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UNIVERSITY OF CALIFORNIA SAN DIEGO

Effects of optogenetic stimulation of the medial septal area on hippocampal firing patterns in the
spatial alternation task

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Deborah Chen

Committee in charge:

Professor Stefan Leutgeb, Chair
Professor Marcus Benna
Professor Jill Leutgeb

2024

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University of California San Diego

2024

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LIST OF ABBREVIATIONS

WM	Working memory
HPC	Hippocampus
MSA	Medial septal area
CA	Cornu Ammonis
DG	Dentate gyrus
EC	Entorhinal cortex
PV+	Parvalbumin-positive
LFP	Local field potential
SWR	Sharp wave ripple
AP	Anterior posterior
DV	Dorsal ventral
ML	Medial lateral
AAV	Adeno-associated virus
ChR2	Channelrhodopsin-2
eYFP	Yellow fluorescent protein
eGFP	Green fluorescent protein
PFA	4% Paraformaldehyde
PBS	Phosphate-buffered saline

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ABSTRACT OF THE THESIS

Effects of optogenetic stimulation of the medial septal area on hippocampal firing patterns in
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by

Deborah Chen

Master of Science in Biology

University of California San Diego, 2024

Professor Stefan Leutgeb, Chair

The hippocampus (HPC) is a brain region known for playing a role in learning and memory. Theta oscillations (7-9 Hz) are found in the HPC and are believed to be linked to the sequential firing pattern of HPC place cells through phase precession. Studies have demonstrated that accelerated theta oscillation frequencies resulted in spatial working memory deficits, but the role that HPC theta oscillations play in memory encoding and retrieval is still unclear. Our study utilized an optogenetic technique that targeted GABAergic

PV cells in the mouse MSA, the pacemakers for HPC theta oscillation. We entrained the HPC theta oscillation frequency to both below and above the endogenous range. We then performed electrophysiological recordings from cells in the HPC CA1 layer while mice were completing a spatial alternation task with variable delays. We saw memory impairments in trials with lower frequencies (4 Hz) and long delays (10-s). Follow-up analyses can reveal how the relative timing between cells has changed.

CHAPTER 1: INTRODUCTION

Memory

Memory is one of the primary brain functions, used anywhere from knowing how to tie shoelaces to remembering complex concepts during an exam. Memory is broadly defined as the encoding of sensory information before storing or retrieving it in the future. Memory can first be broken down into working, short-term, and long-term, depending on the amount of time they're retained in the brain. Working memory allows information to be temporarily maintained when the attention is directly on the event, and that information can be used to form actions or plans (Baddeley, 2010). Short-term memory stores information for a limited amount of time, and the information is retained decays over time. Therefore, short-term memories can only be recalled temporarily. Long-term memories have both a larger capacity and storage time, where vast amounts of information are retained and can be recalled (Cowan, 2008). Memory can be further classified as declarative or non-declarative. Declarative memories are ones that people are conscious of, such as events that have occurred (episodic) and places one has visited (spatial). Non-declarative memories are unconscious and are often shown through skills or learned habits. Non-declarative memories are also called procedural memories (Squire & Zola, 1996). Given the importance of memory function, extensive research has gone into studying the circuits and regions underlying storing, retrieving, and processing different types of memories.

In one landmark study, patient H.M. who had epilepsy underwent a lobectomy that removed his hippocampus (HPC) and adjacent regions in the medial temporal lobe (MTL). Afterward, H.M. maintained his long-term memory and procedural memories but lost the ability to form new episodic memories (Scoville & Milner, 1957; Squire, 2009). This study indicates that regions in the MTL are critical for episodic memories. The study's observations were

confirmed by additional anatomical studies on monkeys, where memory is assessed with the removal of either the amygdala, HPC, or both structures. Severe deficits only occurred with lesions of both structures, which together with the patient H.M. study demonstrated that regions in the MTL are crucial for forming and retrieving memories (Mishkin, 1978). Specifically, for the HPC within MTL, there has been evidence that it is involved in spatial memory and learning. Rodents with HPC lesions showed impaired learning for tasks that required spatial information (Morris et al., 1982).

Rodents as a model organism

While cases such as patient H.M. have proved useful in studying brain regions involved in memory, human studies have many limitations. Recording neurons from outside the skull is difficult due to noise, and studies on patients can raise ethical concerns. Therefore, many memory studies have transitioned to rodent models. Structurally, rodents have similar structures and organizations in their hippocampus compared to humans (Bienkowski et al., 2021). Further, similar neuronal activities in both rodents and humans have also been observed (Axmacher et al., 2008).

Networks in the Hippocampus

The hippocampus serves as a central hub for declarative memories and cognitive processing, and the rodent HPC can be subdivided into the dentate gyrus (DG) and Cornu Ammonis fields (CA1, CA2, and CA3). The entorhinal cortex is adjacent to the HPC, receives inputs from the cortex, and sends projections to the DG (i.e., perforant path). Through the mossy fiber pathway, granule neurons in DG send information to the CA3 layer pyramidal neurons. Cells in the CA3 layer then project to CA1 pyramidal neurons via the Schaffer collateral pathway. These connections together form the tri-synaptic pathway. Finally, CA1 neurons export

information to the subiculum and loop back to the entorhinal cortex. Other than information flow to each other, regions such as the dentate gyrus and CA3 also send recurrent projections onto themselves (Deshmukh & Knierim, 2012). Pyramidal cells are the major cell class in the HPC. They send excitatory projections through high-frequency bursting firing (Hartley et al., 2014). GABAergic interneurons are also present in HPC and send local projections to regulate pyramidal cell firing (Klausberger & Somogyi, 2008).

Oscillations in the Hippocampus

Oscillations are electrical activities that occur with a certain repeating pattern. In the HPC, several distinct oscillations have been observed, which are sharp wave ripples (SWRs), gamma oscillations, and theta oscillations (Colgin, 2016). Each oscillation occurs at a unique range of frequencies. SWRs (150-200 Hz) are large local field potential (LFP) deflections coupled with bursts of high-frequency firing, while the animal is stationary or in periods of slow-wave sleep (Joo & Frank, 2018). SWR are mostly observed in the HPC and EC. SWRs were found to show a replay of previous experiences. The same firing sequences that occurred when a mouse was running on a wheel were observed again, at a faster timescale, when it is resting or in slow wave sleep (Nádasdy et al., 1999). Further, suppressing SWRs after rats have learned a memory task has resulted in worsened performance in the following days. The impaired learning compared to control rats indicates deficits in memory consolidation (Girardeau et al., 2009). SWRs have therefore been hypothesized to support memory consolidation by transferring memory information to long-term memory. Gamma oscillations (25-100 Hz) are present in the CA1 and CA3 layers of HPC and consist of three different bands (fast, mid frequency, and slow) (Belluscio et al., 2012). Gamma oscillations are hypothesized to help filter and process sensory

inputs (Colgin et al., 2009). Theta oscillations (4-12 Hz) are characterized by sinusoidal waves during locomotion as well as rapid eye movement (REM) sleep (Buzsáki et al., 1983).

Theta Oscillation Generation and Function

Theta oscillations are present in all subregions of the HPC and are generated by the medial septal area (MSA). Theta oscillations are prominent in the CA1 cell layer and the theta amplitude changes across the depth of the layer (Bullock et al., 1990). Early lesion studies that examined theta generation showed that rabbits with MSA lesions had no theta rhythm despite stimulations (Green & Arduini, 1954). Further, when MSA GABAergic neurons were eliminated, rats also showed near-complete loss of theta oscillation while running and during REM sleep (Gerashchenko et al., 2001). Subclasses of MSA neurons that either contain parvalbumin or express the HCN channel directly project to the HPC and express bursts of firing preceding theta oscillation observed in HPC. GABAergic neurons in the MSA therefore act as pacemakers for theta oscillations, responsible for rhythm generation (Buzsáki, 2002). To assess what effects MSA lesions have on memory performance, rats were first trained on a goal-learning task and then received MSA neuron lesions. Two lesion groups had either a reduced number of cholinergic or GABAergic neurons. Rats with GABAergic neuron lesions showed severely impaired learning of the goal location, while rats with cholinergic lesions maintained learning (Dwyer et al., 2007). Taken together, these studies indicate that MSA GABAergic neurons are critical inputs for generating theta oscillation and for theta's function in memory encoding.

Theta oscillations have been implicated in encoding new episodic memories when testing the formation of new associations between objects. In one study, rats were first trained on a T-maze task with food rewards presented only in the left arm before receiving a fornix lesion that

abolished their HPC theta rhythms. Lesioned and control rats were then faced with a new phase where rewards were only given in the right arm, which required reversing the previous association made between a left turn and food rewards. Rats were evaluated for their performance, which checks for their ability to encode a new association between food and location. Lesioned rats showed significant memory impairments and made more errors compared to control rats (Hasselmo et al., 2002).

Theta waves have been shown to be disrupted in neurodegenerative disorders, such as Alzheimer's disease (Ego-Stengel & Wilson, 2010). Therefore, understanding the mechanisms underlying theta waves and their relation to other brain regions could be critical in furthering the understanding of memory disorders.

Place cells

Place cells are cells characterized by the encoding of locations and are mainly located in the HPC and EC (O'Keefe & Dostrovsky, 1971). Place cells tend to fire at increased rates when the animal reaches a specific location in an environment. The location where each place cell fires preferentially at is called a place field (Wood et al., 2000).

The firing patterns of place cells are found to have a relation with theta oscillations. When the animal enters and passes through a specific place field, place cell spiking initially occurs at a late phase and then shifts towards progressively earlier phases of the theta wave (O'Keefe & Recce, 1993). This phenomenon is called phase precession, and it suggests that phase-related information may support place cell function. One study recorded rats running on a linear track and attempted to use cell firing rate and phase to reconstruct the rat's running path and location. They found that compared to only using rate-coded information, adding phase-coded information significantly improved the prediction accuracies. Thus, the association

between cell firing and the theta oscillation phase may code for information about the animal's position. However, it is not clear whether a functional theta wave is required to maintain the timing of cells' firing.

