Interrogation of Thalamocortical Circuits during Ketamine/Xylazine-Induced Slow-Waves with High-Resolution Electrophysiological Methods

Ph.D. thesis

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1. Introduction

The current state of neuroscientific knowledge is still unclear even with regard to a seemingly straightforward question such as what the function of sleep is. Nevertheless, it is beyond question that sleep is essential for a number of cognitive processes, including memory consolidation, and plays a crucial role in maintaining homeostasis. The list of its functions is extensive. My thesis investigate the nature of propagating waves and their spatiotemporal dynamics in the predominant slow oscillation observed during anaesthesia and natural slow-wave sleep. I also aimed to determine if slow-wave propagation patterns in the thalamus resemble those in the neocortex and to explore how they differ from cortical propagation.

The second study in my doctoral thesis is concerned with the creation of a publicly accessible, high-density dataset of in vivo electrophysiological recordings from the rat neocortex, presented in a standardised format with the intention of enhancing reusability among the neuroscience community. The high-density in vivo datasets may assist in addressing neuroscientific questions by facilitating a comprehensive examination of cortical neuron electrical signatures and an investigation of layer-specific neuronal activity associated with slow oscillations.

2. Objectives

Objective 1: Detect propagating patterns of Multi-Unit Activity in the thalamus of anesthetized rodent species, similar to those observed in the cortex.

The objective of this research was to investigate whether Multi-Unit Activity (MUA) propagating patterns, which have been well-documented in the cortex, can also be detected in the thalamus of anaesthetised rodents. Given the thalamus's pivotal role in neural processing and its extensive connectivity with the

cortex, the study aimed to investigate the existence of similar spatiotemporal propagation dynamics within the thalamic nuclei. By employing state-of-the-art high density electrophysiological recording techniques, our research aimed to shed light on whether these thalamic patterns mirror those observed in the cortex. Such insights could provide deeper understanding of the thalamus's role in neural network activity and its potential contributions to cortical processing under anaesthesia.

Objective 2: Identify the predominant relationships between the different thalamic propagation patterns and to determine their proportions of occurrence.

The aim of this research was to investigate the predominant relationships between various thalamic propagation patterns and to determine their proportions of occurrence during spontaneous up-states in anaesthetised rats. By conducting a systematic analysis of the direction, speed, and frequency of these propagation patterns, the study aimed to identify whether certain sequences, such as ventral-to-dorsal propagation, were more common and to better understand how these patterns operate within the thalamic network. This exploration included comprehensive measurements across different thalamic nuclei, with the objective of capturing the diversity of propagation dynamics and providing a comprehensive understanding of how these patterns contribute to the overall thalamocortical activity during anaesthesia.

Objective 3: Determine whether a temporal relationship exists between cortical and thalamic up-state propagation patterns.

The objective of this research is to explore whether a temporal relationship exists between cortical and thalamic up-state propagation patterns in anesthetized rodents. By conducting simultaneous recordings of multi-unit activity (MUA) from both cortical and thalamic regions, the study aims to identify how the

timing of up-states in these two brain areas correlates. Specifically, it seeks to determine whether cortical up-state initiation influences the propagation patterns within the thalamus, or if distinct, synchronized patterns emerge independently within the thalamic nuclei that mirror those observed in the cortex. Understanding these temporal dynamics could provide insights into the coordination and interaction between the cortex and thalamus during slow-wave activity.

Objective 4: Determine whether thalamic multi-unit activity propagation occurs in naturally sleeping rats and, if so, how its properties differ from those recorded under anesthesia.

The research aims to determine whether thalamic multi-unit activity (MUA) propagation occurs in naturally sleeping rats and, if so, how its properties differ from those recorded under anesthesia. By conducting chronic recordings in naturally sleeping rats, the study seeks to compare the frequency, speed, and spatiotemporal patterns of thalamic MUA during natural sleep with those observed under anesthetic conditions. This comparison is crucial for understanding how anesthesia alters neural dynamics and how closely these induced states mirror natural sleep, potentially revealing insights into the thalamus's role in sleep-related brain activity

Objective 5: Provide a large electrophysiological dataset collected from the neocortex of twenty anesthetized rats with a 128-site silicon probe providing high spatial resolution. The continuous raw and wideband recordings, along with metadata, are packaged in the Neurodata Without Borders (NWB) format, a standardized data format for ease of use.

