

Mechanisms of neuronal rhythmogenesis by synchronization in the rodent medial septum



KOCSIS Barnabás

Pázmány Péter Catholic University

Faculty of Information Technology and Bionics

Roska Tamás Doctoral School of Sciences and Technology

Supervisor: **ULBERT István, DSc**

Theses of the PhD Dissertation

Budapest, 2025

1 Introduction

Exploratory behaviors are accompanied by a 4-12 Hz theta oscillation in the hippocampus of rodents, carnivores, and primates, including humans (Buzsáki, [2002](#); Buzsáki & Moser, [2013](#); Kahana et al., [1999](#); Rutishauser et al., [2010](#)). This theta oscillation provides a temporal reference signal for hippocampal networks, which is crucial for episodic learning and memory retrieval (Buzsáki, [2006](#)). GABAergic neurons of the medial septum have been identified as putative pacemakers of hippocampal theta rhythms (Freund & Antal, [1988](#); Hangya et al., [2009](#); Petsche et al., [1962](#); Yoder & Pang, [2005](#)). However, the mode of theta-frequency synchronization within the septal GABAergic network has not been tested experimentally.

Theoretical studies proposed that individual pacemaker neurons may oscillate at different frequencies and synchronize in the frequency domain upon increased depolarizing input (Ujfalussy & Kiss, [2006](#); Wang, [2002](#)), a mechanism termed “Huygens synchronization” in the physics of coupled oscillators (Oliveira & Melo, [2015](#); Ramirez et al., [2016](#)). Two influential models assumed the presence of two separate GABAergic populations that inhibit each other through a so-called ping-pong mechanism, either within the medial septal network (Cutsuridis & Poirazi, [2015](#); Ujfalussy & Kiss, [2006](#)) or involving the hippocampo-septal GABAergic projection neurons (Wang, [2002](#)). Theta amplitude modulation through increased medial septal firing rates was proposed as a direct layer of control (Denham & Borisyuk, [2000](#); Oddie et al., [1996](#); Tokuda et al., [2019](#)), whereas a change in medial septal bursting patterns could also impact theta generation (Joshi et al., [2017](#); Simon et al., [2006](#); Sotty et al., [2003](#)). The role of the hyperpolarization-activated and cyclic nucleotide-gated channels was emphasized as part of the potential underlying ionic mechanisms (Kocsis & Li, [2004](#); Varga et al., [2008](#)). However, beside frequency synchronization, other mechanisms are also plausible, including synchronization in the phase domain without changes in oscillation frequency (Borhegyi et al., [2004](#); Ujfalussy & Kiss, [2006](#)). Additionally, little is known about the exact role of different medial septal cell types in theta generation (Lee et al., [1994](#); Robinson et al., [2016](#); Vandecasteele et al., [2014](#)).

Therefore, we investigated potential theta generation mechanisms experimentally by recording single neurons from the medial septum, with concurrent local field potentials from the hippocampus using multichannel silicon electrode arrays. To address the generality of results across theta oscillations observed in awake or anesthetized rodents (Kramis et al., [1975](#); Stewart & Fox, [1989](#)), we obtained three data sets: from urethane-anesthetized rats, urethane-anesthetized mice and awake, drug-free mice. We found that hippocampal theta onset was accompanied by Huygens (frequency) synchronization of medial septal pacemaker neurons otherwise rhythmic at more distinct frequencies in the theta range. We confirmed the pacemaker role of PV-expressing GABAergic neurons (Freund &

Antal, [1988](#); Hangya et al., [2009](#); Varga et al., [2008](#)) by optogenetic tagging (Kvitsiani et al., [2013](#); Lima et al., [2009](#)), whereas glutamatergic neurons showed strong firing rate increase during theta without much rhythmicity, suggesting they may contribute to the theta-associated increase of tonic drive to the pacemakers (Ford et al., [1989](#); Green & Arduini, [1954](#); Oddie et al., [1996](#); Vandecasteele et al., [2014](#); Yang et al., [2014](#)). Indeed, optogenetic stimulation of medial septal glutamatergic neurons induced theta oscillation in the CA1. These results were consistent with a circuit model of the medial septum in which a network of inhibitory neurons was capable of synchronizing and desynchronizing in the frequency domain upon changing levels of tonic excitatory input.

2 Experimental choices

The aim of this chapter is to justify the rationale behind the experimental choices made, including the identification of phenomena, models, methodologies, and specific brain areas. These aspects are crucial for addressing fundamental questions regarding theta rhythm generation and synchronization.

Phenomena

Oscillatory activities underlie numerous brain functions, coordinating information flow, segmenting neural processing, and dynamically linking distributed brain regions. Categorized by their frequency into delta (0.5–4 Hz), theta (4–12 Hz), alpha (8–12 Hz), beta (13–30 Hz), and gamma (30–100 Hz) bands, these rhythms are believed to support distinct cognitive and behavioral functions.

Oscillations are not mere byproducts of neural activity. Brain rhythms are integral to neural computation and communication, enabling the temporal organization of neural ensembles. Investigating specific oscillations offers critical insights into how the brain orchestrates its vast networks to achieve complex functions.

Theta rhythm is the most regular oscillatory activity in the hippocampus, associated with exploratory behavior, learning, and memory. It serves as a temporal reference for hippocampal network activity (for a detailed description of its functions please refer to the “*Role of theta oscillation*” chapter in the dissertation). Despite nearly 90 years of extensive research and an ever-expanding body of literature, the precise dynamics of how medial septal (MS) neurons synchronize to generate theta rhythms remain unclear. The examination of such a phenomenon is critical in resolving discrepancies between theoretical constructs and experimental observations, particularly with regards to processes like Huygens synchronization, a foundational concept in coupled oscillator theory. A deeper understanding of theta mechanisms requires methods that selectively block theta rhythm generation with minimal side effects (e.g., loss of neuromodulatory inputs, changes in mean firing rates) to reveal causal links.

Studying theta rhythms could also shed light on fundamental brain processes and offer potential applications in treating neurological disorders such as schizophrenia, epilepsy and Alzheimer's disease (see *“Oscillopathies: a rationale behind this study”* chapter in the dissertation).

Models

2.1.1 Relevance of animal research

Animal research holds a pivotal role in neuroscience, offering detailed insights into neuromodulatory systems facilitating the development of new pharmacological strategies to enhance or restore neural synchronization. Modern experimental techniques enable the exploration of synchronization mechanisms in both wild-type and transgenic animals, supporting the identification of critical neuronal contributors and developing models of synchronization that can be validated across species.

2.1.2 Why rodents?

Rodents, especially rats and mice, are widely used in neurosciences, providing particularly useful models for studying brain function and neural dynamics. Much of the foundational neuroanatomical data in neuroscience has been derived from rodent studies, including detailed mappings of the hippocampal formation. Rodents play a prominent role in functional analyses of the hippocampus, making them an indispensable model for investigating oscillatory activities like theta rhythms. Their hippocampal theta oscillations are broadly comparable to those in humans, as they occur during exploratory behaviors, spatial navigation, and memory processing, providing a translational framework for understanding theta's role across species.

Rodents also provide excellent genetic and anatomical accessibility, enabling precise experimental manipulations. Techniques such as in vivo electrophysiology and optogenetics are well-established in rodent models, providing powerful tools to dissect the contributions of specific neuronal populations and circuits to theta rhythm generation and synchronization.

Another advantage of rodents is their versatility in expressing theta oscillations. Theta is not only present during active wakefulness but also during REM sleep, indicating its essential role across behavioral states. Moreover, theta can be elicited under anesthesia in response to noxious stimuli.

2.1.3 Why urethane anaesthetized and freely moving conditions?

Using both anesthetized and freely moving rodent models ensures that findings are not limited to a single physiological state, providing a more general understanding of theta rhythm generation and synchronization.

Anesthetized models offer a controlled environment for stable recordings, minimizing variability caused by behavioral factors. Urethane-induced anesthesia is particularly advantageous as it maintains robust and long-lasting oscillatory activity without requiring continuous administration, unlike other anesthetics such as isoflurane or barbiturates. Urethane preserves the functional connectivity between the medial septum and hippocampus, ensuring physiologically relevant theta rhythms are sustained throughout the experiment. Moreover, urethane has a minimal impact on cardiovascular and respiratory systems, thus providing a stable physiological baseline. Finally, the uniform and reproducible effects of urethane across experiments reduce inter-animal variability, enhancing the comparability and reliability of results.

In contrast, freely moving conditions are essential for investigating the functional relevance of theta rhythms during natural behaviors such as navigation and memory encoding. By combining anesthetized and freely moving models, we ensure that findings are robust across different physiological states and can generalize to both controlled and naturalistic scenarios.

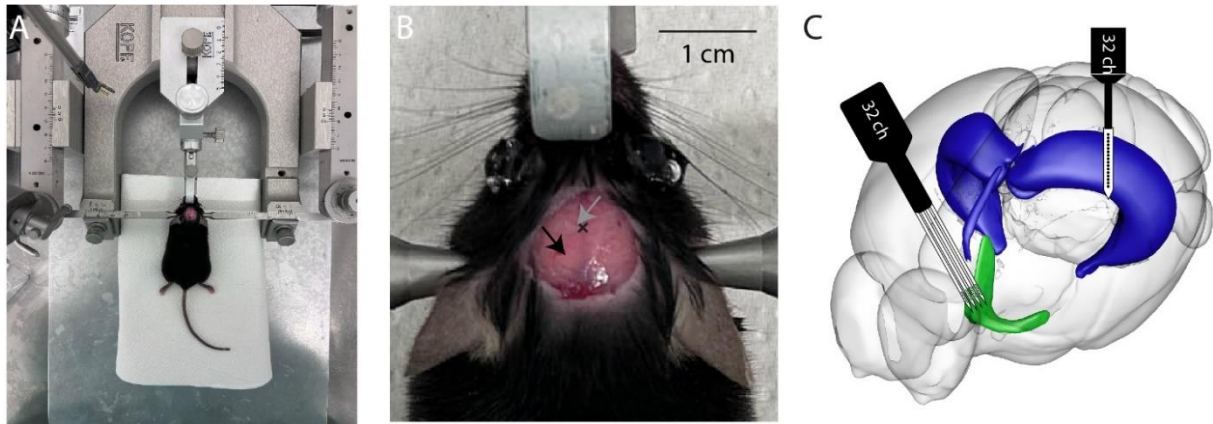
2.1.4 Relevance of computer models

Computer models complement animal studies by allowing researchers to test hypotheses about network dynamics under controlled conditions. These models enable systematic manipulation of parameters like excitation levels, synaptic strength, or their variability among neurons to explore synchronization mechanisms that are difficult to isolate experimentally. Network simulations allowed us to study network dynamics under various conditions, confirming that frequency synchronization suffices for theta rhythm generation, independent of additional specialized mechanisms.

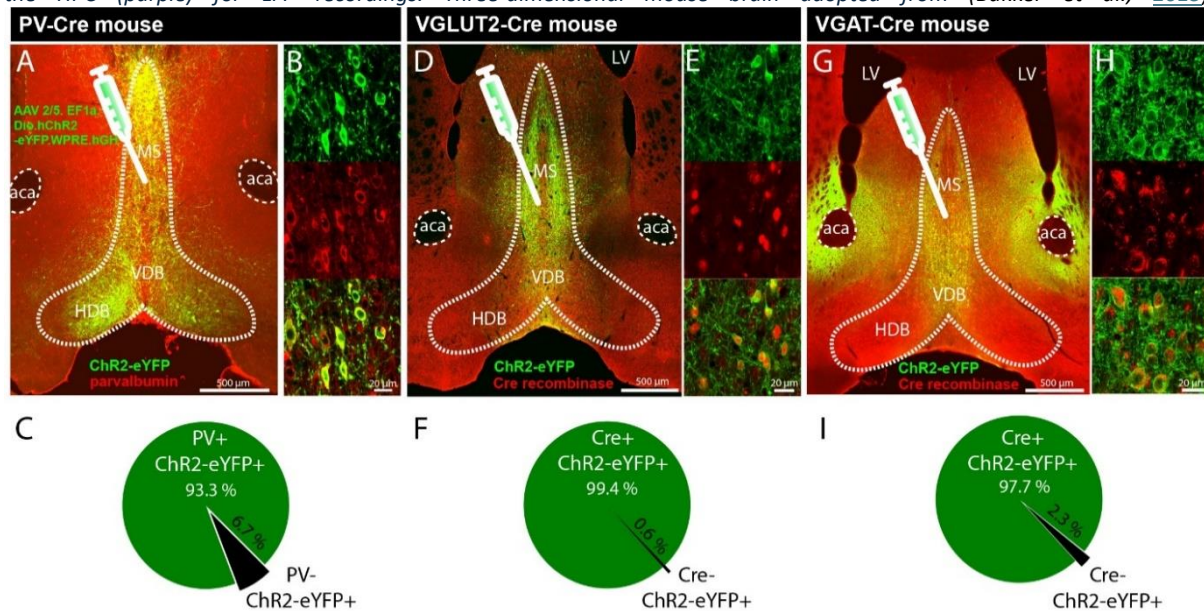
By combining *in vivo* and *in silico* approaches, we achieved a holistic understanding of synchronization mechanisms that extend beyond species-specific differences.

Techniques

To study theta rhythm generation and synchronization, we employed high temporal resolution methods capable of capturing millisecond scale changes (*Figure 1C*). Electrophysiology provided direct recordings of neuronal activity and local field potentials, allowing observations of synchronization at both the single-cell and network levels (*Figure 3C*). On the other hand, optogenetics enabled precise identification and manipulation of specific neuronal subtypes, such as parvalbumin-expressing neurons, helping to establish their roles in pacemaking and synchronization (*Figure 2*).



1. Figure. Stereotaxic surgery in rodents (anaesthetized mouse). (A) Nose and ear bones are fixed by gently fitted metal bars. Now the three-dimensional coordinate system of the frame can be utilized in the stabilized skull. (Photo was taken by Vivien Pillár). (B) The intersection of the coronal and sagittal sutures is used as a reference landmark (Bregma, cross). Exact penetration sites of the electrodes (MS: grey arrow, HP: black arrow) are computed based on the detailed anatomical atlas of the mouse brain (Paxinos et al., 2001). (C) Silicon probes are implanted in the MS (green) for single neuron recording and in the HPC (purple) for LFP recordings. Three-dimensional mouse brain adopted from (Bakker et al., 2015).



