulation the transition between the awake state and slow wave sleep is arguably initiated by other mechanisms. Mammalian sleep can be triggered by electrical stimulation of the thalamus. First experiments were carried out using cats while later experiments confirmed that the effect exists for dogs as well (Akert, Koella, and Hess Jr, 1951; Akimoto et al., 1956). A stimulation of the intralaminar thalamus of cats with a duration of 30-60 seconds and pulses at 4-8Hz leads to a transition to sleep. This transition occurs in different stages. Sleep spindles occur while the animal is still awake. After several minutes a full transition to slow-wave sleep can be observed (Akert, Koella, and Hess Jr, 1951). These early studies indicate that thalamocortical sleep spindles play an important role for the transition to sleep.

Early lesion studies indicated that brainstem nuclei are required for both normal awake activity and REM sleep. In animals with a Cerveau isolé preparation only slow waves can be observed. In named preparation ascending fibers between pons and midbrain are dissected. Effectively large parts of the reticular formation and several nuclei that use specific neurotransmitters are disconnected from midbrain, thalamus and cortex. In contrast an encéphale isolé preparation in which the brain and the spine are separated does not have named effects but normal sleep patterns can be observed. This highlights the importance of brain stem for the regulation of sleep and alertness. Named nuclei include serotonergic neurons in the raphe nucleus, norepinephrine neurons in locus coeruleus and glutamate neurons in the pedunculopontine nucleus. They represent the most important excitatory neurotransmitters. These nuclei are known as the source of the ascending reticular activation system (ARAS).

Similar to target sites in thalamus electrical stimulation of the raphe nuclei induces sedation and sleep in rats (Kostowski et al., 1969). The raphe nuclei are brain stem areas that represent the origins of the ascending serotonergic pathways. Together with several neighboring cores they provide excitatory input to large populations of cortical neurons and hence facilitate firing via different pathways. If the activity of the raphe nuclei is reduced by electrical stimulation the arousal of neocortex arguably consequently decreases (see 2.3.2). Mechanisms that lead to a transition to sleep modulate the activity of ascending pathways including the serotonergic system which increase cortical excitability

It shows that the mechanisms that regulate sleep decrease the excitability of cortical neurons under the contribution of chemical messengers including melatonin and an inhibition of the ascending arousal system. Subcortical nuclei can produce oscillatory patterns that reflect the circadian cycle whereas thalamus plays a key role in the transition from an awake state to deep sleep. Deep sleep represents a bistable and is characterized by a switch between up and down states of cortical activity. Thalamic nuclei are known to produce sleep spindles which are considered a switch for the transition between light and deep sleep (Montagna, 2005). Besides thalamic regions generate rhythmic activity that oscillates in the delta range. Neocortical slow waves that arise during sleep arguably interact with these rhythms. The underlying processes are more closely examined in the next section.

2.3.2 Neural oscillators

Both the transition to the bistable state of slow wave sleep and fluctuations in the delta range include ascending signals that originate from subcortical and hippocampal areas. Several centres have been identified that generate oscillatory patterns of spiking activity. These patterns include (1) thalamocortical sleep spindles, (2) hippocampal short wave ripples (3) delta waves of thalamic origin and (4) neocortical slow waves. Distinct pacemakers have been identified that act as neural oscillators and cause named rhythms in the brain.

The circumstance that slow waves were present in the Cerveau isolé preparation indicates that slow waves are initiated in brain regions above pons. Thalamus is considered a relay for ascending signals in the awake state. However it also contains nuclei that act as neural oscillators especially under certain conditions, most notably sleep. "During these states, the behavior of thalamic cells is characterized by long-lasting hyperpolarizations and phasic burst discharges recurring rhythmically" (Steriade and Deschenes, 1984, p. 21). Chirurgical removal of thalamus in cats was found to lead to a decrease of the amount of non-REM sleep from 38% to 11.8%. In contrast diencephalic cats still showed sleep spindles (Montagna, 2005). Further support comes from findings from fatal familial insomnia which indicate that thalamic atrophy causes a lack of sleep spindles and delta sleep which implied that "thalamus in the origin of slow wave sleep" (Montagna, 2005, p. 339).

Both sleep spindles and EEG delta waves are present during slow wave sleep. Sleep slow waves in the theta range are generated in distinct nuclei of thalamus. Rostral midline thalamus (RMT) contains neurons which show alternating patterns of spiking activity that oscillate in the theta range. RMT projects unspecifically to cortex.