The research is focused on creating a comprehensive and high-resolution electrophysiological dataset by recording neural activity from the neocortex of twenty anesthetized rats using a

128-site silicon probe. This approach allows for capturing intricate details of neural dynamics with high spatial precision, which is crucial for understanding the complex interactions within cortical networks. The continuous raw and wideband recordings are not only extensive but also enriched with detailed metadata, ensuring that every aspect of the experimental conditions is documented.

To enhance the utility of this dataset, it is packaged in the Neurodata Without Borders (NWB) format—a standardized format widely recognized in the neuroscience community. This format is designed to be easily accessible and compatible with various data analysis tools, making it a valuable resource for researchers. The significance of this dataset lies in its potential to facilitate new insights into cortical function, provide a reliable basis for comparative studies, and support the development of new analytical methods. By making this high-quality data readily available, the research aims to advance our understanding of neural processes and foster collaboration across different research groups in the field.

3. Methods

In this study, spontaneous thalamic activity in anesthetized rodents was recorded using silicon-based probes to explore spatial scales of activity spread. Multi-shank probes (64 sites for rats, 128 for mice) captured activity across various thalamic nuclei at multiple dorsoventral depths. Additionally, Neuropixels 1.0 probes, with 960 sites, provided finer spatial resolution. A total of 82 recordings from 14 rats and 18 from 5 mice were made using multi-shank probes, and 46 from 20 rats and 11 from 4 mice using Neuropixels. Each session lasted 30 to 60 minutes, and the data was stored for later analysis to understand thalamic activity propagation during anesthesia.

3.2. Simultaneous electrophysiological recordings from the thalamus and neocortex

To study the relationship between thalamic and neocortical activity during sleep, simultaneous recordings were conducted in rats using two types of probes. A Neuropixels probe was inserted into the thalamus at a depth of 7-7.5 mm, targeting both first-order and higher-order nuclei. Concurrently, a NeuroNexus Buzsaki64 probe was implanted into the neocortex at a depth of 1.1 mm to record layer 5 activity. The multi-shank probe was positioned laterally to align perpendicularly with cortical layers. The recordings from both regions were synchronized with TTL pulses and collected at a 30 kHz sampling rate, allowing detailed analysis of their interaction during sleep.

3.3. Chronic in vivo experiments in freely moving, naturally sleeping rats

In the chronic in vivo experiments on freely moving, naturally sleeping rats, a similar surgical procedure to the acute experiments was used for probe implantation. Adult Wistar rats were anesthetized, and a Neuropixels 1.0 probe was implanted into the thalamus using a 3D-printed fixture attached to the skull. The surgery involved precise craniotomy, careful probe insertion, and sealing of the exposed cortex. After implantation, signal quality was checked before a recovery period with antibiotics and analgesics. Chronic recording sessions began five days post-surgery, with rats placed in a soundproof Faraday cage for daily sessions over two weeks, capturing thalamic activity during sleep-wake cycles using the same data acquisition parameters as in the acute experiments.

3.4. Thalamic data analysis

The analysis of thalamic data in this study involved several key steps. First, up-state onsets were detected in thalamic and cortical recordings by processing multiunit activity (MUA) signals, which were filtered, rectified, and downsampled to create a smoothed population activity (SPA) signal. This SPA signal was used to identify up- and down-state transitions and calculate MUA averages to study thalamic and cortical dynamics during up-states. Preferred propagation patterns of MUA within the thalamus were analyzed based on the sequence of up-state onset times across different locations, and these patterns were correlated with cortical activity. Sleep stages in chronically implanted animals were scored by analyzing local field potentials, focusing on theta and delta frequencies to distinguish between NREM and REM sleep. Finally, thalamic nuclei were identified by verifying probe positions through histological analysis and comparing them to brain atlases.

3.5. High-density electrophysiological recordings.

In the second study, high-density silicon probes were used to record spontaneous cortical activity in anesthetized rats with a custom-made NeuroSeeker probe. This probe had a single shank with 128 titanium nitride electrodes for detailed neuronal recordings. Data was acquired at 20 kHz per channel, collecting about 30 minutes of multichannel data at each site. Recordings were analyzed for noise, power line contamination, and signal quality. Spike sorting was done using Kilosort2, with manual curation in Phy, resulting in a refined dataset. Post-experiment histological analysis verified probe tracks and recording sites, ensuring data accuracy.

