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Disruption of Hippocampal Rhythms via Optogenetic Stimulation During the Critical Period for Memory Development Impairs Spatial Cognition

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Abstract

Background: Hippocampal oscillations play a critical role in the ontogeny of allocentric memory in rodents. During the critical period for memory development, hippocampal theta is the driving force behind the temporal coordination on neuronal ensembles underpinning spatial memory. While known that hippocampal oscillations are necessary for normal spatial cognition, whether disrupted hippocampal oscillatory activity during the critical period impairs long-term spatial memory is unknown. Here we investigated whether disruption of normal hippocampal rhythms during the critical period have enduring effects on allocentric memory in rodents.

Objective/Hypothesis: We hypothesized that disrupt of hippocampal oscillations via artificial regulation of the medial septum during the critical period for memory development results in long-standing deficits in spatial cognition.

Methods: After demonstrating that pan-neuronal medial septum (MS) optogenetic stimulation (465 nm activated) regulated hippocampal oscillations in weanling rats we used a random pattern of stimulation frequencies to disrupt hippocampal theta rhythms for either 1Hr or 5hr a day between postnatal (P) days 21–25. Non-stimulated and yellow light-stimulated (590 nm) rats served as controls. At P50–60 all rats were tested for spatial cognition in the active avoidance task. Rats were then sacrificed, and the MS and hippocampus assessed for cell loss. Power spectrum density of the MS and hippocampus, coherences and voltage correlations between MS and hippocampus were evaluated at baseline for a range of stimulation frequencies from 0.5 to 110Hz

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and during disruptive hippocampal stimulation. Unpaired t-tests and ANOVA were used to compare oscillatory parameters, behavior and cell density in all animals.

Results: Non-selective optogenetic stimulation of the MS in P21 rats resulted in precise regulation of hippocampal oscillations with 1:1 entrainment between stimulation frequency (0.5–110Hz) and hippocampal local field potentials. Across bandwidths MS stimulation increased power, coherence and voltage correlation at all frequencies whereas the disruptive stimulation increased power and reduced coherence and voltage correlations with most statistical measures highly significant ($p < 0.001$, following correction for false detection). Rats receiving disruptive hippocampal stimulation during the critical period for memory development for either 1Hr or 5hr had marked impairment in spatial learning as measured in active avoidance test compared to non-stimulated or yellow light-control rats ($p < 0.001$). No cell loss was measured between the blue-stimulated and non-stimulated or yellow light-stimulated controls in either the MS or hippocampus.

Conclusion: The results demonstrated that highly robust regulation of hippocampal oscillations can be achieved with non-selective optogenetic stimulation of the MS in rat pups. A disruptive hippocampal stimulation protocol, which markedly increases power and reduces coherence and voltage correlations between the MS and hippocampus during the critical period of memory development, results in long-standing spatial cognitive deficits. This spatial cognitive impairment is not a result of optogenetic-induced cell loss.

Graphical Abstract

There is increasing evidence that there is a critical period for processing memories which depends on activity and plasticity mechanisms within the developing hippocampus. Whereas normal hippocampal EEG patterns are required for the normal development of spatial cognition, it is not known if disruption of EEG activity during this critical period of memory development has detrimental effects on cognition. Compared to rats with normal hippocampal oscillations during the third postnatal week, rats with optogenetically-disrupted hippocampal oscillations have substantial cognitive defects when the rats are tested as young adults in the active avoidance test. As demonstrated by the heat (dwell) maps demonstrating position of the rat during the testing, with hippocampal disruption rats do not avoid the shock zone (golden triangle) whereas control rats learn not to enter the shock zone. These results show that abnormal hippocampal oscillations during the critical period of memory have dire consequences on subsequent spatial cognition.

Keywords

memory; development; cognition; optogenetic; spectral power

Introduction

Spatial memory is the aspect of memory responsible for encoding and retrieval of information regarding one's environment and spatial orientation. In rodents, spatial memory is considered equivalent to declarative memory in humans [1–3]. The ontogeny of spatial memory in rodents is highly orchestrated with stereotyped patterns of activity propagating through developing circuits to establish connections that optimizes information processing [4, 5]. Organized expression of hippocampal network oscillations in the theta, gamma, and ripple frequency bands are believed to play a central role in the generation and coordination of memory [6–10].

Hippocampal oscillatory activity emerges and develops within the first three postnatal weeks in rodents [11–14]. This patterned oscillatory activity disseminates through the medial entorhinal cortex-hippocampal circuit and finely tunes the firing rates of emerging grid and place cells [15–19]. The maturing of oscillatory activity in the hippocampus parallels allocentric spatial learning and memory which arises approximately between postnatal (P) day 21 and P25 in the rodent [20–27].