Another thalamic nucleus is arguably the source of sleep spindles. Sleep spindles manifest as patterns of damped oscillations in EEG. These patterns in EEG arise from synchronous firing of cortical pyramidal cells. It is known however that sleep spindles originate from the thalamic reticular nucleus (Lüthi, 2014). Thalamocortical sleep spindles are considered to be a switch for the transition between light and deep sleep and precede many neocortical slow oscillations (Niethard et al., 2018).

Neocortical oscillations occur spontaneously during deep sleep and deep anaesthesia with distinct frequencies below one Hz. The hypothesis that neocortex contains networks that act as a neural oscillator has been confirmed both empirically and by means of simulation (see section 2.2). Dissection. Name example for empirical finding. Figure 2.1 illustrates the location of oscillatory nuclei which arguably play a role in slow wave sleep and shows the

ventral and dorsal pathway of the ascending reticular activation system. Interaction effects between signals that arise from thalamus and those in cortex have been identified. Thalamus is evidently the source of sleep spindles and theta waves, the most important patterns of slow wave sleep. However it shall be mentioned that thalamus is strongly interconnected with cortex. Electrical stimulation of the reticular core of thalamus was found to cause an increased theta power in hippocampus. In contrast rhythmical stimulation of cortical area 7 evokes periodic oscillations in thalamic neurons that sustain over extended periods of time after stimulation offset. The resulting thalamic rhythm can in return enhance synchronization (Steriade and Deschenes, 1984, p. 21). This finding indicates not only that entrained rhythms exist in thalamus but also that oscillations in thalamus and both archiocortex and neocortex strongly relate to each other.

Proposed function of Medial nucleus and ventrolateral nucleus for sleep initiation. production and autonomic balance.

While the centers can be assumed to produce oscillations independantly it must be noted that signals do not occur fully independantly and indirect projections exist between most cortical and many subcortical areas. Up and down states of neocortical slow waves arise in cortex while interactions with signals from neural oscillors in archiocortex and thalamus can be assumed. The presumed role of cortico-hippocampal synchrony for memory consolidation is discussed in the next section.

thalamic stimulation (dLGN) in transgenic mice produced Ca^{2+} waves that were initiated in V1 During non-REM sleep as well as during many forms of anesthesia, slow oscillations occur spontaneously (Haider et al., 2006), but they can also be evoked by brief sensory stimulation (Stroh 2013)

2.3.3 Neocortical slow waves during sleep and anaesthesia

Thalamus gateway to consciousness. In awake. Relay. However it was found that the thalamus can also act as neural oscillator. In sleep. "During these states, the behavior of thalamic cells is characterized by long-lasting hyperpolarizations and phasic burst discharges recurring rhythmically" (Steriade 1984, p. 21). Thalamic -> Athalamic cat: non-REM sleep 38% -> 11.8% of total observation time. Diencephalic cats (ablation of the entire neocortex and striatum): Still Sleep spindles (after Montagna 2005).

- lesioning the reticular nucleus also decreased delta waves and led the animals to sudden death.36

Preferential thalamo-olivary degeneration. "paramedian thalamus acts as 'final common pathway'""??

Spindles under anaesthesia with barbiturates.

This gives rise to the question: What is sleep and how is it different from anaesthesia? what is sleep. Explains dynamics during anaesthesia not sleep. Sleep.... hippocampus. Long up states → long down states. during anaesthesia.

Functions of sleep. short. Ending with the role of memory formation. Question whether it is different is unclear. The circumstance that it is debated whether sleep-slow waves and slow-waves under anaesthesia are comparable highlights the importance of an approach that allows to automatically characterize these patterns of neural signals.

Methodological triangulation.

2.4 Sleep slow-waves and memory consolidation

According to the hippocampal-neocortical dialogue model of slow-waves, the interaction of hippocampal sharp wave ripples and neocortical slow-waves fosters memory consolidation (Buzsáki 1989, Walker et al. 2009): Recently it was shown that generating neocortical slow-waves in prefrontal networks such that they are coupled to the occurrence of short wave ripples in the hippocampus increases the performance of rats in a memory task (Maingret 2018). Coherently, a correlation of slow-wave activity and the brain-derived neurotrophic factor was found for humans (Duncan, 2013). Spontaneously occurring slow-waves arguably play a similar role for memory. They predominantly occur phase locked to sleep spindles (Demanuele et. al. 2017). However, the exact mechanisms that orchestrate this synchrony are unknown (Sanda et. al 2020). Moreover, it was found that at least two different types of slow-waves exist that potentially relate to distinct synchronization processes (Bernardi 2018). Because of its high spatial resolution fluorescence microscopy can provide more fine-grained distinctions and may potentially reveal trajectories of neural signal transduction during slow wave anesthesia. This highlights the importance of methods that allow to capture the variance of temporospatial patterns of neural slow-waves.