Manipulating theta oscillations

Earlier studies have found optogenetics to be an effective method to manipulate theta waves in HPC. In particular, optogenetically stimulating MSA PV neurons that express channel rhodopsin (CHR2) with blue light lasers has been shown to result in reliable control of the theta oscillation frequency in mice (Zutshi et al., 2018a). Incorporating the optogenetic technique, a previous study shifted theta oscillation frequency in mice to above and below the endogenous range and looked for changes in memory performance. It was found that reducing theta wave frequency resulted in deficits in spatial working memory, which suggests that theta waves are essential in a functioning spatial memory circuit (Quirk et al., 2021a). These findings make theta waves a potentially useful target for memory disorders, as theta waves may help with developing diagnoses or treatments. To verify and build upon previous findings, more work with manipulations of theta oscillations is needed.

To identify how theta waves support neural computations in the hippocampus, I will investigate the relationships between theta waves and place cell firing patterns. The mice will perform a memory task by running in a figure-8 maze. The maze task will include a range of delays that test the mice's HPC-dependent and independent memories. As altered theta oscillation frequencies are linked to memory deficits, we will manipulate the pacemaker cells in PV-Cre transgenic mice MSA using laser stimulation, thereby altering HPC theta waves' frequency. As the mice run with typical or altered theta oscillation frequency, HPC LFP signals will be recorded and signals will be further processed to identify specific cells during analysis.

Memory performance will also be recorded and analyzed to check for deficits or improvements in relation to the shifted theta frequencies. Combining the overall brainwave and single neuron recordings with the mice's performance in the maze, I can examine which changes in the neuron firing pattern are related to changes in memory performance. Therefore, I can then observe whether normal theta waves are necessary for HPC neurons to fire in meaningful or functional sequences. I expect that altering the theta frequency will make mice perform worse on the maze, as their HPC neurons will lose the coordination they had. By investigating this question, I hope to shed light on previously unknown mechanisms of how memories are formed and retrieved. An understanding of the role of theta waves in memory processing will hopefully lead to a more complete comprehension of memory disorders, such as Alzheimer's disease, and to new therapies that can restore oscillatory and sequential brain activity patterns, which are known to be disrupted in those diseases.

CHAPTER 2: METHODS

Approvals

All animal experiments were approved and supervised by the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego. All procedures were performed per the National Institutes of Health's (NIH) Guide for the Care and Use of Laboratory Animals.

Subjects

Genetically modified heterozygous PV-Cre mice were used for experiments. Training and recordings were done using 3-12 months old male and female mice. The mice were kept in the vivarium on a 12-hour reverse light-dark cycle, with the lights turned on at 9:00 pm and off at 9:00 am. During the light-off hours, red lights were available to use. Before any surgeries, mouse chew and water were provided *ad libitum*. The mice were group-housed until the first surgery (i.e., injection with viral vectors when they were at least 3 months old) and were then separated into individual cages. The first surgery was done before the start of the behavioral procedures.

Behavioral Procedures

Apparatus

The behavior task was performed on a figure-8 maze (Fig. 1). The maze consisted of one 72 cm long stem arm in the center and two 72 cm long return arms on the sides, connected by 50 cm long perpendicular arms on the top and bottom. The delay zone was located at the beginning of the stem arm, and the two reward zones at opposite corners. Training and recordings were done in the room while it was dimly lit, and the location of the light source was kept constant.

During training and recording days, the mice were food-deprived to 85% to 95% of their baseline weight while water was available *ad libitum*. Before each session, the mice were weighed to monitor their health and to ensure their weight was within range.

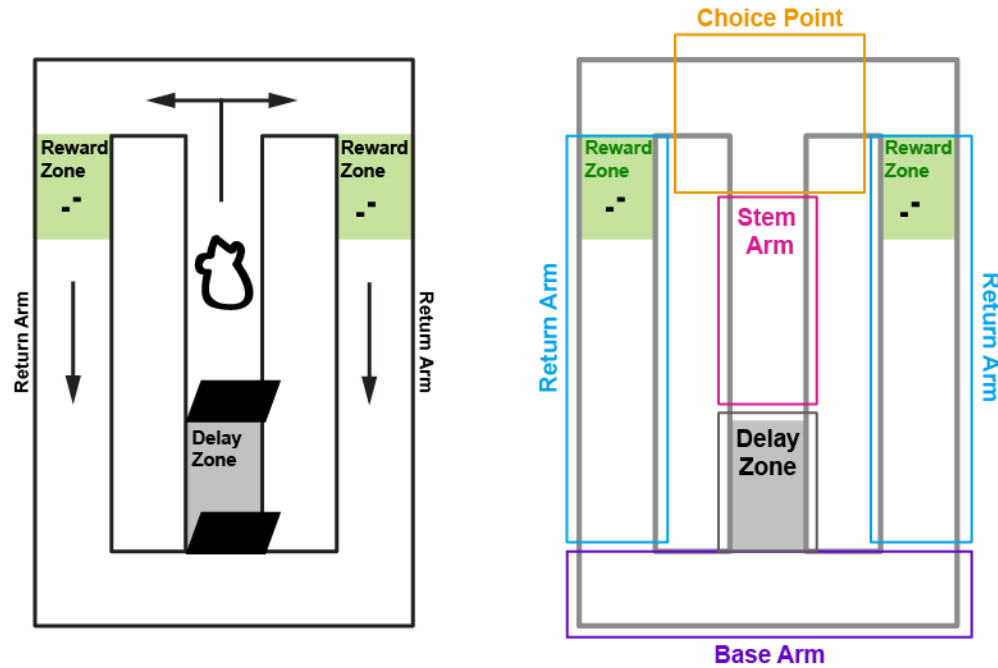


Figure 1 | Figure-8 maze for spatial working memory task.

The maze is divided into a base arm, the choice point, a stem arm, the delay zone, and two reward zones on the return arms. The path that mice were trained to run is marked by arrows.

Handling and Habituation

Before training for the maze task, mice were placed on the maze in the behavior room to familiarize them with the environment as well as with handling by researchers. Chocolate sprinkles would be placed in various locations on the maze to habituate the mouse to running and eating on the maze. When mice were eating all the sprinkles on the maze within 10 minutes, they proceeded to the next training stage.

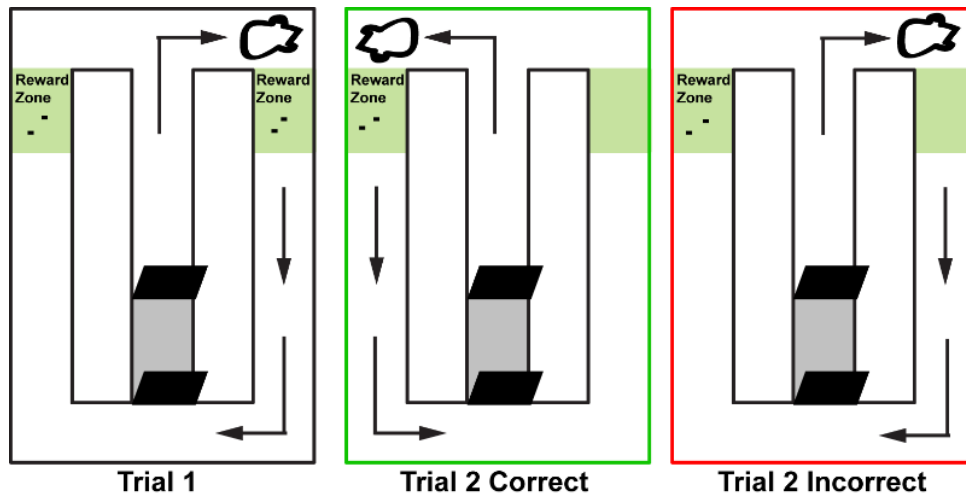


Figure 2 | Figure-8 delayed spatial alternation task.

Mice were trained on the spatial alternation task with delays of either 0, 2, or 10 seconds. On each trial / lap at the choice point, the mice can turn to either the same side or opposite side of the previous lap. Rewards are only given if the mice alternate between making left and right turns.

Forced Alternation Phase

Mice were guided by barriers to make alternate left and right turns for either 40 trials or 20 minutes. Barriers covered with black duct tape would block either the left or right choice arm to force a correct turn during each trial. Chocolate sprinkle rewards were given in the reward area of the return arms. When not in the reward area, mice were prevented from stopping for long periods of time or turning around by gently tapping them. The forced alternation phase was conducted for at least 3 consecutive days until mice were eating the rewards consistently.

Continuous Alternation Task and Retraining

In this task phase, the barriers blocking the incorrect choice arm were removed and mice were free to turn to whichever arm they chose. One recording day session lasted for 80 trials, and each trial was marked as correct or incorrect. The mice would only receive rewards if they made a correct turn. Training in the continuous alternation phase continued until the mice made at least 80% correct choices for 2 of 3 consecutive days. They were then housed until surgical procedures were performed. After drive implantation surgery and recovery from surgery, the mice were retrained to the same criterion as before surgery. Once they passed the criterion, the next training phase began on the following day.

Delayed Alternation Task

Delay trials were added to the task, such that the task now included trials with no delay, a 2-s delay, or a 10-s delay. Each session consisted of 60 trials, which were divided into 6 blocks of 10 trials with each of the three conditions repeated twice. The delay order of the first 3 blocks was repeated for the last 3 blocks. For each training session, the order of the 3 delays was pseudorandomized to ensure that all permutations were used and that the order of the last session was not repeated. The addition of delays makes the behavior task HPC-dependent (Ainge et al., 2007a). The mice completed this training phase when they reached a criterion of at least 80% correct for 2 out of 3 consecutive days.

Surgical Procedures

Two major survival surgeries were performed, a viral vector injection in the MSA and implantation of electrodes targeting the hippocampus. At least one day before either surgery, mice were given food and water *ad libitum* to ensure a quicker recovery. Mice were placed in an

induction chamber and anesthetized with 3.0% isoflurane vapor at 1L oxygen per minute (Isoflurane Vaporizer, VetEquip). After anesthesia, mice were transferred to and mounted onto a stereotaxic frame, where they were fixed with a nose cone and two ear bars. Anesthesia was maintained through isoflurane inhalation through the nose cone. The isoflurane concentrations were slowly reduced to 1.5% or 2.0% during the surgeries. Mice were also placed on top of a 38°C water heating pad throughout the surgeries to maintain their body temperatures. The mice's breathing rate and reflexes were checked immediately after anesthesia, and every 30 minutes thereafter. The mice's eyes were coated with a lubricating ointment to prevent drying.

