Summary

1 Introduction

As an engineer, I chose biology as the application of my skills. I found that fundamental research in neuroscience in particular is in great need of technical background, from experimental design, onto signal processing implementation and development and finally in understanding models and analogies with electrical and computational systems.

Here lies my main motivation for the development of the thesis: the combination of curiosity towards a new field, the diversity of necessary skills and the overall potential of the results, with implications in human welfare fascinate me. With these arguments in mind, I defined my research goal to provide theoretical support, practical implementation and experimental evaluation of the dynamics of neural networks in vivo.

2 Background

Brain processes arise as a result of function and connectivity of cellular networks generating complex patterns of activity (Arieli et al., 1996; Friedrich and Laurent, 2001). Traditionally, the study of these processes relied on the correlations between sensorial stimuli (provided as input), the activity of individual neurons or populations and further towards the motor output of behavior (Brown et al., 2004). However, recent studies zoom in on the activity of neuronal networks with the goal of defining precisely how a pattern of activity arises from the interaction of neurons, neuronal sub-populations and cortical networks (Dhawale et al., 2010; Jurjuţ et al., 2011; Laurent, 2002).

The link between physiology and network dynamics is still elusive, since it holds a great level of complexity. The multiple "degrees of freedom", factors which influence the way networks react to input is one of the reasons why there are still so many missing pieces of this puzzle. For example, the architecture of neural networks has been documented extensively, both at small (Grillner et al., 2005) and large scale (Felleman and Van Essen, 1991; Hilgetag and Kaiser, 2004; Sporns et al., 2004). Morphological features of neurons, membranes and synapses have been described in many studies throughout the years (Colquhoun and Sakmann, 1998; White and Keller, 1989; Armstrong and Hille, 1998; Bertolino and Llinas, 1992). Despite of such thorough evaluation of composing factors, adding them together to build a coherent concept by which they govern network dynamics proves to be quite difficult. Several attempts have been made to describe how network function is defined by network connectivity (Wang and Buzsáki, 1996) and synaptic properties (Bartos et al., 2007; Maex and De Schutter, 2003) and further, how the activity of these networks interact on a larger scale to give rise to dynamic cortical states (Battaglia et al., 2012). However, fewer studies looked at the effect of intrinsic neuronal properties on neural network dynamics (Moca et al., 2014; Muresan and Savin, 2007). Despite our familiarity with the neuronal membrane: the pool of expressed channels, their kinetics (Armstrong and Hille, 1998; Bertolino and Llinas, 1992; Hutcheon and Yarom, 2000), electrical currents (Holmes et al., 1992; Rall, 1969) and their sub- and supra-threshold activity (Holmes et al., 1992; Rall, 1969), the differential effect of these properties on network-scale cortical activity has not been coined.

Our research group is interested in oscillatory patterning of neuronal activity (Moca and Mureşan, 2011). Specifically, we focus on the stability and responsiveness of cortical activity (Muresan and Savin, 2007) and look for the underlying mechanisms that could account for the wide variety of features of network dynamics (Moca et al., 2014). One line of research was to look at the implications of neuronal membrane properties on network dynamics: neuronal membranes which behave as passive low-pass filters (integrators) or membranes that exhibit a frequency preference (resonators) (Hutcheon and Yarom, 2000). These two types of membranes have been defined experimentally in the thalamus and cortex (Gray and McCormick, 1996; Hutcheon et al., 1996; Llinás et al., 2007) and their interaction is believed to have some implication in oscillatory activity in those areas. One important experimental finding is that the resonant property of membranes is not a fixed, but can be altered via voltage (Hutcheon et al., 1996) and neuromodulatory pathways (Steriade et al., 1991) in a dynamic way, thus making resonance a great candidate for changing the stability and responsiveness of oscillatory behavior in the brain.

In-silico studies have looked closer into the effects of resonance on network activity (Muresan and Savin, 2007; Moca et al., 2014). Resonance was shown to promote the development and stability of oscillatory transients (Muresan and Savin, 2007). Networks where excitatory cells are integrators and inhibitory cells are resonators are capable of entraining more stable and robust gamma oscillations, compared to purely integrator networks (Moca et al., 2014). Therefore, we hypothesize that intrinsic neuronal properties dramatically influence cortical network dynamics.

3 Research question

The goal for this thesis is to characterize oscillatory responses in the visual cortex and to further investigate the potential effects of neuronal membrane properties on cortical responses in vivo. We look at visual responses to a wide pallet of stimuli and evaluate the relationship between network and unit activity with respect to input. Also, we manipulate arousal pathways with the aim of perturbing cortical state and observe the resulting perturbations in response properties. In this way, we look for hallmarks of resonance, both at the level of neuronal membranes and at that of networks.

