The Role of Brain Oscillations in Memory Processing in Siberian Hamsters
Adrienne Thom
May 2016
The Role of Brain Oscillations in Memory Processing in Siberian Hamsters

An Honors Thesis Submitted to
the Department of Biology
in partial fulfillment of the Honors Program
STANFORD UNIVERSITY

by
Adrienne Thom
May 2016
The Role of Brain Oscillations in Memory Processing in Siberian Hamsters
by
Adrienne Thom

Approved for submittal to the Department of Biology
for consideration of granting graduation with honors:

Research Sponsor
H. Craig Heller [Signature] Date 5/2/16

Second Reader
Russell Fernald [Signature] Date 5/3/16
Acknowledgements

I would like to thank Dr. Craig Heller and Dr. Russell Fernald for their insights and helpful edits during this process. I would also like to thank Dr. Norman Ruby for sparking my interest in neuroscience and teaching me all I know about research. Lastly, I would like to thanks Dr. Jamie Imam for her kindness and support throughout my time at Stanford.
Table of Contents

I. LIST OF FIGURES .................................................................................................................. 6
II. ABSTRACT .............................................................................................................................. 7
III. INTRODUCTION .................................................................................................................. 8-10
IV. MATERIALS AND METHODS ............................................................................................ 11-14
V. RESULTS ............................................................................................................................... 15-17
VI. DISCUSSION ......................................................................................................................... 17-21
VII. FIGURES ............................................................................................................................ 22-27
VIII. BIBLIOGRAPHY .................................................................................................................. 28-30
List of Figures

Figure 1: Novel Object Exploration Elicits Theta

Figure 2: Power Spectrum Analysis
   a. ENT Power Spectrum
   b. DPS Power Spectrum
   c. SCNx Power Spectrum

Figure 3: Theta Delta Ratio and Theta Dominated Epochs
   a. Theta Delta Ratio
   b. Number of Theta-Dominated Epochs

Figure 4: Peak Theta Frequency and Power
   a. Peak Theta Frequency
   b. Peak Theta Power

Figure 5: Bout Duration and Fragmentation
   a. Theta-Dominated Average Bout Duration
   b. Theta Delta Ratio Binary

Figure 6: High Gamma Power
   a. ENT Power Spectrum (Gamma)
   b. DPS Power Spectrum (Gamma)
   c. SCNx Power Spectrum (Gamma)
   d. High Gamma Power
Abstract

This study investigated the role of the suprachiasmatic nucleus (SCN) in the expression of hippocampal theta rhythms by electroencephalographic (EEG) recordings in freely behaving Siberian hamsters (*Phodopus sungorus*) in response to novel object exploration. The SCNs of these animals were made circadian-arrhythmic in one of two ways. The first way involved a disruptive phase shift (DPS) protocol that induced arrhythmia while leaving the animals neurologically and genetically intact. The DPS protocol consisted of administering a phase-advancing light signal on one night and a phase-delaying signal on the following night. The second method made hamsters arrhythmic through surgical ablation of the SCN (SCNx). Hippocampal theta rhythms are necessary for encoding spatial information and for providing spatiotemporal information to hippocampal place cell assemblies. Because DPS-arrhythmic Siberian hamsters exhibit substantial impairments in hippocampal-dependent memory tests, we recorded EEG signals during an object exploration task and investigated how certain properties of their EEG signals differed from SCNx and entrained (ENT) animals. Deficits in cognitive performance caused by disrupted circadian timing have become a growing concern among health care professionals. Additionally, identifying EEG markers of impaired memory may benefit clinicians as an early non-invasive screening technique to identify early stages of mild cognitive impairment (MCI) or Alzheimer’s.
Introduction

Circadian rhythms are endogenous daily cycles that provide daily timing cues to an organism and coordinate physiological processes over long time scales. These rhythms originate in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus, which is a bilateral structure comprised of approximately 20,000 neurons\(^1\). SCN cells are autonomous oscillators that are synchronized to produce a rhythm that is close to, but not exactly, 24 hours\(^2\). These rhythms are synchronized to the environment by daily exposure to light so that the organism’s internal rhythms match the day-night cycle. All living organisms, except for a few species that live at high northern latitudes, have circadian timing mechanisms\(^3\). The core of the cellular oscillator in the SCN consists of a number of “clock” genes. Although the specific genes differ across taxonomic categories, the basic feedback mechanism of the clock has been conserved across evolutionary history. Because this function is so highly conserved, it is thought that clocks have a fundamental role in biological systems that ultimately conserve energy by allowing organisms to anticipate rather than react to changes in their environment\(^4\).