Viral Vector Injection Surgery

Mice were randomly selected to receive the control (AAV-hSyn-eGFP) or experimental (AAV-EF1a-DIO-hChR2-eYFP) virus vector injection. The top of the mouse's head was shaved and the scalp was disinfected with povidone iodine and 70% ethanol. The skull was then exposed with an incision on the midline and leveled at the midpoint between bregma and lambda. A hole was drilled above MSA [+1.0 mm anterior-posterior (AP) axis, -0.7 mm medial-lateral (ML) axis]. A glass pipette connected to a micro syringe pump was inserted into the brain angled at 10 degrees towards the midline in the coronal plane, and 400 nl virus was injected at 400 nl per minute at each of three depths [-4.8 mm, -4.6 mm, and -4.2 mm dorsal-ventral (DV)] (Micro4 MicroPump, World Precision Instruments). There was at least a two-minute waiting period after injection at each depth to prevent virus backflow. After the final injection at -4.2mm DV, the pipette was left in place for 5 minutes before getting withdrawn slowly. The skin above the scalp was sutured and secured with Vetbond, and the mouse was removed from the stereotaxic frame. When the mouse's reflexes returned, 0.03mL buprenorphine was provided. After the surgery, the

mouse was left to recover in the vivarium with food and water *ad libitum* for a minimum of 5 days or until fully recovered.

Electrode Implantation Surgery

After mice passed the criterion for continuous alternation performance, electrodes and an optic fiber were implanted. Anesthesia induction, mounting, and leveling were performed as described for the viral vector surgery. The surgery then proceeded with attaching a cage base with a thin layer of Metabond (C&B metabond, Parkell). Craniotomy holes were then marked and drilled above the left hemisphere, above the MSA, and above the right dorsal hippocampus (dHPC). These were for inserting the optic fiber, electrodes, and ground screw respectively. The ground screw hole was drilled first and dura was removed with a bent needle. The ground screw was plugged or screwed into the hole and a layer of Metabond was applied to the screw. Next, the optic fiber was inserted with a 10-degree tilt towards the midline in the coronal plane and was lowered 3.5 mm from the brain surface [+0.9 mm AP, -0.6 mm ML] into the MSA. The fiber was secured with UV-cured cement (UNIFAST LC Light-Cured Acrylic Resin). For the craniotomy above right dHPC, two craniotomy holes were drilled and connected into one larger hole for probe insertion [-1.9 mm AP, +1.9 mm ML and -2.5 mm AP, +2.5 mm ML].

The implanted probes had either 32 or 64 electrode channels arranged into 6 or 8 shanks, respectively. The probes were mounted on a moveable microdrive (Vöröslakos et al., 2021), and the shanks and moveable portions of the drive were covered with wax to avoid contact with cement. DiI dye was applied to the tips of the shanks to help visualize shank tracks in histological material. The array of probe shanks was angled at 45 degrees from the AP axis, and the probe shanks were inserted 500 μ m from the brain surface. A cage was assembled on top of

the cage base to protect the drive and electrodes, stabilize the optic fiber, and hold the plug of the probe. The cage walls consisted of metal mesh, and ground wire was soldered onto mesh for shielding. After cage assembly, the mouse was taken off isoflurane and injected with buprenorphine. Postoperative recovery procedures were the same as those after the virus injection surgery. Each day after implantation surgery until perfusion, the mouse was monitored for health conditions.

Recordings in the Delayed Alternation Task

The drives were lowered in increments of 1/8 turns (35 μm) to 1/4 turns (70 μm) until they were approaching the CA1 cell layer, which would be signified by the appearance of SWRs. If mice had passed the criterion for retraining in the delayed alternation task and had visible SWRs, the recording phase could begin. Recordings of spikes of single cells and of LFP were done using either a Neurolynx Cheetah system or an Intan system, depending on the microdrive type that the animal was implanted with (32 or 64 channels). On each recording day, one stimulation frequency (4, 8, 12, or 20 Hz) and delay order were pseudorandomly selected. Each recording session consists of 60 trials divided into 6 blocks of 10 trials. Laser stimulation would be turned on every other block (blocks 2, 4, and 6) to optogenetically stimulate the MSA. During the recording phase, the tetrodes continued to be gradually lowered in increments of 35 μm to record cells at different depths of the CA1 and CA3 cell layers.

Probe Extraction and Perfusion Surgery

Recordings are finished after the electrodes pass through cell layers or if signal quality is poor. After the mice finished the recording phase, food restriction would end to prepare the mice for perfusion. Before perfusion, all mice were anesthetized and mounted for surgical procedures.

Soldering was then undone, and the cage components were removed. Probes were recovered by slowly lifting them out of the brain. The probes were then cleaned with warm saline before soaking them in 1% tergazyme solution for at least 4 hours. After probes were extracted, mice were injected intraperitoneally with 0.13 ml Fatal-Plus, and gas anesthesia was ended. The mice were perfused with 25 ml 0.1 M phosphate-buffered saline (PBS) followed by 25-30 ml 4% paraformaldehyde (PFA) in PBS solution. When the perfusion procedures were complete, their brains were extracted. For post-fixation, the brains were stored at 4 degrees C for a minimum of 24 hours in 4% PFA in PBS solution away from light. Then the brains were transferred to 1xPBS solution before sectioning.

Histology

All mouse brains were sectioned using a vibratome. The brains were sliced coronally into 40 μ m thick slices. Slices from and near the MSA and hippocampus regions were collected and mounted onto glass slides. The slides were dried in the dark for 24 hours and then coverslipped with DAPI solution (DAPI Fluoromount g, SouthernBiotech). Fluorescence images were taken using the Olympus VS120 microscope. For slides with MSA, filters were used to visualize DAPI and GFP, and for slides with HPC, to visualize DAPI and DiI. The images throughout MSA were used to confirm the virus expression, while the images with HPC were used to confirm the location of the probe traces. Recording sessions were used for further analysis after confirming that electrode tracts passed through the hippocampal CA1 and CA3 layers.

Statistical Analysis

Overview

Statistical analysis was performed with Microsoft Excel and MATLAB (R2020a). Analysis only included recording sessions with successful pacing, or with good behavioral performance for control animal sessions. Data were visualized using MATLAB, Python, and Adobe Illustrator.

Video and Behavioral analysis

MATLAB 2020b was used to perform the preprocessing of video data to express the animal running path and position during recordings. Animal positions were visualized and mapped onto the idealized predefined maze template and on the different zones on the maze (reward and delay zones, stem, center and return arms). Animal performance for training and recording sessions was recorded in Microsoft Excel and averaged across sessions for each stimulation frequency and delay condition.

Spike sorting and cell type classification

Spike sorting was done on electrophysiological data with an edited version of KiloSortWrapper (Petersen et al., 2020). Phy was used for manual curation of spike-sorted results. Spike amplitude, waveform, and inter-spike intervals (ISI) were first used as parameters to extract clusters with good signals, identify identical clusters, and exclude noisy signals from clusters. After curation, MATLAB scripts plotted the mean waveforms of the selected clusters and calculated waveform metrics to aid in / confirm the neuron classification. The duration between the mean peak and trough was calculated in milliseconds. The ISI of each spike within a cluster was also calculated and compared to a threshold of 0.002 seconds. An ISI violation ratio

was generated to check if the identified cluster contained noise from other neurons. MATLAB was used to calculate the average and peak firing of each cluster and to map the firing rate onto the figure-8 maze. Cells with peak firing consistently at one location on the maze throughout a recording session were classified as place cells.

CHAPTER 3: RESULTS

In order to investigate the role of theta oscillations in organizing the temporal firing patterns of hippocampal neurons, we replicated a procedure that was previously found to be effective in modulating theta oscillation frequency (Zutshi et al., 2018). PV-Cre knock-in mice were injected with Cre-dependent viral constructs into the MSA. Experimental mice were injected with AAV-EF1a-DIO-hChR2-eYFP, and controls were injected with AAV-hSyn-eGFP. As the viral vectors were Cre-dependent, ChR2 channels or eGFP would only be expressed in PV cells. In the case of ChR2 expression, the cells would become responsive to light stimulation, and rhythmic blue light (473nm) stimulation delivered through an optic fiber implanted in the MSA would activate the PV cells. The artificial firing of PV cells would result in additional neuronal activity, and such signaling would override the endogenous pacing of theta oscillation and result in a frequency that corresponds to the frequency of optical stimulation.

Histological images confirmed viral construct expression and probe implant location

In mice with AAV-EF1a-DIO-hChR2-eYFP injection ('ChR2 mice'), viral constructs co-expressed ChR2 and eYFP, which allowed us to confirm the expression of ChR2 in PV+ cells by imaging eYFP (Fig. 1A). Mouse brains were sectioned coronally throughout the extent of the MSA and HPC. Slices that contained MSA were imaged to visualize eYFP (or in controls, eGFP) expression across the entire AP extent. In an example mouse, virus injection was found throughout the MSA, with high expression levels indicated by the strong yellow-green fluorescence (Fig. 1A). We reviewed the scanned images of all mice that we recorded from to confirm that viral vector expression is selectively present in MSA PV cells. In control mice, we confirmed the presence of eGFP expression.

After confirming the expression of viral vectors in PV neurons, we checked for probe traces in the HPC CA1 layer. Moveable recording probes were implanted above the dorsal HPC, and a microdrive was used to gradually lower the probes towards the hippocampal cell layers in increments of 35 μm . The drive and probe design was developed by the Buzsaki lab and has been shown to allow for the recording of spikes at different levels of the brain (Vöröslakos et al., 2021). Before implantation, drive shanks were coated with DiI dye, which can be visualized to confirm probe location. Accordingly, histological slides that contained the dorsal hippocampus were scanned for DiI. To better visualize the cell layers, the slices were counterstained with DAPI, which was visualized along with DiI. Alternatively, slices were stained with Cresyl Violet to visualize track marks that were left by the shanks. In the same example mouse as the one that shows eYFP expression, marks made by probe shanks were visualized from anterior to posterior, confirming the location of all shanks (Fig. 1B). The HPC images of all mice from which recordings were taken were reviewed, and only mice with shank locations that were confirmed to be either in the hippocampal CA1 or CA3 layer were used for further analysis.