Recent evidence has indicated there is a critical period for processing memories which depends on activity and plasticity mechanisms within the developing hippocampus [10, 28, 29]. Whereas normal activity patterns are required for circuit maturation, there is less known about the role of aberrant neuronal activity during the critical period in causing spatial memory impairment [27]. There are several genetic and acquired encephalopathic disorders that manifest during the critical period of memory development in children that cause abnormal hippocampal rhythms resulting in impaired declarative memory. Determining whether aberrant hippocampal oscillations are causally related to impaired cognition has important clinical implications in the treatment of neurological disorders in children.

Here we hypothesized that disruption of endogenous hippocampal oscillations during the critical period would result in lasting effects on spatial cognition. To address this hypothesis, we disrupted hippocampal theta by optogenetic stimulation of the medial septum (MS) during the critical period for memory development and then assessed spatial memory when the rats were adults.

Methods

All procedures were approved by the University of Vermont's Institutional Animal Care and Use Committee and conducted in accordance with guidelines from the National Institutes of Health. Male (n=14) and female (n=11) Sprague-Dawley (Charles River, Montreal) pups were subjects in this study. Pups were weaned at P21 and group housed until approximately P35–40, at which time they were individually housed. Rats were maintained on a 12-hour light/dark cycle. Experimental design, performance and analysis aimed to adopt guidelines

for rigor and reproducibility in science [30]. Group sizes were determined a priori, and animals were randomly assigned to experimental groups.

Experimental Overview:

The study design is provided in Fig. 1. To artificially regulate hippocampal oscillations, we used pan-neuronal MS stimulation. The MS, a midline structure that projects bilaterally to the hippocampus, acts as a pacemaker for theta oscillations in the hippocampus [31–34] by generating rhythmic inhibition through long-range connections to hippocampal GABAergic neurons [35–40]. After showing that MS optogenetic stimulation regulates hippocampus oscillations, pups received either 1 or 5Hr of optogenetically-induced disruptive blue light (BL) stimulation or inert yellow light (YL) stimulation for 4 days during the critical period for memory development (P21–25). To determine whether such disruptive stimulation resulted in long-term adverse effects rats were then tested at P60 for spatial memory in the active avoidance task.

Viral Injection:

At P7–8 rats were injected with a viral vector expressing channelrhodopsin (ChR2) into the MS. Under isoflurane anesthesia, the skull was exposed and a burr hole was placed in the skull (AP=0.7mm; ML=0mm) that allowed for access to the MS and diagonal band of Broca with a Hamilton injection syringe. The needle was then lowered 6.0mm into the brain. A total of 0.8μL of an adeno-associated virus expressing humanized ChR2 fused to EYFP driven by human Synapsin I promoter for optogenetic activation (AAV2-hSyn-ChR2(E123A)-EYFP; 5.7×10^{12} virus molecules/ml) (UNC Vector Core, Chapel Hill, NC) was injected slowly (0.1 μl/min) into 5 sites (0.1μl, 0.2μl, 0.2μl, 0.2μl and 0.1μl) with the syringe raised by 0.2mm before each injection. The scalp was closed with sutures and the pups returned to their dam once they were fully ambulatory.

Hippocampal and Septal Implants:

At P16–17, 8–9 days following the viral injection, two custom-made electrode arrays were implanted in both the MS and the dorsal hippocampus (CA1). The MS implant included an optic fiber with 2 recording electrodes glued to the surface that extended 0.25–0.5mm from the end of the optic fiber. A 230μm multimode optic fiber (Thorlabs, CFLC230-10; Montreal, Canada) was used to provide light stimulation of the MS septum. The percent transmittance of light through the fiber was tested using 100% BL (465nm) transmittance generated from Spectralynx LED source and measured by a light meter with a photodiode sensor (Thorlabs; Model PM100D). Using a 50μm patch cable for testing, only fibers that allowed >70% light transmittance at approximately 0.5mm from the tip of the optical fiber were used in the implants.

The optical/recording ensemble was lowered into the MS along the same path previously of taken by the Hamilton injection syringe, with the end of the optical fiber also lowered to a final depth of 5.8mm below the brain surface. The hippocampal implant consisted of 3 tetrodes with 50μm diameter stainless steel EEG electrodes (California Fine Wire, CA, USA) that were stereotactically placed in CA1 in the left hippocampus (AP=−3mm; ML=−2.5 mm; DV = 2.2mm; bregma reference). Two skull screws (FHC Inc) were inserted, one

screw was anterior to bregma while the other screw placed on the right side mirrored to the hippocampal implant bore hole. Grounding was achieved via connection to the right cerebellar screw while a signal reference wire was placed through a small burr hole over the cerebellum. Both implants were fixed to the skull via the skull screws and dental cement. The wound was sutured and topical antibiotic applied. The interval between surgery and the beginning of electrophysiological recordings and stimulation was 4 days.