In this thesis, we propose an experimental approach to investigate how electrical membrane properties of neurons influence network dynamics. We have formulated three intermediate objectives to guide us along the accomplishment of the project:

- First, our goal is to document, design and build the necessary infrastructure for performing a series of experimental recordings and manipulations for investigating cortical dynamics in vivo. This process includes hardware configuration, adaptation and building, the implementation of software solutions for hardware control, acquisition, data processing and analysis and the development of algorithms for optimal extraction of information form our data.

- Secondly, we aim to identify network dynamics characteristics that can serve as indirect evidence of membrane resonance. For this, we choose an in vivo experimental model, we designed and built our experimental setup and analysis methods and performed an extensive characterization of primary visual cortex gamma oscillatory network response. We characterize circuit-level behavior as a function of periodic input to probe for integrator/resonator circuit responses, in relation to theoretical predictions.

- Thirdly, we aim to investigate the potential modulator of mechanisms membrane resonance. For this, we performed a series of modulations on cortical state in order to observe a switch in network dynamics that could be generated by individual neuron membrane properties. We varied levels of anesthesia to simulate arousal and we applied cholinergic modulation to simulate the ac-

tivation of arousal pathways leading to the visual cortex. Our aim is to investigate the influence of neuromodulatory pathways, such as the noradrenergic and cholinergic systems, on local and global circuit properties. We seek to understand if neuromodulation produces dynamic changes of membrane properties and how these local changes alter the dynamics of entire cortical circuits.

These three objectives are reached through a series of methods that will be described in detail in the section that follows.

4 Experimental strategy

In a first step we implement a setup of electrophysiological recordings via use multi-electrode silicon probes in anesthetized rodents, while delivering various input types. Sensorial stimulation is achieved via a palette of visual stimuli consisting of drifting gratings of various orientations, with fixed or variable contrast. Direct stimulation using light is implemented, using optogenetics and cortical modulation is applied through a novel neurotransmitter delivery method. We establish a pipeline for data processing and analysis, both by implementing novel time and frequency-domain analyses (Jurjuţ et al., 2009, 2011; Mureşan et al., 2008; Nikolić et al., 2012), and developing new algorithms for neuronal response characterization.

In the second step we aim to establish the responses of cortical circuits to input delivered via sensory stimulation. Extracellular recordings monitor the activity of a large number of neurons and we focus on the response of the circuit to different stimulation parameters. We use multi-electrode extracellular recordings and optogenetics in anaesthetized rodents to establish the responses of cortical circuits to periodic input delivered via optical stimulation. This is performed in transgenic mice whose neurons express light-activatable ion channels, e.g. based on Channelrhodopsin-2 (Cardin et al., 2009). We use periodic stimulation in the form of simple sinusoidal signals with various frequencies. Extracellular recordings monitor the activity of a large number of neurons and we focus on the response of the circuit to different stimulation frequencies. Previous studies indicate that cortical circuits have a resonant circuit response to periodic inputs (Cardin et al., 2009) and results show that this property is manifested differently when interneurons are integrators versus the case when they are resonators (Moca et al., 2014). Purely integrator circuits that produce oscillations via a Pyramidal INterneuron Gamma (PING) mechanism behave differently from circuits with integrator pyramids and resonator interneurons, which produce oscillations via the Resonance INduced Gamma (RING) mechanism (Moca et al., 2014). Both PING and RING networks exhibit resonant circuit property, but their frequency response profiles to periodic input are dramatically different. The theoretical prediction is that resonance enhances gamma responses to slow inputs and impairs them at fast inputs, whereas integration favors the opposite (Moca et al., 2014) this will be extensively tested in vivo using simultaneous stimulation and recording. By exploiting these observations, we intend to probe for the presence of the RING mechanism during in vivo stimulus-induced gamma oscillations.