2. Figure. Optogenetic identification of specific neuronal subtypes of the MS. Channelrhodospin-2 expression is confined to targeted PV+, VGLUT2+ and VGAT+ neuronal populations. (A) Confocal image of ChR2-eYFP (green) and PV (red) expression in the MS of an infected PV-Cre mouse. (B) High magnification images of ChR2-eYFP expression in the MS (top) along with PV expression (middle). Composite image shown on the bottom support strong specificity of ChR2 expression in PV+ cells. (C) 93.25 percent of cells expressing ChR2-eYFP were positive for PV (n = 267 cells from 3 animals). (D) Expression of ChR2-eYFP (green) and Cre recombinase (red) in the MS of an infected VGLUT2-Cre mouse. (E) High magnification images of ChR2-eYFP expression in the MS (top) along with Cre expression in VGLUT2-Cre mouse line (middle). Composite image is shown on the bottom. (F) 99.43 percent of cells expressing eYFP were positive for Cre recombinase (n = 529 cells from 3 animals). (G) Image of ChR2-eYFP (green) and Cre recombinase (red) expression in the MS of an infected VGAT-Cre mouse. (H) High magnification images of ChR2-eYFP expression in the MS (top) along with Cre expression in VGAT-Cre mouse line (middle). Composite image is shown on the bottom. (I) 97.65 percent of cells expressing eYFP were positive for Cre recombinase (n = 256 cells from 3 animals), supporting the high specificity of the viral targeting of ChR2 in GABAergic cells. (Photos and Figure was made by Daniel Schlingloff.)

Target brain areas and signals

2.1.5 CA1 LFP

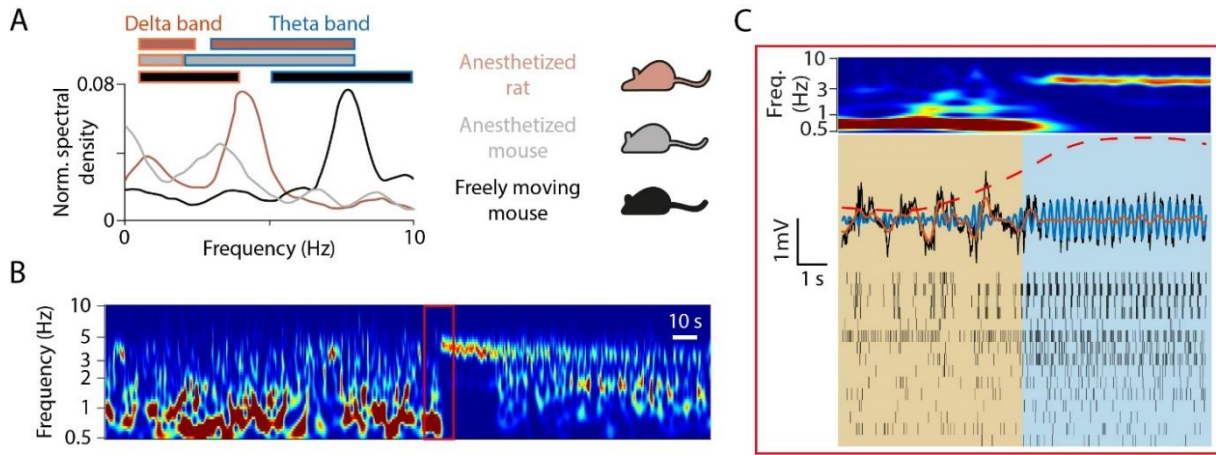
The hippocampal formation is particularly well-suited for field potential (LFP) studies due to its histological arrangement. The dense packing of the cell bodies, the roughly parallel position of the apical dendrites and their capacity for synchronous activation collectively contribute to the prominence and clarity of hippocampal field potentials.

Despite the similar cytoarchitecture and similar inputs of the CA1 and CA3 regions, the extracellular theta currents in the latter are considerably smaller than those in the former. This difference arises partly due to variations in the distal dendritic arbor sizes and the interference of mossy fibers induced excitatory currents with opposite phase perforant path and recurrent collateral generated currents.

2.1.6 Medial septal multiple single units

One of the earliest findings regarding hippocampal theta rhythm, discussed in *“Electrophysiology of the hippocampal theta, historical models”* chapter in the dissertation, was that lesion of the medial septum abolish theta oscillations (Petsche et al., [1962](#)). The MS plays a central role in orchestrating oscillatory neuronal activities throughout the brain, contributing to functions such as sensory and emotional salience, locomotion, mood regulation, and the governance of learning and memory.

The hippocampus is the principal target of septal theta-generating circuits, and its local field potentials provide a direct measure of network oscillatory activity. Concurrent recordings of medial septal unit activity and hippocampal LFPs allowed us to correlate individual neuron dynamics with population-level rhythms, revealing mechanisms of frequency synchronization and phase relationships (*Figure 3A*). This dual-focus approach established the medial septum's role as a pacemaker for hippocampal theta, aligning experimental observations with theoretical predictions.



3. Figure. Hippocampal theta and non-theta states. (A) Theta and delta frequency band boundaries were determined based on Fourier spectra of HPC LFPs for each model separately (representative recordings), indicated by color-coded horizontal bars on top. (B) Representative HPC LFP wavelet spectrogram from an anesthetized rat recording. Non-theta - theta state transition (red box) is enlarged on panel C. (C) Top: Theta onset from panel B. Middle: Raw LFP (black), LFP filtered in the theta (blue) and delta frequency band (orange), and theta-delta power ratio (red dashed line). Bottom: Raster plot of individual units ($n = 15$) recorded from the MS concurrently. Note the change in network activity at theta onset.

3 Methods

Hippocampal theta oscillations have been investigated in both urethane-anesthetized and awake rodents (Borhegyi et al., 2004; Buzsáki, 2002; Klausberger & Somogyi, 2008; Kramis et al., 1975; Mikulovic et al., 2018; Stewart & Fox, 1989). To explore the commonalities and differences across different theta model systems, we collected three independent data sets (Figure 3). We recorded multiple single neurons from the medial septum (MS) using silicon probes from anesthetized rats ($n = 903$ neurons from $N = 7$ rats; 416 and 636 neurons active during both theta and non-theta states or at least one of them, respectively), anesthetized mice ($n = 1155$ neurons from $N = 11$ mice; 617 and 950 active during both or at least one of the states) and awake mice ($n = 312$ neurons from $N = 4$ mice; 265 and 289 active during both or at least one of the states). Simultaneously, we recorded local field potentials (LFP) from all layers of the dorsal CA1 area of the hippocampus using linear silicon probe electrodes (Figure 3). For a detailed description of the surgical procedures, recordings, and data preprocessing see “Surgery and electrophysiological recordings – Methods” chapter in the dissertation.

Hippocampal recordings were segmented into **dominant theta and non-theta sections** based on the amplitude ratio of theta to slower frequency bands. LFP traces were bandpass filtered in theta and delta bands and were subsequently Hilbert-transformed to extract the amplitude and phase values of the respective frequency components (Figure 3; for a details, see “Hippocampal state detection” in the dissertation).

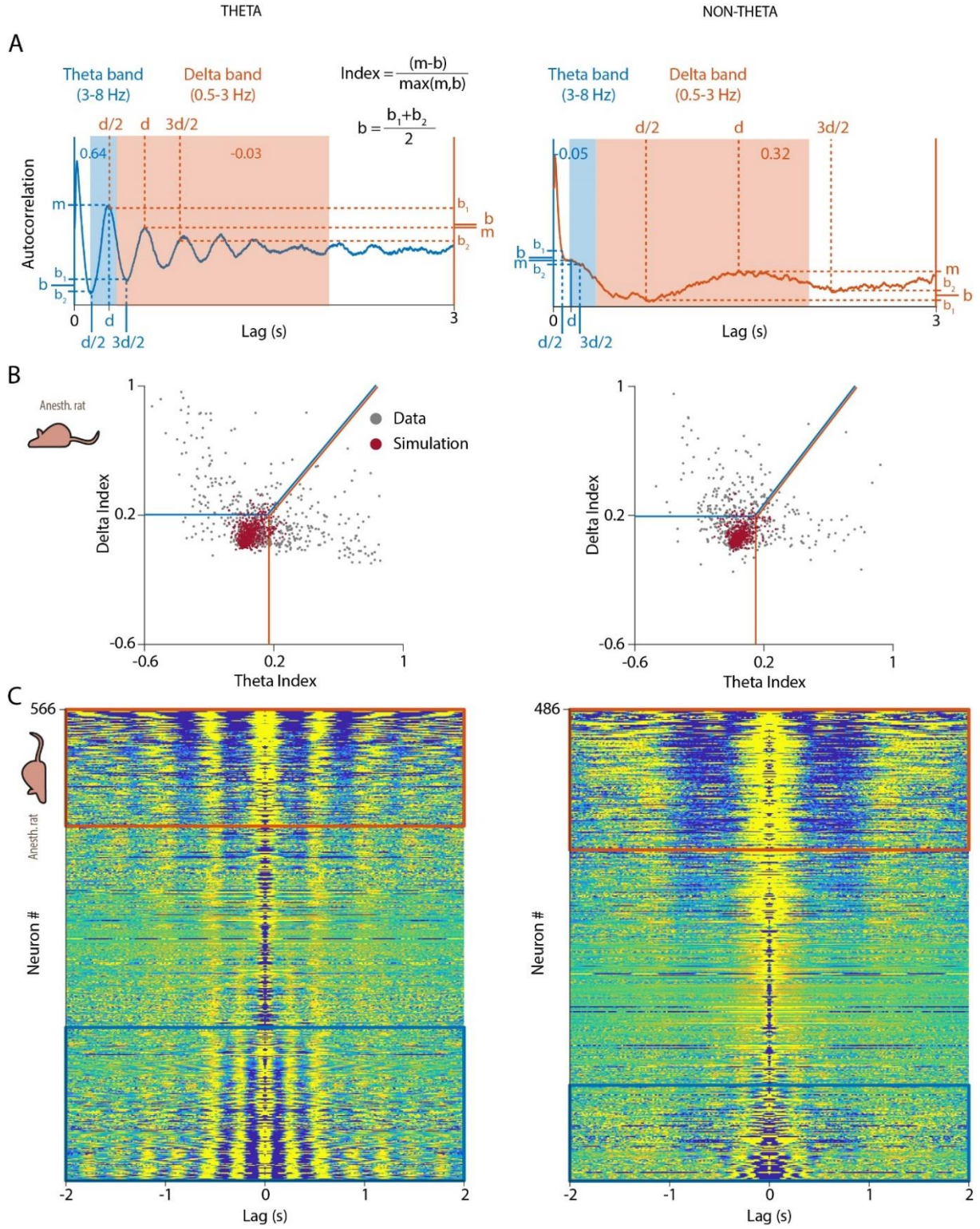
Next, the firing patterns of medial septal neurons were analyzed for **theta-rhythmic, delta-rhythmic (or non-rhythmic) modulation** in both hippocampal states (Figure 4). Autocorrelograms of spike trains

of the neurons were calculated and rhythmicity indices were derived based on the different frequency bands. Significant rhythmic modulation was determined by a bootstrap permutation test to ensure unbiased classification. Poisson neurons were simulated based on the firing rates of the recorded units. Rhythmicity Indices were calculated for the simulated neurons using the same procedure applied to the biological recordings, then Rhythmicity Index thresholds were determined corresponding to $p < 0.05$ significance levels based on this bootstrap null distribution (*Figure 4*; for details, see the “*Rhythmicity Indices of septal neurons*” chapter in the dissertation).

Based on the rhythmicity profiles of MS neurons, distinct **rhythmicity groups** were established. The firing properties and phasic relations of these groups were characterized across all three datasets (see the “*Hippocampal phase of septal spikes and phase-coupling*” chapter of the dissertation).

To investigate **synchronization** within and between rhythmicity groups, pairwise crosscorrelations were computed for MS cell spike trains ($n = 527$ simultaneously recorded pairs, anesthetized rats). Synchrony strengths was quantified and statistically compared within and across the rhythmicity groups (see the “*Hippocampal phase of septal spikes and phase-coupling*” and “*Statistical testing*” chapters of the dissertation).

Crosscorrelation analysis suggested that **pacemaker neurons play a guiding role in theta synchronization**. To examine how these neurons achieve synchrony, several parameters were calculated and compared between theta non-theta episodes (*Figure 8*). These includes firing rates, rhythmicity frequencies (estimated from autocorrelograms), intraburst interspike intervals, and theta skipping (the proportion of “silent” cycles within the neuron’s theta cycle-binned edge vector). To explore frequency synchronization mechanism, differences in rhythmicity frequencies were calculated between concurrently recorded pairs of putative pacemaker neurons. Additionally, all concurrently recorded pacemaker frequencies were examined to determine whether they converge to a tighter distribution (see the “*Huygens synchronization of pacemaker cells*” chapter of the dissertation). Theta vs. non-theta parameter pairs were compared with the Wilcoxon signed-rank test (see the “*Statistical testing*” chapter of the dissertation).



4. Figure. Determining theta and delta rhythmicity of medial septal units. (A) Autocorrelations of spike trains were calculated for theta (left) and non-theta states (right) for each neuron. Theta Index was calculated by normalizing the theta-band peak (m at lag d) with the average of pre- and post-peak troughs (b , mean of b_1 and b_2 at lags $d/2$ and $3d/2$, respectively) for both states (blue numbers above the traces). Delta Index was calculated using a similar algorithm for the delta band (red numbers). (B) Theta and Delta Index was calculated for all recorded neurons during theta (left) and non-theta segments (right; grey, recordings from anesthetized rats are shown) and for simulated Poisson-neurons with matching firing rate (red). Significant rhythmicity was defined by the 0.05 percentile of the Theta and Delta Index distributions of the Poisson-neurons (vertical and horizontal lines). If both indices exceeded the threshold, the larger of the two rhythmicity indices was considered (identity line). (C) Autocorrelograms (left, theta segments; right, non-theta segments) for all neurons with sufficient firing rate, sorted

by the larger of the two rhythmicity indices: delta-rhythmic on top and theta-rhythmic on bottom; significantly delta- and theta-modulated neurons are above and below the respective colored lines (anaesthetized rat).

To determine the genetic and neurotransmitter identity of MS neurons **optogenetic tagging** was performed in PV-IRES-Cre (N = 4, genetic background FVB/AntFx), VGAT-Cre (N = 4, genetic background Bl6Fx) or VGlut2-Cre (N = 5, genetic background C57BL/6J) mice. These animals were transduced by a Cre-dependent Channelrhodopsin (ChR2) construct (adeno-associated virus vector) injected in the MS to identify parvalbumin-expressing, GABAergic and glutamatergic MS neurons, respectively. Around two weeks after the virus injection, the animals were anesthetized with urethane, and the silicon probes were positioned in the HPC and MS. An optic fiber was inserted into the MS to allow selective activation of the targeted cell types. Light pulses (20 Hz) were delivered at the beginning and end of each recording depth (for a detailed description, see the *“Surgery and optogenetic tagging protocol”* chapter of the dissertation).

Hippocampal state detection, rhythmic modulation and synchronization measures were calculated using the same methods described previously for the “wild type” dataset (see the *“State detection and spike sorting”* chapter in the dissertation).