Optogenetic Stimulation:

Between P21–P25 rats were placed in a 13cm high ceramic flowerpot that was 10.5cm wide at the base and lined with home cage bedding and connected to a custom-made adapter for the Neuralynx head stage with preamplifiers and a fiber optic optogenetic light-emitting diode (LED) driver (Neuralynx, Montana, USA). If the quality of the signals assessed using the cerebellar reference was sufficient, subsequent recordings were done using the cerebellar reference. Otherwise, the ground reference was used. The purpose of the flowerpot was to limit animal movement without restraint to measure resting hippocampal oscillations while minimizing potential sources of stress. At P21 rats were tested for MS optogenetic regulation of hippocampal oscillations using a ramp stimulation protocol in which optogenetic stimulations were administered for 300sec from 5–12Hz (Fig. 2A–C). The EEGs were visually inspected to determine if there was optogenetic regulation of the EEG. Depending on the state of control found the previous day, the rats were given one of the following stimulation protocols over the following days (P22–P25): i) 1Hr optogenetic stimulation disruption with BL (n=5 with visually confirmed optogenetic regulation of EEG); ii) 5Hr optogenetic stimulation BL (n=4 with visually confirmed optogenetic regulation of EEG); iii) 1Hr stimulation with yellow light (YL; 590 nm) (n=6); iv) no stimulation due to broken or defective implants (n=6). The disruption protocol consisted of 1Hr or 5hr loops of 13 frequencies of 275sec durations: 2, 4, 25, 12, 90, 50, 8, 0.5, 75, 1, 6, 35, and 110Hz; all stimulus frequencies were delivered as a sine wave. All rates were determined via random number generator and specifications with a 2sec interval between stimulation frequencies. Rats were stimulated between the hours of 08:00 to 17:00 with the lights on. During the ramp stimulation rats remained awake. During the disruptive stimulation rats were predominately awake (based on behavior observation and EEG evaluation), although some sleep was recorded during portions of the 5Hr stimulation.

EEG Recordings:

All EEG analyses were performed using NeuroExplore 4 software (Nex Technologies, Madison, AL). Power spectrum density (PSD), coherences and EEG correlations were obtained for each rat at each optogenetic stimulation frequency using methods previously described in our laboratory [41, 42]. The following oscillatory properties using local EEG were calculated for each animal:

PSD: After a single taper with the Hamming windowing function the fast Fourier transform (FFT) of the EEG was calculated for from 0–50 or 0–100Hz depending on the stimulating condition. The number of frequency values was 65,536 with a 50% window overlap. Normalization of the log of the PSD (dB) was performed using the following formula:

$$\text{power_spectrum}[i] = 10 \cdot \log_{10}(\text{raw_spectrum_matlab}[i])$$

Waveform frequencies were classified as follows: delta = 0–4Hz, theta 4–13Hz, beta 13–30Hz and gamma 30–50Hz. Coherence was computed between the hippocampus and MS. The FFT of data segments was obtained as described above and individual and cross densities were calculated:

$$P_{xx} = \text{FFT}(X) * \text{Conj}(\text{FFT}(X))$$

$$P_{yy} = \text{FFT}(Y) * \text{Conj}(\text{FFT}(Y))$$

$$P_{xy} = \text{FFT}(X) * \text{Conj}(\text{FFT}(Y)),$$

where $\text{Conj}(z)$ is a complex conjugate of z , X and Y refer to electrodes in the hippocampus and MS respectively. P_{xx} , P_{yy} , and P_{xy} values were averaged across all intervals, and coherence values were calculated as:

$$\text{Mean}(P_{xy}) * \text{Mean}(P_{xy}) / (\text{Mean}(P_{xx}) * \text{Mean}(P_{yy})).$$

Coherence values for bandwidths during ramp stimulation and the disruption protocol were analyzed. Voltage correlations. Cross-correlograms between EEG in the hippocampus and MS were obtained for 2 sec intervals and averaged over the EEG recording during ramp and disruption protocols (bin size = 3.4×10^{-4}). The EEG voltages within the specified bin size were calculated and then the correlations between the reference MS and other electrode computed. If $x[i]$, $i=1, \dots, N$ is the reference voltage and $y[i]$, $i=1, \dots, N$ is another voltage, then $c[t]$ = Pearson correlation coefficients (PCC) between vectors $\{ x[1], x[2], \dots, x[N-t] \}$ and $\{ y[t+1], y[t+2], \dots, y[N] \}$.