The third step of the experimental part deals with the manipulation of membrane properties via arousal pathways. We attempt to modulate arousal via anesthesia or by using controlled stimulation of neuromodulatory pathways. Resonance, for example, depends on voltage-gated ion channels (Hutcheon and Yarom, 2000), which are influenced by several neuromodulators (Hutcheon et al., 1996) like norepinephrine, dopamine and acetylcholine. Norepinephrine modulates the outwardly rectifying K^+ current (I_K) and the hyperpolarization-activated cation current (I_H) (Banks et al., 1993), dopamine the persistent sodium current I_NaP (Gorelova and Yang, 2000), and acetylcholine the M-current (Halliwell and Adams, 1982). All these currents are involved in producing membrane resonance (Hutcheon and Yarom, 2000). To causally control the resonance-involved

currents, we will couple the above-mentioned recording paradigms with simultaneous manipulations of neuromodulatory pathways. The cholinergic stimulation is controlled by administration of acetylcholine on the cortical surface, close to the recording site. Cholinergic modulation is known to predictably enhance expression of gamma oscillations (Munk et al., 1996). The controlled modulation of membrane properties via neuromodulators with concomitant sensory stimulation and extra-cellular recording from cortical circuits allows a more clear understanding of the causal role of membrane properties, such as integration and resonance, in circuit dynamics.

5 Experimental setup construction

Here, we tackle the issues we have met along the way in the consolidation of our neuroscience laboratory. The elementary pieces themselves have been either acquired from specialized producers or have been custom built as part of this PhD project, in which case their design and prototyping has been a main contribution for this thesis. The engineering side of the project is at its peak, as the understanding, adaptation and design of both hardware and software components required a broad view on electronics, signal acquisition and processing theory.

The experimental work developed for this thesis consists of recording electrophysiological signals from the visual cortex of anesthetized mice while presenting a series of visual stimuli.

5.1 Hardware design and construction

For visual stimulation, we used a monitor that was placed within the visual field of the animal. The stimuli consisted of sinusoidal bars, called gratings, of different orientations, moving across the monitor. Therefore, local luminance of the stimulus consisted the first order stimulus parameter. We generated different contrast levels defined by the difference between bright and dark pixels, determining a second order stimulus parameter. And third, we changed the contrast dynamically within an experimental trial, first increasing and then decreasing, causing a variation in contrast rate, thus generating a third order stimulus parameter.

This was the input delivered to the network. To measure the output, the activity, we used an electrophysiology recording setup. An array of electrical contacts etched on a silicone substrate was inserted in the visual cortex. The arrangement of these contacts varied across probes and recorded electrical activity coming from cells in the volume in the proximity of the contacts. The signal was then preamplified close to the probe, then amplified with a programmable gain amplifier and converted into digital signals that we can observe real time on the computer.

To position the probe precisely at the coordinates of the visual cortex and to move it finely in order to find the area of interest, the animal is fixed in a stereotactic frame. We built a custom device to hold the skull of the animal so that it would not induce vibration or movement and would allow access to the visual cortex and not block the field of view of the animal. The probe was fixed to a hydraulic mechanism, which needed a custom built adapter to provide a firm grip.

We also implemented a setup for optogenetic manipulation. Our mice had a genetic mutation which induced the presence of particular ion channels in neuronal subpopulations which opened when light was shined on them. This caused the direct activation of these neurons, providing a known input directly on the network elements. We used this input delivery method for measuring the frequency characteristic of the cortical circuit. For this, a diode-pumped blue laser was used. We custom-built a mechanical shuttering system which would be able to generate the necessary frequency range of pulses (5-105 Hz). We used a voice coil actuator from a disabled hardware and build a driver using an H-Bridge and a resistive-capacitive parallel circuit for adjusting the damping coefficient. This was useful in counteracting the mechanical inertia of the arm of the actuator. The laser light was coupled to a 200 um fiber optics via a fiber coupler and placed on top of the cortical area of interest using one of the stereotactic arms.

During the experiment, the physiological parameters of the animal were constantly monitored. Temperature was kept constant using a heating pad. For monitoring heart and respiration rate, we built our custom solution, using a passive piezoelectric sensor placed under the animal. Vibrations induced by heard beats and respiration bouts generated voltage fluctuations which were acquired, via a voltage limiter, by the same acquisition hardware that acquired the electrophysiological signals. In software, we detected the respiration peaks and measured the heart rate between each two successive breaths.

For anesthesia delivery, we used a vaporizer connected to a pressurized oxygen tank. Air flow was controlled via a flow meter and the anesthetic concentration was controlled mechanically. We built a custom part for delivering the anesthetic to the animal in a small volume, while minimizing the blockage of the mouse field of view.

For perturbing the cortical state of the animal, we applied a neurotransmitter on the surface of the brain. We custom-built a system that would continuously perfuse liquid above the surface of the brain. In this way, we could easily switch solutions during the experiment and ensure constant concentration of modulator agent within experiment.

5.2 Data analysis algorithms - development and implementation

The raw signal was processed in order to extract the relevant data for our analysis. Low frequency components represent the local field potential(LFP), the sum of electrical activity within a larger volume around the electrodes. High frequency components contain action potentials generated by neurons in the close proximity. These action potentials were detected via an amplitude threshold and were treated as binary events (MUA).