Because of their fundamental role in biological systems, circadian clocks influence all major physiological systems in animals, including learning and memory. Rhythms are known to affect memory in three different ways. First, performance on memory tests varies throughout the day within each species. For example, when mice are trained in a task, they have peak performance times at 24 hour intervals following the learning phase, indicating a link between time of day and performance on a task\(^5\). Second, disruptions in circadian rhythms in humans, such as shift workers or people who experience jet lag, lead to memory deficits and impairment in cognition\(^6\). Third, recent clinical studies have shown that as we age, most people experience a breakdown in circadian timing, which can lead to mild cognitive impairment. In cases where
there is already some cognitive impairment, the breakdown in circadian timing accelerates the onset of Alzheimer’s disease\textsuperscript{7,8,9,10,11}. Thus, there is great interest in elucidating how the circadian system interacts with neural structures that control learning and memory.

The mechanism by which the SCN regulates and affects learning and memory is not well studied, but the most probable mechanism is through the septo-hippocampal pathway. Most of the SCN projections target the hypothalamus area immediately surrounding the SCN with very few projections extending outside of the hypothalamus. However, the SCN does project outside of the hypothalamus to the septal nuclei, which in turn project to the hippocampus, specifically the dentate gyrus\textsuperscript{12}. The dentate gyrus is the main target of input pathways to the hippocampus, and is one of the only areas of the brain that undergoes adult neurogenesis and is thought to play a role in the formation of new memories. Thus, the SCN has the potential to directly influence the septo-hippocampal pathway and alter memory function, and yet, no one has ever studied this connection. This pathway is comprised of both excitatory (cholinergic, glutamatergic) and inhibitory (GABAergic) projections. The excitatory pathways stimulate the hippocampus’ Cornu Amonis (CA) fields, which are necessary for memory consolidation, whereas the inhibitory pathways determine the frequency and phasing of theta oscillations\textsuperscript{13,14}.

Theta oscillations are brain waves that coordinate information processing across large brain regions. These waves oscillate intrinsically in the 5-12 Hz range and are generated by the medial septum. The connection between the medial septum and the dentate gyrus (as well as the reciprocal connection from the hippocampus) forms the core of theta oscillations in the brain. Theta waves are associated with complex behaviors and are required for spatial exploration, working memory, and navigation. These oscillations are responsible for moving information across brain regions and integrating learned information between the hippocampus and cortex\textsuperscript{15}. 
Short-term memory tasks elicit theta rhythms in waking animals, and theta activity is also seen during memory retrieval. Additionally, studies have shown a link between theta oscillations during task performance and cognitive impairment. Specifically, cognitively impaired individuals (mild cognitive impairment (MCI), Alzheimer’s) show a decrease in phasic (event-related) theta activity\textsuperscript{16,17}.

Another important class of oscillations in the brain is gamma oscillations, which coordinate interactions between the hippocampus and prefrontal cortex\textsuperscript{18}. Additionally, gamma oscillations are linked with working memory maintenance as well as long-term memory formation\textsuperscript{19,20,21}. Gamma oscillations co-occur with theta oscillations and may be theta regulated\textsuperscript{22}.

The central thesis of my project is that disruptions in circadian timing impair memory by interfering with the expression of theta oscillations. Specifically, disrupted SCN activity may impair neural transmission from the medial septum, which in turn could affect the inhibitory pathways that provide rhythmic stimulation (theta rhythms) to the dentate gyrus. To investigate this possibility, I used an animal model of circadian arrhythmia and recorded theta rhythms while the animals explored a novel object in their home cages. In mice and rats, circadian rhythms can only be eliminated by surgical ablation of the SCN or by clock gene knockouts; however, neither of these manipulations impair memory. In contrast, Siberian hamsters (\textit{Phodopus sungorus}) that are circadian-arrhythmic but have a genetically and structurally intact SCN have severe memory deficits. I used this species for my project because they can be rendered arrhythmic via a non-invasive environmental light exposure method\textsuperscript{23}. Furthermore, I was able to compare the impact of circadian arrhythmia in these animals to those made arrhythmic by surgical ablation of the SCN.
Materials and Methods