4. Results

4.1 Propagation of neuronal population activity is common during spontaneous up-states in dorsal thalamic nuclei of rats under anesthesia

In our study involving 14 rats, we observed the apparent propagation of multiunit activity (MUA, 500–5000 Hz) during

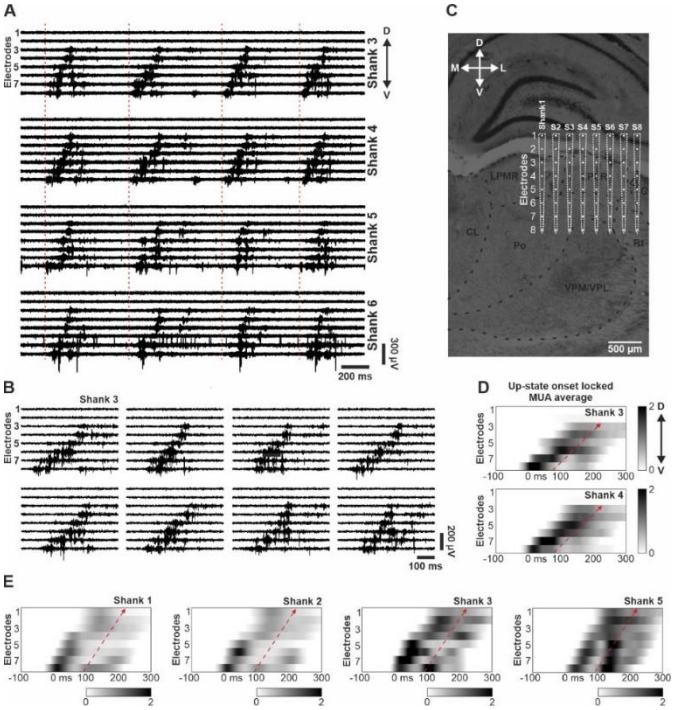

Figure 1. Propagating thalamic population activity observed with multi-shank silicon probes under ketamine/xylazine anesthesia in rats. (A) Spontaneous MUA traces on four adjacent shanks in Po and LP nuclei, with up-state onsets marked by red dashed lines. Propagation from ventral (bottom electrodes) to dorsal (top electrodes) regions of the thalamus is visible on shanks 3 and 4. (B) Propagating up-states recorded on shank 3 at different times. (C) Nissl-stained section showing probe location relative to thalamic nuclei. (D) Normalized MUA depth profiles showing ventral-to-dorsal propagation on shanks 3 and 4. (E) Normalized up-state onset MUA profiles on four shanks in dorsal thalamus of another rat