2.5 A working definition

A distinction between slower and shorter waves has already been made in the pioneering electroencephalographic study almost a century ago (Berger, 1929, p. 550). Shortly later the term delta wave was coined to describe oscillations in the recorded voltage in the respective frequency band of around 1 to 4 Hz. The term slow-wave is typically also used to describe oscillatory patterns in EEGs. "Slow-wave activity (SWA) is defined as the EEG power in the slow wave frequency band" (Furrer et al., 2019, p. 1). This frequency band relates to the delta band. Hence the term delta wave which is oftenly used in EEG research can be considered a synonym for slow waves. However, when speaking of slow-waves one oftenly refers to slow wave sleep or anaesthesia where delta waves occur largely independent from higher frequency components.

As explained above slow waves are however more than an oscillation in the scalp potential relative to a reference electrode. The term is also used in relation to the neural phenomenon, its functions and its signatures that can be measured using various methods. Slow-waves are short periods of above baseline activity that occur in bistable states such as deep sleep and anaesthesia which are characterized by reduced neural excitability. Slow-waves are dynamic patterns of recurrent activity of large potentially distinct populations of neurons. They can differ with respect to the pathway of neural flow and the set of recruited cells. Sleep slow waves represent an important mechanism for memory consolidation. Evidence suggests that memory replay occurs both for sparse hippocampal codes and the associated dense cortical manifestations of the previously encoded learning experience. If thalamic slow wave ripples correlate with cortical up-states one may speak of a thalamocortical slow wave event. Analogously one could form subcategorizations for slow-waves that incorporate deviant sets of temporally connected neurons. One may hypothesize that slow waves show neural associations in temporal isolation.

If slow-waves are understood as short periods of above baseline activity they can hence incorporate multiple peaks or slow oscillations in the strict sense. This is especially important for the detection and separation of events. Instead of relying on local minima alone this definition requires to detect in addition whether activity went back to baseline. While conceptualizing slow waves as slow oscillations in the strict sense this approach is chosen. It bears potentials of revealing the temporal dynamics of slow waves that incorporate several stages.