Active avoidance:

Animals underwent testing in the active place avoidance task (Biosignal; Brooklyn, New York) at P50–P60. In this task, animals must attend to their ever-changing position in the room frame lest they be rotated into a pre-determined zone where they receive a mild electrical shock [43–45]. The test requires that the rats attend to their ever-changing position in the room frame lest they be rotated into a pre-determined shock zone where they receive an aversive electrical shock.

One day prior to the active avoidance task rats were anesthetized and implanted with a stainless-steel swivel in the skin between the shoulders. The swivel was attached to a cable with an LED at the end allowing for automated tracking and the delivery of shock.

The arena consists of a steel disc 82cm in diameter lighted from both above and below. The arena is centered in a room where it is approximately 50cm from black curtains on the S and

E sides and 50cm from white walls on the N and W sides. The N and W walls have an 11cm gray power-strip that forms a continuous line 50cm above the floor of the arena. Two rectangular spatial cues (30cm×43cm) depicting a red star (centered at W position) and a black circle (centered at N position), both on a white background, were placed 18cm above the arena floor. An additional rectangular polarizing cue (53cm×84cm) made of white paper with five 2.5cm wide diagonal black stripes centered at the N position, 5cm above the gray power strip.

On the first day of training, the animal was connected to the shock cable and introduced to the rotating arena for a 10min habituation without shock. On all subsequent sessions rats received a 0.4mA shock in an unmarked 876cm² wedge-shaped sector covering a 60° arc in the NE sector of the arena. The shock zone was stable in the room frame while the arena rotated. The entrance latency of the shock was 1ms, the shock duration was 0.5sec and the inter-shock latency was 2sec. Rats were trained in eight 10min sessions per day for two days (16 sessions). Performance measures were recorded and analyzed using custom software (Biosignal; Brooklyn, New York). Estrus cycle was not assessed in the female rats during active avoidance testing.

Tissue Processing and Imaging.

Following all experiments, rats were deeply anesthetized and perfused with ice-cold PBS followed by 4% paraformaldehyde. Brains were removed and postfixed for 24–48hrs, then incubated in 30% sucrose until fully impregnated. Slide-mounted sections (40µm thick) were taken from the MS and hippocampus on a cryostat. Slides were permeabilized with 0.5% Triton X-100 in PBS and blocked with 10% fetal goat serum prior to overnight incubation with rabbit anti-NeuN primary antibody (1:500; ThermoFisher Scientific, Massachusetts, USA). Following primary antibody incubation, slides were incubated in a FITC anti-rabbit secondary antibody (Jackson ImmunoResearch, Pennsylvania, USA) and cover-slipped with DAPI-containing mounting medium (Invitrogen, California, USA). All images were obtained with a Nikon C2 laser scanning confocal microscope (Nikon, Tokyo, Japan). Images were processed using FIJI/ImageJ (NIH).

Image Analysis:

Image processing was performed blinded to the stimulus condition. In tissue sections containing the hippocampus, cell counts were obtained manually in FIJI/ImageJ – the density of DAPI and NeuN-labelled neurons led automated counting tools to be inaccurate. Individual NeuN- and DAPI-labeled structures were visually identified by the experimenter based on shape, size, and presence of defining dark pixels denoting unlabeled extracellular space. The CA1 pyramidal cell layer was outlined by a region of interest (ROI), which was consistent across all tissue sectioned ($0.23 \pm 0.002 \text{ mm}^2$). For tissue sections containing the MS, a separate group of consistently sized ROIs were used to normalize neurons and total cells counted from the MS ($2.14 \pm 0.23 \text{ mm}^2$). In the MS ROI, NeuN and DAPI-labelled structures were clearly defined and therefore the plugin “Analyze Particles” was used to count both neurons and total cell nuclei (minimum shape detection size 20µm²). All automated counts were visually verified for accuracy of the analysis by the experimenter. Cell counts were obtained manually in the hippocampus and using the “Analyze Particles”

plugin in the MS (all automatic counts were verified by the experimenter for accuracy). Total neuron and total cell counts in each CA1 and MS ROI included in the analysis were normalized by measurement area to account for minor variations in ROI shape/size.

Sample size/power calculations and statistics.