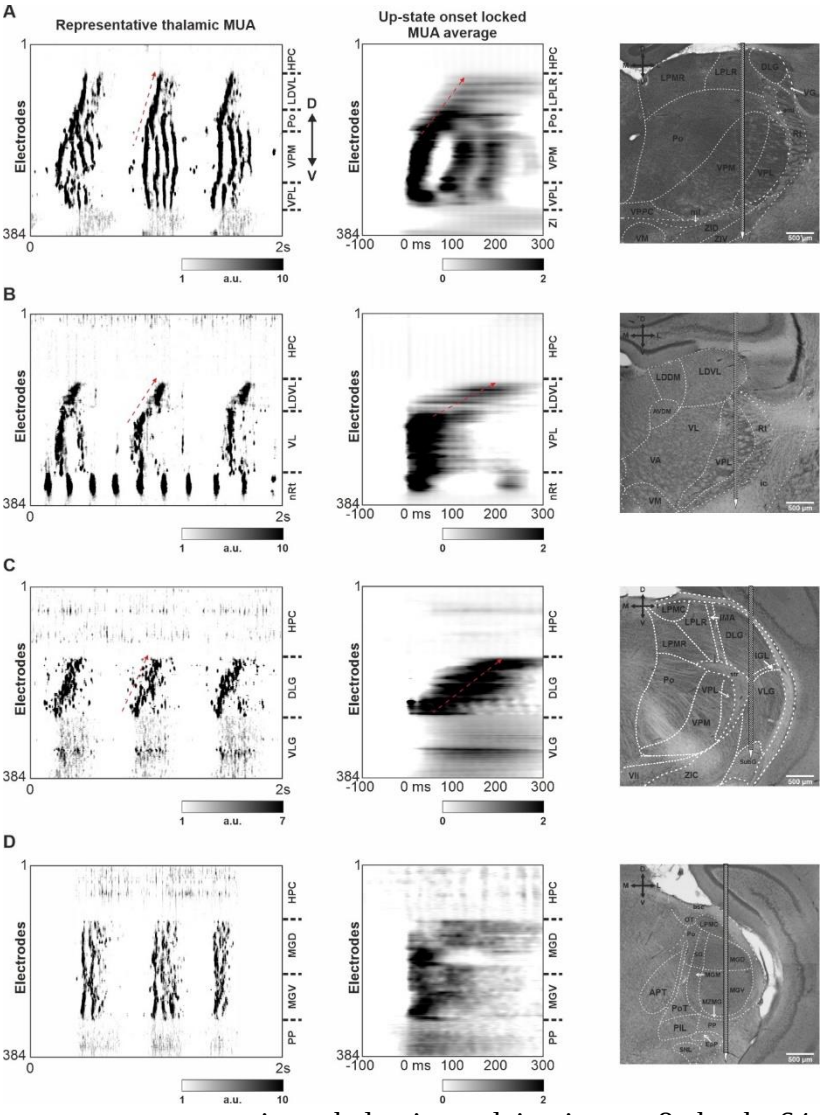

Figure 11. Thalamic population activity recorded with single-shank high-density probes under ketamine/xylazine anesthesia in rats. (A-C) Left: Two-second recordings of spontaneous MUA from different thalamic regions showing ventral-to-dorsal propagation during up-states. MUA maps are smoothed in time and space, with boundaries between nuclei marked by dashed lines. Red arrows indicate propagation direction. Middle: Normalized up-state onset locked MUA profiles. Right: Nissl-stained sections showing probe location. (D) Thalamic recording without visible MUA propagation.

electrode silicon probe. This propagation, characterized by a visible delay in the onset of up-states across adjacent channels, extended several hundred micrometers and could result in delays of up to several hundred milliseconds. Notably, the propagation was more prevalent in higher-order dorsal thalamic nuclei (e.g., Po, LP, LD) and followed a highly stereotypical dorsoventral pattern in about 50% of recordings, with less frequent alternative patterns like posterior-to-anterior and medial-to-lateral propagation. The ventral thalamic structures, in contrast, exhibited minimal or no up-state propagation except for the propagation of sleep spindles. Our focus remained on the dorsoventral propagation due to its consistency and dominance in the observed patterns.

4.2 Up-states in the dorsal thalamus tend to propagate primarily in the ventral to dorsal direction

Our study revealed that the propagation of multiunit activity (MUA) in the thalamic nuclei predominantly follows a ventral-to-dorsal direction along the dorsoventral axis, as confirmed by visual evaluations. This pattern was the most common, occurring in approximately 71% of cases across 15 recordings. However, other propagation patterns, including the opposite dorsal-to-ventral direction, were also observed, albeit less frequently, with occurrence rates between 3% and 10%. The propagation speeds varied significantly, averaging around 15 millimeters per second. The analysis of MUA initiation sequences at different up-state positions further confirmed the presence of multiple distinct propagation patterns, some of which were more complex.

4.3. Spontaneously arising dorsal thalamic up-states are synchronized with cortical up-state activity.

In our study, we explored the relationship between cortical and thalamic multi-unit activity (MUA) propagation patterns during up-states in the thalamocortical system, particularly under anesthesia in rodents. Using simultaneous recordings from the neocortex and thalamus in five rats, we found that while cortical