To evaluate the effect of MS photostimulation on hippocampal oscillations, spectral power was calculated from the wavelet spectrograms in the delta and theta frequency bands by averaging across within-band frequencies. Average theta-to-delta power ratios during stimulation and off-stimulation periods were compared with the Wilcoxon signed-rank test (see the *“Statistical testing”* chapter of the dissertation).

To test if a simple network model of a homogeneous MS pacemaker population could produce in vivo dynamics, we simulated MS neurons based on a minimalistic **single-compartment conductance-based model** constructed to produce fast spiking burst dynamics (Golomb et al., [2007](#)). This model included a transient sodium channel (nas) that is necessary for action potential generation, the delayed rectifier potassium channel (kdr) for repolarization during fast spiking and the slowly inactivating d-type potassium channel (kd) for bursting behavior through controlling the duration of the after-hyperpolarization. HCN-channel (hcn) responsible for the hyperpolarization-activated H-current, was also added (generic HCN model from (Káli & Zemankovics, [2012](#))). Parameters of the ‘kd’ and ‘hcn’ channels were fine-tuned to achieve the target rhythmicity frequency and sag response (*Figure 11*; for details, see the *“Septal pacemaker neuron model”* chapter of the dissertation).

Following the development of the rhythmic MS neuron model, the MS **network** was simulated. Twenty pacemakers were connected with inhibitory synapses, with reversal potentials and exponential decay factors set to mimic GABA-A receptor kinetics. All neurons received non-rhythmic (‘tonic’) somatic excitatory current to emulate the excitatory drive typically observed in the MS pacemaker population.

To assess the network's synchronization and desynchronization capabilities, a pattern of weak, strong (1.4-fold), and weak stimulation was applied on the neurons. The extent of increase in excitation strength was based on the average firing rate increase of MS glutamatergic neurons during theta (*Figure 10*). For additional details on network simulations, see the *"Modeling the septal pacemaker network"* chapter of the dissertation.

The theoretical output signal of the simulated pacemaker network was modelled as a proxy for the septal input to the hippocampus. Hippocampal state detection, rhythmic modulation and synchronization measures were calculated using the same procedures described previously for the "wild type" dataset.

To test the robustness of the theta-generating mechanisms a series of simulations was performed. The **parameter space** was spanned by the connection rate (0-100% in increments of 20%), mean synaptic delay (1, 4, 7, 11, 15 ms), mean absolute synaptic strength (1, 3, 6, 9, 12 nS), mean (40-80 pA in increments of 10 pA) and variance (0-20% in increments of 5%) of baseline stimulation strength. The variance of synaptic weights, delays and decay times was kept at 10%. The simulated models were evaluated by calculating a simple synchronization score, which quantified the ratio of the total simulation time the network spent in the expected state based on the state detection described above. For further information on the parameter space examination, see the *"Exploring the parameter space of the pacemaker network model"* chapter of the dissertation.

Contributions

I will now describe my contributions to the pillars of this dissertation (Király et al., [2023](#); Kocsis et al., [2022](#)). In addition to the following, I will indicate the contributions of other researchers along with their affiliations at the end of each relevant chapter in the dissertation.

Animal surgeries, electrophysiological **recordings** and **histological verifications** of the recording locations were performed by my colleagues Balázs Hangya, Richárd Fiáth, Andor Domonkos and Sergio Martínez-Bellver. **Immunostaining** for the verification of the specificity in optotagging experiments was conducted by Dániel Schlingloff. Detailed descriptions of these methods are included in this report for technical completeness.

Preprocessing of the recorded data entailed the selection of hippocampal channel with highest amplitude and spike sorting of septal neurons. These tasks were carried out by me, except for the acute rat dataset, spike sorted by Péter Barthó.

The identification of state dependent **rhythmicity classes** in the MS and **analysis** of their **synchronization** were entirely performed by me under the guidance of Balázs Hangya.

The septal rhythmic **cell model** was developed using an existing model (Golomb et al., [2007](#)) and was extended with the H current using an existing generic HCN model (Káli & Zemankovics, [2012](#)). For the sake of completeness, I will share the differential equations of the activation and inactivation gates that control transmembrane currents.

The construction of the **network** and the study of model synchronization were conducted entirely by me. The fine tuning of the Golomb model parameters and the network modelling were performed under the guidance of Szabolcs Káli and Balázs Hangya.

Data processing and the result analysis were carried out using self-developed Matlab codes (MATLAB 2016a and 2020a (Mathworks, Natick, US)). Model simulations were executed using NEURON software. Both the Matlab and NEURON software code have been deposited at Zenodo and is publicly available at <https://doi.org/10.5281/zenodo.6798310>. The GitHub repository of the code is available at https://github.com/hangyabalazs/ms_sync_analysis.

4 Results

This dissertation rests on two pillars. In the article by Kocsis et al. we defined septal rhythmicity groups and investigated their synchronization mechanisms and their role in the generation of hippocampal theta frequency oscillations (Kocsis et al., [2022](#)). The research and results presented here are largely related to this first author article.

The second pillar is my co-authored paper with Kiraly et al. (Király et al., [2023](#)). This work studied the behavior of septal cells in relation to hippocampal theta spectral components (tSCs). The preprocessed data and classifier codes were partly derived from the first project. In Király et al. we reported the tSC-dependent behavior of septal rhythmicity groups (Kocsis et al., [2022](#)). The other results of this paper are not addressed in this dissertation.

The two papers are connected by the finding that the rhythmicity groups defined in the first paper were also distinct functional groups in the second paper. Therefore, the firing patterns of septal cells suggest that they play an active role in generating not only hippocampal theta but also gamma frequency oscillations.

Thesis group I.

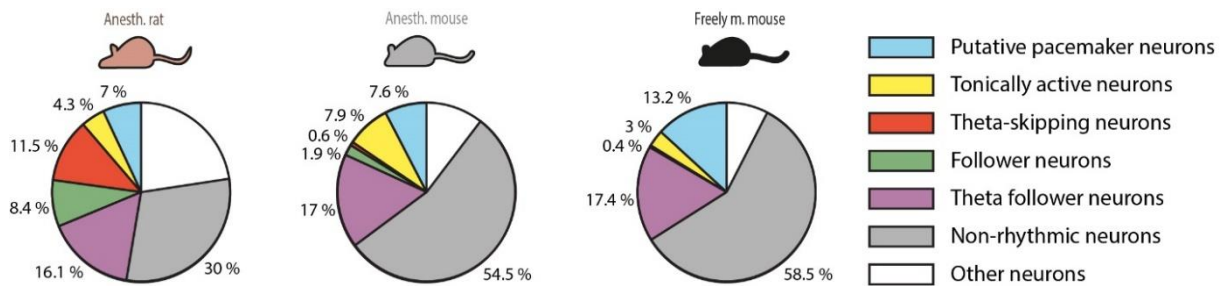
Related to the hippocampal-state-dependent classification of medial septal rhythmic neurons and the exploration of their synchronization.

Thesis Ia. My semi-automatic method identifies functional neuron classes based on rhythmic firing properties.

Corresponding publications: Király et al., [2023](#); Kocsis et al., [2022](#).

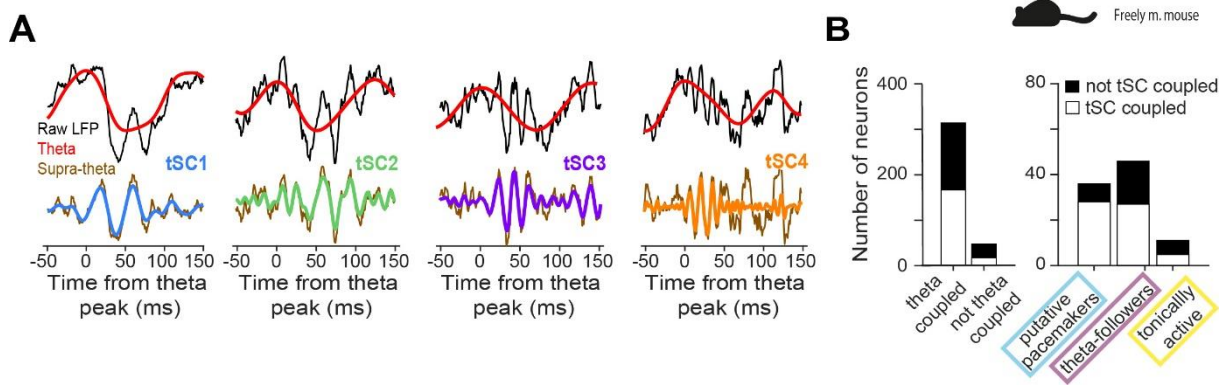
I have developed a Matlab code package for the rhythmicity-based classification of neurons. Following some preprocessing steps and manual specification of the global variables corresponding to the examined rodent model, the software autonomously classifies cells and calculates group means, as well as intra- and intergroup synchronization measures.

In our experiments four main categories of rhythmic behaviors were observed: pacemaker, theta follower/ follower, theta-skipping and tonically active neurons (*Figure 5*). Firing properties and phasic relations of these classes were characterized across all three datasets. For a detailed description of the rhythmicity groups, see the “*Rhythmic neuron classes of the medial septum*” chapter of the dissertation.



5. Figure. Proportions of medial septal neurons in the different rhythmicity groups in anesthetized rats (left), anesthetized mice (middle) and freely moving mice (right).

When septal rhythmic cells were examined in relation to hippocampal tSCs, significant firing modulation was observed (*Figure 6*; see the “*Septal rhythmic cells contribute to hippocampal supra-theta oscillations*” chapter of the dissertation). Putative pacemaker and theta-follower neurons exhibited increased firing rate and theta phase coupling in cycles with gamma-band tSCs, whereas tonically active cells also increased their firing rate. The majority of these neurons were coupled to specific tSC phases. Additionally, tSC-coupled putative pacemakers not only preceded all types of tSCs, but also emerged as the earliest forecasters among all tSC-coupled cells. Thus, my semi-automatic method identified functional neuron classes involved in rhythm genesis beyond theta frequency as well.

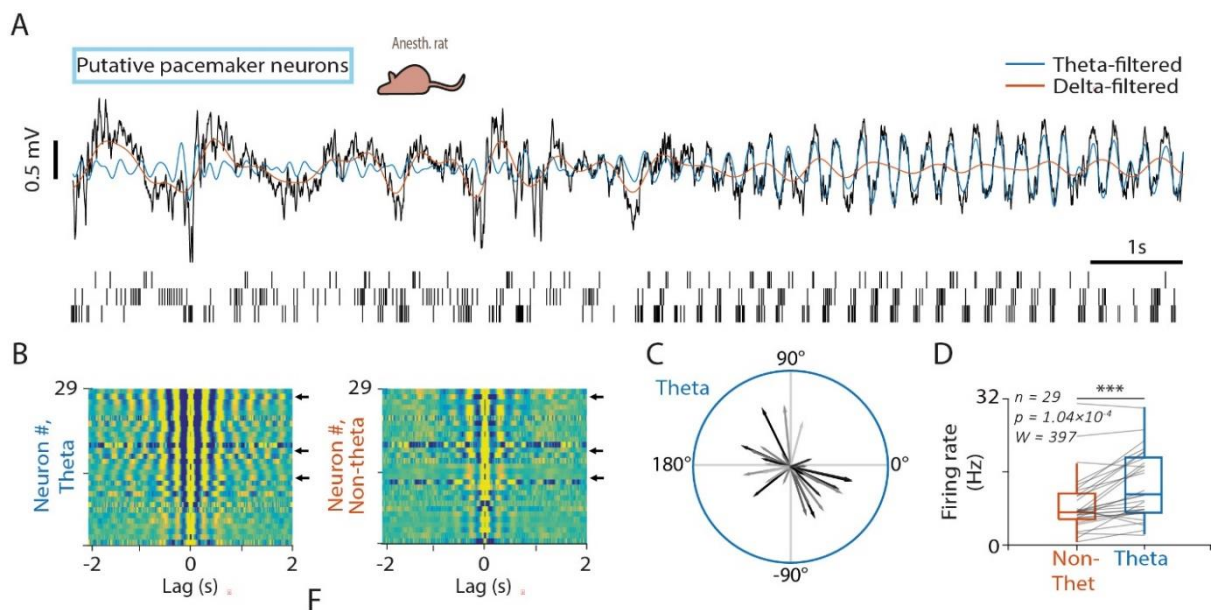


6. Figure. Activity of rhythmic septal cells during CA1 supra-theta spectral components, recorded from freely moving mice. Figures are taken from (Király et al., 2023) (A) “Example theta cycles dominated by different tSCs”. (Király et al./ Figure 1./e). (B) “Stacked bar chart showing the proportion of tSC-coupled neurons (empty bars) as a function of their theta-coupling (left) and rhythmic firing properties (right)”. (Király et al./Figure 3./f).

Thesis Ib. Pacemakers synchronize their burst frequencies.

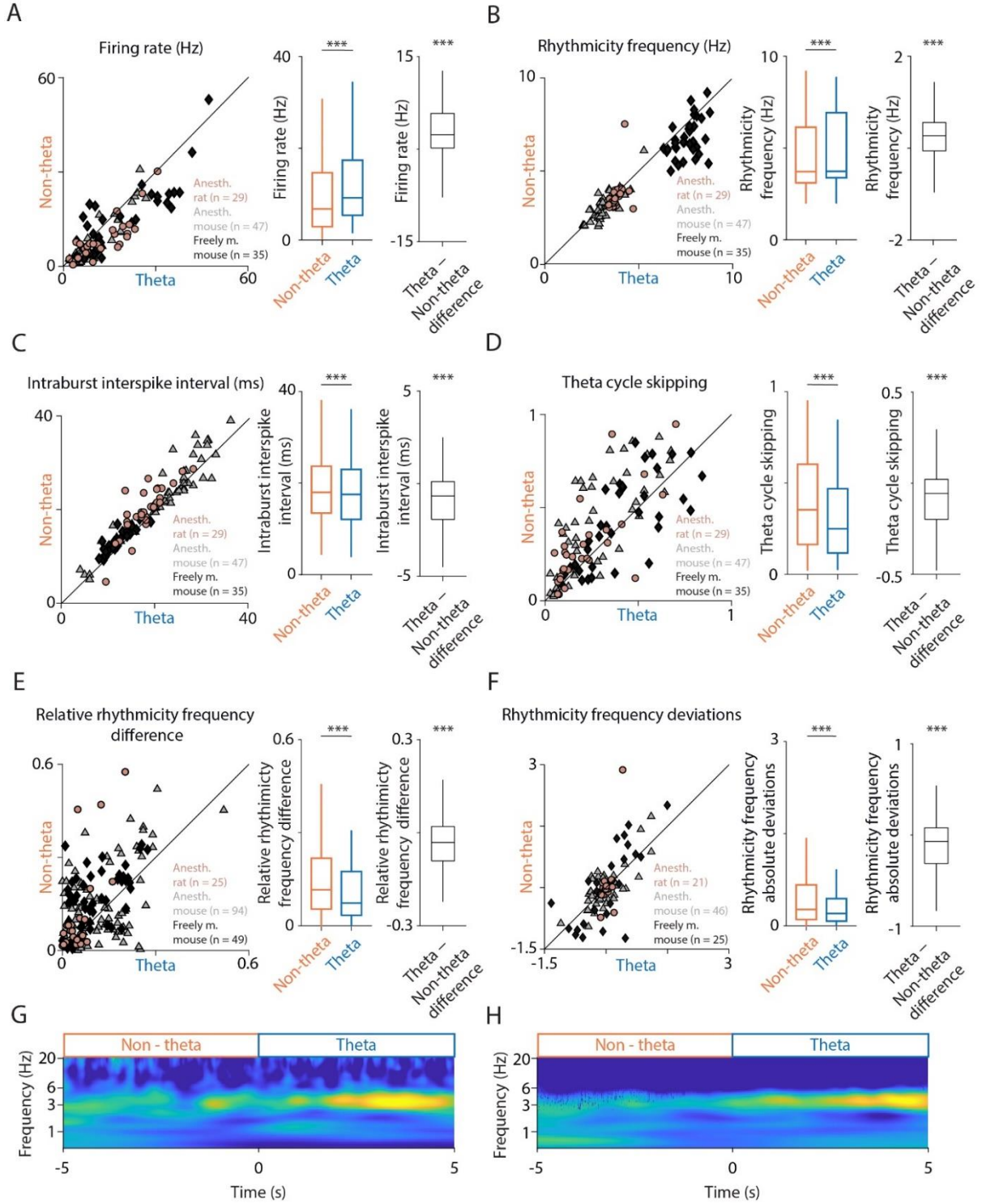
Corresponding publication: Kocsis et al., 2022.