The primary outcome goal was to address the hypothesis that disruptive hippocampal rhythms induced by optogenetic stimulation during the critical period (P21–P25) would result in long-standing impairments in spatial cognition as measured in the active avoidance task. Using number of shocks as the primary outcome measure, a sample size of 10 rats in both the control and stimulated groups would detect a 20% (90% power, 5% α) difference in number of shocks over the 16 trials. While both a 1Hr and 5Hr blue-light stimulation was used to develop exploratory data for duration of stimulation and outcome, the study was not powered to detect a difference between the two stimulation paradigms. Likewise, the study was not powered to detect gender differences.

Between 4–6 channels of hippocampal EEG which demonstrated MS stimulation regulation of hippocampal oscillations and was devoid of artifact were analyzed for each rat. Total power and coherence, the mean of all evaluated EEG signals, and bandwidth (delta, theta, beta and gamma) were calculated. PSD was normalized and baseline power compared to power during each of the stimulation frequencies using the unpaired or paired Student's t test depending upon whether all data points were matched. Coherences were not normalized and analyzed similarly to the PSD. Both normalized PSD and coherences during baseline recordings and during the disruption protocol were compared.

A 2-way ANOVA for two independent variables (shocks vs days) was used to determine differences between the controls and rats receiving blue stimulation. Total number of shocks, entrances into the shock zone and time spent in each quadrant across 16 days were compared with a 1-way ANOVA with Tukey's multiple comparisons test used to compare each group. The 1-way ANOVA was also used to compare cell densities in groups of total cells (glia and neurons) and neurons in the hippocampus and MS. Data is present as mean $\pm$ standard error (SE). False detection rate was assessed using the Holm-Sidak statistic and the corrected p values are presented.

Results

A total of 25 rats (14 males/11 females) had the virus injected and had electrodes placed. Nine rats had visually confirmed regulation of hippocampal EEG with optogenetic stimulation; 16 rats either did not have regulation of EEG with stimulation ($n=6$) or had defective stimulating LEDs or recording electrodes ($n=9$). The following groups were studied: i) 1Hr optogenetic stimulation disruption with BL ($n=5$; 4 males/1 female, with visually confirmed optogenetic regulation of EEG); ii) 5Hr optogenetic stimulation BL ($n=4$; 2 males/2 females with visually confirmed optogenetic regulation of EEG); iii) 1Hr stimulation with yellow light (YL; 590 nm) ($n=6$; 4 males/2 females); iv) no light stimulation due to broken or defective implants ($n=6$; 4 males; 2 females).

Optogenetic MS stimulation of the MS results in Regulation of Hippocampal Oscillation Frequency

Optogenetic stimulation of the MS resulted in clear frequency-matched EEGs in CA1 of the hippocampus. At baseline P21–P25 rats demonstrate 5–7Hz theta when awake and exhibiting limited movement (Fig. 2A). As shown in Fig. 2A–C, stimulation at 5–12Hz resulted in dominance of on-going hippocampal oscillations by the optogenetic stimulation, i.e. MS stimulation at 8Hz resulted in 8Hz theta oscillations. PSD showed increased power and voltage correlations demonstrated well-modulated frequencies at the stimulated frequency. Stimulation at other than the “ramp” frequencies also dominated the hippocampal frequencies at the stimulation elicited at low and high frequencies (Fig. 3).

Normalized power measurement at baseline and during optogenetics stimulation (5–12Hz) showed increases in total, delta and theta, and to a lesser degree beta power in both the hippocampus and MS (Fig. 4). Coherences between the MS and hippocampus increased with ramp stimulation in total, theta and beta bandwidths (Fig. 5). Harmonics were noted with each of the stimulation frequencies. To demonstrate the harmonics for each of the stimulation frequencies the coherences were normalized across all frequencies for each rat and displayed in Fig. 5. Of note, subharmonics (frequencies less than the optogenetically-induced fundamental frequency) were not observed.

In the groups with no light or YL stimulation no changes were measured in the EEG following any of the frequencies studied. Normalized PSD and coherences across the total, delta, theta, beta and gamma oscillations showed no differences from baseline recordings (Suppl. Fig. 1).

During hippocampal disruption there was an increase in power and loss of amplitude correlations during the BL but not with the YL (Fig. 6). Significant differences were found in both normalized PSD and coherence with the BL stimulation, but not the YL stimulation when examined over 1 and 5Hr. The hippocampal disruption procedure significantly increased power and decreased coherence during the 1Hr or 5Hr stimulation (Fig. 7). Stimulation with no light or YL had no discernible effect on PSD, coherence or voltage correlations. No behavioral changes were noted during or after any of the stimulations.