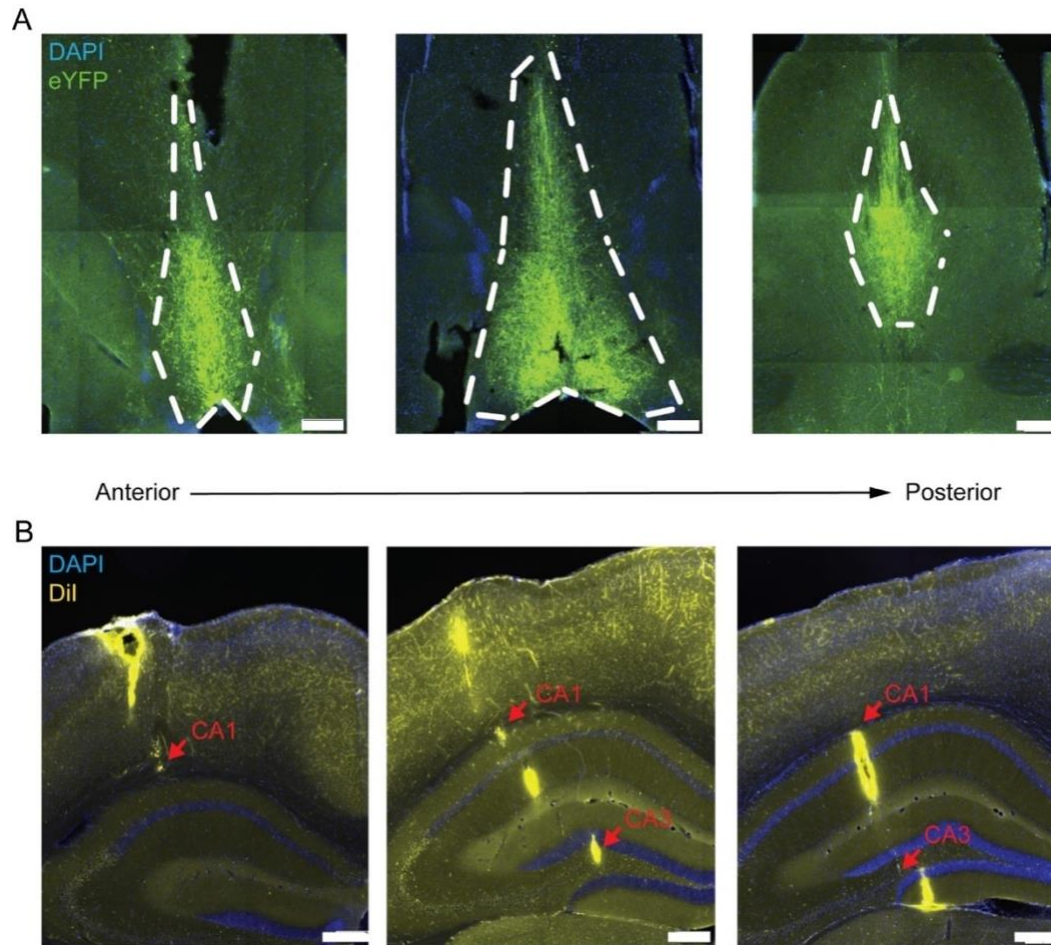


Figure 3 | Imaging of histological material confirmed channelrodopsin (ChR2) expression in the MSA and accurate electrode probe placement in the HPC.

(A) AAV vectors expressing Cre-dependent ChR2 or green fluorescent protein (eGFP) were targeted to the medial septal area (MSA) of PV-Cre transgenic mice. Areas with PV cells in the MSA are circled by white dashed lines. Viral expression is imaged at different levels along the anterior-posterior axis of an example mouse. The scale bar is 200 μ m. (B) Electrode shafts were coated with DiI, which resulted in tracks that are shown in yellow in an example mouse. Probes were implanted and lowered into the CA1 cell layer of HPC, and were in a subset of mice, advanced to the CA3 layer. The visualization of the probe tracks confirmed the placement of the probes in the HPC CA1 and CA3 layers.

Efficacy of pacing in ChR2 animals verified with LFP frequency spectrograms

We performed optogenetic stimulation with a blue light laser, delivering 2mW of stimulation to MSA PV cells during memory task performance. We then examined changes in the LFP in order to verify that theta oscillation frequency was indeed overridden while rhythmic stimulation was performed during recording sessions. With timestamps marking the beginning and end of laser stimulation within a recording session, we were able to compare the baseline LFP theta oscillations to stimulated LFP. From the 6 blocks of 10 trials (i.e., stimulation and no stimulation with each of the 3 delay conditions), we chose to plot and analyze the spectrograms from the two no-delay blocks because they are the least likely to show recording artifacts from the rest during delay. Spectrograms were created for each channel within a session, and the channel with the highest endogenous theta amplitude was selected to quantify the pacing effect.

For quantification, the LFP frequency spectrogram was calculated for every 2-second segment, and the power in different frequency bands was measured. For sessions with stimulation frequencies below or above the endogenous range (4, 12, and 20 Hz), there is a clear shift in LFP frequency. The endogenous theta amplitude decreased and was replaced with a peak at the stimulation frequency. For sessions with 8 Hz stimulation, we expected that the stimulated oscillations remained within the endogenous range, but that a narrower peak compared to the endogenous theta would emerge. Inspection of the spectrograms was also performed to exclude any channels that showed no baseline theta or high noise levels. Channels with high noise or that appeared to be defective were excluded from further neuron clustering and spiking analysis.

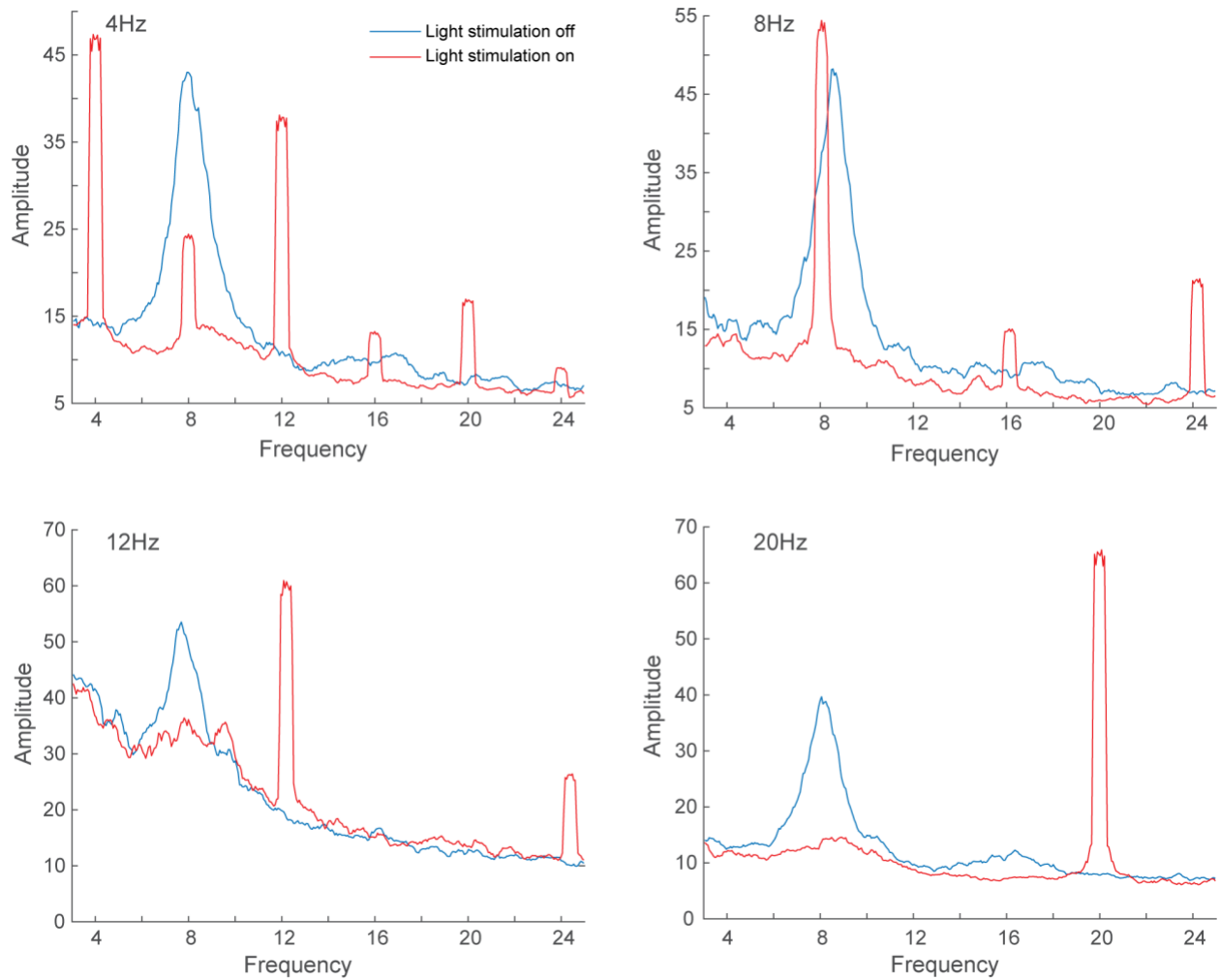


Figure 4 | Spectrograms of LFP signals recorded from an electrode in hippocampus, shown separately for trials with rhythmic optical stimulation on (in red) or off (in blue).

Blue light stimulation was applied through an optic fiber inserted into the MSA, which is expected to activate MSA pacemaker cells that control theta oscillations. Selective activation of these cells controlled the hippocampal oscillation frequency throughout the stimulation period. The LFP frequency corresponded to the optical stimulation frequency, and endogenous theta oscillations in the 7-9 Hz were superseded by oscillations at the stimulation frequency.