Crosscorrelation analysis of rhythmicity groups indicated the central role of putative pacemaker neurons in theta synchronization. Putative pacemakers fired theta rhythmic bursts of action potentials, irrespective of the hippocampal state, and exhibited hippocampal theta phase coupling (Figure 7; for details, see the “Synchronization of rhythmicity groups” chapter of the dissertation).



7. Figure. Putative pacemaker neurons of the anaesthetized rat. This group of neurons fired theta rhythmic bursts of action potentials, irrespective of the hippocampal state. (A) Top: Raw LFP (black) from the CA1 shows a delta-to-theta state transition. Bottom: spike raster of three examples of putative pacemaker neurons from the same recording session. (B) Autocorrelograms of all putative pacemaker neurons during theta (left) and non-theta (right) segments. Arrows indicate the example neurons (panel A). (C) Phase-locking of all putative pacemaker neurons to HPC theta oscillation in polar coordinates. Angle: circular phase; length: mean vector length; greyscale corresponds to average firing rate (black, higher firing rate). Note that neurons formed two opposite phase clusters during theta (147.28° and 342.57°, see text) (D) Firing rates of putative pacemaker neurons during non-theta and theta segments. Boxes and whiskers represent interquartile ranges and non-outlier ranges, respectively. Lines correspond to individual neurons. Firing rate was significantly higher during theta (Wilcoxon signed-rank test).

To decipher the mechanisms of medial septal pacemaker synchronization I examined four alternatives, not excluding each other: tonic drive change, burst properties change, theta skipping and frequency synchronization. Applied metrics for these mechanisms revealed increased tonic drive, reduced cycle skipping and relatively stable burst properties during theta (*Figure 8*; see the “*Huygens synchronization of pacemaker cells*” chapter of the dissertation). Additionally, pacemakers synchronized their bursts frequencies in a manner similar to coupled pendulum clocks, a phenomenon known as Huygens synchronization (*Figure 8*; see “*The sympathy of clocks*” chapter of the dissertation).



8. Figure. Septal pacemakers synchronize their burst frequencies. (A) Firing rates of MS putative pacemakers were higher during theta oscillation then during non-theta segments. Left, scatter plot shows the firing rate of all putative pacemaker neurons, color coded according to the rodent theta models. Middle and right, box-whisker plots showing statistics on pooled data (**p < 0.001; $p = 1.402 \times 10^{-9}$, $W = 5166$, $n = 111$, Wilcoxon signed-rank test). See Table S3 in the dissertation for statistics on the three datasets separately. All box-whisker plots show median, interquartile range and non-outlier range in this figure. (B) Left, scatter plot of rhythmicity frequency of putative pacemakers measured by the time lag of the first autocorrelation peak in the theta band. Middle and right, statistics on pooled data indicated higher rhythmicity frequency during hippocampal theta (**p < 0.001; $p = 2.64 \times 10^{-7}$, $W = 4857.5$, $n = 111$, Wilcoxon signed-rank test). (C) Left, scatter plot of average intraburst interspike intervals (ISIs) for putative pacemaker neurons. Middle and right, the moderate decrease of ISIs during theta oscillation indicates a slight elevation of intraburst frequency (**p < 0.001; $p = 7.336 \times 10^{-5}$, $W = 1275.5$, $n = 111$, Wilcoxon signed-rank test). (D) Left, scatter plot of the ratio of skipped theta cycles of putative pacemakers during non-theta and theta segments. Middle and right, statistics on pooled data indicated that putative pacemaker neurons skipped

more theta cycles during non-theta segments ($***p < 0.001$; $p = 3.021 \times 10^{-6}$, $W = 1521$, $n = 111$, Wilcoxon signed-rank test). (E) Left, scatter plot of relative frequency difference of individual pacemaker neurons for simultaneously recorded pairs, normalized to the larger of the frequencies. Middle and right, statistics on pooled data indicated that individual pacemakers became more similar in frequency during theta oscillation ($***p < 0.001$; $p = 3.17 \times 10^{-6}$, $W = 4156$, $n = 168$, Wilcoxon signed-rank test). (F) Left, deviation from the session mean rhythmicity frequency during theta vs. non-theta segments for putative pacemaker neurons (sessions in which at least two putative pacemakers were co-recorded are included). Middle and right, statistics on pooled data indicated that the absolute deviation from the mean rhythmicity frequency was significantly larger during non-theta segments ($***p < 0.001$; $p = 4.189 \times 10^{-4}$, $W = 1233$, $n = 92$, Wilcoxon signed-rank test). (G) Mean wavelet coherence spectrogram of an example putative pacemaker neuron pair (spike trains were convolved with a 50-ms Gaussian window, from anesthetized rat) averaged around non-theta to theta transitions. (H) Mean wavelet coherence spectrogram averaged across pairs of putative pacemaker neurons recorded in urethane-anesthetized rats (time 0 corresponds to non-theta to theta transitions; sessions in which at least two putative pacemakers were co-recorded are included, $n = 25$ pairs).

Thesis group II.

Related to the optogenetic tagging of pharmacological neuron types in the medial septum.

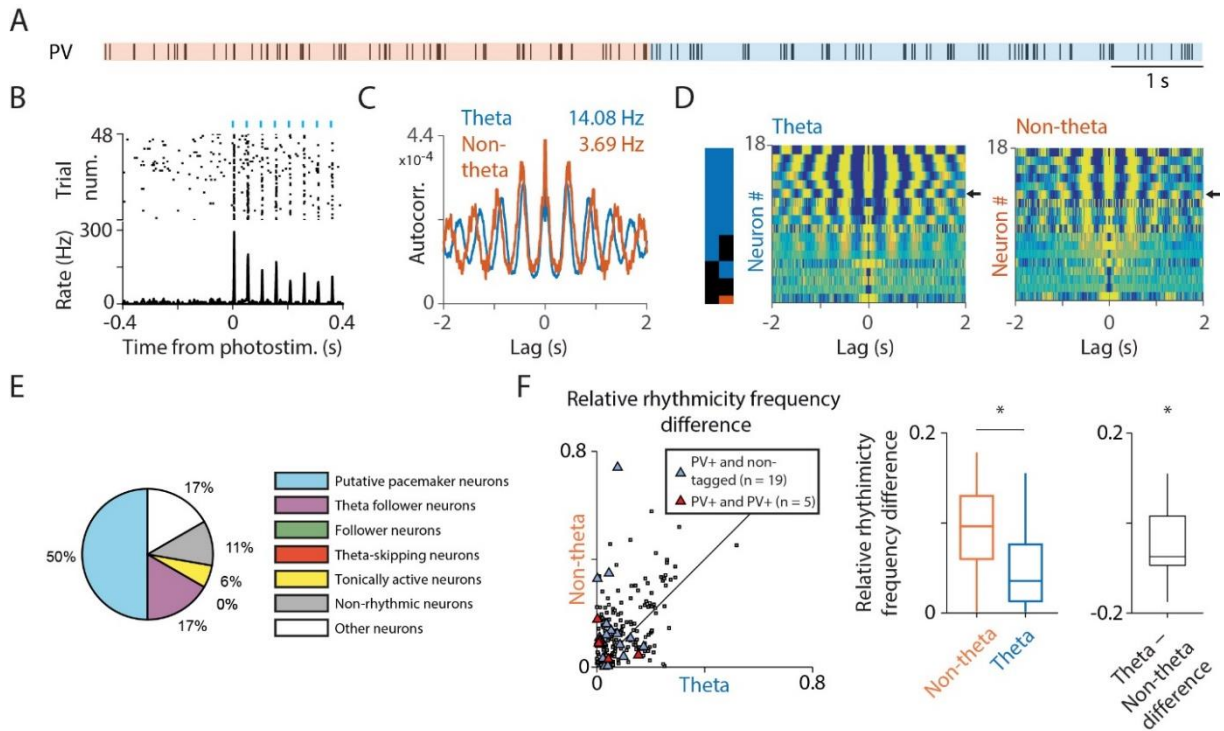
To investigate the genetic and neurotransmitter identity of medial septal neurons, optogenetic tagging was performed in urethane-anesthetized mice. Following the successful tagging of PV+ ($n = 18$ from 2 animals), GABAergic ($n = 20$ from 2 animals) and glutamatergic ($n = 30$ from 3 animals) neurons (*Figure 2*), I performed the same battery of the previously described rhythmicity analyses on these recordings. Finally, I evaluated the proportions of rhythmicity classes in each tagged group.

Thesis IIa. PV expression marks the theta-generating septal population.

Corresponding publication: Kocsis et al., [2022](#).

I found the highest proportion of putative pacemaker cells in the PV-expressing group ($n = 9/18$ neurons), suggesting that PV expression marks the theta-generating population (*Figure 9*).

When I calculated the theta-associated change in relative frequency differences between pairs of putative pacemakers where at least one of the cells was tagged as PV+, I observed similar frequency synchronization ('Huygens synchronization') as shown for unlabeled pairs of putative pacemakers (*Figure 9*; for details, see the "PV+ pacemaker cells synchronize their frequencies" chapter of the dissertation).

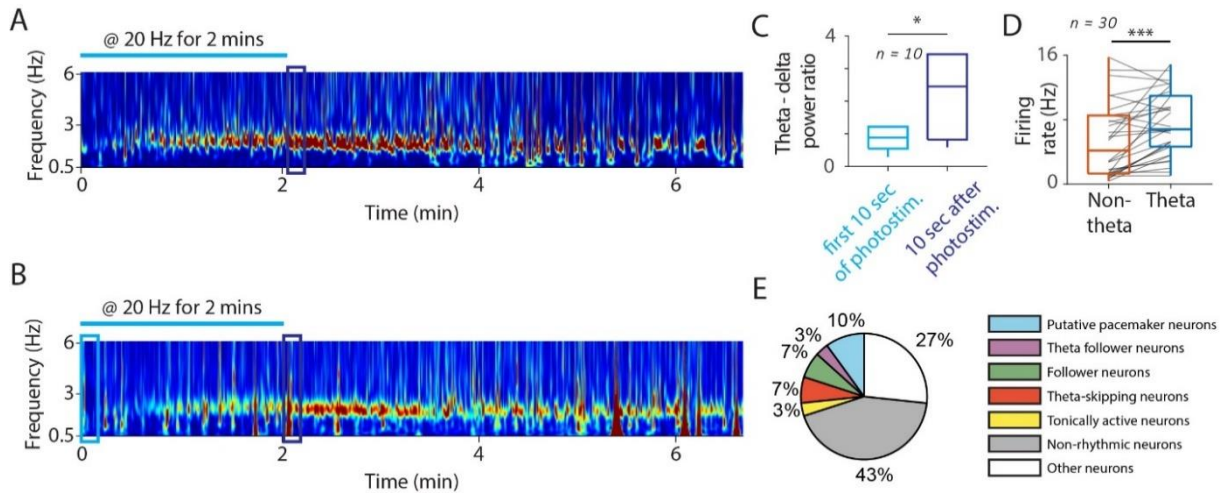


9. Figure. Rhythmicity and synchronization of parvalbumin+ cells. (A) Example spike raster of a PV-expressing MS neuron during non-theta (orange) and theta (blue) segments. (B) Raster plot (top) and corresponding peri-stimulus time histogram (bottom) of an example PV-expressing light-sensitive MS neuron aligned to the onset of 20 Hz photostimulation (blue ticks). (C) Autocorrelograms of the example neuron during non-theta (orange) and theta (blue) segments. Numbers in the top right corner indicate average firing rate during non-theta (orange) and theta (blue). (D) Autocorrelograms of all identified PV-expressing MS neurons, sorted by rhythmicity index. Left: Color bar indicates significant theta-rhythmicity (blue) and delta-rhythmicity (orange) during theta (left) and non-theta (right) segments. Middle: Autocorrelograms during theta segments. Right: Autocorrelograms during non-theta segments. The arrows indicate the example neuron. Most PV-positive MS neurons were constitutively theta-bursting putative pacemakers. (E) Proportion of rhythmicity types within 18 identified PV+ neurons. (F) Left: Relative rhythmicity frequency difference of putative pacemaker MS neurons during theta vs. non-theta segments. Pairs of two optogenetically identified PV+ neurons (red triangles) and pairs of an identified PV+ and an untagged putative pacemaker neuron (blue triangles). Black squares, pooled data from the anesthetized rat, anesthetized mouse and awake mouse experiments. Middle and right: The relative frequency difference of pairs containing at least one identified PV+ neuron significantly decreased during theta oscillation compared to non-theta segments (*, $p < 0.05$; $p = 1.293 \times 10^{-2}$, $W = 63$, $n = 24$ pairs containing at least one PV+ neuron, Wilcoxon signed-rank test).

Thesis IIb. Stimulation of glutamatergic cells evokes theta in the hippocampus.

Corresponding publication: Kocsis et al., [2022](#).

