Final report

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Investigation of spontaneous and evoked propagating slow waves in the thalamocortical network during sleep and anesthesia

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Aims of the study

The main objectives of this research project were

1. to examine whether propagating neuronal activity patterns can be observed during spontaneously occuring slow waves in the thalamus of anesthetized rodents (rats and mice)
2. to investigate which parts (nuclei) of the thalamus exhibit propagating slow waves
3. to assess the properties of spontaneous thalamic propagating activity patterns (e.g., preferred direction of propagation, propagation speed, proportion of different propagation patterns)
4. to investigate the relationship between propagating cortical and thalamic slow waves
5. to examine whether spontaneous propagating neuronal activity patterns can be detected during slow wave sleep in the thalamus of naturally sleeping rats
6. to examine whether propagating slow waves can be evoked by sensory stimulation
7. to make the recorded dataset freely available by depositing it in a research data repository

Results related to the first five research goals are described in detail in our recently published bioRxiv preprint (https://doi.org/10.1101/2023.08.31.555472; manuscript currently under review in Neuroimage). Here, I provide only a brief summary of the methodology and the findings of this study. Results related to the last two research goals are discussed after this summary.

Methods

In acute experiments, thalamocortical recordings were obtained from adult Wistar rats (n = 34; weight: 285.22 ± 81.21 g, mean ± SD; n = 19 female) and adult C57BL/6J mice (n = 9; weight: 26.33 ± 7.00 g; n = 4 female). Two adult Wistar rats (259 g, female; 476 g, male) were used for chronic slow-wave sleep recordings. Two types of silicon-based probes were used to record spontaneous thalamic activity in anesthetized rodents. To determine whether activity propagation in the thalamus occurs on a larger spatial scale, we used multi-shank probes that provide a larger thalamic coverage, and allow mapping the simultaneous activity of multiple nuclei (A8x8-10mm-200-200-177 probes with 64 recording sites for rats, and A8x16-Edge-5mm-100-200-177 probes with 128 recording sites for mice). From 14 rats, 82 spontaneous recordings were acquired with multi-shank silicon probes with an average duration of about 30 minutes, while 18 spontaneous recordings were obtained from 5 mice. In 10 out of 14 rats (and in all mice), the orientation of the probe shanks was parallel to the medial-lateral axis, while in the remaining four animals the probe shanks were aligned along the anterior-posterior axis. To record thalamic activity on a finer spatial scale, we used high-density single-shank Neuropixels 1.0 probes with 960 recording sites from which 384 sites can be selected for simultaneous recording. From 20 rats, 46 spontaneous recordings were acquired with Neuropixels high-density probes, while 11 recordings were obtained from 4 mice. In a subset of rat experiments (n = 5 animals), besides the thalamically positioned Neuropixels probe, a multi-shank silicon probe (NeuroNexus Buzsaki64) was inserted into the neocortex to simultaneously record spontaneous population activity from cortical and thalamic regions. For chronic implantations (n = 2 rats), we used Neuropixels probes and reusable 3D-printed single-probe fixtures. For the chronic recording sessions, the animal was transferred from the animal facility to a dark and soundproof Faraday cage, then it was connected to the Neuropixels recording system. We recorded thalamic activity on a daily basis for about two weeks starting on the fifth day after probe implantation. The recording sessions varied in duration (0.5 – 3 hours) but a single recording session usually included several sleep-wake cycles. To detect the tracks of silicon probes in the brain tissue and to identify thalamic nuclei, we used Nissl staining. For both acute and thalamic recordings, thalamic multiunit activity (MUA; i.e., population activity) was assessed during data analysis. To assess the properties of MUA propagation, we detected to onset of up-states and calculated the up-state onset locked MUA depth profile averages. Furthermore, we computed the proportion of different MUA propagation patterns both in the cortex and thalamus. In chronic recordings, slow-wave sleep periods were identified based on spectral properties of hippocampal local field potentials.