Disruption of hippocampal oscillations during the critical impairment results in spatial cognitive deficits

There were group differences in number of shocks administered during active avoidance (Fig. 8A) with a 2-way ANOVA showing a significant interaction between shocks and trials ($F(15,270)=3.230$, $p<0.0001$) with a significant difference in groups ($F(2.613, 235.1)=25.41$, $p<0.0001$). Tukey’s multiple comparisons test showed significant differences between no light vs. 1Hr BL and 5Hr BL ($p<0.0001$) and YL vs 1Hr BL and 5Hr BL ($p<0.0001$) but no differences between 1hr and 5hr BL ($p=0.8209$) and No light and YL ($p>0.9999$). Total number of shocks and entrances were greater in the BL stimulated group compared to no light or YL. Group differences in total number of shocks were found (Fig. 8B) with the ANOVA showing a significant difference between groups ($F(3,332)=14.33$, $p<0.0001$) with Tukey’s multiple comparisons test showing significant differences between groups: no light

vs. 1Hr BL ($p < 0.0001$); no light vs. 5Hr BL ($p < 0.0001$), YL vs. 1Hr BL ($p = 0.0112$); and YL vs 5Hr BL ($p = 0.0013$). No differences were seen between no light vs YL or 1HR vs 5Hr BL. Group differences in number of entrances to the shock zone were also found (Fig. 8C) with an ANOVA showing a significant difference between groups ($F(3,332) = 11.72$, $p < 0.0001$) with Tukey's multiple comparisons tests showing significant differences between groups: no light vs. 1Hr BL ($p = 0.0048$); no light vs. 5Hr BL ($p < 0.0001$), and YL vs. 5Hr BL ($p = 0.002$). No differences were seen between no light vs YL or 1HR vs 5Hr BL.

Heat (dwell) maps showing the position of the rat during the active avoidance demonstrated that rats receiving no light or YL spent less time in the shock zone than animals receiving 1Hr or 5Hr of BL ($F(3,356) = 14.38$, $p < 0.0001$) with the Tukey's multiple comparison test showing the no light and YL groups spending significantly less time in the shock zone than both the 1Hr and 5Hr BL groups ($p < 0.0001$) (Fig. 9).

Disruption of hippocampal oscillations by optogenetic stimulation is not caused due to cell loss

NeuN and DAPI-labelled cells were counted in the hippocampus and MS to assess whether BL or YL caused neuron or total-cell loss, respectively. In the hippocampus (BL N = 5; YL N = 5; No-Stim N = 7), there was no difference in neurons ($p = 0.4857$) or total cells ($p = 0.4026$); suggesting that both neuron and glia population densities likely were unaffected by BL or YL stimulation of septohippocampal axons (Fig. 10). Similarly, in the MS (BL N=4; YL N=5; No-stim N=7), there was no measurable difference between neuron quantity ($p = 0.9331$) and total-cell quantity ($p = 0.2133$); demonstrating that direct exposure of MS cells to BL or YL does not induce cell loss (Fig. 10).

Discussion

There are two main findings in this study. First, non-selective optogenetic stimulation of the MS in weanling rats resulted in precisely regulated hippocampal oscillations. Secondly, the hypothesis that disruption of hippocampal rhythms during the critical period would result in subsequent spatial cognitive deficits was correct; rats receiving optogenetically-induced disruptive activity for either 1Hr or 5Hr from P22–P25 showed spatial cognitive deficits when tested at P60. Remarkably, this spatial cognitive impairment occurred in rats with limited periods of disruption. Only 1Hr a day of disruptive oscillatory activity for 4 days resulted in significant spatial memory deficits.

The hippocampal response to MS stimulation in weanling rats was precise and robust. As with previous studies in adult rats from our laboratory [34, 42] and others [46–49], optogenetic stimulation resulted in robust regulation of hippocampal oscillations with precise 1:1 entrainment of stimulation to hippocampal EEG at a wide range of frequencies. Stimulation at 5–12Hz resulted in increases in power in the theta bandwidths, but also in the delta and beta bandwidths in both hippocampus and MS electrodes. Coherence was likewise significantly increased in multiple bandwidths, most markedly at each stimulation frequency and its corresponding harmonics. As reported by others [50], these findings suggest that effects of optogenetic stimulation of the MS on hippocampal oscillations extend beyond the precise 1:1 stimulation to band frequency.