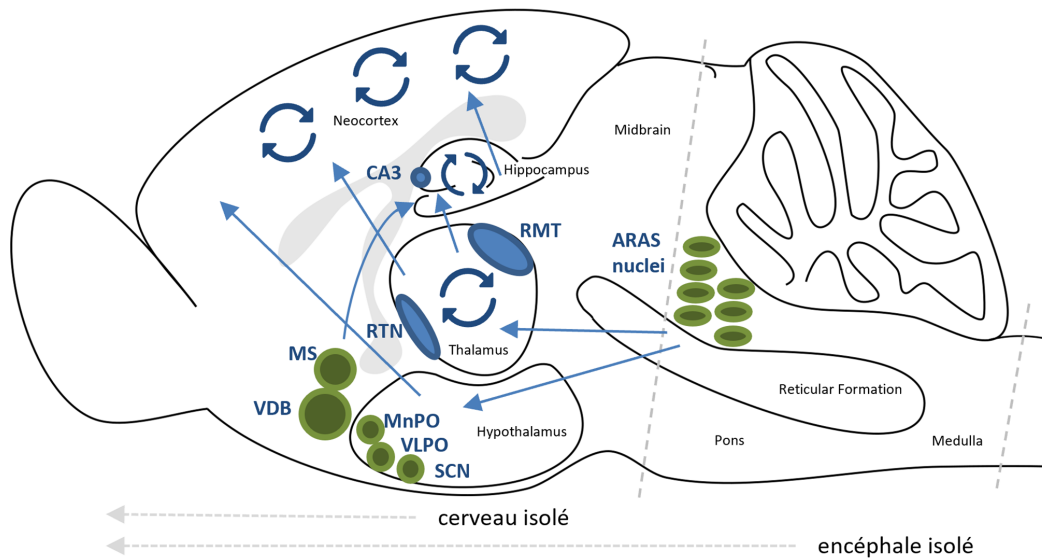


Figure 2.1: Oscillatory centres and the ascending reticular activation system. Circular arrows illustrate sites that act as neural oscillators. Thalamocortical sleep spindles are produced in the reticular thalamic nucleus. Rostral midline thalamus (RMT) contains calcium channels (Cav3.1) that are necessary for the generation of delta waves. It projects to cortex in a non-specific manner. Hippocampal short-wave ripples arise in the subfield CA3 of hippocampus proper. Slow cortical oscillations (<1Hz) and high frequency waves arguably arise in neocortex. Straight arrows indicate the lateral and dorsal pathways of the ascending reticular activation system. Note that centres in the basal forebrain (BF) may suppress the generation of hippocampal short-wave-ripples. Arrows do not relate to the anatomical location of projection fibres and indicate no preferential sites within the target structure. The transections of the preparations “cerveau isolé” and “encéphale isolé” are indicated by dotted lines. Slow waves occur in the cerveau isolé while normal sleep patterns have been observed for encephala isolé. Abbreviations: Reticular thalamic nucleus (RTN), Medial nucleus of the preoptic area MnPO and ventrolateral nucleus of the preoptic area (VLPO), Basal forebrain including the Medial septum (MS) and the Vertical Limb of the Diagonal Band (VDB), suprachiasmatic nucleus (SCN), rostral midline thalamus (RMT), Nuclei of the ascending reticular activation system (ARAS nuclei) including serotonergic neurons in the raphe nucleus, norepinephrine neurons in locus coeruleus and glutamate neurons in the pedunculopontine nucleus. While oscillatory centres are isolated inter projections allow for interaction that can cause patterns to occur at the same time. Own depiction inspired by (Brown et al., 2012, p. 1099).

Chapter 3

Methods

3.1 Dense Optical Flow

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Aliquam ultricies lacinia euismod. Nam tempus risus in dolor rhoncus in interdum enim tincidunt. Donec vel nunc neque. In condimentum ullamcorper quam non consequat. Fusce sagittis tempor feugiat. Fusce magna erat, molestie eu convallis ut, tempus sed arcu. Quisque molestie, ante a tincidunt ullamcorper, sapien enim dignissim lacus, in semper nibh erat lobortis purus. Integer dapibus ligula ac risus convallis pellentesque.

3.2 Helmholtz-Decomposition

3.3 Helmholtz-Decomposition of Dense Optical Flow

Chapter 4

Results

4.1 Main Section 1

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Aliquam ultricies lacinia euismod. Nam tempus risus in dolor rhoncus in interdum enim tincidunt. Donec vel nunc neque. In condimentum ullamcorper quam non consequat. Fusce sagittis tempor feugiat. Fusce magna erat, molestie eu convallis ut, tempus sed arcu. Quisque molestie, ante a tincidunt ullamcorper, sapien enim dignissim lacus, in semper nibh erat lobortis purus. Integer dapibus ligula ac risus convallis pellentesque.

4.1.1 Subsection 1

Nunc posuere quam at lectus tristique eu ultrices augue venenatis. Vestibulum ante ipsum primis in faucibus orci luctus et ultrices posuere cubilia Curae; Aliquam erat volutpat. Vivamus sodales tortor eget quam adipiscing in vulputate ante ullamcorper. Sed eros ante, lacinia et sollicitudin et, aliquam sit amet augue. In hac habitasse platea dictumst.

4.1.2 Subsection 2

Morbi rutrum odio eget arcu adipiscing sodales. Aenean et purus a est pulvinar pellentesque. Cras in elit neque, quis varius elit. Phasellus fringilla, nibh eu tempus venenatis, dolor elit posuere quam, quis adipiscing urna leo nec orci. Sed nec nulla auctor odio aliquet consequat. Ut nec nulla in ante ullamcorper aliquam at sed dolor. Phasellus fermentum magna in augue gravida cursus. Cras sed pretium lorem. Pellentesque eget ornare odio. Proin accumsan, massa viverra cursus pharetra, ipsum nisi lobortis velit, a malesuada dolor lorem eu neque.