Control animals showed no pacing during stimulation periods

Control GFP animals were injected with a viral construct containing expressing only eGFP. They underwent the same surgical and recording procedures, with an optic fiber implanted and with rhythmic stimulation turned on during task periods. As for ChR2 mice, the LFP frequency spectrogram was calculated for every 2 s segment, and data were further analyzed to ensure that the presence of blue light stimulation did not result in any major changes in the LFP oscillation amplitude and frequency. As expected, optical stimulation at 4, 8, 12, or 20 Hz in control mice showed no effects on the theta oscillations. The amplitude and the peak at 8 Hz both remained approximately the same, and plotted curves with stimulation were similar to those without stimulation (Fig. 3).

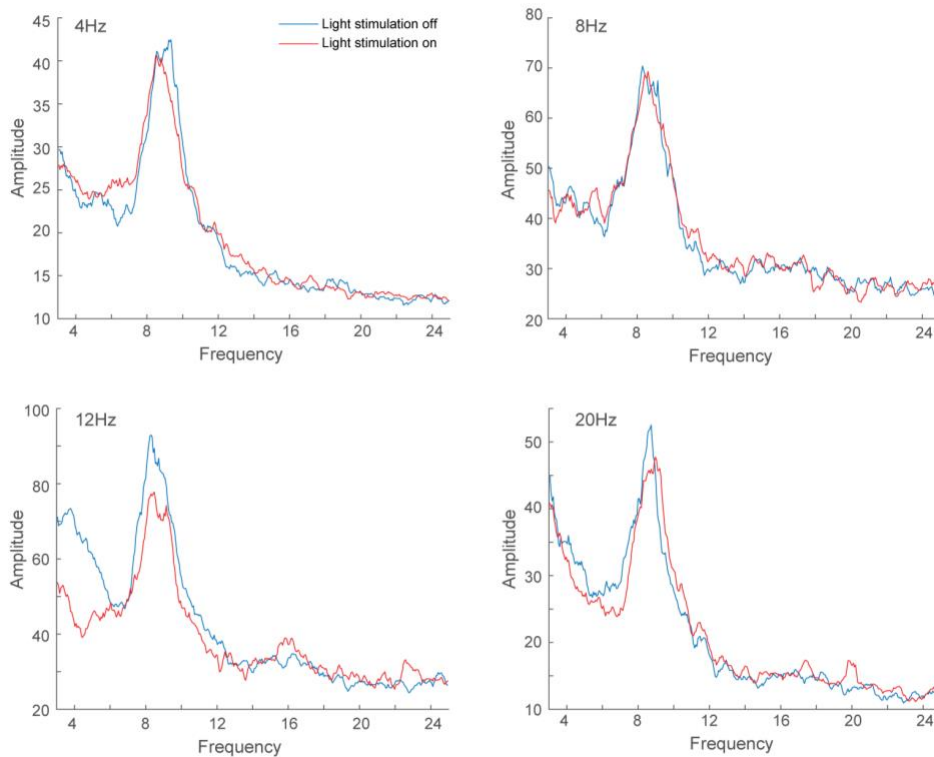


Figure 5 | LFP signal spectrograms of control sessions.

Blue light stimulation in control animals was performed as for ChR2 animals. LFP spectra during stimulation-on and stimulation-off periods are plotted. The endogenous theta oscillations were retained during periods with laser stimulation at various frequencies.

Overview of the dataset that was generated and prepared for further spike timing analysis

Depending on variations in the effectiveness of virus expression, the accuracy of targeting electrodes to the hippocampus, the quality of the recording probes, and optimization of the recording and optical stimulation procedures, a subset of ChR2 mice and/or sessions from ChR2 mice were excluded from further analysis. The excluded mice/sessions did not have sufficiently strong pacing of theta oscillations, sufficient electrophysiological signal quality, or probe tracks that could be confidently located within the hippocampus. Mice with histological images that showed good ChR2 expression, good pacing of hippocampal oscillations, and accurate probe locations in the hippocampus were kept.

Twelve to 20 recording sessions were performed per mouse – with each session consisting of 6 blocks of 10 trials in the spatial alternation task as well as sleep sessions before and after the behavior. We used several criteria to select for sessions that were suitable for further analysis (Table 1). First, probe placements in CA1 or CA3 were verified in histological slides from each mouse. Next, from all recording sessions, we selected those with visible spikes in the raw electrophysiological traces and with sharp wave ripples (SWRs) detectable on multiple probe shanks. Furthermore, the spiking of hippocampal principal cells was expected to be distributed across multiple channels of the same shank and to show a burst pattern that consists of multiple spikes with slightly decreasing amplitudes across the sequence. In addition, strong pacing of hippocampal oscillations was an additional criterion for the inclusion of a recording session and was verified by a shift of the frequency distribution in the frequency spectra, as shown in Fig. 2. Furthermore, sessions where animals were visibly agitated and overactive during sleep were excluded. Similar criteria were used for the inclusion of recording sessions of control animals. For pacing spectra of control animals, it was required that

endogenous theta was present in most channels and that there was no major change in LFP frequency with laser stimulation.

Overall, histological imaging confirmed viral construct expression and probe shank placement in 16 mice. Out of those, 47 sessions from 10 mice (4 female, 6 male) fulfilled the criteria for inclusion in further analyses. The selected sessions are listed in Table 1, along with key information for each session. Each session is labeled by animal ID, animal sex, probe type, session date, depth from brain surface in μm , stimulation frequency delivered, delay order, and number of functional channels at the time of recording. Two ChR2 animals (P99-1 and P100-1) were treated as controls as they consistently had insignificant pacing but good spiking signals.

Table 1 | Summary table of recording sessions selected for further analysis.

Mouse ID	Sex	Probe Type	Session ID	Depth	Delay Order	Functional Channels
Training and recording with laser stimulation on and off (N = 29 sessions)						
12 Hz Sessions						
P95-1	F	Cambridge64	02/06/2024	1445	2 0 10	31 / 64
		Cambridge64	02/16/2024	1445	10 0 2	29 / 64
P86-1	M	Cambridge64	01/03/2024	1585	0 10 2	63 / 64
		Cambridge64	01/11/2024	1795	0 10 2	48 / 64
P84-4	F	Cambridge64	09/01/2023	1325	10 2 0	56 / 64
P76-2	M	Buzsaki64	05/23/2023	815	2 0 10	64 / 64
P76-1	M	Buzsaki64	08/29/2023	1830	2 10 0	29 / 64
P70-2	F	NeuroNexus32	04/26/2023	1060	0 10 2	23 / 32
4 Hz Sessions						
P95-1	F	Cambridge64	9/3/2024	2215	2 10 0	25 / 64
P84-4	F	Cambridge64	09/05/2023	1360	2 0 10	58 / 64
		Cambridge64	09/08/2023	1395	2 10 0	58 / 64
P76-2	M	Buzsaki64	05/24/2023	815	10 2 0	64 / 64
		Buzsaki64	05/26/2023	815	2 10 0	64 / 64
P76-1	M	Buzsaki64	09/14/2023	1900	10 2 0	43 / 64
P70-2	F	NeuroNexus32	04/30/2023	1130	10 2 0	22 / 32
8 Hz Sessions						
P95-1	F	Cambridge64	02/15/2024	1445	0 2 10	30 / 64
		Cambridge64	03/08/2024	2215	0 2 10	26 / 64
P86-1	M	Cambridge64	01/10/2024	1795	10 2 0	64 / 64
		Cambridge64	01/12/2024	1795	10 0 2	56 / 64
P84-4	F	Cambridge64	09/07/2023	1395	10 0 2	63 / 64
		Cambridge64	09/14/2023	1430	0 10 2	50 / 64
P76-2	M	Buzsaki64	05/22/2023	815	0 10 2	64 / 64
P70-2	F	NeuroNexus32	05/02/2023	1165	0 10 2	22 / 32
20 Hz Sessions						
P95-1	F	Cambridge64	02/08/2024	1445	2 10 0	19 / 64
		Cambridge64	02/17/2024	1445	2 0 10	19 / 64
P84-4	F	Cambridge64	09/06/2023	1360	0 10 2	63 / 64
		Cambridge64	09/11/2023	1395	0 2 10	63 / 64
		Cambridge64	09/12/2023	1395	10 2 0	63 / 64
P76-2	M	Buzsaki64	05/25/2023	815	0 2 10	64 / 64

Table 1 | Summary table of recording sessions selected for further analysis, continued.

GFP Controls						
Mouse ID	Sex	Probe Type	Session ID	Depth	Delay Order	Functional Channels
Training and recording with laser stimulation on and off (N = 18 sessions)						
P65-2	M	Cambridge64	11/21/2023	1795	0 10 2	63 / 64
			11/23/2023	1795	10 2 0	63 / 64
			11/28/2023	1865	2 10 0	63 / 64
			11/30/2023	1865	0 10 2	63 / 64
			12/01/2023	1865	0 2 10	63 / 64
P65-1	M	Cambridge64	09/13/2023	1025	0 2 10	61 / 64
			10/17/2023	1445	0 2 10	59 / 64
			10/19/2023	1480	10 0 2	58 / 64
			10/25/2023	1550	0 10 2	58 / 64
P99-1	M	Cambridge64	04/20/2024	1480	2 10 0	38 / 64
			04/29/2024	1620	2 10 0	38 / 64
			05/07/2024	1690	2 10 0	38 / 64
			05/08/2024	1690	10 0 2	38 / 64
P100-1	F	Cambridge64	05/31/2024	1410	10 0 2	64 / 64
			06/03/2024	1480	2 10 0	64 / 64
			06/06/2024	1515	10 2 0	64 / 64
			06/10/2024	1515	0 10 2	64 / 64
			06/12/2024	1585	10 0 2	54 / 64