We implemented a series of algorithms to analyze cortical response. The event related potential is the average potential across multiple stimulus presentations, aligned to stimulus onset. Peri-stimulus time histogram represents its analog for multi unit activity, where the number of events per time bin are averaged across trials.

Correlation analysis was implemented for binary, continuous and binary-continuous correlations. We used cross-correlation and auto-correlation analysis to detect oscillatory coupling and activity bursts which are transient or non-sinusoidal. To quantify the oscillatory nature of binary events in multi unit activity, we implemented the oscillation score algorithm, which computes the normalized spectral power of the smoothed autocorrelation histogram of the binary signal.

For computing the spectrum, we used the Welch method of the Fourier transform in order to minimize the noise in the average spectrum and to have a time-resolved estimation of spectral features. The spectrogram was z-scored against a baseline period, where no stimulation was applied, to reduce the 1/f characteristic of spontaneous activity and better visualize stimulus specific spectral features.

To dissociate between frequency and power fluctuation in the three-dimensional time-resolved spectrum, we designed an algorithm of defining the trajectory of the response. The response crest is defined via an iterative process, following the local maxima across time. Therefore, power, spectral bandwidth and frequency of the response can be analyzed independently.

Since we used multiple orders of stimulus parameters, luminance, contrast and contrast rate, at different timescales, we needed a way to dissociate between modulatory actions. For this, we adapted an algorithm for empirical mode decomposition, which has the advantage of correctly separating spectral components in the time domain, without making assumptions about sinusoidal shape, and stability of the component. The method consists of the iterative subtraction of the mean of the top and bottom envelopes of the signal until an intrinsic mode function remains. We adapted this method for extracting two mode functions, one related to luminance variation and the other to contrast.

At the end of this part of the project we had a functional electrophysiology experimental rig and a processing pipeline that would render relevant metrics for evaluating oscillatory activity in

neural networks.

6 Characterizing cortical oscillatory responses to visual stimuli

6.1 Fixed contrast

Cortical processing exhibits a complex trajectory through a multidimensional space. The induction of surgical anesthesia limits the states of this trajectory, as the animal is not capable of providing feedback, make decisions or generate behavior as a reaction to the perceived stimulus. However, the study of visual responses in the primary visual cortex when displaying a relatively simple, documented stimulus reveals a wide palette of interesting features. Responses to stimulus change are present, such as onset and offset responses described above. Slow modulation of amplitude signals the presence of adaptation to luminance and contrast of the presented stimuli. All in all, the anesthetized cortex still preserves much of the relevant functions in the primary visual areas.

However, these features are generally ignored in investigations of cortical processes. A repeated stimulus is presented and the average features in a steady-state regime are considered, but this disregards two main issues: firstly, the cortex is designed to process changes. It is in the response difference between one stimulus and the next that the highest quantity of information lies. Ignoring all response variability across stimulation period or providing constant stimulation as input to the cortical network, equipped for differential processing, leads to the analysis of merely amplified noise and redundant information. Secondly, gratings and other visual stimuli used in vision studies are not static stimuli. They elicit a time-course of input drive upon reacting cellular populations that generate a response which is highly complex. For example, response frequency varies with grating position. An averaging across multiple orientations and even across the entire stimulus presentation leads to a smearing of the spectral component of interest, increasing its bandwidth and decreasing its power. Interpretation of these results is therefore biased.

Here, we performed a detailed characterization of visual responses to drifting gratings, taking into account the time course of visual stimulation. We dissected the evolution of responses in time, by extracting independent parameters of population activity and studied the covariance of these parameters with respect to input, with an emphasis on gamma-band oscillations. Our aim was to determine features of network dynamics which provide insight on underlying mechanisms of gamma oscillations in the mouse visual cortex.

A first observation concerns the reliability of visual responses in the mouse visual cortex. We found that both anesthetic type and genetic strain of the animal can have a great impact on the observed activity. Visual responses were barely observable when using ketamine as an anesthetic, whereas under isoflurane anesthesia, responses were robust and reliable. Moreover, the usage of BALB albino mice was considered suboptimal for our experiments, due to their reduced visual acuity. Therefore, the use of C7BL6 mice under isoflurane anesthesia was the chosen configuration for subsequent experiments.