Results

The results obtained in this project demonstrate, for the first time, that propagating patterns of spontaneous population activity occur in the rodent thalamus in vivo, both under anesthesia and during slow-wave sleep. Additionally, our findings revealed that neuronal activity is most likely to propagate within dorsal, particularly higher order thalamic nuclei (such as the posterior or the laterodorsal nucleus), where up-states generally travel along the dorsoventral axis with a preference for the ventral-to-dorsal direction. The distance of propagation ranged from several hundred micrometers to up to 1.8 millimeters (1.14 ± 0.39 mm), while the propagation delay was approximately 100 ms, resulting in a propagation speed of 14.6 ± 8.6 mm/s. While, in general, the thalamus exhibited a rich variety of spatiotemporal activity patterns both during natural sleep and anesthesia, thalamic neuronal activity in anesthetized animals was less complex and more stereotyped. This difference in complexity is indicated, for instance, by our observation that the majority of up-states occurring under anesthesia display ventral-to-dorsal propagation with minimal variations in their propagation trajectories. During natural sleep, although still present, ventral-to-dorsal propagation was less frequent, and the spreading of MUA was faster compared to thalamic activity propagation under anesthesia. Furthermore, ketamine/xylazine anesthesia-induced cortical population activity, obtained with multi-shank silicon probes, also exhibited stereotypical propagation patterns. A notable proportion of cortical up-states arrived first at the anterior shanks, then traveled in the posterior direction. The proportion of these anterior-to-posterior propagating cortical up-states was similar to the proportion of predominant ventral-to-dorsal propagating thalamic up-states (~50% of up-states), whereas proportions of other propagation patterns were notably lower (3-10%). Ventral-to-dorsal propagation of thalamic activity was more pronounced in thalamic MUA depth profiles locked to cortical up-states than in those aligned to thalamic up-states. This suggests that the neocortex may play an influential role in shaping population activity within the thalamus. Our findings related to cortical slow wave propagation are in good agreement with previous studies. Even within a localized cortical area (~1.5 mm), we were able to observe a tendency for up-states to travel in the anterior-to-posterior direction with a propagation speed (~38 mm/s) within the range reported in earlier studies.

Propagating thalamic activity during evoked up-states

Using somatosensory (whisker) stimulation we were able to reliably evoke up-states in the thalamus, as was also previously observed in the neocortex. Futhermore, similar to cortical up-states, thalamic up-states have a refractory period, that is, we could not evoke up-states with somatosensory stimuli during ongoing up-states or shortly after the termination of spontaneous up-states. Thus, the collision of propagating population activity in the thalamus during anesthesia may be very rare. Qualitatively, evoked up-states were similar to spontaneous up-states, exhibiting ventral-to-dorsal propagation within the dorsal part of the thalamus, but the activity in the Po started more synchronously. We hypothesize that somatosensory stimulation evokes new slow waves in the somatosensory cortex which then spreads rapidly to Po and to adjacent cortical areas, but then the propagation of population activity slows down and continues to travel with properties similar to spontaneous slow waves.

Publication and dissemination activity

During the three years of the project, six peer-reviewed journal articles, one preprint and four poster presentations were published related to the topic of the research. Furthermore, one manuscript is under review and another in preparation. Additionally, two oral presentations were given at an international conference. The cumulative impact factor of the published papers is 49.964. All six articles were published in journals ranked Q1 (four are ranked D1). The principal investigator (PI) of the project is the first/last author of two publications and of the preprint (which is under review). Two MSc theses and one TDK work have been completed on the topic of this project at the Faculty of Information Technology and Bionics of the Pázmány Péter Catholic University (PPKE ITK) and at the Faculty of Science of the Eötvös Loránd University (ELTE TTK). The majority of the project dataset (about 200 files with a total size of approximately 3 TB) will be made publicly available in the CONCORDA (ARP) Hungarian research data repository when our submitted manuscript (see the bioRxiv preprint) is accepted in a peer-reviewed journal (project goal 7). However, the dataset has already been uploaded and can be viewed at the following private URL: PD20_dataset. The original raw multi-shank and Neuropixels recordings, along with rich metadata have been packaged in the Neurodata Without Borders: Neurophysiology version 2.0 format (NWB:N 2.0; www.nwb.org), which is a data standard for neurophysiology.

Future project related research and publication plans

A graduate student and the PI are still analyzing the large dataset collected during this three-year project. We plan to prepare and submit another manuscript (around Q1/Q2 of 2024) discussing the differences in the spatiotemporal dynamics (activity propagation) of the thalamic MUA in rats anesthetized with different anesthetics (e.g., ketamine/xylazine, urethane and isoflurane). The experiments related to the perturbation of cortical activity by local manipulation of the cortical temperature (warming and heating) yielded interesting but inconsistent results. The spatiotemporal dynamics of thalamic population activity was clearly affected by changes in the cortical temperature, but futher experiments and analysis are still needed to publish these results. We intend to pursue this part of the project further and it could be used as a research topic for new graduate students. In the longer term, we also plan to publish a review on the propagation of neuronal activity related to slow waves emerging in the thalamocortical system. Finally, my graduate student (Csaba Horváth) is writing his dissertation on the results of this topic, and he is expected to defend his dissertation at the end of next year.

Summary

The research project yielded interesting and previously unknown findings related to the spatiotemporal dynamics of sleep slow waves in the thalamus. The report prepared from these findings is currently under review in a highly ranked neuroscience journal. Furthermore, several articles with the PI as the first/last author or co-author have been published in prestigious neuroscience journals. Additionally, the invaluable dataset acquired during the project will soon be made publicly available in a research data repository. Finally, several Master's theses on the topic of this research have been completed under the supervision of the PI, and a PhD dissertation will also be completed next year.