Animals and Housing Conditions

Breeding of the Siberian hamsters (*Phodopus sungorus*) was done in a 16:8-hour light-dark (LD) cycle at an ambient temperature of 22°C. After weaning, males and females were separated and housed two to four per cage. At the beginning of the experiment, animals that were 2-3 months of age were housed individually with food and tap water available ad libitum and given cotton batting for nesting. Passive infrared motion detectors were used to record activity of the hamsters. Activity bout summaries, which can be used to assess rhythmicity, were compiled and stored every 10 minutes. All experimental procedures were approved by Stanford University’s Administrative Panel on Laboratory Animal Care (Animal Use Protocol Number: 14988) and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Disruptive Phase Shift Protocol

A disruptive phase-shift (DPS) protocol was used to eliminate circadian timing. Animals were housed individually for fourteen days to establish baseline activity levels and confirm that activity was entrained to the LD cycle. Once confirmed, the lights in the activity recording chambers were turned on for 2 h beginning 5 h after lights-off (i.e., a 2-h light pulse). The next day, the LD cycle was phase delayed by 3 h so that dark onset occurred 3 h later than on the previous night. The animals remained in this LD cycle (16:8) from then on with continuously recorded locomotor activity. Four weeks after the DPS protocol was initiated, animals’ activity records were screened to determine which animals were circadian-arrhythmic. Arrhythmia was confirmed by visual inspection of their actograms and quantified by time-series
analysis with a chi-square periodogram (ClockLab, Actimetrics, Evanston, IL). Any periodogram peaks that exceeded the 99.5% confidence interval were deemed significant and confirmed the presence of circadian rhythms. To establish arrhythmia, the periodogram must lack any significant peaks, and locomotor activity had to be evenly distributed during the day and night. Arrhythmia induced in this way does not affect the sleep cycle, allowing us to disregard disruption of the sleep cycle as a cause for memory impairment. Additionally, arrhythmicity lasts indefinitely even once returned to a normal daily light-dark cycle.

**SCN Lesion Surgery**

Animals were anesthetized with a cocktail of 100 mg/kg ketamine and 5 mg/kg xylazine. The skull was secured and held level in a stereotaxic device. Two small holes were drilled in the skull and a stainless steel electrode was lowered into the brain, targeting the SCN. Lesions were made by passing a 6 mA current through the tip of a stainless steel electrode for 10 s bilaterally. Electrodes were insulated except for 0.1 mm at the tips. Lesion coordinates were 1.0 mm anterior to bregma, ±0.2 mm lateral to the sagittal sinus, and 6.9 mm ventral to dura. The animals were administered meloxicam (5 mg/kg) as an analgesic during recovery from surgery. Post-surgery, locomotor activity was monitored in the animals’ home cages to determine whether or not the animals were arrhythmic.

**EEG Implant Surgery**

Animals were anesthetized as described above and held in a stereotaxic frame. Two electrodes (0.4mm screw) were soldered to a multichannel electrical connector and screwed into the skull through two holes (0.4 mm) located in the frontal (1 mm lateral and anterior to bregma)
and parietal (1 mm lateral to the midline at the midpoint between the bregma and lambda) cortices. This assembly was anchored to the skull using Super6-Bond (Sun Medical Co., Shiga, Japan) and dental cement.

**EEG Recording**

After recovery from EEG surgery, the hamster’s implant was connected to an electrical cable above the cage. Embla, an EEG recording software, recorded EEG signals from the animals. The EEG signal was amplified, passed through an analog-to-digital converter sampling at 200 Hz, and digitally filtered and separated based on frequency range (EEG, 0.5 – 40 Hz). The power spectra of the EEG signal were subjected to a fast Fourier transformation (FFT) analysis using 4 s epochs to determine the peak spectral component, which was then used to quantify theta activity. A mean EEG spectrum was obtained for baseline and exploration for each animal by averaging spectra of all 4 s epochs of the considered state. Total EEG power was calculated for each state as the sum of EEG densities in each frequency bin. Theta activity was defined as EEG power density in the 5-8 Hz range while delta activity corresponded to the 1-4 Hz range. The ratio of power in the theta and delta (T:D) ranges was used to quantify theta activity. For each band, the power was calculated as the sum of the corresponding power densities. For each individual and state, T:D was calculated and then submitted to further statistical analysis.