Behavior performance in the memory task changed with optical stimulation

From all the recording sessions, those with good observed behavior (i.e., consistent running) and LFP signals (i.e., low noise levels) were selected for behavioral analysis and evaluation of the effects that emerge from disruption of theta oscillations. The recording sessions for behavioral analysis also included sessions (47 sessions from 10 mice) in which no clear single-unit signals could be detected, but in which the LFP signals could be used to ensure that pacing was efficient. Using the Shapiro-Wilk Test, we tested the behavior performance scores (mean percent correct and the difference between stimulation and no stimulation blocks) for normality, with $p = 0.05$ as the threshold. We found that data for the ChR2 and control animals both contained non-normal distributions, thus we decided to move forward with non-parametric testing for all recorded sessions. We performed a two-sided Wilcoxon paired signed-rank test to

compare the behavioral performance across different delay and stimulation conditions. In the trials with no delay, which are non-hippocampal dependent, we found no differences in memory performance [n(4, 8, 12, 20 Hz) = 7, 8, 9, 5; W = 7, 6.5, 10.5, 4.5, p = 0.63, 1.00, 1.00, 2.00, two-sided Wilcoxon signed rank tests]. The 2-s delay also showed no significant differences [n(4, 8, 12, 20 Hz) = 7, 8, 9, 5; W = 16, 16.5, 25.5, 2, p = 0.34, 0.84, 0.08, 1.00, two-sided Wilcoxon signed rank tests). In the 10-s delay trials, 4 Hz stimulation produced statistically significant differences [n(4 Hz) = 7; W = 21, p < 0.05, two-sided Wilcoxon signed rank tests], while all other stimulation conditions did not show any significant impairment [n(8, 12, 20 Hz) = 8, 9, 5; W = 15, 13, 1.5, p = 1.00, 0.25, 0.75, two-sided Wilcoxon signed rank tests]. The same analysis was done on GFP controls to control for any effects from the presence of laser light in the brain and from the visual perception of flashing light. Control animals expressed no effect in response in stimulation [n(4, 8, 12, 20 Hz) = 2, 8, 4, 4; no delay: W = 3, 19, 6, 3.5, p = 0.50, 0.52, 0.25, 1.00; 2-s delay: W = 1, 10, 3, 2, p = 1, 1, 0.5, 1; 10-s delay: W = 2, 9, 3, 1, p = 1, 0.52, 1, 1; two-sided Wilcoxon signed rank tests], demonstrating that any behavioral changes in ChR2 animals would be due to theta disruptions and not from light delivery.

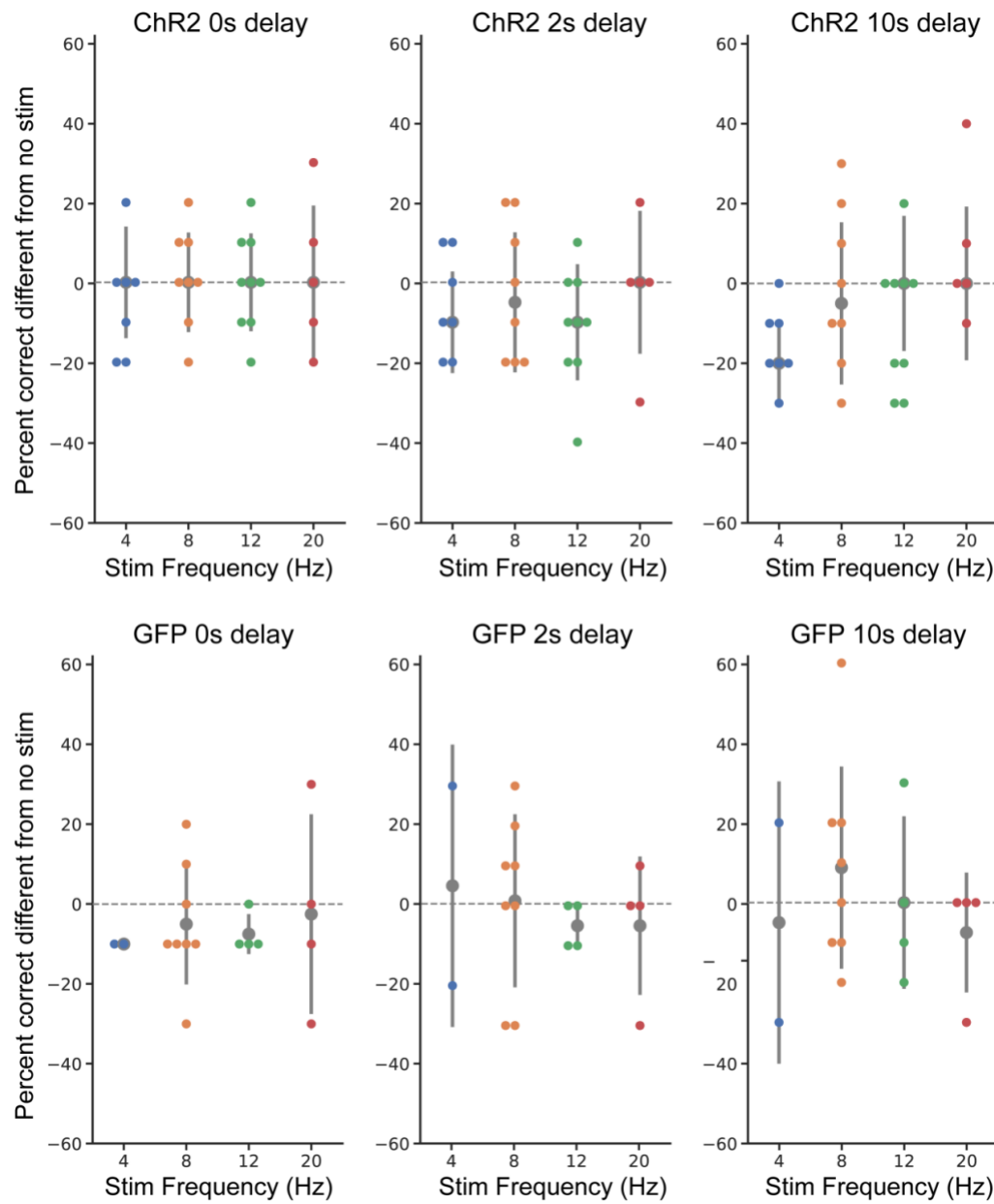


Figure 6 | Difference in percent correct trials. Performance in stimulation (stim) periods is subtracted from performance in no stimulation (no stim) periods.

Each panel depicts a different delay condition, with sessions from mice expressing the ChR2 viral construct on top and sessions from mice expressing the GFP construct on the bottom. Data points for each session are shown as small colored dots (blue, 4 Hz; orange, 8 Hz; green, 12 Hz; red, 20 Hz), and averages across sessions (i.e., median % change in behavioral performance) are shown as large gray dots.

Cluster cutting and curation identified CA1 principal cells and interneurons

From selected recording session, electrophysiological data was processed for cell spiking analysis. Spikes were automatically grouped with KiloSort before manual curation with phy or phy2. In general, clusters with amplitudes at or above 30mV were considered for further clustering. The clusters were checked to show constant firing across the session during which they were recorded. The spike amplitude distribution should also be normal and distinct from the noise distribution. The ISI violation metric, which is the proportion of spikes with ISI less than 2 ms, was used to indicate contamination in the cluster. We set the violation threshold arbitrarily at 30%. Any clusters with ISI violations above the threshold percentage were considered to be noisy or over-merged. They would either undergo more splitting or be rejected as a suitable unit for analysis.

Clusters were further classified as putative principal cells or interneurons according to waveform and spiking metrics. For an example putative pyramidal neuron, we show that the waveform is characterized by a sharp positive peak followed by a downward deflection and a slow return to baseline (Fig. 5A). The calculated mean duration is expected to be more than 0.4 ms, which separates them from fast-spiking interneurons, which show shorter waveforms. The ISI, which reflects how closely spikes of a single-unit fire after each other, is expected to show a distribution with a peak of less than 20 ms and more than 2 ms. Spike train auto-correlograms can also be used to identify principal cells using characteristics such as a clear refractory period, a sharp peak at 3 to 5 ms, and a fast decay (Csicsvari et al., 1998). These characteristics result from the bursting firing pattern of pyramidal neurons. In contrast to principal cells (see Fig. 5A), the mean waveform of a putative interneuron shows an initial positive peak before a negative deflection and rapid repolarization to a second positive peak (Fig. 5B). The second positive peak is expected to have higher amplitude compared to the first one (Csicsvari et al., 1998).

Interneuron clusters usually contain a much larger number of spikes, as they fire more regularly and at higher rates. Thus, their auto-correlograms display a slow decay compared to that of pyramidal cells.

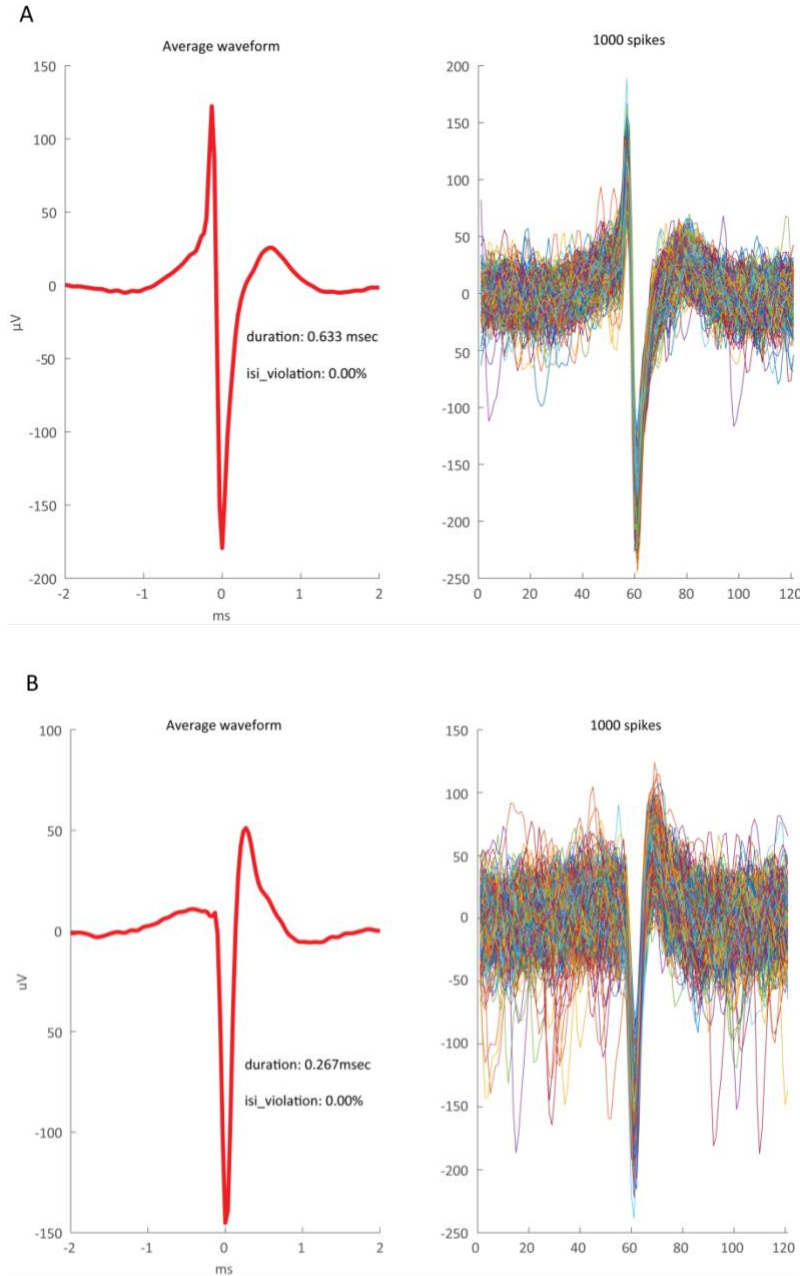


Figure 7 | Average waveforms from a putative pyramidal cell and interneuron of an example ChR2 mouse.