An interesting experimental consideration was that visual responses were always associated with gamma-band oscillations, which is not the case for most studies in larger mammals such as cats (Rodriguez et al., 2010). We found that we always had gamma oscillations when firing rate was modulated by the stimulus. This increase in incidence in gamma oscillations could be due to the different cortical organization of mouse brain compared to larger mammals. Even though retinotopy is preserved in the mouse visual cortex, orientation preference maps are no longer ordered, but have a salt and pepper organization (Ohki et al., 2005). These differential features change the way neuronal activity is sampled by the silicon probe: the parameters of the cells within the volume of interest are more diverse and therefore, the average type of responses

obtained are more stable as variability is averaged.

When looking at the time-evolution of visual responses, we found that onset and offset responses elicit a reproducible pattern in a three-dimensional time-frequency-power space in LFP and MUA. Specifically, both onset and offset response generated an increase in frequency, but gamma power was only increased at onset. The onset response firing gave little information about stimulus features, such as orientation, whereas offset response showed higher selectivity, rendering a short term memory mechanism at primary cortical levels.

Across the duration of visual stimulation, luminance change, generated by the passage of the grating through the receptive fields of the cells within the recorded area triggered an increase in both LFP frequency, power and MUA oscillation strength, but not necessarily in a correlated manner. Frequency was highest at a different phase then power and oscillation strength with respect to luminance variation. Therefore, frequency and power of gamma oscillations in LFP do not always covary, fact which is not explained in existing theories behind gamma oscillatory behavior in cortical circuits.

The characterization of gamma oscillations in response to visual stimulation has the potential of revealing features which are highly relevant for understanding network dynamics. A more detailed and independent analysis of response parameters reveal that not all response features can be explained by current models of gamma oscillation generation and enhancement and there is a need in going deeper into the investigation of the revealed features, until the origin of these phenomena is nailed into place and a coherent context for gamma dynamics and function is revealed.

6.2 Variable contrast

The effects of smooth contrast variation on visual response patterns of LFP and MUA reveal high level computation in the anesthetized primary visual cortex. Even though transient onset and offset responses were reduced by the gradual increase of visual input strength, we observed a number of interesting features regarding modulatory effects of contrast and luminance.

First of all, local luminance and contrast modulation both altered response patterns, but they evolve through different physiological mechanisms. When separating the two, contrast modulated event-related potentials and gamma frequency in a different, if not opposite, correlation strength compared to luminance.

Secondly, the variation of stimulus response to contrast reveals a nonlinear dependence of oscillation score with contrast. Oscillation strength was highest at intermediate contrast levels, whereas it decreased at contrast limits. This indicates a state change in the network, a switch between processing modes: a putative passive mode, where contrast is below perception threshold, an active, prediction generating mode and an idle mode, where prediction and incoming stimulation coincide.

And third, contrast rate appears to be one of the relevant parameters for primary visual processing. The change in contrast rate triggered a slow voltage deflection in extracellular ERP. Differences in other parameters such as LFP gamma power and MUA firing rate confirm that there is an effect of changing the rate of contrast variation from positive to negative.

All of these features reveal the fact that contrast response in the visual cortex is a complex mechanism, independent of that of luminance modulation. Nonlinearities indicate state changes putatively correlated to perception. The stimulation protocol is therefore well tuned for analyzing underlying mechanisms of gamma oscillatory activity in a wide variety of cortical states and processing conditions.

6.3 Computing the frequency characteristic of the cortical circuit

The direct manipulation of neuronal activation is a useful tool for cortical circuitry investigations. Here, we showed that optogenetics can be used to describe the frequency characteristic of a cortical volume, by periodically activating subsets of neurons at various frequencies. We showed that circuits in the primary visual cortex respond to slow input with an oscillatory response in the gamma range, similar to physiological stimulation conditions. When input frequency approaches the preferred response frequency, it enhances oscillatory activity, without increasing the effective firing rate, but rather through synchronization mechanisms. At high input frequencies, the network is not capable to maintain intrinsic oscillatory mechanisms due to the perturbing input. Therefore, neuronal activation follows stimulation pattern.

Such features of neuronal response are highly informative when investigating underlying mechanisms of gamma oscillations. For example, different frequency characteristics arise when different neuronal populations receive the input. For example, if fast-spiking neurons are driven optogenetically, the increase in power in the gamma range is significantly higher than when regularly spiking neurons are targeted, underlying the primary importance fast-spiking cells have in the generation and stabilization of gamma oscillations (Cardin et al., 2009). On the other hand, the frequency characteristic could give hints about the intrinsic properties of neurons within the recorded population. Our group revealed that intrinsic membrane resonance expressed in the inhibitory population of a cortical network could alter the frequency response of the surrounding network (Moca et al., 2014). Specifically, when resonance is expressed in the inhibitory population, responses are stronger at slow stimulation frequencies compared to passive integrative membranes, wheres response power is smaller for high frequencies.