**Experimental Protocol**

Two groups of animals were used (n=8), 2-3 months old at the start of the experiment. The first group of entrained (ENT) animals underwent the EEG implant surgery described above, recovered for 4 weeks before EEG recording during periods of novel object exploration.
(described below). The ENT group was then made arrhythmic using the DPS protocol described above, and allowed 4 weeks to establish arrhythmia. EEG signals of these now arrhythmic (ARR) hamsters were recorded during novel object exploration. The second group of animals underwent a SCN lesion surgery as described above. After 4 weeks of recovery, animals with confirmed circadian arrhythmia underwent an EEG implant surgery and were allowed to recover for another 4 weeks. These SCNx animals were then recorded during novel object recognition.

**Novel Object Exploration**

Each hamster’s skull implant was connected to a lightweight recording cable and the hamster was allowed to habituate for 4 days, during which time the experimenter interacted with the animals by gently handling them in their cages so that the presence of the experimenter would not be a novel experience on the day of testing. On the 5th day (test day), the animal’s baseline data were recorded for 15 minutes, at which point a novel object (small, closed tea strainer with apple enclosed) was placed into the center of the cage. The experimenter stood 4 feet away from the cage, and observed the animal during the entirety of the EEG recording. The animals were allowed to explore the object for 5 minutes.

**Statistical Analysis**

The effects of exploration on various EEG parameters were compared against baseline measures using a T-test. Bouts of exploration were compared across all three groups of animals using a one-way ANOVA with Dunnett’s post-hoc correction applied for pair-wise comparisons.
Results

Efficacy of Novel Object Exploration in Eliciting Theta

Power spectra were obtained through FFT analysis and total power was calculated by averaging the power in each frequency range (in .25Hz buckets). For each bucket, the power was calculated as a percent of the total power. Delta was defined as 1-4 Hz while theta was defined as 5-8 Hz. The first goal of this study was to determine a protocol that would successfully elicit theta during exploration of an object. As seen in the power spectra of entrained (ENT) animals (Fig.1), the novel object exploration protocol used in this study successfully elicits theta in the EEG of these animals during the exploration period. Additionally, there is a decrease in delta power during exploration, consistent with previous findings.

Power Spectrum Analysis

All three experimental groups (ENT, DPS, and SCNx) were able to elicit theta during exploration of the novel object (Fig. 2A, 2B, 2C). Both ENT and DPS animals also showed a decrease in delta power during exploration as compared to baseline. In SCNx animals, the baseline data differed from ENT and DPS, showing a more flattened appearance due to variation in baseline state (theta, delta) (Fig. 2C).

Theta Delta Ratio and Theta-Dominated Epochs

Theta to delta ratio (T:D) was calculated by taking the ratio of average theta power to average delta power during the experimental phase (BL or EXP). Given that theta and delta rhythms are interdependent, the ratio is a good indicator of theta domination. Epochs (4 seconds) were defined as theta dominated if the T:D ratio for that epoch was greater than one. All three
experimental groups showed a significant increase in T:D ratio from the baseline to exploration phase (ENT, SCNx p < 0.05, DPS p < 0.01; Fig. 3A). Additionally, all groups showed a significant increase in the number of theta dominated epochs from the baseline to exploration (ENT, SCNx p < 0.05, DPS p < 0.01; Fig. 3B).

**Peak Theta Frequency and Power**

Peak theta frequency was calculated as the frequency within the theta range that had the highest power in the power spectrum for each experimental phase (BL or EXP). Peak theta power was calculated as the power of the peak theta frequency. Both the DPS and SCNx groups showed a significant increase in peak theta frequency from baseline to exploration (p < 0.05; Fig. 4A). However, in ENT animals, there was no significant peak frequency shift due to the higher peak frequency seen in baseline of this group compared to DPS and SCNx animals. Even though there was no significant shift in peak theta frequency in ENT animals, there was a significant increase in peak theta power between baseline and exploration (p < 0.05; Fig. 4B). The DPS and SCNx groups did not show a significant shift in peak theta power.