(A) Waveforms from an example putative principal cell and (B) waveforms from an example putative interneuron. (Left) Average spike waveform computed from an identified cluster, plotted as time (ms) by amplitude (μV). The spike duration in milliseconds and the proportion of spikes violating the 2-ms ISI threshold are listed in the lower right corner. (Right) Waveform illustrated from 1000 randomly selected spikes.

Spatial spiking patterns and firing rate maps were generated from alignment of spike trains to maze coordinates

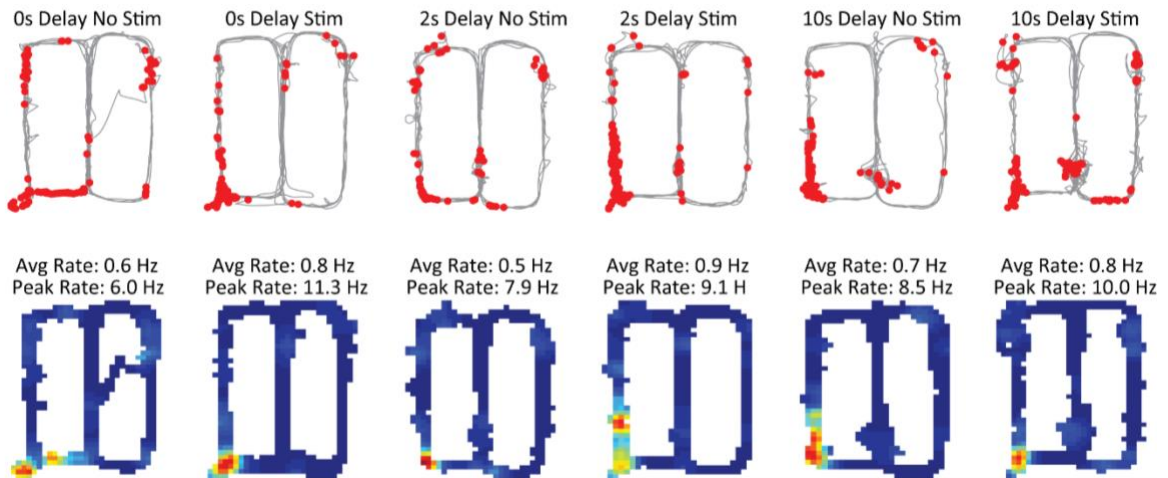
After units were classified and curated, their firing was mapped onto the maze. The animal's running path was matched to a template of the maze, and errors in tracking were corrected. For each identified cluster, spiking activity was visualized by plotting the spikes at the location where they occurred and by binning the firing into pixels which are then displayed as a heat map that depicts the firing rate in each pixel (Fig. 6).

In one example session, 34 clusters were identified. 6 were classified as single place field principal cells, 6 were interneurons, 10 were multiple place field principal cells, and the remaining 12 were either noise or over-merged clusters. For putative pyramidal cells ($n = 6$), firing was mostly concentrated at one location on the maze across the different stimulation and delay conditions. For single place field principal cells in the example session mentioned, their average firing rate varied between 0.1 and 1.5 Hz. Their firing locations were just before the delay zone on either arm, in the delay zone, and at the reward zones (Fig. 6A). For interneurons, there is high-frequency firing throughout all areas of the maze. The average firing rate ranged from 11.6 to 37.8 Hz for the identified interneurons ($n = 6$). Spiking of the example interneuron seemed to be at a higher rate at the decision, delay, and reward zones (Fig. 6B). In addition, a subset of identified pyramidal cells showed firing at multiple place fields on the maze ($n = 10$). These cells' average firing rates ranged from 0.1 to 6.1 Hz. In one example, there was more frequent firing just before the delay zone on one arm and before the reward zone of the other arm (Fig. 6C).

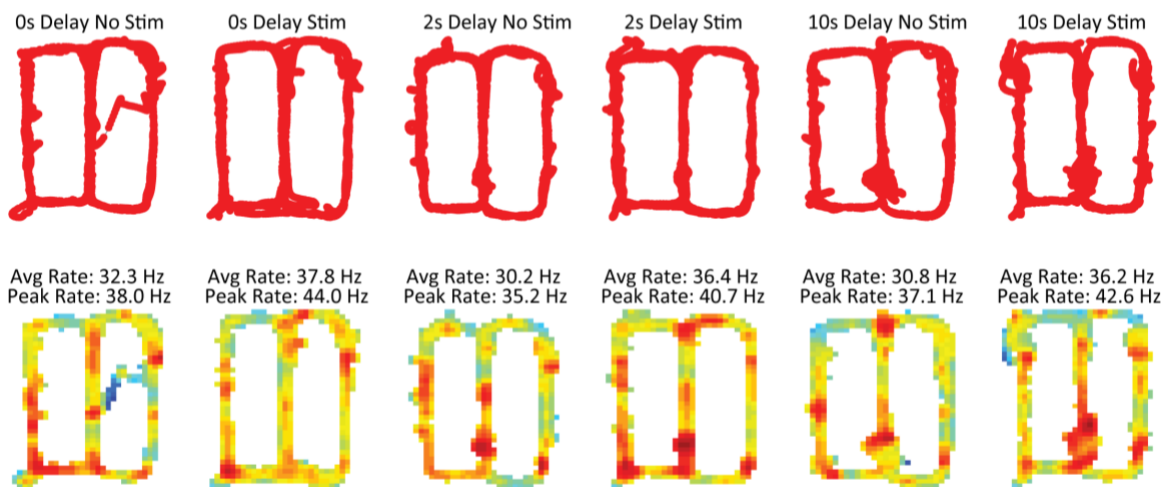
Figure 8 | Firing rate maps from a putative principal, interneuron, and multiple place field principal cell of an example ChR2 mouse.

(A) An example putative pyramidal neuron with a single place field, (B) an example putative interneuron, and (C) an example putative principal neuron with multiple place fields. (Top) Plots depict the path of the mouse in gray and each spike of the cell as a red dot. The six different delay/stimulation combinations of one recording session are shown. (Bottom) Firing rate as heat maps across a recording session. The color code is from blue (0 Hz) to red (peak rate). The average and peak firing rates in each delay/stimulation condition are indicated on top of the plot.

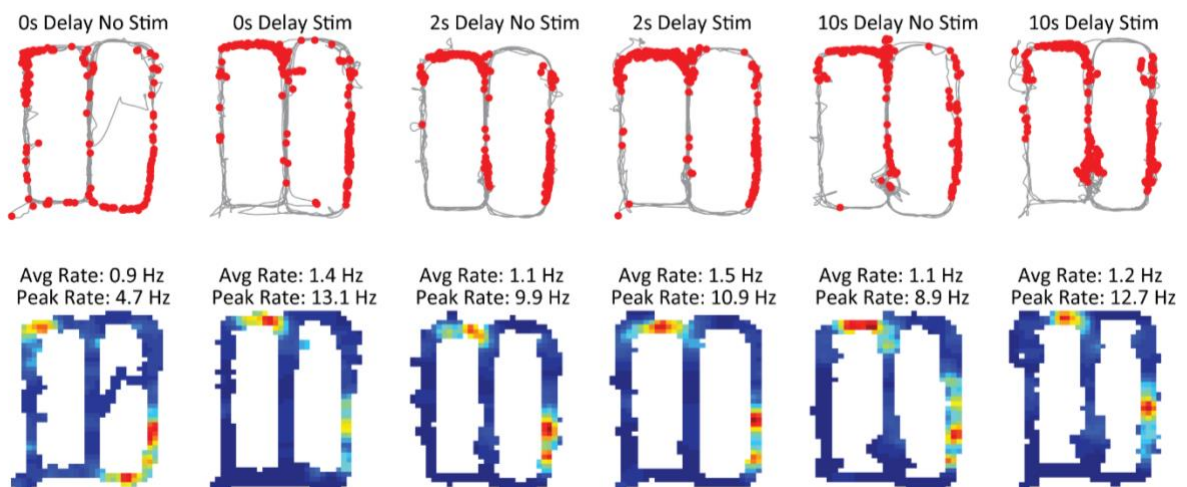
A



B



C



CHAPTER 4: DISCUSSION

Theta oscillations are prominent in the HPC and have been found to play an important role in organizing neuronal activity patterns and in supporting spatial working memory. Specifically, it has been reported that theta oscillations are associated with establishing well-timed sequential firing of place cells (O'Keefe & Recce, 1993). A phenomenon that is tightly linked to sequential firing is the occurrence of phase precession – across successive theta cycles, place cells tend to fire at progressively earlier phases of the cycle. Blocking septal connections to HPC reduces or removes the theta rhythm and leads to spatial memory deficits, but these studies have not definitely established whether functional theta is required in the encoding and retrieving memory process because they also have effects on other neuronal firing patterns, such as firing rates.

To more directly test the role of theta oscillations, methods to modulate the theta oscillation frequency were devised based on the presumptive role of GABAergic MSA cells to function as a pacemaker for hippocampal theta. In particular, optogenetic stimulation of GABAergic cells through light-activated channels has proved successful in overriding the endogenous theta oscillation frequency and resulting in frequencies above and below the endogenous range (Quirk et al., 2021). This technique allowed for studies that examine the role of theta oscillations for working memory performance as well as HPC neuron firing properties. In a previous study, working memory deficits occurred with pacing above the endogenous frequency range (10, 12, and 20Hz) on trials that required 2-s or 10-s delays (Quirk et al., 2021).