4.2 Main Section 2

Sed ullamcorper quam eu nisl interdum at interdum enim egestas. Aliquam placerat justo sed lectus lobortis ut porta nisl porttitor. Vestibulum mi dolor, lacinia molestie gravida at, tempus vitae ligula. Donec eget quam sapien, in viverra eros. Donec pellentesque justo a massa fringilla non vestibulum metus vestibulum. Vestibulum in orci quis felis tempor lacinia. Vivamus ornare ultrices facilisis. Ut hendrerit volutpat vulputate. Morbi condimentum venenatis augue, id porta ipsum vulputate in. Curabitur luctus tempus justo. Vestibulum risus lectus, adipiscing nec condimentum quis, condimentum nec nisl. Aliquam dictum sagittis velit sed iaculis. Morbi tristique augue sit amet nulla pulvinar id facilisis ligula mollis. Nam elit libero, tincidunt ut aliquam at, molestie in quam. Aenean rhoncus vehicula hendrerit.

Chapter 5

Discussion

5.1 Main Section 1

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Aliquam ultricies lacinia euismod. Nam tempus risus in dolor rhoncus in interdum enim tincidunt. Donec vel nunc neque. In condimentum ullamcorper quam non consequat. Fusce sagittis tempor feugiat. Fusce magna erat, molestie eu convallis ut, tempus sed arcu. Quisque molestie, ante a tincidunt ullamcorper, sapien enim dignissim lacus, in semper nibh erat lobortis purus. Integer dapibus ligula ac risus convallis pellentesque.

5.1.1 Subsection 1

Nunc posuere quam at lectus tristique eu ultrices augue venenatis. Vestibulum ante ipsum primis in faucibus orci luctus et ultrices posuere cubilia Curae; Aliquam erat volutpat. Vivamus sodales tortor eget quam adipiscing in vulputate ante ullamcorper. Sed eros ante, lacinia et sollicitudin et, aliquam sit amet augue. In hac habitasse platea dictumst.

5.1.2 Subsection 2

Morbi rutrum odio eget arcu adipiscing sodales. Aenean et purus a est pulvinar pellentesque. Cras in elit neque, quis varius elit. Phasellus fringilla, nibh eu tempus venenatis, dolor elit posuere quam, quis adipiscing urna leo nec orci. Sed nec nulla auctor odio aliquet consequat. Ut nec nulla in ante ullamcorper aliquam at sed dolor. Phasellus fermentum magna in augue gravida cursus. Cras sed pretium lorem. Pellentesque eget ornare odio. Proin accumsan, massa viverra cursus pharetra, ipsum nisi lobortis velit, a malesuada dolor lorem eu neque.

5.2 Main Section 2

Sed ullamcorper quam eu nisl interdum at interdum enim egestas. Aliquam placerat justo sed lectus lobortis ut porta nisl porttitor. Vestibulum mi dolor, lacinia molestie gravida at, tempus vitae ligula. Donec eget quam sapien, in viverra eros. Donec pellentesque justo a massa fringilla non vestibulum metus vestibulum. Vestibulum in orci quis felis tempor lacinia. Vivamus ornare ultrices facilisis. Ut hendrerit volutpat vulputate. Morbi condimentum venenatis augue, id porta ipsum vulputate in. Curabitur luctus tempus justo. Vestibulum risus lectus, adipiscing nec condimentum quis, condimentum nec nisl. Aliquam dictum sagittis velit sed iaculis. Morbi tristique augue sit amet nulla pulvinar id facilisis ligula mollis. Nam elit libero, tincidunt ut aliquam at, molestie in quam. Aenean rhoncus vehicula hendrerit.

Appendix A

Frequently Asked Questions

A.1 How do I change the colors of links?

The color of links can be changed to your liking using:

```
\hypersetup{urlcolor=red}, or
```

```
\hypersetup{citecolor=green}, or
```

```
\hypersetup{allcolor=blue}.
```

If you want to completely hide the links, you can use:

```
\hypersetup{allcolors=.}, or even better:
```

```
\hypersetup{hidelinks}.
```

If you want to have obvious links in the PDF but not the printed text, use:

```
\hypersetup{colorlinks=false}.
```