The majority of glutamatergic neurons showed a strong increase in the firing rate -without any theta rhythmicity- during hippocampal theta state, suggesting their contribution to the increased tonic excitatory drive on pacemakers during theta synchronization (Figure 10). Indeed, I observed a significant increase in theta-band activity in the hippocampal frequency power distributions after the tagging protocol (Figure 10). Thus, photostimulation of glutamatergic cells induced hippocampal theta oscillations (for details, see the “Glutamatergic stimulation evokes theta” chapter of the dissertation).



10. Figure. 20 Hz stimulation of glutamatergic cells generates theta in the CA1. (A) Wavelet spectrogram of an example CA1 LFP segment during and after 20-Hz stimulation of MS glutamatergic neurons. The photostimulation induced lasting theta oscillation. (Note the lower frequency of theta typical under anesthesia). (B) Average of all recording sessions ($n = 10$) of 20 Hz glutamatergic stimulation. (C) Box-whisker plot showing significant increase in theta-delta ratio upon glutamatergic stimulation. Median, interquartile range and non-outlier range are shown ($*p < 0.05$; $p = 3.71 \times 10^{-2}$, $W = 7$, $n = 10$, Wilcoxon signed-rank test). (D) The majority of optogenetically identified MS glutamatergic neurons showed a strong increase in the firing rate during theta ($***p < 0.001$; $p = 8.94 \times 10^{-4}$, $W = 394$, $n = 30$. Fold increase median $\pm$ standard error of the median, 1.42 ± 0.34). (E) Proportion of rhythmicity types within 30 identified glutamatergic neurons.

Thesis group III.

Related to the modeling of the medial septal theta generation.

The analysis of electrophysiological recordings suggested that increased tonic excitation induces frequency synchronization of septal pacemakers, leading to the generation of hippocampal theta oscillations. To deepen our understanding of theta synchronization, I have developed a minimalistic network model, allowing direct testing of in vivo findings.

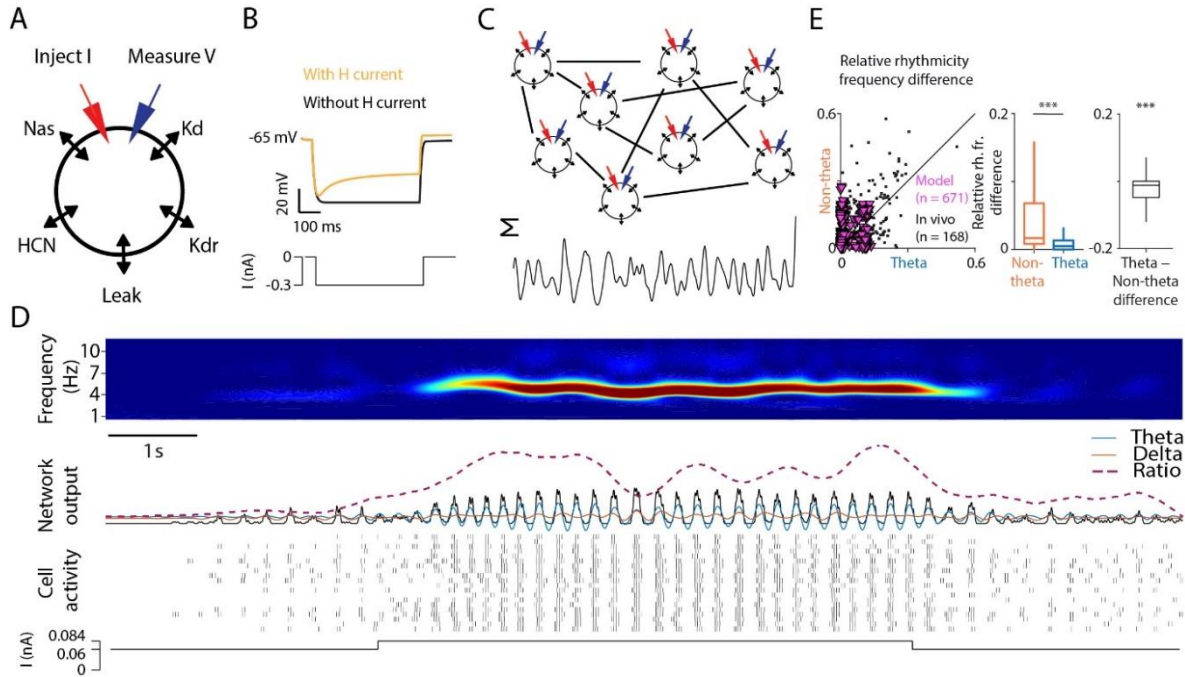
Thesis III. My homogeneous tonically driven network model is sufficient for theta generation.

Corresponding publication: Kocsis et al., 2022.

I designed a model of pacemaker cell based on a single compartment, rhythmically bursting model neuron (Golomb et al., 2007). After implementing the mechanism for H-current in my model I could reproduce the described sag response of some MS neurons (Figure 11). Following this, I connected the pacemaker model neurons randomly with inhibitory synapses without introducing network-level biases. All neurons received controllable tonic excitation as the only input, allowing direct intervention to the network dynamics during simulation (Figure 11).

After optimizing static parameters for theta generation, I performed the same set of analyses for pacemaker synchronization as I did for the electrophysiological and optotagging data. I observed similar synchronization metrics, including frequency synchronization mechanism ('Huygens synchronization') of pacemaker cells (Figure 11; for details, see the "Septal network model confirms

Huygens-synchronization” chapter of the dissertation). Thus, the model successfully reproduced several aspects of in vivo synchronization.



11. Figure. A simple conductance-based network model of the MS pacemaker circuit is capable of theta-synchronization upon tonic excitation. (A) Schematic of the single-compartment model neuron. The inhibitory neuron was equipped with transient sodium (Nas), slowly inactivating D-type potassium (Kd), delayed rectifier potassium (Kdr), HCN channels and passive leak channels. An electrode delivering current is attached to the cell. Adopted from (Golomb et al., 2007). (B) Adding H current caused a sag response to hyperpolarizing current, similar to MS GABAergic neurons reported in the literature (see e.g. (Xu et al., 2004, Figure 1: C). The moderate rebound effect did not induce rebound spikes. (C) Schematics of the network. Twenty model cells were connected with simulated GABA-A-mediated inhibitory synapses. Spike trains were summed and convolved with a 50-ms Gaussian window to model network output. (D) Simulation of network behavior (60% connection rate; 7-ms synaptic delay, 3-nS mean synaptic strength; 2-ms synaptic decay; 10% variance for the latter three synaptic parameters; 60-pA mean baseline stimulation with 10% variance). Neurons synchronized in the theta frequency band in response to a tonic increase of stimulation ($\times 1.4$, adjusted by glutamatergic firing rate increase, see Figure 10/ D) and desynchronized when stimulation was reset to baseline. (Increased excitation was applied with jittered onset latency to avoid synchronous phase reset). Top: Wavelet spectrogram of network output. Middle: network output (black: raw; blue: filtered in the theta band; orange: filtered in the delta band; dashed: theta-delta ratio) and simulated spike raster (see Figure 3C for in vivo recording). Bottom: Injected current. (E) The pacemaker network model reproduces Huygens-synchronization properties of the MS neural circuit (See Figure 8 for in vivo synchronization). Scatter plot of relative frequency difference of individual pacemaker neurons for simultaneously simulated pairs, normalized to the larger of the frequencies. Statistics indicated that individual model pacemakers became more similar in frequency during theta (***, $p < 0.001$; $p = 2.20 \times 10^{-30}$, $W = 51519$, $n = 671$). Box-whisker plots show median, interquartile range and non-outlier range.

5 Potential applications and scientific contributions

Oscillopathies

Understanding the synchronization mechanisms medial septal pacemaker neurons use to generate coordinated theta rhythms is important for understanding how the brain achieves temporal coordination of neural activity, a process essential for cognitive functions such as attention, memory, dynamic grouping, and awareness. Disruptions in synchronization are associated with specific

cognitive deficits and implicated in various neurological and psychiatric disorders, collectively termed 'oscillopathies'. Oscillopathies are considered significant contributors to the pathophysiology of these disorders. Although reduced neural synchronization can result from disconnection, it may also act as a causal factor. Regardless of whether oscillation abnormalities are a cause or consequence of symptoms, they hold clinical relevance as potential biomarkers or therapeutic targets. Beyond diagnosis there is also potential in monitoring disease progression and evaluating treatment efficacy.

Identifying the neuronal correlates responsible for generating oscillatory activity is therefore crucial. Apparently, all major neuromodulatory systems are involved in this process. For instance, gamma oscillations depend on several key mechanisms: GABAergic neurons play a central role in primary generation and local synchronization; glutamatergic connections regulate the strength, duration, and long-range synchronization; and cholinergic modulation facilitates state-dependent high-frequency oscillations and associated response synchronization. However, further research is needed to elucidate how GABAergic, glutamatergic, cholinergic, serotonergic, dopaminergic, and noradrenergic systems contribute to oscillogenesis and their interplay in oscillopathies.

Recent research has also shifted focus toward the relationships between different frequency rhythms. Slow oscillations are believed to facilitate interareal communication by temporally organizing higher-frequency rhythms, which are essential for cognitive processes. Some recent studies have reported MS involvement in generating supra-theta band oscillations, presenting a non-negligible therapeutic potential. This includes, for instance, high-amplitude gamma waves, implicated in seizure-induced automatism following temporal lobe seizures and schizophrenia-like symptoms after ketamine injection (Leung & Ma, 2022). Conversely, inactivation or lesions within the septohippocampal-Nucleus Accumbens (NAC) system could reduce the dose of anesthetic required to suppress gamma oscillations and induce loss of consciousness in animals. In this dissertation I report a previously unknown control of HPC beta and gamma band tSCs by the MS rhythmic cells.

These findings highlight the therapeutic potential of MS stimulation. If disrupted oscillations are regulated by the MS, patterned stimulation could potentially compensate for or mitigate abnormal oscillatory activity, thereby modulating symptoms associated with oscillopathies (Takeuchi et al., [2021](#)). Emerging research has explored various intervention strategies targeting the MS for conditions such as dementia. These approaches include pharmacological treatments (e.g., dopamine-based therapies), instrumental techniques (e.g., Deep Brain Stimulation and Vagus Nerve Stimulation), and behavioral interventions (e.g., locomotor exercise and cognitive enhancement).

For more details on oscillopathies related to cognitive deficiencies, see the *"Oscillopathies: a rationale behind this study"* chapter of the dissertation.

Rhythmic neurons of the medial septum

We first performed an unbiased physiological characterization of MS units, extending previous electrophysiology studies (Apartis et al., [1998](#); Borhegyi et al., [2004](#); Duque et al., [2000](#); Ford et al., [1989](#); Hassani et al., [2009](#); Simon et al., [2006](#); Stewart & Fox, [1989](#); Varga et al., [2008](#)). Bland and colleagues characterized MS neurons as theta-on versus theta-off based on their firing rate changes, and phasic (theta-bursting) versus tonic (non-rhythmic) based on their firing patterns. Petsche et al. described A- and B-units, where B-units were phase locked to theta with a stable phase relationship (Petsche et al., [1962](#)). Many studies have since investigated B-units (Apartis et al., [1998](#); Borhegyi et al., [2004](#); Joshi et al., [2017](#); King et al., [1998](#); Stewart & Fox, [1989](#)), but A-units received less attention. Other studies indicated the presence of lower frequency rhythms, including “slow oscillations” and rhythmic activity related to breathing (Buzsaki et al., [1986](#); Tsanov, [2015](#); Tsanov et al., [2014](#); Wolansky et al., [2006](#)). In accordance, we also observed rhythmic activity in the delta frequency band, especially in urethane-anesthetized rats. Therefore, we characterized MS neurons based on their rhythmicity during hippocampal theta and non-theta episodes as non-rhythmic, delta-rhythmic or theta-rhythmic. While this resulted in nine theoretical combinations, most MS neurons belonged to a few characteristic types.

Stewart and Fox found that, after eliminating theta oscillation in the hippocampus of urethane-anesthetized rats using atropine, a subset of MS neurons remained theta rhythmic (Stewart & Fox, [1989](#)). In another study, 15% of septal neurons were found phasic bursting even during non-theta epochs under anesthesia (Ford et al., [1989](#)). We identified putative pacemaker neurons that fired theta-rhythmic bursts regardless of whether theta oscillation was present in CA1. This group comprised 13% of MS neurons in freely moving mice; however, we found a lower percentage of rhythmic bursting neurons in anesthetized rodents, in line with some previous reports (King et al., [1998](#); Stewart & Fox, [1989](#)). The differences in percentage of pacemakers across reports may stem both from biased sampling, as the MS can be identified during recording based on the presence of theta-rhythmic activity, as well as from strictness of definitions for rhythmicity. We applied relatively conservative inclusion criteria for significant rhythmicity (*Figure 4*) to ensure data quality necessary for analyzing mechanisms of rhythmic synchronization, which may have resulted in a lower percentage of putative pacemakers in our datasets. These results also suggest that some MS neurons may be identified as putative pacemakers under awake but not in anesthetized conditions.

We referred to MS neurons only showing theta-rhythmic bursting when hippocampal theta was present as follower neurons; their firing patterns tended to correspond with hippocampal network states. However, this property does not exclude them from participating in theta genesis. Indeed,

these neurons tended to strongly synchronize with each other and putative pacemaker neurons both during theta and non-theta states, and optogenetic tagging experiments revealed an enrichment of both putative pacemaker and follower neurons in the PV+ GABAergic group, suggesting that this functional distinction based on rhythmicity may occur within the anatomically defined PV-expressing group.

Previous studies demonstrated theta-skipping behavior of rhythmic neurons both in the entorhinal cortex (EC) (Brandon et al., [2013](#); Jeffery et al., [1995](#)) and in the MS (Kay et al., [2020](#); King et al., [1998](#); Varga et al., [2008](#)). Theta skipping was characterized by rhythmic activity with one-half the frequency of ongoing hippocampal theta, by neurons firing rhythmically in every second theta cycle. It has been demonstrated that MS input was necessary for theta-skipping behavior in the EC in rats (Brandon et al., [2013](#)), and that theta-skipping behavior in the EC may rely on inhibitory rebound mechanisms, potentially crucial to the formation of characteristic grid cell firing fields (Hasselmo, [2014](#)). We confirmed the presence of theta-skipping neurons in the MS of urethane-anesthetized rats. While some of these neurons fired regularly in every second cycle, other neurons showed a less regular pattern, occasionally skipping more than one cycle. Interestingly, we found very few theta-skipping neurons in anesthetized and freely moving mice. Therefore, theta-skipping in the MS may not be necessary for grid pattern formation in mice.