**Bout Duration and Fragmentation**

A “bout” was defined as one or more theta dominated epochs (4 seconds per epoch) and the bout duration was expressed in seconds. ENT animals showed a very significant increase in bout duration during the exploration phase as compared with the baseline (p < 0.01; Fig. 5A). DPS animals also showed a significant increase during exploration (p < 0.05; Fig. 5A), whereas SCNx animals did not. Both the DPS and SCNx groups showed a significant decrease in theta bout duration during exploration as compared with ENT animals (F (2,18) = 3.523, p < 0.05; Fig.
5A). This is visually seen when the T:D ratio is graphed as a binary. ENT animals show longer sustained theta dominated bouts (Fig. 5B), while DPS and SCNx animals have much shorter bouts during exploration, termed fragmentation (Fig. 5C, 5D).

Effect of Object Exploration on Gamma Oscillations

High gamma power was calculated in the same way as theta power above, with gamma defined in the 70-90Hz range. ENT animals showed a significant increase in high gamma power during exploration, indicating successful long-term communication between brain regions (p < 0.05; Fig. 6A, 6D). SCNx animals also showed this significant increase during exploration (p < 0.05; Fig. 6 B, 6D). However, DPS animals do not show a significant increase in high gamma from baseline to exploration (Fig. 6 C, 6D).

Discussion

A malfunctioning SCN impairs memory processing, and this research represents a first attempt to propose a mechanism that explains this effect. Because the SCN innervates the septal nuclei, I investigated whether changes in SCN function could impair neural function in the septohippocampal pathway. To achieve this goal, I examined theta oscillations in animals that were made circadian-arrhythmic by two different methods that either left the SCN intact (DPS-ARR) or ablated this structure (SCNx-ARR). Through a comparison of the DPS animals to the ENT and SCNx animals, I determined which EEG characteristics are consisted with impaired memory function. My main finding was that DPS-ARR animals’ EEG showed a blunted increase in gamma power during object exploration compared to ENT and SCNx animals. This suggests that an arrhythmic SCN may inhibit gamma oscillations in the hippocampus via the septo-
hippocampal pathway, ultimately leading to decreased cortical communication and long term memory formation.

To identify the influences of the SCN on the septohippocampal pathway, I first characterized ARR animals’ EEG parameters, including theta and delta power as well as the theta:delta ratio. If this ratio was greater than one, then a bout was considered “theta dominated,” indicating substantial theta activity (a hallmark of spatial memory encoding). Additionally, I quantified the number of epochs that were theta dominated as well as the average duration of those bouts (sections of continuous theta-dominated epochs). I also quantified the peak theta frequency and the power of that frequency for both the baseline and exploration phases. Lastly, I looked at the high gamma frequency (70-90 Hz), which has been linked to higher prefrontal cortex activity and long term encoding of hippocampal information. ENT animals showed low theta:delta ratio in baseline (BL), which substantially increased during exploration (EXP) by an increase of theta power and a reduction of delta power. This result is consistent with previous studies linking an increase in theta power with memory encoding. Similarly, during EXP there were many long bouts of theta-dominated epochs as well as an increase in the high gamma power, indicating prefrontal cortex activity. The peak theta power also showed the characteristic increase during exploration. However, the peak theta frequency did not shift significantly higher, which was unexpected given that theta frequency shift is a marker of increased memory load. Overall, our experimental setup successfully elicited theta during novel object exploration in these control animals.

