

OSNABRÜCK UNIVERSITY

MASTER THESIS

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

A new approach for temporospatial pattern detection in neuroimaging

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## 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:

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- Where I have consulted the published work of others, this is always clearly attributed.
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# *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.



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# List of Abbreviations

**LAH** List Abbreviations **Here**  
**WSF** What (it) Stands For





# Physical Constants

Speed of Light  $c_0 = 2.997\,924\,58 \times 10^8 \text{ m s}^{-1}$  (exact)



# List of Symbols

$a$	distance	m
$P$	power	W (J s <sup>-1</sup> )
$\omega$	angular frequency	rad



*For/Dedicated to/To my...*



## 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. At intermediate concentrations many agents cause propagating low-frequency waves that occur in close succession and manifest as delta oscillations in EEG. Similar patterns can be observed during deep sleep. 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. 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 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 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 slow-waves of different shapes and signal strengths.

Hence a new approach was developed that allows to distinguish and characterize 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 anesthetics change important properties of neurons most prominently their excitability which arguably explains why bistable states of network level activity can be observed empirically (section 21). While important differences exist between anaesthesia and sleep, slow-waves can be observed in both states (section 2.2). Here slow-waves are understood as extended periods of recurrent neural activation that manifests as above baseline activity (section 23).

The methods to analyze 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 presented 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 shown.

Finally 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 flow of neural activity. 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. However, the approach allows presented here for more fine grained distinctions and reveals a complex topology 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 <sup>1</sup>.

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

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<sup>1</sup>For 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).

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 of thalamic circuits plays 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 circadian 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 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. 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.

The exact pharmacological mechanisms of action of inhalant anaesthetics such as isoflourane remain uncertain (Miller et al. 2020). Effects on several ion channels have been reported including both chemically gated  $\text{Cl}^-$  channels (GABA receptors and glycine receptors) and  $\text{K}^+$  channels (Glutamate receptors). For example isoflourane is known to reduce the hyperpotentiation that results from  $\text{Cl}^-$  influx as a consequence of in vitro GABA administration (Jenkins 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  $\text{K}^+$  channel currents resulting in smaller electrically triggered peak amplitudes of action potentials (Buljubasic 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 dependance (Mogdahan 2019). 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 isoflurane 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 implausible 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.<sup>2</sup>

Arguably the abovementioned effects explain important features of population level dynamics. Marshall (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 = .56% 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 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

<sup>2</sup>Note that other anaesthetics were found to increase the membrane capacitance

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 unapacifically by a release or an accumulation of disperse chemical messengers. Second processes exist that incorporate electrical signalling and 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 activity of both other types of processes. However oscillatory activity also occurs in the delta range. 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  $\text{Ca}^{2+}$  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 above-mentioned 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. 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. Similar to intralaminar thalamus the electrical stimulation of the raphe nuclei induces sedation and sleep in rats. The raphe nuclei are brain stem areas that represent of origin of the ascending serotonergic pathways and hence play an important role in the arousal system. The mechanisms that lead to a transition to sleep include an inhibition of the ascending arousal system and hence decrease the excitatory input to cortical neurons in the sense of the second of the abovementioned systems.

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).

### 2.3.2 The ascending reticular activation system and neural oscillators

Both the transition to the bistable state of slow wave sleep and fluctuations in the delta include ascending signals that originate from subcortical areas. Several centres that generate different oscillatory patterns have been identified. These patterns include thalamocortical sleep spindles, hippocampal short wave ripples and slow waves in the delta range that are of thalamic origin.

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. The nuclei mentioned above including serotonergic neurons in the raphe nucleus, norepinephrine neurons in locus coeruleus and glutamate neurons in the pedunculopontine nucleus - the most important excitatory neurotransmitters. These nuclei are known as the source of the ascending reticular activation system.