We found a group of tonically active neurons both in rats and mice, which may be cholinergic, since (i) their firing patterns resemble cholinergic neurons in previous publications (Brazhnik & Fox, [1997](#); Mamad et al., [2015](#); Tsanov, [2015](#)), (ii) my colleagues identified cholinergic neurons with similar rhythmic and synchronization properties from other basal forebrain structures (Laszlovszky et al., [2020](#)), and (iii) rhythmic basal forebrain cholinergic neurons were not phase locked to hippocampal theta in a former study (Lee et al., [2005](#)), as we also found for the tonically active group. Theta-rhythmic cholinergic neurons may be more linked with a neocortical theta oscillation that can be recorded from the posterior cingulate cortex (Destradé & Ott, [1982](#); Lee et al., [2005](#)), and my colleagues have also reported behavior-specific synchronization of nucleus basalis cholinergic neurons with theta-band activity in the auditory cortex (Laszlovszky et al., [2020](#)). This would confirm that the role of MS cholinergic neurons in hippocampal theta generation is mostly indirect through GABAergic neurons, controlling theta amplitude but not participating in rhythmic theta pacing (Dannenberg et al., [2015](#); Lee et al., [1994](#); Yang et al., [2014](#), [2017](#)). We expect that it will be one of the most exciting future directions to better understand how multiple theta rhythms interact dynamically in the brain, including synchronization with active sensing processes (Moore et al., [2013](#); Ranade et al., [2013](#); Semba & Komisaruk, [1984](#)), entraining local pacemaker groups (Manseau et al., [2008](#)) and interacting with cellular resonance properties (Zemankovics et al., [2010](#)).

Huygens synchronization of septal pacemakers

In the seventeenth century, Christiaan Huygens observed that two pendulum clocks, coupled through a house beam, tended to synchronize over time, which he referred to as “sympathy of two clocks” (Huygens, [1673](#); Oliveira & Melo, [2015](#); Ramirez et al., [2016](#); Willms et al., [2017](#)). Such synchronization of coupled oscillators, where individual oscillators become closer to each other in their frequency and period time, has been found in many dynamical systems (Equihua & Ramirez, [2018](#); Ramirez et al., [2016](#)). We identified this mechanism among MS pacemaker neurons, suggesting that a Huygens synchronization mechanism underlies the generation of a strong theta rhythmic output transmitted to the hippocampus. The two dominant oscillatory behaviors that emerge in coupled oscillators during Huygens synchronization are in-phase and anti-phase synchronization (Korteweg, [1906](#); Ramirez et al., [2014](#)), and either one can sufficiently underlie frequency synchronization (Czolczynski et al., [2011](#); Ramirez et al., [2014](#), [2016](#)). This may, at least in part, explain the observation of anti-phase firing in the MS of urethane-anesthetized rats. Experiments and modeling studies also observed less common modes of Huygens synchronization, including partial synchronization, when oscillators synchronize with a phase difference of less than 180 degrees; “quenching”, when the two oscillators suppress each other; “beating death”, in which one oscillator is suppressed but remains oscillatory owing to the influence of the other; and amplitude modulation, a consequence of mutual influence (Ramirez et al., [2014](#)). It will be exciting to study whether and how the broad theory behind Huygens synchronization, often investigating limit behavior of complex non-linear ordinary differential equation systems, can be applied to neural circuits. For instance, “modulating” behavior can theoretically be related to forms of cross-frequency coupling (Buzsáki & Wang, [2012](#); Lisman & Jensen, [2013](#)), whereas quenching and beating death can be viewed as synchronization failures, which may be relevant to oscillopathies, e.g. gamma oscillation failures in patients with schizophrenia (Hunt et al., [2017](#))(See chapter “*Oscillopathies: a rationale behind this study*”). Theoretical studies may explain how and why the output oscillation period may differ from the eigenperiods of the coupled individual oscillators (Dilão, [2009](#); Ramirez et al., [2016](#)), also observed in the context of neuronal oscillations, possibly serving as a basis for phase precession (Geisler et al., [2007](#); O’Keefe & Recce, [1993](#); Zutshi et al., [2018](#)).

PV+ pacemaker cells

The expression of PV and HCN, the channel protein that underlies the H-current, has been associated with rhythmic pacemaking properties of MS GABAergic neurons (Borhegyi et al., [2004](#); Kocsis & Li, [2004](#); Varga et al., [2008](#); Xu et al., [2004](#)). However, other studies suggested that rhythmic MS glutamatergic or cholinergic neurons may act as pacemakers (Manseau et al., [2005](#); Mysin et al., [2015](#); Smythe et al., [1992](#); Ujfalussy & Kiss, [2006](#)), but see (Vandecasteele et al., [2014](#)). Here, we confirmed

by optogenetic identification of multiple MS cell types that PV-expressing GABAergic neurons probably constitute most of the constitutive theta-rhythmic group. At the same time, we found that GABAergic neurons as a population contained only few theta-rhythmic neurons, suggesting that their subset that were rhythmic may have been PV expressing. We confirmed that septal pacemakers formed two groups with opposing phase preference with respect to the hippocampal theta oscillation in urethane-anesthetized rats, as reported before (Borhegyi et al., [2004](#); Joshi et al., [2017](#); Varga et al., [2008](#)).

Glutamatergic activation evokes theta

We found a substantial increase in the firing rates in MS pacemaker neurons upon theta onset. This finding is in line with previous studies suggesting that an increased tonic excitatory drive can switch the MS network into theta state (Korvasová et al., [2021](#); Lu et al., [2011](#); Müller & Remy, [2018](#); Oddie et al., [1996](#); Ujfalussy & Kiss, [2006](#)). Multiple cell types and pathways were proposed to contribute to this excitatory drive, including MS cholinergic, glutamatergic, and ascending brainstem inputs (Hajszan et al., [2004](#); Leranth & Frotscher, [1989](#); Müller & Remy, [2018](#); Oddie & Bland, [1998](#); Zaborszky et al., [2012](#)). We found that MS glutamatergic neurons strongly and non-rhythmically increased their firing rates at the onset of CA1 theta, suggesting they may be the dominant relay that conveys changing levels of tonic excitation to the MS pacemaker network, in agreement with anatomical findings showing that local excitatory neurons provide the majority of excitatory inputs to MS GABAergic neurons (Hajszan et al., [2004](#)).

In line with this, the optogenetic activation of MS glutamatergic neurons induced theta oscillation in the CA1. Importantly, we evoked theta rhythm by using a stimulation frequency of 20 Hz well above the theta range, demonstrating that VGLUT2-expressing MS neurons are capable of theta induction not only when stimulated at theta-range frequencies (Robinson et al., [2016](#)). These results are also consistent with the growing body of evidence showing that these neurons are involved in conveying locomotion-related activation to other cell types of the MS and beyond (Fuhrmann et al., [2015](#); Justus et al., [2017](#); Korvasová et al., [2021](#); Oddie et al., [1996](#); Teitelbaum et al., [1975](#)).

Pacemaker network model of theta generation

Previous models of the septal pacemaker network either explored GABAergic (Denham & Borisyuk, [2000](#); Ujfalussy & Kiss, [2006](#); Wang, [2002](#)) or glutamatergic (Ujfalussy & Kiss, [2006](#)) pacing mechanisms. These previous works already contained indirect reference to potential Huygens synchronization mechanisms. For instance, Wang suggested that desynchronized septal pacemaker units may oscillate at different frequencies (see Figure 4 in (Wang, [2002](#))). Additionally, Ujfalussy et al. wrote that “blocking the GABAergic synapses in our ping-pong model causes desynchronization of

these neurons but the cells remain theta periodic.” However, Huygens synchronization has neither been experimentally tested nor directly modelled, and previous models mostly focused on exploring the ways different oscillator networks may interplay, such as two GABAergic MS groups with opposing firing phase (Ujfalussy & Kiss, [2006](#)), MS and CA1 GABAergic groups (Denham & Borisyuk, [2000](#); Wang, [2002](#); Zou et al., [2011](#)) or MS GABAergic and glutamatergic neurons (Mysin et al., [2015](#)). Thus, in previous ping pong models, the synchronization mechanism required the presence of at least two dedicated inhibitory groups, with biased, non-random connectivity between the groups. In contrast, we found that even a minimalistic, homogeneous network of MS GABAergic pacemakers was sufficient to generate coherent theta-rhythmic output upon increased tonic excitation. While desynchronization of the MS network was rarely addressed in previous theoretical work, we also found that our MS model network showed rapid spontaneous desynchronization when tonic excitation was decreasing. This MS model network could recapitulate multiple features of the in vivo synchronization process, including Huygens synchronization, increased firing rate, faster rhythmic firing, decreased theta skipping and increase in intra-burst firing frequency.

Future perspectives

One of the most intriguing open questions concerns the composition of rhythmicity groups: are we dealing with genetically distinct cell types, cells with different network connectivity, or state-dependent group transitions between groups?

For example, examination of the computer model revealed that, in certain cases, cells exhibiting follower-like behavior emerged, even though all elements of the network originated from the same pacemaker cell model. It is therefore plausible that the 'follower' property is also a function of a cell's embeddedness within the network, which may depend on either connectivity or state.

To investigate state dependence, chronic rodent experiments would be necessary: a series of recordings spanning sleep and wakefulness (active and inactive), and preferably including a final urethane anesthesia condition, would enable tracking of rhythmicity group stability across different experimental states. However, it should be noted that, even with multi-channel recording, it is unlikely that all rhythmicity groups can be captured at a single recording depth.

From this perspective, the most valuable data would likely come from optotagging experiments. Recordings in rats are more likely to capture the full range of rhythmicity, including delta rhythmic activity. Additionally, identifying cholinergic cells would be particularly important.

Following the increase in recorded cell counts, analysis of firing metrics and waveforms of the identified rhythmic and optogenetically defined groups could provide further insight. Identification of

hippocampal state-dependent groupings within the septal rhythmic populations based on alternative parameters would obviate the need for concurrent hippocampal LFP recordings. Establishing such a database would not only simplify the process and solve the problem of sometimes noisy hippocampal signals, but would also be advantageous due to the reduced invasiveness and lower disruption to the neural network.

However, as some firing metrics are state-dependent, it would be difficult to control for variability between experiments, for example that arising from different levels of anesthesia. If only septal recording were available, without knowledge of the dominant hippocampal activity and relative proportions of theta and non-theta (e.g. whether theta was successfully evoked by tail pinch), then one could only rely on the average septal population activity. In other words, a general classification scheme could not be proposed; instead, it would need to be derived separately for each recording or experimental session, which would in turn require a large number of simultaneously recorded cells.

In contrast, waveform-based clustering is less state-dependent. Furthermore, the existence of such clustering patterns may reflect differences in intrinsic properties, such as ion channel composition.

An exciting future research direction is the patch-seq approach, which combines electrophysiology, morphology and transcriptomics, although at present it is only limitedly applicable to large scale recordings.

My research does not include inhibition-controlled experiments. Optogenetic inhibition can significantly reduce hippocampal theta activity, making it less suitable for observing finer-scale, Huygens-type synchronization phenomena. It may therefore be preferable to use specific excitatory stimuli to demonstrate stronger causal relationships. For instance, in my co-authored paper, we describe 'whole-cell patch clamp' and acute optogenetic experiments conducted on awake mice (Király et al., [2023](#)). These experiments demonstrate that hippocampal PV cells consistently respond to theta-modulated stimulation in the 22–80 Hz range. Future experiments of this type could provide more direct evidence for the mechanism described here.

An interesting direction for future research is the clarification of the role of glutamatergic cells in theta generation. For instance, it would be valuable to investigate whether theta oscillations generated by stimulation of septal glutamatergic cells differ in frequency, amplitude, or spatial characteristics from spontaneously occurring theta, or from theta evoked by nociceptive stimulation.

I have presented a minimal network model capable of generating theta waves through a frequency synchronization mechanism, consistent with in vivo findings. However, the biological system is likely to be considerably more complex, and specific aspects will therefore require more detailed modeling of the underlying phenomena. Future modelling directions could be the investigation of tSCs (theta

spectral components), the incorporation of follower and theta-follower cell types, the inclusion of septal non-rhythmic but hippocampal theta phase linked cells as inputs. Further extensions could also examine the effects of hippocampal feedback and hippocampal phase precession.

Hippocampal theta activity is closely linked to certain behaviors, such as exploration and memory encoding. Although I analyzed sleep and wakefulness recordings, I did not have data derived from specific behavioral tasks. While such experiments could provide valuable insights, they are technically challenging and time-consuming to conduct. From a methodological perspective, two-electrode recordings, potentially combined with optogenetics, are difficult to implement reliably, even when the target areas are successfully accessed. Moreover, variability introduced by behavioral factors can further complicate the interpretation of data. It is also worth noting that, during behavior, analysis would primarily capture urethane-sensitive type 1 theta, which is associated with higher-frequency components. Nevertheless, conducting a medial septum-dependent working memory study would be highly valuable, ideally one that is dissociated from the navigational component (e.g. an adaptive associative learning paradigm).

I propose that the findings presented in this dissertation are at least indirectly relevant to the human context. Pathological animal models could be employed to investigate alterations in the composition, frequency and synchronization mechanisms of the cell groups identified here, both in chronic conditions and in relation to abnormal activity (e.g. seizures). Subsequently, human electrophysiological data could be analyzed, either from tissue sections (in vitro) or from recordings obtained during surgery (in vivo). Data from both healthy and pathological tissue may be informative. Non-invasive human recordings may also be of interest; however, their spatial resolution is considerably lower than that of invasive methods. If certain lesion patterns could be identified, they might contribute to the currently limited toolkit available for the early diagnosis of conditions such as Alzheimer's disease.

Even more compelling results have emerged from mouse studies in recent years, as discussed in the "Oscillopathies" chapter of the dissertation. For instance, Etter et al. showed how, in a J20-APP AD model, supra-theta stimulation of PV+ cells in the medial septum can restore slow gamma amplitude and theta phase coupling in the hippocampus (Etter et al., [2019](#)). Notably, this intervention also rescued impaired memory traces despite substantial amyloid plaque accumulation.

The medial septum also appears to be a promising target for antiepileptic (Takeuchi et al., [2021](#)). There is evidence that certain epileptic seizures are preceded by alterations in hippocampal theta activity, and that induction of theta can exert antiepileptic effects. In optogenetic animal models of pathology, the effects of glutamatergic stimulation could be investigated in relation to seizure prevention,

attenuation and postictal consequences such as behavioral hyperactivity. It is important to note, however, that such deep brain regions are difficult to access in humans, and that optogenetic techniques are currently limited to animal models. Nevertheless, sonogenetics may offer considerable therapeutic potential for neurodegenerative disorders (Fan et al., [2021](#)).

Furthermore, another intriguing idea is the interpretation of seizure-related synchronization mechanisms in the context of Huygens synchronization. For instance, the cessation of septo-hippocampal theta generation around seizures could be analogous to the 'quenching' ('amplitude death') phenomenon observed in physical systems, where the interaction of oscillators causes oscillations to cease. At the same time, the dynamics of intermittent ('modulatory') and partial synchronizations can provide a framework for describing state-dependent and unstable dynamics. Within this framework, parameters such as coupling strength, damping factor and cross-sectional parameters of the suspensions mentioned in the "The Sympathy of Clocks" section may be mapped onto biological features, including connection strength, excitatory/inhibitory balance, and the level of tonic input strength.