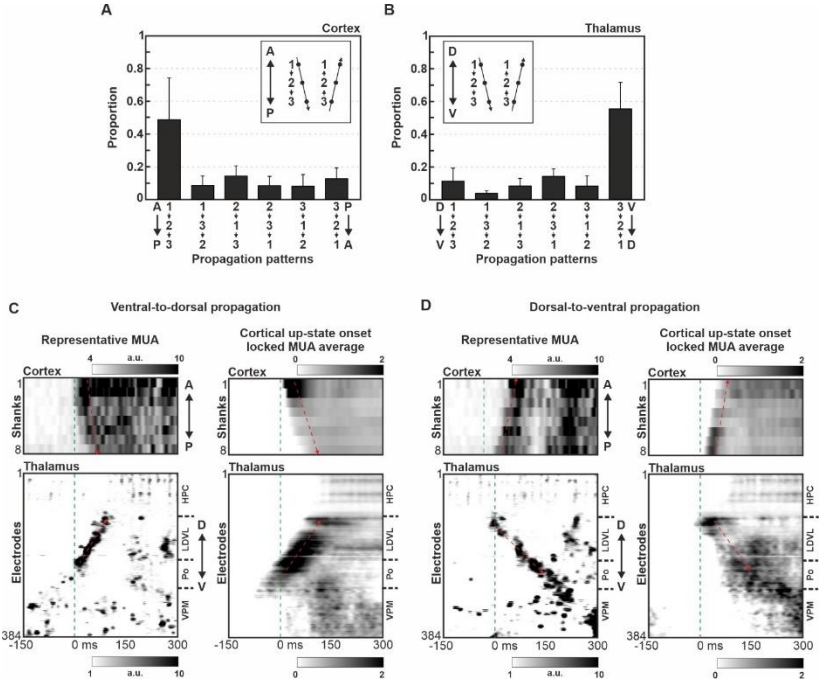

Figure 2. Cortical and thalamic propagation patterns during up-states are weakly correlated. (A) Proportion of cortical propagation patterns (n = 5 thalamocortical recordings): Sequence 1-2-3 for anterior-to-posterior, and 3-2-1 for posterior-to-anterior propagation. (B) Proportion of thalamic propagation patterns in the same recordings: Sequence 1-2-3 for dorsal-to-ventral, and 3-2-1 for ventral-to-dorsal propagation. (C) Left: Thalamic ventral-to-dorsal propagation during an anterior-to-posterior cortical up-state. Right: Normalized MUA averages for these up-states (n = 887). (D) Left: Thalamic dorsal-to-ventral propagation during a posterior-to-anterior cortical up-state. Right: Normalized MUA averages for these up-states (n = 190). Dashed lines mark boundaries and propagation directions.

MUA typically propagated along the anterior-posterior axis, thalamic MUA predominantly followed a ventral-to-dorsal

pattern. Interestingly, in most cases, thalamic MUA began in the ventral Po nucleus, preceding cortical up-states by about 50 ms, suggesting a significant influence of cortical activity on thalamic propagation. The propagation speed was slower in the thalamus (~12.4 mm/s) compared to the cortex (~37.9 mm/s). The most frequent cortical pattern, anterior-to-posterior propagation, often corresponded with the dominant ventral-to-dorsal thalamic propagation (~52% of up-states). However, we also observed less common patterns, such as posterior-to-anterior cortical propagation linked with dorsal-to-ventral thalamic MUA. Overall, while each cortical propagation pattern could be associated with different thalamic patterns, the correlation between the two was generally weak.

4.4. Thalamic activity propagation is less frequent and faster during natural sleep in rats.

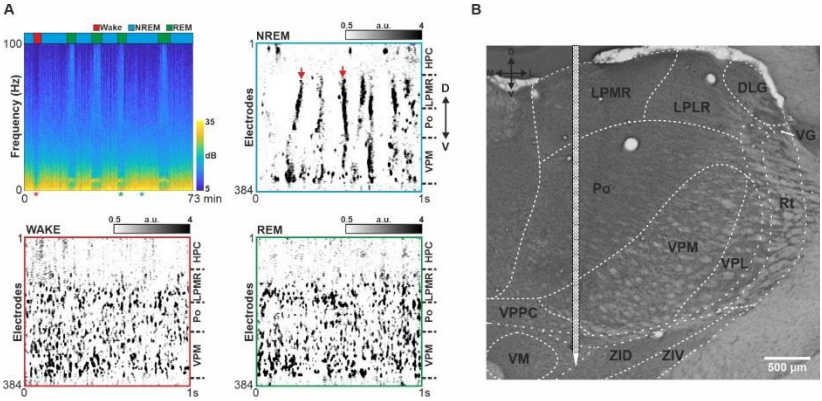

Figure 17. Thalamic MUA propagation occurs only during NREM sleep in naturally sleeping rats. (A) Spectrogram (top left) of a 73-minute recording from a chronically implanted rat, showing periods of NREM, REM sleep, and wakefulness. MUA snippets for each state are displayed (NREM: top right, wake: bottom left, REM: bottom right). Red arrows in the NREM snippet indicate propagating activity in the thalamus. Asterisks mark the snippet time points on the spectrogram. Color bars indicate sleep/wake periods. (B) Nissl-stained section showing probe location relative to thalamic nuclei.

In this study, we compared the properties and spatiotemporal patterns of thalamic multi-unit activity (MUA) during ketamine/xylazine-induced anesthesia and natural slow-wave sleep (SWS) in rats. Using chronically implanted Neuropixels probes targeting thalamic nuclei (Po and LP), we found that while MUA propagation was prominent under anesthesia, it was significantly reduced during natural NREM sleep, occurring at a much lower rate (1.89 events/min versus ~45 events/min under anesthesia). During wakefulness and REM sleep, thalamic activity was desynchronized, showing no propagation. Although both anesthesia and natural sleep exhibited a ventral-to-dorsal propagation pattern in the thalamus, the events during NREM sleep were shorter and faster. Despite these differences, the overall MUA depth profiles were remarkably consistent between anesthesia and natural sleep, as well as between the two rats studied, indicating that stereotyped activity patterns are present in the thalamus under both conditions.