We conclude that the characterization of network dynamics using variable input frequencies is a relevant factor when dissecting underlying mechanisms of gamma oscillations. The features we have revealed could be indicative of specific features that lead to the generation, enhancement and stabilization of gamma activity in the cortex.

7 Modulating cortical dynamics via arousal

7.1 *In silico* modeling

Our project set off with the goal of determining the characteristics of gamma oscillations in the mouse visual cortex, for determining whether this is a good experimental model for investigating the effect of intrinsic cellular properties on the oscillatory behavior of the neuronal network.

We have previously shown via *in silico* modeling that cortical state dependent expression of gamma resonance in interneurons could explain strong state dependence of gamma oscillations under anesthesia (Moca et al., 2014). In the presence of interneuronal resonance, gamma oscillations exhibit large power and stable frequency with respect to stimulus intensity.

We have determined thus far that mechanisms underlying gamma oscillatory activity are complex and existing hypotheses cannot explain all the experimentally determined response features. In these conditions, we turned to in silico modeling to further investigate the effects of neuronal properties on network dynamics. Therefore, the investigation of underlying mechanisms of gamma oscillations becomes an iterative process of experimental observations, hypothesis generation, in silico testing and again experiment.

Specifically, we wanted to test two particular hypotheses using neuronal models: One is to investigate the effects of resonance on response patterns in the visual cortex. The experimental findings in chapter **??** show that response patterns show features which are poorly described by theories involving the interaction of inhibition and excitation only. We wanted to see whether our model could reproduce the effects we see in vivo, where gamma frequency and power are decoupled and if resonance can explain any of the effects observed.

Experimental findings of visual responses suggest that the passage of a grating within the receptive field generates a response that varies both in power and in frequency. We have developed a method of defining the trajectory of the response in the three-dimensional space of time, frequency and power to investigate the projection of the response on each of these dimensions for an independent analysis.

In experimental data, we found that grating passage did not modulate power and frequency of gamma oscillations in the same manner. Most importantly, a peak in power was not necessarily accompanied by a peak in frequency. The correlation between these features was relatively low ($r = 0.37$) when the spatial and temporal frequency of gratings was low (0.1 cycles/degree, 1.75 cycles/s). This fact is not consistent with theories of underlying mechanisms of gamma oscillations that imply that power and frequency covary as a result of the same generator mechanism.

We also looked at the correlation between frequency and power in the case of contrast modulation. For the same stimulation conditions, contrast modulation induced a higher correlation between frequency and power than grating position, suggesting the possibility of frequency and power to be modulated by different factors within the gamma generation mechanism.

We applied the same analysis on our in silico cortical network model in two different configurations, with the aim to investigate frequency and power relationship under the assumptions of our artificially derived networks. Previously, we looked at the effect of intrinsic membrane resonance on the stability and robustness of gamma oscillations considering slow-varying stimuli (Moca et al., 2014). Now, we changed the frequency of the input to simulate moving gratings and look at the pattern of gamma power and frequency across time in two networks of interest: Excitatory neurons were modeled as pure integrate-and-fire (IF) neurons, while interneurons were either IF or Izhikevich neurons exhibiting membrane resonance (RES). By switching the interneuron model, we obtained two network types: IF-IF and IF-RES.

The input was sinusoidal, with a frequency of 1 Hz to resemble our fixed contrast stimulation setup. The input current was delivered to both excitatory and inhibitory neurons, weighted accordingly to reach physiological firing rates for both populations, as determined empirically (Moca et al., 2014).

Our results show that indeed resonance leads to a stronger stability of oscillation frequency. In the case of IF-IF networks, both power and frequency followed the sinusoidal input. The range of covariance in frequency was within 20 Hz when power varied by 1.2 uV^2/Hz. In IF-RES networks, even if power variability was the same as in IF-IF networks, frequency varied only within a range of 6 Hz. When looking at the correlation between frequency and power across response patterns, the correlation was stronger for IF-IF networks than for IF-RES.

When looking at the shape of the response bursts, we see that in the case of IF-IF networks, the response burst is longer than in the case of IF-RES, even though the maximum power value is similar in the two cases. The response in the case of integrator circuits seems to be modulated by input in a stronger fashion. On the other hand, when resonance is present, the transition from no response to strong power is binary at a certain threshold of input drive. The network transitions abruptly over the power threshold, after which the power continues to increase to a high value. In this high activity state, response power is not strongly modulated by input.