Bibliography

- Akert, K (1965). "The anatomical substrate of sleep". In: *Progress in brain research*. Vol. 18. Elsevier, pp. 9–19.
- Akert, Konrad, WP Koella, and R Hess Jr (1951). "Sleep produced by electrical stimulation of the thalamus". In: *American Journal of Physiology-Legacy Content* 168.1, pp. 260–267.
- Akimoto, Haruo et al. (1956). "On the sleep induced through electrical stimulation on dog thalamus". In: *Psychiatry and Clinical Neurosciences* 10.2, pp. 117–146.
- Aulinas, Anna (2019). "Physiology of the Pineal Gland and Melatonin". In: *Endotext [Internet]*. MDText. com, Inc.
- Berger, Hans (1929). "Über das elektroencephalogramm des menschen". In: *Archiv für psychiatrie und nervenkrankheiten* 87.1, pp. 527–570.
- Bernardi, Giulio et al. (2018). "Local and widespread slow waves in stable NREM sleep: evidence for distinct regulation mechanisms". In: *Frontiers in human neuroscience* 12, p. 248.
- Brown, Ritchie E et al. (2012). "Control of sleep and wakefulness". In: *Physiological reviews*.
- Buljubasic, Nediljka et al. (1992). "Effects of halothane and isoflurane on calcium and potassium channel currents in canine coronary arterial cells". In: *Anesthesiology: The Journal of the American Society of Anesthesiologists* 76.6, pp. 990–998.
- Buzsáki, György (1996). "The hippocampo-neocortical dialogue". In: *Cerebral cortex* 6.2, pp. 81–92.
- Celotto, Marco et al. (2020). "Analysis and model of cortical slow waves acquired with optical techniques". In: *Methods and Protocols* 3.1, p. 14.
- Choi, Tae-Yong et al. (2014). "Melatonin inhibits voltage-sensitive Ca²⁺ channel-mediated neurotransmitter release". In: *Brain research* 1557, pp. 34–42.
- De Berardis, Domenico et al. (2011). "The melatonergic system: Effects on sleep and implications for the treatment of psychiatric disorders". In: *ChronoPhysiology and Therapy* 1, pp. 59–67.
- Eger, Edmond I (1981). "Isoflurane: A Review". In: *Anesthesiology: The Journal of the American Society of Anesthesiologists* 55.5, pp. 559–576.
- Furrer, Melanie et al. (2019). "Sleep EEG slow-wave activity in medicated and un-medicated children and adolescents with attention-deficit/hyperactivity disorder". In: *Translational psychiatry* 9.1, pp. 1–8.

- Gemignani, Angelo et al. (2015). "Linking Sleep Slow Oscillations with consciousness theories: new vistas on Slow Wave Sleep unconsciousness". In: *Archives italiennes de biologie* 153.2-3, pp. 135–143.
- Jenkins, Andrew, Nicholas P Franks, and William R Lieb (1999). "Effects of Temperature and Volatile Anesthetics on GABA_A Receptors". In: *Anesthesiology: The Journal of the American Society of Anesthesiologists* 90.2, pp. 484–491.
- Jercog, Daniel et al. (2017). "UP-DOWN cortical dynamics reflect state transitions in a bistable network". In: *Elife* 6, e22425.
- Koella, WP (1984). "The organization and regulation of sleep". In: *Experientia* 40.4, pp. 309–338.
- Kostowski, W et al. (1969). "Electrical stimulation of midbrain raphe: biochemical, behavioral and bioelectrical effects". In: *European Journal of Pharmacology* 7.2, pp. 170–175.
- Lüthi, Anita (2014). "Sleep spindles: where they come from, what they do". In: *The Neuroscientist* 20.3, pp. 243–256.
- Miller, Amanda L, Danny Theodore, and Jason Widrich (2020). "Inhalational anesthetic". In: *StatPearls [Internet]*. StatPearls Publishing.
- Moghadam, Hadi et al. (2019). "A Comparative Study of Cell Specific Effects of Systemic and Volatile Anesthetics on Identified Motor Neurons and Interneurons of *Lymnaea stagnalis* (L.), Both in the Isolated Brain and in Single Cell Culture". In: *Frontiers in Physiology* 10, p. 583.
- Montagna, Pasquale (2005). "Fatal familial insomnia: a model disease in sleep pathophysiology". In: *Sleep medicine reviews* 9.5, pp. 339–353.
- Nghiem, Trang-Anh E et al. (2018). "Two types of slow waves in anesthetized and sleeping brains". In: *bioRxiv*, p. 430405.
- Niethard, Niels et al. (2018). "Cortical circuit activity underlying sleep slow oscillations and spindles". In: *Proceedings of the National Academy of Sciences* 115.39, E9220–E9229.
- Qazzaz, Munir M and William Winlow (2017). "Modulation of the passive membrane properties of a pair of strongly electrically coupled neurons by anaesthetics". In: *EC Neurol* 6, pp. 187–200.
- Steriade, M and M Deschenes (1984). "The thalamus as a neuronal oscillator". In: *Brain Research Reviews* 8.1, pp. 1–63.