The circumstance that slow waves were present in the Cerveau isolé preparation indicated 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 which is especially under certain conditions, most notably for 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). 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 (after Montagna 2005). Further support



comes from findings from fatal familial insomnia which indicate that thalamic atrophy is "associated with lack of sleep spindles and delta sleep implicate the thalamus in the origin of slow wave sleep" (Montagna 2005). 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. Another thalamic nucleus is arguably the source of sleep spindles i.e. sequences of synchronized high frequency bursts. They are considered to be a switch for the transition between light and deep sleep and precede many neocortical slow oscillations.

Neocortical oscillations occur spontaneously during deep sleep and deep anaesthesia with distinct frequencies of below 1 Hz. The hypothesis that neocortex contains networks that act as a neural oscillator themselves has been confirmed both empirically and by means of simulation (see also section 2.2). Dissection. Name example for empirical finding. Figure 1 illustrates the location of oscillatory nuclei and shows the ventral and dorsal pathway of the ascending reticular activation system. While ... interconnected

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

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 1984). This finding indicates not only that entrained rhythms exist in thalamus but also that oscillations in thalamus and both archicortex and neocortex strongly relate to each other.

### 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 -> A thalamic 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.<sup>36</sup>

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 independant from higher frequency components.

As explained above slow waves are however more then 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 short 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.

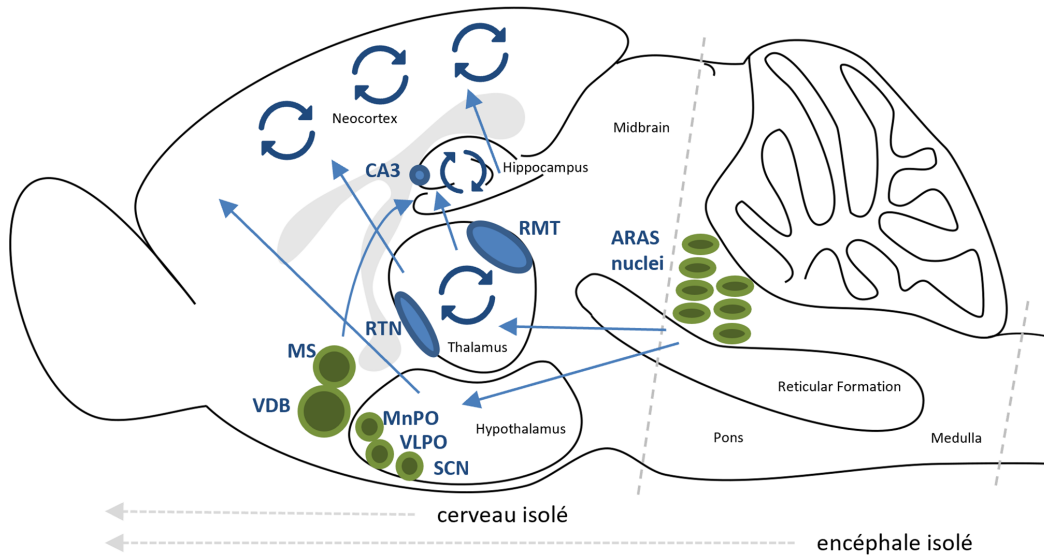


FIGURE 2.1: Circular arrows illustrate sites that act as neural oscillators. Thalamocortical sleep spindles are produced in the reticular thalamic nucleus. Rostral midline thalamus (RMT) contains calcium channels (Cav3.1) that are necessary for the generation of delta waves. It projects to cortex in a non-specific manner. Hippocampal short-wave ripples arise in the subfield CA3 of hippocampus proper. Slow cortical oscillations ( $<1\text{Hz}$ ) 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” ad “encephale 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

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### 3.2 Helmholtz-Decomposition

### 3.3 Helmholtz-Decomposition of Dense Optical Flow



## Chapter 4

# Results

### 4.1 Main Section 1

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#### 4.1.1 Subsection 1

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#### 4.1.2 Subsection 2

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### 4.2 Main Section 2

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## Chapter 5

# Discussion

### 5.1 Main Section 1

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#### 5.1.2 Subsection 2

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## 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}.
```



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