Another question we had originates from the optogenetic study of frequency characteristic of cortical networks. We had a number of available transgenic mouse lines where Channelrhodopsin was expressed in different neuronal populations. However, the frequency characteristic dramatically depends on the population which receives input drive. Therefore we were interested to study, in a controlled in silico model, how the targeted stimulation of different populations of cortical neurons leads to differential properties of oscillatory entrainment.

When either E or both E and I neurons were periodically stimulated, we found circuit resonance for all networks. At low stimulation frequencies (<15 Hz), both IF-IF and IF-RES networks sustained oscillations, with a frequency in the range of 18-30 Hz, irrespective of the input. For stimulation frequencies in the range of 18-40 Hz, networks were locked to input frequencies. At

higher stimulation frequencies, the oscillation stabilized within the 18-30 Hz range and while frequency was higher for the IF-RES network, power was higher for IF-IF networks. The latter exhibited circuit resonance at multiples of the main peak frequency when only E neurons were stimulated, indicating m-to-n locking. This was not the case for IF-RES networks, where interneuron resonance constrained the circuit resonance to a limited domain. When input was delivered to I cells only, IF-IF networks were not able to sustain oscillations, while IF-RES nets exhibited a circuit resonance peak matching interneuron resonance frequency.

Our results indicate complex network responses to periodic stimulation, dependent on input populations and their properties, with important implications for optogenetic studies of cortical oscillations.

7.2 Anesthesia level modulation

Anesthesia is known to induce the depression of cortical functions through its action on neuronal processes via neurotransmitters. This action leads to reduced responsiveness and unconsciousness. However, intermediate values of anesthesia have been documented to have a differential effect on neuronal function. Generally, at low levels of anesthesia, the arousal level of the animal is modulated. However, it is not known what the effect of anesthesia level variation is when operating under surgical anesthesia range.

We hypothesized that, even under deep anesthesia, changes in the level of anesthetic will have an effect on the cortical state of the animal. For this, we performed electrophysiological recordings of visual responses generated by fixed contrast moving gratings at different anesthetic levels. We chose 3 levels for each animal: light anesthesia was determined as the point right after the animal lost the pedal reflex, deep anesthesia was determined as the point where heart rate and respiration rate were stable and the animal was not showing signs of overdose, and a middle level was selected between these two values. This assessment was performed individually for each animal to account for variations in animal size, age and fitness. Anesthetic levels were changed in a random order. We acquired data from 3 C57Bl/6 mice, 6 sessions each comprising of 2 iterations through the 3 anesthetic levels.

However, our results showed no consistent effect of anesthetic level on neuronal responses. Both LFP and MUA features were compared and no effect was visible in gamma power, frequency or firing rate.

One explanation for this negative finding could be that the cortex has a series of mechanisms which keep its state relatively constant within a given range of perturbation. Homeostasis is responsible for keeping the organism in functional parameters regardless of the temperature, humidity or other variations, as long as they are in a physiological range. In our case, the available anesthetic range which would not wake the animal nor would it produce an overdose may have been within the range of homeostasis. Therefore, whatever perturbation was brought to the brain, it was able to adapt and resist the effect in a reliable manner by using compensatory mechanisms. These in turn could have changed the properties of the neural circuit and decorrelate its dynamics from the depth of anesthesia in the concentration range we explored

Therefore, we concluded that anesthesia manipulations within surgical range is not sufficient to induce a cortical state change in a reliable manner.

7.3 Cholinergic modulation

The molecular mechanism underlying the enhancement in gamma induced by ACh is not clear. However, there is evidence that acetylcholine modulates the M-current, involved in the generation of intrinsic membrane resonance (Halliwell and Adams, 1982). Building on this fact, we conducted a study which tested the potential implications of intrinsic membrane resonance in the mechanism of cholinergic enhancement of gamma oscillations. We found that indeed gamma power was

enhanced by application of acetylcholine on the surface of the brain and that this enhancement comes together with features consistent with the presence of intrinsic resonance, as determined via in silico modeling (Moca et al., 2014). Moreover, we observed that cholinergic activation has a positive effect on putative predictive coding mechanisms in the visual cortex for 3rd order stimulus characteristics such as contrast rate variation. All these things together point to a scenario directed by the inhibitory subnetworks and support the observation that membrane properties of this subpopulation is of great relevance for the nature of generated oscillatory activity.

8 Conclusions

In this chapter, we will go down the red thread of the research performed and remind the reader about the aims of each chapter, about the author's contribution from each perspective and about the importance of these contributions for the scientific community.