Aside from increases in power coherence at the stimulation frequency, there were concomitant increases of power at harmonics of the stimulation frequency. Harmonics have been noted previously in adult rats undergoing optogenetic stimulation in a variety of anatomical sites [34, 46, 50, 51]. Harmonics are neuronal in origin and play an integral role in generating the waveform of the EEG, although their biological significance is unclear [46]. Harmonics have been described as a Fourier decomposition of the waveform, rather than originating in a separate neuronal process or response [46]. Mouchati et al [42] showed that prominent 12 Hz harmonics following 6 Hz MS stimulation had no effect on theta phase preference of hippocampal neurons.

Voltage correlations showed precise modulation of the hippocampus at a 1:1 ratio to the MS stimulation at all frequencies studied. Taken together these findings demonstrate that optogenetic-stimulation of the MS in weanling pups results in precise temporal regulation of hippocampal rhythms.

Our goal of achieving disruption of hippocampal oscillatory activity was achieved. Our disruption protocol consisted of optogenetically-induced physiological frequencies administered randomly over a course of 1 or 5Hr resulting in temporal disruption of oscillations. When examined over the entire 1 or 5 hrs this scrambled stimulation paradigm resulted in significant increases in power, reductions in coherence and marked alterations in voltage correlations compared to the no light or YL-stimulated animals during the same time period. The mechanism by which disruptive stimulation for as little as 1Hr a day resulted in long-standing cognitive impairment is not known. It is established that in the mature hippocampal network theta activity modulates the firing of action potentials [52, 53]. For example, hippocampus place cells, a subset of pyramidal neurons in the hippocampus that fire action potentials that correspond to the animal's location within its environment, have a precise temporal firing relationship within hippocampal theta oscillations [54, 55]. This temporal coding of action potentials generated by theta underpins mnemonic and spatial memory [56–59]. It seems likely that the disruptive oscillations interferes with temporal coding of grid and place cells which emerge during the critical period of memory development [14, 60–64].

In this study, the disruptive stimulation was administered at a time when the animals were predominately awake and not involved in any tasks requiring cognitive demand. It is known that the interplay between MS-generated oscillations and its downstream target, the hippocampus, is dynamic and complex [65, 66]. The temporal organization of cell firing in the hippocampus is paced by theta oscillations and the timing of cell activity occurs in relation to behavioral variables such as speed [34, 67, 68], novelty [69, 70] and spatial and cognitive demands [42, 44, 71, 72]. Likewise, there is an interaction between optogenetic-induced MS oscillations and hippocampal oscillations. Using low-theta frequency MS optogenetic stimulation administered to rats performing a spatial accuracy task, we found that hippocampal receptivity to MS stimulation and performance in the task was determined by speed and task demands [71]. Whether disrupting hippocampal oscillations during the critical period when the animal is engaged in a cognitive task or while asleep, at a time memory consolidation occurs, has long term consequences is not known, but warrants further investigation.

This study has several limitations. This study is descriptive and does not provide a mechanism by which disruptive oscillations impairs spatial cognition. Future work should evaluate how cells such as grid and place cells and oscillatory activity come “on-line” as well as the temporal coordination of cell firing with theta following disruptive stimulation [27, 44]. Since disrupted hippocampal oscillations were administered during only one age range, we cannot say whether stimulation at other ages would have had the same or different effects. While the hippocampal and MS electrodes remained in place while the animals matured, EEG recordings at the time active avoidance was done were not of sufficient quality to be analyzed due to electrode movement or dysfunction because of the length of time the electrodes were in place. Assessing long-term changes in the EEG following hippocampal disruption warrants investigation but will require additional surgery or a different study design.

There are clinical indications that aberrant oscillatory activity during the critical period of memory development can have long-lasting changes on spatial cognition. In children, allocentric spatial memory abilities in children emerge around 22 months of age and are in place by the age of 4 years [73–76]. There are many genetic and acquired disorders occurring during this critical period that are associated with impaired learning and memory. It is known that early-life seizures, which cause massive bursts of synchronized network activity, occurring prior to or during the critical period can have profound effects on learning and memory [77–79]. Clinical studies have found that cognitive outcome in children with epilepsy is related more to EEG background frequencies than seizures or epileptiform activity [80, 81]. For example, there is evidence that Dravet syndrome, a childhood epilepsy disorder associated with devastating effects on cognitive development, the strongest predictor of cognitive outcome is not frequency or duration of seizures but abnormal hippocampal EEG rhythms during the critical period of memory development [82–85]. While extrapolation of results from weanling rats to children is very difficult, our results raise the possibility that disruption of normal oscillations contributes to adverse outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Optogenetic stimulation of the medial septum in rat pups results in precise EEG regulation.
- Optogenetic stimulation can severely disrupt normal oscillatory activity.
- Disrupting endogenous EEG activity during the critical period impairs spatial cognition.
- Cognitive dysfunction after optogenetic disruption of EEG activity is not due to cell loss.