The DPS-ARR animals have an intact but dysfunctional SCN which renders them arrhythmic. Additionally, they have been shown to perform poorly on memory tests. These animals were subjected to the same protocol as the ENT animals, including analysis of their
EEGs during BL and EXP. The DPS and ENT animals displayed the same engaging behavior during the novel object exploration. Additionally, DPS animals were able to elicit theta and suppress delta during exploration, leading to a similar increase in T:D ratio as seen in the control (ENT) animals, indicating that these animals had no trouble generating theta. However, DPS animals did not show a significant increase in peak theta power during exploration as seen in the control animals, indicating that DPS animals are unable to generate as high a theta power. Additionally, although the number of theta-dominated epochs during EXP was roughly the same as ENT animals, the DPS group had significantly shorter theta dominated bouts during exploration as compared to ENT. These data hint that a normally functioning SCN may be necessary to elicit high and sustained theta power during exploration. Given that the number of theta dominated epochs is the same in DPS as ENT, arrhythmicity likely does not affect the exploratory behavior or the total time spent encoding information. High gamma was not increased during EXP in DPS animals, in contrast to the ENT animals, demonstrating decreased prefrontal cortex activity and possibly a lack of memory information transfer to long-term storage. These data indicated that there might be a disruption in encoding ability due to the DPS animals’ inability to sustain high power theta as well as a diminished ability to transfer information to the cortex.

The ideal intermediate experimental group would be animals that are arrhythmic but have intact memory. This would allow us to specifically identify which EEG markers are linked to impaired memory and which are a by-product of arrhythmicity. SCNx animals fall into this category: many previous studies have shown SCNx-ARR animals to have intact and, in some cases, improved memory. In 1996, Mistlberger et al. showed in rats that SCN ablation did not impair learning and SCN ablated rats performed as well as intact SCN rats on the T-maze
discrimination task\textsuperscript{36}. Additionally, in 2014, Fernandez et al. showed that SCN lesioned hamsters performed just as well as ENT on novel object recognition and spontaneous alternation tests\textsuperscript{37}. In 1978 Stephan et al. showed that SCN lesioned rats did not show the same deficit in retention of test performance at 18 and 30 hours post-training that ENT animals did, indicating that in some cases, SCN ablation may improve memory\textsuperscript{38}. Through analyzing SCNx-ARR animals EEGs, we were able to identify possible markers that are linked to intact memory, which are shared by the SCNx-ARR and ENT animals and are impaired in the DPS-ARR animals.

The SCNx animals showed power in theta and delta similar to that of ENT and DPS animals in both BL and EXP. Additionally, the T:D ratio in these animals increased significantly during exploration, consistent with the data from ENT and DPS animals. Similar to the DPS animals, the SCNx group did not show a significant increase in peak theta power during exploration and showed significantly shorter theta dominated bouts duration during exploration as compared to ENT animals. Combined with the DPS data, this indicates that a fully functioning, rhythmic SCN is required for high, sustained theta during exploration of a novel object. Nonetheless, these attributes may not be necessary for successful memory encoding given that SCNx animals lack high, sustained theta while having intact memory. Lastly, similar to ENT animals, these SCNx animals showed a significant increase in high gamma power during exploration as compared to baseline. This is in contrast to the lack of a significant increase in gamma power seen in the DPS animals. Given that SCNx and ENT animals have intact memory formation while DPS animals do not, these data indicate that high gamma may be the key player in successful memory encoding. High gamma has been linked repeatedly to long-term memory formation and communication between the hippocampus and the prefrontal cortex. This lack of gamma increase during exploration in the DPS animals may reflect a lack of cortical activity.
Potentially, chronic GABA-ergic output from an arrhythmic SCN may inhibit the long distance transfer of information via suppression of gamma oscillations.

The dramatic lack of gamma power in DPS animals was an unexpected result, and one that will require future research to understand. Gamma plays an important role in the spatial and temporal organization of hippocampal place cells, which have spatially linked firing (they fire when the animal is in a specific location). Phase precession is a phenomenon in which a place cell fires earlier and earlier with respect to the phase of the underlying theta cycle as the animal gets closer to the cell’s place field. Gamma is involved in both this theta phase precession phenomenon as well as the sequence retrieval of place cell information. To better understand gamma’s role in memory formation and spatial navigation, it would be useful to gather EEG information directly from the hippocampus of ENT, DPS, and SCNx animals. The theta-gamma relationship during memory and spatial tasks could be monitored with an electrode placed in the hippocampus.