6 Publications

1. **Barnabás Kocsis**, Sergio Martínez-Bellver, Richárd Fiáth, Andor Domonkos, Katalin Sviatkó, Dániel Schlingloff, Péter Barthó, Tamás F. Freund, István Ulbert, Szabolcs Káli, Viktor Varga, Balázs Hangya (2022). Huygens synchronization of medial septal pacemaker neurons generates hippocampal theta oscillation. *Cell Reports*, Volume 40, Issue 5, 2022, 111149, ISSN 2211-1247, <https://doi.org/10.1016/j.celrep.2022.111149>.
2. Bálint Király, Andor Domonkos, Márta Jelítai, Vítor Lopes-dos-Santos, Sergio Martínez-Bellver, **Barnabás Kocsis**, Dániel Schlingloff, Abhilasha Joshi, Minas Salib, Richárd Fiáth, Péter Barthó, István Ulbert, Tamás F. Freund, Tim J. Viney, David Dupret, Viktor Varga & Balázs Hangya (2023). The medial septum controls hippocampal supra-theta oscillations. *Nature Communications*, ISSN 2041-1723, <https://doi.org/10.1038/s41467-023-41746-0>.

7 References

1. Apartis, E., Poindessous-Jazat, F. R., Lamour, Y. A., & Bassant, M. H. (1998). Loss of rhythmically bursting neurons in rat medial septum following selective lesion of septohippocampal cholinergic system. *Journal of Neurophysiology*, 79(4), 1633–1642. <https://doi.org/10.1152/jn.1998.79.4.1633>
2. Bakker, R., Tiesinga, P., & Kötter, R. (2015). The Scalable Brain Atlas: Instant Web-Based Access to Public Brain Atlases and Related Content. *Neuroinformatics*, 13, 353–366.
3. Borhegyi, Z., Varga, V., Szilagyi, N., Fabo, D., & Freund, T. F. (2004). Phase segregation of medial septal GABAergic neurons during hippocampal theta activity. *The Journal of Neuroscience*, 24(39), 8470–8479.
4. Brandon, M. P., Bogaard, A. R., Schultheiss, N. W., & Hasselmo, M. E. (2013). Segregation of cortical head direction cell assemblies on alternating theta cycles. *Nature Neuroscience*, 16(6), 739–748. <https://doi.org/10.1038/nn.3383>
5. Brazhnik, E. S., & Fox, S. E. (1997). Intracellular recordings from medial septal neurons during hippocampal theta rhythm. *Experimental Brain Research*, 114(3), 442–453. <https://doi.org/10.1007/PL00005653>
6. Buzsáki, G. (2002). Theta oscillations in the hippocampus. *Neuron*, 33(3), 325–340. [https://doi.org/10.1016/s0896-6273\(02\)00586-x](https://doi.org/10.1016/s0896-6273(02)00586-x)
7. Buzsáki, G. (2006). *Rhythms of the Brain*. Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780195301069.001.0001>
8. Buzsáki, G., Czopf, J., Kondakor, I., & Kellenyi, L. (1986). Laminar distribution of hippocampal rhythmic slow activity (RSA) in the behaving rat: Current-source density analysis, effects of urethane and atropine. *Brain Research*, 365(1), 125–137.
9. Buzsáki, G., & Moser, E. I. (2013). Memory, navigation and theta rhythm in the hippocampal-entorhinal system. *Nature Neuroscience*, 16(2), 130–138. <https://doi.org/10.1038/nn.3304>
10. Buzsáki, G., & Wang, X.-J. (2012). Mechanisms of gamma oscillations. *Annual Review of Neuroscience*, 35, 203–225. <https://doi.org/10.1146/annurev-neuro-062111-150444>
11. Cutsuridis, V., & Poirazi, P. (2015). A computational study on how theta modulated inhibition can account for the long temporal windows in the entorhinal-hippocampal loop. *Neurobiol. Learn. Mem.*, 120, 69–83.
12. Czolczynski, K., Perlikowski, P., Stefanski, A., & Kapitaniak, T. (2011). Huygens' odd sympathy experiment revisited. *International Journal of Bifurcation and Chaos*, 21(7), 2047–2056. <https://doi.org/10.1142/S0218127411029628>
13. Dannenberg, H., Pabst, M., Braganza, O., Schoch, S., Niediek, J., Bayraktar, M., Mormann, F., & Beck, H. (2015). Synergy of direct and indirect cholinergic septo-hippocampal pathways coordinates firing in hippocampal networks. *Journal of Neuroscience*, 35(22), 8394–8410. <https://doi.org/10.1523/JNEUROSCI.4460-14.2015>
14. Denham, M. J., & Borisyuk, R. M. (2000). A model of theta rhythm production in the septal-hippocampal system and its modulation by ascending brain stem pathways. *Hippocampus*, 10(6), 698–716. [https://doi.org/10.1002/1098-1063\(2000\)10:6<698::AID-HIPO1008>3.0.CO;2-Z](https://doi.org/10.1002/1098-1063(2000)10:6<698::AID-HIPO1008>3.0.CO;2-Z)

15. Destrède, C., & Ott, T. (1982). Is a retrosplenial (cingulate) pathway involved in the mediation of high frequency hippocampal rhythmic slow activity (theta)? *Brain Research*, 252(1), 29–37. [https://doi.org/10.1016/0006-8993\(82\)90975-1](https://doi.org/10.1016/0006-8993(82)90975-1)
16. Dillão, R. (2009). Antiphase and in-phase synchronization of nonlinear oscillators: The Huygens's clocks system. *Chaos*, 19(2). <https://doi.org/10.1063/1.3139117>
17. Duque, A., Balatoni, B., Detari, L., & Zaborszky, L. (2000). EEG correlation of the discharge properties of identified neurons in the basal forebrain. *Journal of Neurophysiology*, 84(3), 1627–1635.
18. Equihua, G. G. V., & Ramirez, J. P. (2018). Synchronization of Hindmarsh-Rose Synchronization of Hindmarsh-Rose Synchronization of Hindmarsh-Rose neurons Huygens-like coupling Synchronization of Hindmarsh-Rose neurons Huygens-like coupling Huygens-like coupling. *IFAC-PapersOnLine*, 51(33), 186–191. <https://doi.org/10.1016/j.ifacol.2018.12.115>
19. Etter, G., van der Veldt, S., Manseau, F., Zarrinkoub, I., Trillaud-Doppia, E., & Williams, S. (2019). Optogenetic gamma stimulation rescues memory impairments in an Alzheimer's disease mouse model. *Nature Communications*, 10(1). <https://doi.org/10.1038/s41467-019-13260-9>
20. Fan, C. H., Wei, K. C., Chiu, N. H., Liao, E. C., Wang, H. C., Wu, R. Y., Ho, Y. J., Chan, H. L., Wang, T. S. A., Huang, Y. Z., Hsieh, T. H., Lin, C. H., Lin, Y. C., & Yeh, C. K. (2021). Sonogenetic-Based Neuromodulation for the Amelioration of Parkinson's Disease. *Nano Letters*, 21(14), 5967–5976. <https://doi.org/10.1021/acs.nanolett.1c00886>
21. Ford, R. D., Colom, L. V., & Bland, B. H. (1989). The classification of medial septum-diagonal band cells as σ -on or σ -off in relation to hippocampal EEG states. *Brain Research*, 493(2), 269–282. [https://doi.org/10.1016/0006-8993\(89\)91162-1](https://doi.org/10.1016/0006-8993(89)91162-1)
22. Freund, T. F., & Antal, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature*, 336(6195), 170–173.
23. Fuhrmann, F., Justus, D., Sosulina, L., Kaneko, H., Beutel, T., Friedrichs, D., Schoch, S., Schwarz, M. K., Fuhrmann, M., & Remy, S. (2015). Locomotion, Theta Oscillations, and the Speed-Related Firing of Hippocampal Neurons Are Controlled by a Medial Septal Glutamatergic Circuit. *Neuron*, 86(5), 1253–1264. <https://doi.org/10.1016/j.neuron.2015.05.001>
24. Geisler, C., Robbe, D., Zugaro, M., Sirota, A., & Buzsáki, G. (2007). Hippocampal place cell assemblies are speed-controlled oscillators. *Proceedings of the National Academy of Sciences of the United States of America*, 104(19), 8149–8154. <https://doi.org/10.1073/pnas.0610121104>
25. Golomb, D., Donner, K., Shacham, L., Shlosberg, D., Amitai, Y., & Hansel, D. (2007). Mechanisms of Firing Patterns in Fast-Spiking Cortical Interneurons. *PLoS Computational Biology*, 3(8), e156. <https://doi.org/10.1371/journal.pcbi.0030156>
26. Green, J. D., & Arduini, A. A. (1954). Hippocampal electrical activity in arousal. *J. Neurophysiol*, 17, 533–557.
27. Hajszan, T., Alreja, M., & Leranth, C. (2004). Intrinsic vesicular glutamate transporter 2-immunoreactive input to septohippocampal parvalbumin-containing neurons: Novel glutamatergic local circuit cells. *Hippocampus*, 14, 499–509.
28. Hangya, B., Borhegyi, Z., Szilagy, N., Freund, T. F., & Varga, V. (2009). GABAergic Neurons of the Medial Septum Lead the Hippocampal Network during Theta Activity. *Journal of Neuroscience*, 29(25), 8094–8102. <https://doi.org/10.1523/JNEUROSCI.5665-08.2009>

29. Hassani, O. K., Lee, M. G., Henny, P., & Jones, B. E. (2009). Discharge profiles of identified GABAergic in comparison to cholinergic and putative glutamatergic basal forebrain neurons across the sleep-wake cycle. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 29(38), 11828–11840. <https://doi.org/10.1523/JNEUROSCI.1259-09.2009>
30. Hasselmo, M. E. (2014). Neuronal rebound spiking, resonance frequency and theta cycle skipping may contribute to grid cell firing in medial entorhinal cortex. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1635), 20120523. <https://doi.org/10.1098/rstb.2012.0523>
31. Hunt, M. J., Kopell, N. J., Traub, R. D., & Whittington, M. A. (2017). Aberrant Network Activity in Schizophrenia. *Trends in Neurosciences*, 40(6), 371–382. <https://doi.org/10.1016/j.tins.2017.04.003>
32. Huygens, C. (1673). *Horologium Oscillatorium: Sive de motu pendulorum ad horologia aptato demonstrationes geometricae*. F. Muguet.
33. Jeffery, K. J., Donnett, J. G., & O’Keefe, J. (1995). Medial septal control of theta-correlated unit firing in the entorhinal cortex of awake rats. *NeuroReport*, 6(16), 2166–2170. <https://doi.org/10.1097/00001756-199511000-00017>
34. Joshi, A., Salib, M., Viney, T. J., Dupret, D., & Somogyi, P. (2017). Behavior-Dependent Activity and Synaptic Organization of Septo-hippocampal GABAergic Neurons Selectively Targeting the Hippocampal CA3 Area. *Neuron*, 96(6), 1342-1357.e5. <https://doi.org/10.1016/j.neuron.2017.10.033>
35. Justus, D., Dalügge, D., Bothe, S., Fuhrmann, F., Hannes, C., Kaneko, H., Friedrichs, D., Sosulina, L., Schwarz, I., Elliott, D. A., Schoch, S., Bradke, F., Schwarz, M. K., & Remy, S. (2017). Glutamatergic synaptic integration of locomotion speed via septoentorhinal projections. *Nature Neuroscience*, 20(1), 16–19. <https://doi.org/10.1038/nn.4447>
36. Kahana, M. J., Sekuler, R., Caplan, J. B., Kirschen, M., & Madsen, J. R. (1999). Human theta oscillations exhibit task dependence during virtual maze navigation. *Nature*, 399, 781–784.
37. Káli, S., & Zemankovics, R. (2012). The effect of dendritic voltage-gated conductances on the neuronal impedance: A quantitative model. *Journal of Computational Neuroscience*, 33(2), 257–284. <https://doi.org/10.1007/s10827-012-0385-9>
38. Kay, K., Chung, J. E., Sosa, M., Schor, J. S., Karlsson, M. P., Larkin, M. C., Liu, D. F., & Frank, L. M. (2020). Constant Sub-second Cycling between Representations of Possible Futures in the Hippocampus. *Cell*, 180(3), 552-567.e25. <https://doi.org/10.1016/j.cell.2020.01.014>
39. King, C., Recce, M., & O’Keefe, J. (1998). The rhythmicity of cells of the medial septum/diagonal band of Broca in the awake freely moving rat: Relationships with behaviour and hippocampal theta. *The European Journal of Neuroscience*, 10(2), 464–477.
40. 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, T. F., Viney, T. J., Dupret, D., Varga, V., & Hangya, B. (2023). The medial septum controls hippocampal supra-theta oscillations. *Nature Communications*, 14(1). <https://doi.org/10.1038/s41467-023-41746-0>
41. Klausberger, T., & Somogyi, P. (2008). Neuronal diversity and temporal dynamics: The unity of hippocampal circuit operations. *Science*, 321(5885), 53–57. <https://doi.org/10.1126/science.1149381>