5. Conclusions

Recent studies have shown that slow-waves (SWs), the most prominent electrophysiological events in the thalamocortical system during anesthesia and deep sleep, exhibit complex spatiotemporal dynamics and propagate across neocortical areas. Nevertheless, it is still uncertain whether neuronal activity within the thalamus displays analogous propagation characteristics during SWs. This dissertation presented the propagation of population activity within the thalamus of ketamine/xylazine-anesthetized rats and mice, which is visualized through high-density silicon probes. The propagation of spontaneous thalamic activity during up-states was predominantly detected in dorsal thalamic nuclei, including the posterior (Po), lateral posterior (LP), or laterodorsal (LD) nuclei, in both rodent species. It was observed that the preferred direction of thalamic activity spreading was along the

dorsoventral axis, with over half of the upstates exhibiting a gradual propagation in the ventral-to-dorsal direction. Furthermore, simultaneous neocortical and thalamic recordings collected under anesthesia demonstrated that there is a weak but noticeable interrelation between the propagation patterns observed during cortical up-states and those displayed by thalamic population activity. Furthermore, *in vivo* recordings of chronically implanted silicon probes revealed the presence of propagating activity patterns in the thalamus of naturally sleeping rats during slow-wave sleep. However, in contrast to the propagation patterns observed in the upstates under anesthesia, these patterns exhibited a lower frequency and a faster propagation speed. The results indicate that the propagation of spontaneous population activity is an intrinsic characteristic of the thalamocortical network during synchronized brain states like deep sleep or anesthesia. The findings presented in this thesis may contribute to our understanding of synchronized brain states as complex neural processes during deep sleep and anesthesia. Future research directions include examining the effects of different anesthetics on thalamocortical activity. Moreover, it explores the role of different thalamic nuclei in different brain states. Characterizing the single-unit activities extracted from each thalamic nucleus may help answer both questions.

The scarcity of publicly available neural recordings with high spatial resolution is a significant limitation for neuroscientific advances. The second objective of this thesis is to present an electrophysiological data set recorded from the neocortex of twenty rats anaesthetised with a combination of ketamine and xylazine. The wideband, spontaneous recordings were acquired with a single-shank silicon-based probe having 128 densely-packed recording sites arranged in a 32 × 4 array. The dataset contains the activity of a total of 7126 sorted single units extracted from all layers of the cortex. The data presented here

comprises raw neural recordings, as well as spike times, extracellular spike waveforms, and several properties of units, all packaged in a standardized electrophysiological data format. In order to provide technical validation of our dataset, we present the distributions of derived single unit properties along with various spike sorting quality metrics. The publicly accessible high-density neocortical data set has the potential to yield a wide range of future benefits. On the one hand, it can facilitate a more detailed analysis of individual neurons' different firing patterns and waveforms and assist in classifying neurons based on their electrophysiological characteristics. Furthermore, it is an essential input material for developing different spike-sorting algorithms and electrophysiological data processing methods (259). In addition, the dataset may be employed to investigate the laminar-specific neuronal activity that occurs during the brain rhythm of slow oscillation. At the time of writing this thesis, the ARP data repository of the HUN-REN network indicated that the "Dataset of cortical activity recorded with high spatial resolution from anaesthetised rats" had been downloaded 863 times. The repository also provides access to other research data, including data related to the thalamic study (Study 1).

6. Bibliography of the candidate's publications

Publications related to the thesis:

1. Horváth, C., I. Ulbert and R. Fiáth (2024). "Propagating population activity patterns during spontaneous slow waves in the thalamus of rodents." NeuroImage **285**: 120484.

2. Horváth C, Tóth LF, Ulbert I, Fiáth R. Dataset of cortical activity recorded with high spatial resolution from anesthetized rats. Scientific Data. 2021;8(1):180.

Publications not related to the thesis:

1. C Horváth, K Csikós, B Árkossy, E Klein, P Ruther, I Ulbert and R Fiáth. Polymer-based laminar probes with an ultra-long flexible spiral-shaped cable for in vivo neural recordings. SENSORS *AND ACTUATORS B: CHEMICAL*, 136220, 2024.

∑IF: 21,201