List of publications

Peer-reviewed journal articles

- Fiáth R, Meszéna D, Somogyvári Z, Boda M, Barthó P, Ruther P, Ulbert I: Recording site placement on planar silicon-based probes affects signal quality in acute neuronal recordings, (2021) SCIENTIFIC REPORTS 11: (1) 2028, D1, Impact factor (IF): 4.996
- Horváth C, Tóth LF, Ulbert I, Fiáth R: Dataset of cortical activity recorded with high spatial resolution from anesthetized rats. (2021) SCIENTIFIC DATA 8: (1) 180, D1, IF: 8.501
- Csernyus B, Szabó Á, Fiáth R, Zátonyi A, Lázár C, Pongrácz A, Fekete Z: A multimodal, implantable sensor array and measurement system to investigate the suppression of focal epileptic seizure using hypothermia, (2021) JOURNAL OF NEURAL ENGINEERING 18: (4) 0460c3, Q1, IF: 5.043
- Bod R, Rokai J, Meszéna D, Fiáth R, Ulbert I, Márton G: From End to End: Gaining, Sorting, and Employing High-Density Neural Single Unit Recordings. (2022) FRONTIERS IN NEUROINFORMATICS 16: 851024, doi: 10.3389/fninf.2022.851024, Scimago Journal Rank (SJR): Q1, I: 3.739
- Kocsis B, Martínez-Bellver S, Fiáth R, Domonkos A, Sviatkó K, Schlingloff D, Barthó P, Freund TF, Ulbert I, Káli S, Varga V, Hangya B: Huygens synchronization of medial septal pacemaker neurons generates hippocampal theta oscillation. (2022) CELL REPORTS 40: (5) 111149, D1, IF: 9.995
- Király B, Domonkos A, Jelitai M , Lopes-dos-Santos V, Martínez-Bellver S, Kocsis B, Schlingloff D, Joshi A, Salib M , Fiáth R, Barthó P, Ulbert I, Freund FT, Viney TJ , Dupret D, Varga V, Hangya B: The medial septum controls hippocampal supra-theta oscillations, (2023) NATURE COMMUNICATIONS (accepted manuscript), D1, 17.69

Preprints

- Horváth C, Ulbert I, Fiáth R: Propagating population activity patterns during spontaneous slow waves in the thalamus of rodents, bioRxiv, #2023.08.31.555472, 2023 (submitted to Neuroimage, D1, IF: 5.7)

Poster presentations

- Fiáth R, Horváth Cs, Ulbert I: Propagating slow waves in the thalamus of anesthetized rodents, Virtual FENS Regional Meeting 2021, 25-27 August 2021
- Horváth C, Steinbach M, Ulbert I, Fiáth R: Travelling slow waves in the thalamus of anesthetized rodents, International Neuroscience Meeting, Budapest 2022 – IBRO Workshop, 27-28 January, 2022, Budapest, Hungary
- Horváth C, Steinbach M, Ulbert I, Fiáth R: Propagating spiking activity in the thalamus of anesthetized rodents. FENS Forum 2022, 9-13 July 2022, Paris, France

- Fiáth R, Horváth R, Steinbach M, Ulbert I.: Propagation of spontaneous population activity during slow waves in the thalamus of rodents, FENS Regional Meeting, 3-5 May 2023, Algarve, Portugal

Oral presentation

- Horváth C, Fiáth R: Application of high-density neural probes to explore the complex spatiotemporal dynamics of thalamocortical activity. „Advanced neurotechnologies for brain activity monitoring and modulation" symposium, 12th Neuronus IBRO Neuroscience Forum, 15-17 October, 2022, Krakow, Poland
- Fiáth R: Evolution of silicon-based probes developed for large-scale neuronal recordings, Advanced neurotechnologies for brain activity monitoring and modulation" symposium, 12th Neuronus IBRO Neuroscience Forum, 15-17 October, 2022, Krakow, Poland

MSc theses and TDK work related to the project

- Steinbach Mária. Investigation of spontaneous propagating activity in the thalamus of anesthetized rats. (2022) Pázmány Péter Catholic University, Faculty of Information Technology and Bionics (PPKE ITK), MSc thesis (Supervisor: Richárd Fiáth/István Ulbert).
- Steinbach Mária. Investigation of spontaneous propagating activity in the thalamus of anesthetized rats. (2022) Pázmány Péter Catholic University, Faculty of Information Technology and Bionics (PPKE ITK), (Supervisor: Richárd Fiáth/István Ulbert) TDK work – Special Award
- Omar Al Hajjar. Investigation of thalamocortical slow wave activity in anesthetized rats using the Neuropixels high-density electrophysiology system. (2021) ELTE TTK, MSc thesis (Supervisor: Richárd Fiáth/Tóth Attila)