8.1 Main personal contributions

The list below shows a summary of these contributions, which are expanded in the following sections:

- A detailed overview of the brain and its function, from a mechanistic perspective with the aim of bridging the gap between engineers and neuroscience.

- Systematic literature reviews about key sections of the thesis:
  - Neurotransmitters and their putative modulatory mechanisms.
  - Currents, channels and neuronal interactions involved in the expression of resonance in neuronal systems.
  - Experimental methods for cholinergic modulation.

- Design and construction of a complete, custom experimental setup.
  - Setting up the hardware for electrophysiologic recordings, visual and optogenetic stimulation and temperature monitoring.
  - Building and adapting necessary hardware for the experimental setup, via 3D prototyping, electrical design and software implementations.
  - Configuring artificial neural networks for performing simulations of cortical activity.
  - Establishing a protocol for data management and preprocessing.
  - Custom implementation of existing algorithms for data analysis.
  - Development of novel algorithms for data analysis: Time-resolved pattern analysis and Adapted empirical mode decomposition.

- Characterization of gamma oscillations associated with visual responses in the mouse primary cortex, under isoflurane anesthesia.
  - Investigating the independent evolution of response parameters across time.
  - Analyzing the differential effect of first, second and third order stimulus parameters on response features.
  - Characterizing the frequency characteristic of cortical networks in V1.

- Manipulation of cortical dynamics via arousal

– Analysis of artificial neural network dynamics while altering circuit parameters
– Analysis of the effect of *In vivo* manipulation of arousal mechanisms via anesthesia and cholinergic modulation on cortical responses in the gamma band.

8.2 Research impact and further directions

After three years of intensive work, this endeavor is far from completed. Indeed, one can draw a line and consider what has been done so far as a complete unit in itself, having the purpose of establishing the background, hardware, software and experimental considerations for investigation of underlying mechanisms in oscillatory behavior. The implication of gamma oscillations in a wide variety of complex cognitive functions such as attention, perception formation and motor control, can only tickle the interest of scientists, which are left to decipher the "if"s and "how"s of its mechanisms. Even more importantly, the disruption of oscillatory activity and synchronization has been found to correlate with abnormal brain function found in pathologies such as Epilepsy, Schizophrenia, Autism, Alzheimer's or Parkinson's disease (Uhlhaas and Singer, 2006). The present thesis tackles the interpretation of a series of findings regarding cortical oscillatory mechanisms which can prove to have a great weight in understanding neural processing and its pathologies. But from here on, we are in a position where a large pool of research aims are within our reach.

A first direction of research in direct line with the accomplished results would be a more in-depth evaluation of the stated hypotheses in the current thesis. We found that statistical testing for our data was difficult due to the low number of data points and the high variability across recordings due to the iterative process of setup construction and experimental design. One first step for the future would be to establish an experimental pipeline where all of the tested hypotheses in this thesis are confirmed by a relevant number of subjects and all alternative hypotheses are disproved by control experiments.

Secondly, the quest to pinning intrinsic resonance as an underlying switch in gamma oscillation robustness and stability can only continue by looking at individual cellular units and their membrane properties. For this purpose, a scientific mobility to a laboratory in Berlin is planned, where the author will be able to apply the experimental paradigms detailed in this project while recording potentials within individual neurons. The frequency characteristic of these neurons can be tested in a similar manner as we tested circuit-level frequency characteristics here. Revealing resonance as a key factor of in vivo gamma variability would represent a great advance in the scientific understanding of oscillatory processes and the horizon would be filled with further investigatory niches.

And thirdly, a novel and highly consistent finding in our studies aroused our curiosity. The possibility that the visual cortex has the ability to code third order stimulus parameters such as contrast rate variation is of great interest for us. Above that, the fact that this coding is modulated by gamma enhancement is even mode intriguing. We are interested in investigating this finding even further by designing specific stimulation paradigms that address this question.

Finally, as a general, more philosophical aim, this thesis is a statement that biology and engineering make a great synergy and a good understanding of systems neuroscience requires a significant dose of both, together with other disciplines such as statistics, mechanics, psychology and so on. A future direction for us is encouraging multidisciplinary research in our field by providing support for engineers, computer scientists, physicists or mathematicians that want to find an application of their background in neuroscience, or the other way around, provide the technical skills, like programming, electrical engineering and data analysis for biology, medicine or psychology graduates, thus bridging the conceptual gap between these cohorts. We believe the key to great progress in what the study of the brain is concerned lies in the clash of these fields, which, in the society we live in, are separated by an invisible boundary.

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