There has been a recent push in Alzheimer’s disease research to develop EEG marker screening for early identification of cognitive decline. Specifically, studies have linked decreased theta during memory tasks to cognitive impairment. This opens the doors for developing tools to screen for cognitive decline earlier in life and potentially identify early MCI and Alzheimer’s patients. The ARR-DPS animals used in this study may be good models for developing these tools, given their decreased theta during exploration combined with their poor performance on memory tasks. Through further comparison of ARR-DPS to ARR-SCNx we may be able to identify the specific EEG theta markers that are linked with cognitive decline and may be used as a screening process for early MCI/Alzheimer’s patients.
Fig. 1. Novel Object Exploration Elicits Theta. Power is given for each frequency bin and was calculated as the percent of total power in that bin relative to the total power in the EEG spectrum from 0-100 Hz. Delta is defined as 1-4Hz. Theta is defined as 5-8Hz (n =8).
Fig. 2. Power Spectrum Analysis. A novel object elicited increases in theta and decreases in delta for (A) ENT, (B) DPS, and (C) SCNx animals (n=8).
Fig. 3. Theta Delta Ratio and Theta-Dominated Epochs. White bars represent baseline (BL), black bars represent exploration (Exp). (A) ENT, DPS, and SCNx animals show a significant increase in theta:delta ratio during exploration compared to baseline (* p < 0.05, ** p < 0.01, n = 8). (B) ENT, DPS, and SCNx animals show a significant increase in number of theta dominated epochs during exploration compared to baseline (* p < 0.05, ** p < 0.01, n = 8).
Fig. 4. Peak Theta Frequency and Power. White bars represent baseline (BL), black bars represent exploration (Exp). (A) DPS and SCNx animals show a significant increase in peak theta frequency during exploration compared to baseline (p < 0.05, n = 8). (B) ENT animals show a significant increase in peak theta power during exploration compared to baseline (p < 0.05, n = 8).
Fig. 5. Bout Duration and Fragmentation. White bars represent baseline (BL), black bars represent exploration (Exp). (A) ENT and DPS animals show a significant increase in theta dominated bout duration during exploration compared to baseline (* p < 0.05, n = 8). DPS and SCNx animals show a significant decrease in bout duration during exploration compared to ENT bout duration during exploration (# p < 0.05, n = 8). (B), (C), and (D) show plots from individual animals. (B) ENT animals show less fragmentation during exploration phase (after red bar) compared with DPS (C) and SCNx (D) animals. Red bar indicates placement time of novel object.
Fig. 6. Effect of Object Exploration on Gamma Oscillations. The power was calculated the same as for theta and delta. Gamma is defined as 70-90 Hz. (A) ENT and (C) SCNx animals show a large increase in high gamma power during exploration as compared to baseline. (B) DPS animals show only a slight increase in high gamma power during exploration. White bars represent baseline (BL), black bars represent exploration (Exp). (D) Both ENT and SCNx animals show a significant increase in gamma power during exploration as compared to baseline ($p < 0.05$, n=8).
Bibliography


9 Ying-Hui Wu and Dick F. Swaab, “Disturbance and Strategies for Reactivation of the Circadian Rhythm System in Aging and Alzheimer’s Disease,” Sleep Medicine, Circadian Rhythms in Sleep Medicine, 8, no. 6 (September 2007): 623–36.


19 Marc W. Howard et al., “Gamma Oscillations Correlate with Working Memory Load in Humans,” *Cerebral Cortex* 13, no. 12 (December 1, 2003): 1369–74.


21 Esther Berendina Meeuwissen et al., “Evidence for Human Fronto-Central Gamma Activity during Long-Term Memory Encoding of Word Sequences,” *PLOS ONE* 6, no. 6 (June 29, 2011).


31 Roux et al., “Gamma-Band Activity in Human Prefrontal Cortex Codes for the Number of Relevant Items Maintained in Working Memory.”
32 Meeuwissen et al., “Evidence for Human Fronto-Central Gamma Activity during Long-Term Memory Encoding of Word Sequences.”
33 Klimesch, “EEG Alpha and Theta Oscillations Reflect Cognitive and Memory Performance.”
35 Ruby et al., “Spatial Memory and Long-Term Object Recognition Are Impaired by Circadian Arrhythmia and Restored by the GABAA Antagonist Pentylenetetrazole.”
37 Fernandez et al., “Dysrhythmia in the Suprachiasmatic Nucleus Inhibits Memory Processing.”
39 Colgin, “Mechanisms and Functions of Theta Rhythms.”
41 Klimesch, “EEG Alpha and Theta Oscillations Reflect Cognitive and Memory Performance.”