Fig. 1.

Timeline of the experiments. Following injection of the virus with the channelrhodopsin and placement of electrodes and optic probe the rats had optogenetic stimulation from 5–12Hz (ramp stimulations). Note the spectrogram with the optogenetic stimulation frequencies and harmonics during the “ramp” stimulations. Random optogenetic frequencies were then administered using BL for 1 or 5Hr or YL which resulted in no change in oscillations. Animals were then tested in active avoidance when they were mature.

Fig. 2A-C.

Baseline and optogenetic induced frequencies from 5–12Hz. For each frequency EEG is shown in the top panel, power spectrum and voltage correlations in the bottom panels and spectrograms on the right. The EEG time scale is in seconds. The spectrum is from 1–50Hz. Spectral power is color coded with hot colors represent higher power Arrows show the optogenetically-stimulated bandwidths.

Fig. 3.
Spectrograms from low and high frequency stimulations. Top panel – 0.5, 1 and 2 Hz.
Bottom panel – 55, 70 and 110 Hz. Arrows show the stimulated bandwidths.

Fig. 4. Normalize power at baseline and during optogenetics stimulation (5–12Hz) in hippocampus (A) and MS (B) (^p<0.05; +p<0.01; #p<0.001; *p<0.0001). Note that total power increased in both the hippocampus and MS with stimulation. Significant increases in power were seen at frequencies in the delta and theta, and to a lesser degree beta bandwidth.

A

B

Fig. 5.

Coherences between hippocampus and medial septum. A. Coherences baseline and during ramp stimulations (^ $p<0.05$; + $p<0.01$; # $p<0.001$; * $p<0.0001$). B. Normalized coherences for each of the ramp frequencies demonstrating the harmonics.

Fig. 6. Effect of hippocampal disruption on hippocampal EEG. PSD (A), voltage correlations (B) and spectrogram (C) of EEG during yellow light (A-C). PSD (D), voltage correlation (E) and spectrogram (F) during blue light stimulation. The figures are examples from rats undergoing one hour of YL stimulation or 1Hr of BL stimulation.

Fig. 7.

Effect of disruption protocol on normalized power (A) and coherence between the MS and hippocampus (B) during 1Hr or 5Hr BL stimulation. Oscillatory properties were compared between baseline and during 1Hr and 5hr BL stimulation (* $p < 0.0001$, corrected for false detections).

Fig. 8.

Results of active avoidance task in rats with and without prior optogenetic stimulation. A. Rats that received either 1Hr or 5Hr of BL stimulation received more shocks than rats with YL or no stimulations. B. Total shocks in controls (CTL (No light or YL) and BL (1Hr and 5Hr) groups. C. Total entrances into the shock zone in the (CTL (No light or YL) and BL (1Hr and 5Hr). The two symbols above the GL refers to p value of the no light and YL, respectively (^ $p < 0.05$; + $p < 0.01$; # $p < 0.001$; * $p < 0.0001$).

Fig. 9.

Heat (dwell) map of rat's position in the active avoidance task. A. Top, cartoon of active avoidance apparatus with shock zone in red. Bottom, Heat (dwell) map. Hot/warm colors indicate where the rat spent most of the session (Target = shock zone; CCW – quadrant counterclockwise to the shock zone; OPP – quadrant opposite of the shock zone; CW – quadrant clockwise to the shock zone). B. Examples of heat maps for each of the groups. Rats receiving no light or YL spent most of their time next to the wall in the quadrants outside of the shock zone whereas rats receiving disruptive hippocampal stimulations had no strategy in avoiding the shock zone, visiting many areas of the cylinder, including the shock zone.

Fig. 10.

Histology and cell counts. A. MS showing Yellow Fluorescent Protein (YFP) (green) which is expressed by the viral vector and fused to the CHR2 protein and DAPI (blue) stain of nuclei. B. Hippocampus contralateral to the implant stained with DAPI. Septal axons that are expressing ChR2-YFP (green) are seen throughout the projecting axons in the stratum oriens (arrow). C. MS showing YFP (green), DAPI (blue) and NeuN (red) which stains mature neurons. D. Hippocampus contralateral to the implant stained with YFP, DAPI and neuN. No cell loss was noted with either BL, YL or no stimulation. Scale bar is 500µm. Cell densities are shown in E (a – hippocampus using DAPI stain; b - hippocampus using NeuN stain; c – MS using DAPI stain; d. - MS using NeuN stain).