42. Kocsis, B., & Li, S. (2004). In vivo contribution of h-channels in the septal pacemaker to theta rhythm generation. *European Journal of Neuroscience*, 20(8), 2149–2158. <https://doi.org/10.1111/j.1460-9568.2004.03678.x>
43. Kocsis, B., Martínez-Bellver, S., Fiáth, R., Domonkos, A., Sviatkó, K., Schlingloff, D., Barthó, P., Freund, T. F., Ulbert, I., Káli, S., Varga, V., & Hangya, B. (2022). Huygens synchronization of medial septal pacemaker neurons generates hippocampal theta oscillation. *Cell Reports*, 40(5). <https://doi.org/10.1016/j.celrep.2022.111149>
44. Korteweg, D. (1906). Les horloges sympathiques de Huygens. *Archives Neerlandaises Des Sciences Exactes et Naturelles*, 11, 273–296.
45. Korvasová, K., Ludwig, F., Kaneko, H., Sosulina, L., Tetzlaff, T., Remy, S., & Mikulovic, S. (2021). Locomotion induced by medial septal glutamatergic neurons is linked to intrinsically generated persistent ring. *bioRxiv*, 2021.04.23.441122.
46. Kramis, R., Vanderwolf, C. H., & Bland, B. H. (1975). Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: Relations to behavior and effects of atropine, diethyl ether, urethane, and pentobarbital. *Experimental Neurology*, 49(1), 58–85. [https://doi.org/10.1016/0014-4886\(75\)90195-8](https://doi.org/10.1016/0014-4886(75)90195-8)
47. Kvitsiani, D., Ranade, S., Hangya, B., Taniguchi, H., Huang, J. Z., & Kepecs, A. (2013). Distinct behavioural and network correlates of two interneuron types in prefrontal cortex. *Nature*, 498(7454), 363–366. <https://doi.org/10.1038/nature12176>
48. Laszlovszky, T., Schlingloff, D., Hegedüs, P., Freund, T. F., Gulyás, A., Kepecs, A., & Hangya, B. (2020). Distinct synchronization, cortical coupling and behavioral function of two basal forebrain cholinergic neuron types. *Nature Neuroscience*, 23(8), 992–1003. <https://doi.org/10.1038/s41593-020-0648-0>
49. Lee, M. G., Chrobak, J. J., Sik, A., Wiley, R. G., & Buzsáki, G. (1994). Hippocampal theta activity following selective lesion of the septal cholinergic system. *Neuroscience*, 62(4), 1033–1047. [https://doi.org/10.1016/0306-4522\(94\)90341-7](https://doi.org/10.1016/0306-4522(94)90341-7)
50. Lee, M. G., Hassani, O. K., Alonso, A., & Jones, B. E. (2005). Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 25(17), 4365–4369. <https://doi.org/10.1523/JNEUROSCI.0178-05.2005>
51. Leranth, C., & Frotscher, M. (1989). Organization of the septal region in the rat brain: Cholinergic-GABAergic interconnections and the termination of hippocampo-septal fibers. *The Journal of Comparative Neurology*, 289(2), 304–314. <https://doi.org/10.1002/cne.902890210>
52. Lima, S. Q., Hromádka, T., Znamenskiy, P., & Zador, A. M. (2009). PINP: a new method of tagging neuronal populations for identification during in vivo electrophysiological recording. *PLoS One*, 4, 6099.
53. Lisman, J. E., & Jensen, O. (2013). The Theta-Gamma Neural Code. *Neuron*, 77(6), 1002–1016. <https://doi.org/10.1016/j.neuron.2013.03.007>
54. Lu, C. B., Ouyang, G., Henderson, Z., & Li, X. (2011). Induction of theta-frequency oscillations in the rat medial septal diagonal band slice by metabotropic glutamate receptor agonists. *Neuroscience*, 177, 1–11. <https://doi.org/10.1016/j.neuroscience.2011.01.004>
55. Mamad, O., McNamara, H. M., Reilly, R. B., & Tsanov, M. (2015). Medial septum regulates the hippocampal spatial representation. *Frontiers in Behavioral Neuroscience*. <https://doi.org/10.3389/fnbeh.2015.00166>

56. Manseau, F., Danik, M., & Williams, S. (2005). A functional glutamatergic neurone network in the medial septum and diagonal band area. *Journal of Physiology*, 566(3), 865–884. <https://doi.org/10.1113/jphysiol.2005.089664>
57. Manseau, F., Goutagny, R., Danik, M., & Williams, S. (2008). The hippocamptoseptal pathway generates rhythmic firing of GABAergic neurons in the medial septum and diagonal bands: An investigation using a complete septohippocampal preparation in vitro. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 28(15), 4096–4107. <https://doi.org/10.1523/JNEUROSCI.0247-08.2008>
58. Mikulovic, S., Restrepo, C. E., Siwani, S., Bauer, P., Pupe, S., Tort, A. B. L. L., Kullander, K., & Leão, R. N. (2018). Ventral hippocampal OLM cells control type 2 theta oscillations and response to predator odor. *Nature Communications*, 9(1), 3638. <https://doi.org/10.1038/s41467-018-05907-w>
59. Moore, J. D., Deschênes, M., Furuta, T., Huber, D., Smear, M. C., Demers, M., & Kleinfeld, D. (2013). Hierarchy of orofacial rhythms revealed through whisking and breathing. *Nature*, 497(7448), 205–210. <https://doi.org/10.1038/nature12076>
60. Müller, C., & Remy, S. (2018). Septo–hippocampal interaction. *Cell and Tissue Research*, 373(3), 565–575. <https://doi.org/10.1007/s00441-017-2745-2>
61. Mysin, I. E., Kitchigina, V. F., & Kazanovich, Y. (2015). Modeling synchronous theta activity in the medial septum: Key role of local communications between different cell populations. *Journal of Computational Neuroscience*, 39(1), 1–16. <https://doi.org/10.1007/s10827-015-0564-6>
62. Oddie, S. D., & Bland, B. H. (1998). Hippocampal formation theta activity and movement selection. *Neuroscience and Biobehavioral Reviews*, 22(2), 221–231. [https://doi.org/10.1016/S0149-7634\(97\)00003-1](https://doi.org/10.1016/S0149-7634(97)00003-1)
63. Oddie, S. D., Stefanek, W., Kirk, I. J., & Bland, B. H. (1996). Intraseptal procaine abolishes hypothalamic stimulation-induced wheel-running and hippocampal theta field activity in rats. *Journal of Neuroscience*, 16(5), 1948–1956. <https://doi.org/10.1523/jneurosci.16-05-01948.1996>
64. O’Keefe, J., & Recce, M. L. (1993). Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus*. <https://doi.org/10.1002/hipo.450030307>
65. Oliveira, H. M., & Melo, L. V. (2015). Huygens synchronization of two clocks. *Scientific Reports*, 5, 1–12. <https://doi.org/10.1038/srep11548>
66. Paxinos, G., Franklin, K. B. J., & Franklin, K. B. J. (2001). *The mouse brain in stereotaxic coordinates*. Academic Press.
67. Petsche, H., Stumpf, C., & Gogolak, G. (1962). The significance of the rabbit’s septum as a relay station between the midbrain and the hippocampus I. The control of hippocampus arousal activity by the septum cells. *Electroencephalography and Clinical Neurophysiology*, 14(2), 202–211. [https://doi.org/10.1016/0013-4694\(62\)90030-5](https://doi.org/10.1016/0013-4694(62)90030-5)
68. Ramirez, J. P., Fey, R. H. B., Aihara, K., & Nijmeijer, H. (2014). An improved model for the classical Huygens’ experiment on synchronization of pendulum clocks. *Journal of Sound and Vibration*, 333(26), 7248–7266. <https://doi.org/10.1016/j.jsv.2014.08.030>
69. Ramirez, J. P., Olvera, L. A., Nijmeijer, H., & Alvarez, J. (2016). The sympathy of two pendulum clocks: Beyond Huygens’ observations. *Scientific Reports*, 6(March), 1–16. <https://doi.org/10.1038/srep23580>

70. Ranade, S., Hangya, B., & Kepecs, A. (2013). Multiple modes of phase locking between sniffing and whisking during active exploration. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 33(19), 8250–8256. <https://doi.org/10.1523/JNEUROSCI.3874-12.2013>
71. Robinson, J., Manseau, F., Ducharme, G., Amilhon, B., Vigneault, E., El Mestikawy, S., & Williams, S. (2016). Optogenetic activation of septal glutamatergic neurons drive hippocampal theta rhythms. *Journal of Neuroscience*, 36(10), 3016–3023. <https://doi.org/10.1523/JNEUROSCI.2141-15.2016>
72. Rutishauser, U., Ross, I. B., Mamelak, A. N., & Schuman, E. M. (2010). Human memory strength is predicted by theta-frequency phase-locking of single neurons. *Nature*, 464(7290), 903–907. <https://doi.org/10.1038/nature08860>
73. Semba, K., & Komisaruk, B. R. (1984). Neural substrates of two different rhythmical vibrissal movements in the rat. *Neuroscience*, 12(3), 761–774. [https://doi.org/10.1016/0306-4522\(84\)90168-4](https://doi.org/10.1016/0306-4522(84)90168-4)
74. Simon, A. P., Poindessous-Jazat, F., Dutar, P., Epelbaum, J., & Bassant, M. H. (2006). Firing properties of anatomically identified neurons in the medial septum of anesthetized and unanesthetized restrained rats. *The Journal of Neuroscience*, 26(35), 9038–9046.
75. Smythe, J. W., Colom, L. V., & Bland, B. H. (1992). The extrinsic modulation of hippocampal theta depends on the coactivation of cholinergic and GABA-ergic medial septal inputs. *Neuroscience & Biobehavioral Reviews*, 16(3), 289–308. [https://doi.org/10.1016/S0149-7634\(05\)80203-9](https://doi.org/10.1016/S0149-7634(05)80203-9)
76. Sotty, F., Danik, M., Manseau, F., Laplante, F., Quirion, R., & Williams, S. (2003). Distinct electrophysiological properties of glutamatergic, cholinergic and GABAergic rat septohippocampal neurons: Novel implications for hippocampal rhythmicity. *Journal of Physiology*, 551(3), 927–943. <https://doi.org/10.1113/jphysiol.2003.046847>
77. Stewart, M., & Fox, S. E. (1989). Two populations of rhythmically bursting neurons in rat medial septum are revealed by atropine. *Journal of Neurophysiology*, 61(5). <https://doi.org/10.1152/jn.1989.61.5.982>
78. Takeuchi, Y., Nagy, A. J., Barcsai, L., Li, Q., Ohsawa, M., Mizuseki, K., & Berényi, A. (2021). The Medial Septum as a Potential Target for Treating Brain Disorders Associated With Oscillopathies. *Frontiers in Neural Circuits*, 15. <https://doi.org/10.3389/fncir.2021.701080>
79. Teitelbaum, H., Lee, J. F., & Johannessen, J. N. (1975). Behaviorally evoked hippocampal theta waves: A cholinergic response. *Science*, 188(4193), 1114–1116. <https://doi.org/10.1126/science.175440>
80. Tokuda, K., Katori, Y., & Aihara, K. (2019). Chaotic dynamics as a mechanism of rapid transition of hippocampal local field activity between theta and non-theta states. *Chaos*, 29, 113115.
81. Tsanov, M. (2015). Septo-hippocampal signal processing: Breaking the code. *Progress in Brain Research*, 219, 103–120. <https://doi.org/10.1016/bs.pbr.2015.04.002>
82. Tsanov, M., Chah, E., Reilly, R., & O'Mara, S. M. (2014). Respiratory cycle entrainment of septal neurons mediates the fast coupling of sniffing rate and hippocampal theta rhythm. *European Journal of Neuroscience*, 39(6), 957–974. <https://doi.org/10.1111/ejn.12449>
83. Ujfalussy, B., & Kiss, T. (2006). How do glutamatergic and GABAergic cells contribute to synchronization in the medial septum? *Journal of Computational Neuroscience*, 21(3), 343–357. <https://doi.org/10.1007/s10827-006-9082-x>

84. Vandecasteele, M., Varga, V., Berényi, A., Papp, E., Barthó, P., Venance, L., Freund, T. F., & Buzsáki, G. (2014). Optogenetic activation of septal cholinergic neurons suppresses sharp wave ripples and enhances theta oscillations in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, 111(37), 13535–13540. <https://doi.org/10.1073/pnas.1411233111>
85. Varga, V., Hangya, B., Kránitz, K., Ludányi, A., Zemankovics, R., Katona, I., Shigemoto, R., Freund, T. F. T. F., & Borhegyi, Z. (2008). The presence of pacemaker HCN channels identifies theta rhythmic GABAergic neurons in the medial septum. *Journal of Physiology*, 586(16), 3893–3915. <https://doi.org/10.1113/jphysiol.2008.155242>
86. Wang, X.-J. (2002). Pacemaker Neurons for the Theta Rhythm and Their Synchronization in the Septohippocampal Reciprocal Loop. *Journal of Neurophysiology*, 87(2), 889–900. <https://doi.org/10.1152/jn.00135.2001>
87. Willms, A. R., Kitanov, P. M., & Langford, W. F. (2017). Huygens' clocks revisited. *Royal Society Open Science*, 4(9), 170777. <https://doi.org/10.1098/rsos.170777>
88. Wolansky, T., Clement, E. A., Peters, S. R., Palczak, M. A., & Dickson, C. T. (2006). Hippocampal Slow Oscillation: A Novel EEG State and Its Coordination with Ongoing Neocortical Activity. *Journal of Neuroscience*, 26(23), 6213–6229. <https://doi.org/10.1523/JNEUROSCI.5594-05.2006>
89. Xu, C., Datta, S., Wu, M., & Alreja, M. (2004). Hippocampal theta rhythm is reduced by suppression of the H-current in septohippocampal GABAergic neurons. *European Journal of Neuroscience*, 19(8), 2299–2309. <https://doi.org/10.1111/j.0953-816X.2004.03316.x>
90. Yang, C., McKenna, J. T., Zant, J. C., Winston, S., Basheer, R., & Brown, R. E. (2014). Cholinergic neurons excite cortically projecting basal forebrain GABAergic neurons. *Journal of Neuroscience*, 34(8), 2832–2844. <https://doi.org/10.1523/JNEUROSCI.3235-13.2014>
91. Yang, C., Thankachan, S., McCarley, R. W., & Brown, R. E. (2017). The menagerie of the basal forebrain: How many (neural) species are there, what do they look like, how do they behave and who talks to whom? *Current Opinion in Neurobiology*, 44, 159–166. <https://doi.org/10.1016/j.conb.2017.05.004>
92. Yoder, R. M., & Pang, K. C. H. (2005). Involvement of GABAergic and cholinergic medial septal neurons in hippocampal theta rhythm. *Hippocampus*, 15, 381–392.
93. Zaborszky, L., van den Pol, A., & Gyengesi, E. (2012). The Basal Forebrain Cholinergic Projection System in Mice. In C. Watson, G. Paxinos, & L. Puellas (Eds.), *The Mouse Nervous System* (1st ed., pp. 684–718). Elsevier. <https://doi.org/10.1016/B978-0-12-369497-3.10028-7>
94. Zemankovics, R., Káli, S., Paulsen, O., Freund, T. F., & Hájos, N. (2010). Differences in subthreshold resonance of hippocampal pyramidal cells and interneurons: The role of h-current and passive membrane characteristics. *The Journal of Physiology*, 588(12), 2109–2132. <https://doi.org/10.1113/jphysiol.2009.185975>
95. Zou, X., Coyle, D., Wong-Lin, K. F., & Maguire, L. (2011). Computational study of Hippocampal-septal theta rhythm changes due to Beta-Amyloid-Altered ionic channels. *PLoS ONE*, 6(6). <https://doi.org/10.1371/journal.pone.0021579>
96. Zutshi, I., Brandon, M. P., Fu, M. L., Donegan, M. L., Leutgeb, J. K., & Leutgeb, S. (2018). Hippocampal Neural Circuits Respond to Optogenetic Pacing of Theta Frequencies by Generating Accelerated Oscillation Frequencies. *Current Biology*, 28(8), 1179–1188.e3. <https://doi.org/10.1016/j.cub.2018.02.061>