Our project further investigates the cell firing pattern changes while the theta frequency is periodically shifted to a frequency that is outside of the endogenous range during a working

memory task. We utilized a working memory task with variable delays and recorded cell spiking activities during memory maintenance and retrieval within the WM task. The task allowed for repeated trials of information encoding and retrieval with different delay intervals, making it suitable for looking into memory performance across different conditions while concurrently examining neuronal firing patterns. Importantly, trials with and without stimulation could be performed in the same session, allowing us to track and record reversible changes in cell firing patterns. We found that one combination of stimulation and delay conditions yielded significant deficits in spatial memory performance.

HPC oscillations were paced by selective optical stimulation of MSA PV+ cells

Reproducing the technique used by Quirk et al. (2021), we were able to achieve consistent and precise control of HPC oscillations, which adds support to the role that MSA PV+ neurons serve as pacemakers for theta oscillations. To determine the efficiency of pacing we first verified ChR2 expression in histological images and then examined the prominent LFP frequencies by comparing frequency spectra between stimulation on or off periods. During stimulation periods, we were able to shift CA1 oscillation frequencies to frequencies above and below the endogenous theta range. By plotting the mean LFP frequency in spectrograms, we showed a switch in frequency from ~8 Hz to 4, 12, or 20 Hz during stimulation periods and back to ~8 Hz during no-stimulation periods, which verified that the change is not only effective but also reversible. Checking that no LFP frequency shifts occurred in control animals ensured that any behavioral or cell spiking changes in ChR2 animals can be taken as specific results of theta frequency shifts and did not emerge due to differences in sensory perception, which necessarily results from the flashing laser light.

4Hz pacing and 10-s delay durations resulted in spatial memory impairment

Out of all the delay conditions and stimulation frequencies, we found significant changes in behavioral performance in the 10-s delay trials with 4 Hz stimulation. In this condition, mice showed a lower proportion of correct trials when stimulation was on compared to off. Although not statistically significant, a negative median change in performance was also found with 4 Hz stimulation in the 2-s delay condition. A previous study in rats looked into the effects of delays in the alternation task and found that an intact HPC is required to correctly perform the task with delays of 2-s or more, while a task version without delay can be performed at control levels after HPC lesions. In the continuous version of the spatial alternation task, structures other than the HPC are therefore sufficient to support learning and memory retrieval. For example, it is feasible that mice rely more on motor memory during continuous trials. Because of the significant effects of HPC lesion on 2-s and 10-s delay trials, it can be concluded that the pause that is introduced between trials places more demands on the animal's spatial working memory and that hippocampal computations are required to bridge the delay (Ainge et al., 2007). Our findings in mice corroborate the result that continuous alternation does not require HPC involvement because this condition was unaffected by disrupted HPC theta oscillations. For the 2-s and 10-s delay conditions, we found that a significant deficit with only the 10-s delay, which may indicate that a longer pause puts increased demand on HPC computations and is thus more susceptible to manipulations of hippocampal temporal firing patterns.

With respect to the pacing frequencies that are higher than the endogenous theta frequency, our findings are inconsistent with those of Quirk et al. (2021). Although statistically insignificant, trials with 2-s and 10-s delays showed varying trends in memory performance. Two-second delay trials showed negative median changes in performance for 12 Hz stimulation, but no median change with 20 Hz stimulation. Ten-s delay trials showed no median change with

12 and 20 Hz. This unexpected difference in results could be due to sample size. In our study, we included sessions from 6 ChR2 mice and 4 GFP controls with only 2 to 8 recording sessions per animal. With few sessions per delay and stimulation condition, our data also did not have a normal distribution. In contrast, Quirk et al. used a much larger dataset from 34 ChR2 mice and 16 GFP controls. We could perhaps reach a more definite conclusion on the behavioral influences if we extended our follow-up study to more subjects.

Prospective firing pattern and phase precession analysis

With one example session from a ChR2 animal, we performed spike sorting and cell classification before creating neuron firing rate maps for each identified cell. Signals were referenced to channels with no visible LFP cell spiking to reduce noise, and referenced signals were further sorted into clusters by their amplitude, waveform, and ISI. By then calculating waveform metrics from the spikes that are assigned to clusters, we can label the clusters as pyramidal neurons, interneurons, or noisy signals. The sorted clusters that correspond to pyramidal neurons and interneurons are putatively from isolated single neurons. For each of these neurons, the spike patterns were mapped onto the mouse position in the figure-8 maze, and separate plots were generated for each combination of delay and stimulation condition with each session.

During stimulation trials from the example session, 12 Hz pacing was performed. For identified CA1 pyramidal cells, we observed that all stimulation blocks had a slightly higher average firing rate compared to no stimulation blocks. If rate changes can be confirmed in other 12 Hz sessions, it would fit with one possible scenario for changes in spike timing, which is that the spikes remain at a consistent phase within the theta cycles and that faster oscillations result in more spikes because of the increased number of theta cycles while the mouse traverses the place

field (Fig. 8A and B). Another possible response for spike timing is that the timing between cells firing will stay fixed despite changes to theta oscillation frequency (Fig. 8C). If that is the case, we would expect firing rates to not change and that place cell spiking is no longer correlated in any systematic way to the artificially paced oscillation frequency. However, a complete dissociation of theta oscillation and spike phase is unlikely given that Quirk et al. (2021) found that principal cells remained modulated by the paced LFP frequency.

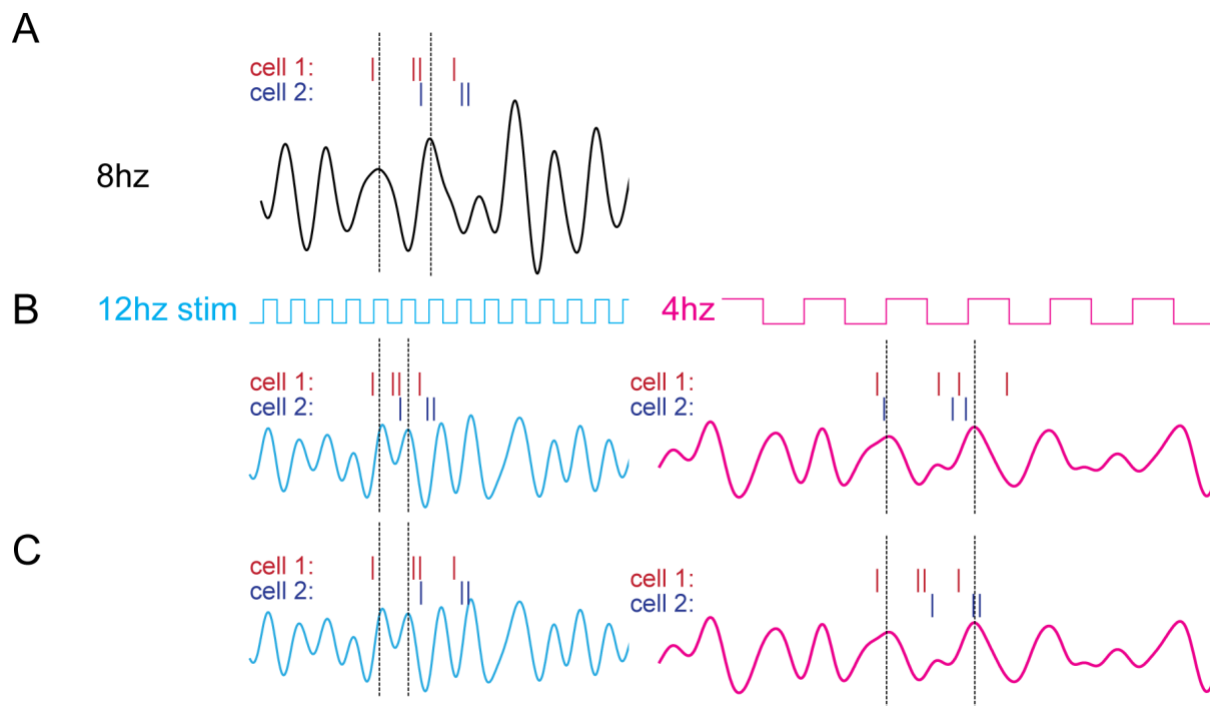


Figure 9 | Hypothesized place cell firing changes in response to slowed or accelerated theta oscillation frequencies.

(A) The schematic shows the phase precession of two principal cells (red and blue) during theta oscillation cycles. (B) Example theta oscillation was entrained to frequencies above (12 Hz, light blue) or below (4 Hz, pink) the endogenous range. In this scenario, spiking remains phase-locked to theta, and cell firing frequency increased or decreased depending on the stimulation frequency. (C) In this scenario, timing between spikes stays the same but relation to theta cycle is scrambled.

CHAPTER 5: CONCLUSION

To investigate the role of theta oscillations for spatial memory, we designed an experiment to examine which aspects of place cell spike timing are altered by a change in theta oscillation frequency. We included stimulation frequencies below (4 Hz), at (8 Hz), and above (12 Hz, 20 Hz) the endogenous theta oscillation frequency and found that stimulation below the endogenous frequency resulted in significant memory deficits in 10-s delay trials. However, we did not reproduce the memory deficits that were found with stimulation above the endogenous theta frequency in a previous study. This may be due to the much smaller sample size that was used here, and follow-up studies with a larger sample size would be needed to more directly compare the results. Overall, we confirmed that optogenetics techniques for manipulating MSA GABAergic cells are a viable method for sustained, but quickly reversible manipulations of theta oscillations. We entrained the HPC theta oscillation to both above and below its endogenous range in behaving mice. Importantly, we have generated a dataset in which a large number of cells were recorded while the mice performed the task, and we have optimized the spike sorting protocol for recordings with silicon probes. This dataset will allow for analyses of spike timing of hippocampal cells with overlapping place fields, which will reveal how the relative timing between cell pairs is altered when the hippocampal theta frequency is shifted. Once fully analyzed, these data will provide further insight on how the precisely timed neuronal spiking patterns support memory computations.

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