Hippocampal Theta and Gamma: Effects of Aging, Environmental Change, Cholinergic Activation and Learning

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Abstract

During aging, hippocampal functioning is impaired; specifically aged humans and rats show reduced performance on spatial memory tasks. An age-related reduction in the neurotransmitter acetylcholine has been postulated to underlie this impairment. Rhythmic oscillations (theta, gamma) may serve to synchronize activity within the hippocampus and across the brain during learning; these may also change with aging.

To determine what aspects of oscillation are important for memory processing, the effects of aging, encountering a novel situation, learning a new task and cholinergic system activation (with physostigmine) were examined. Both age groups showed increased theta, but not gamma activity when encoding a novel situation. Activating the cholinergic system shifted theta power to a lower frequency with no effect on gamma. These results indicate a more prominent role for theta than gamma in processing new spatial information.

In addition, a behavioral paradigm to study changes in hippocampal theta and gamma over trials during single-day learning of a place or response task was designed. Pilot data demonstrates that rats are capable of learning these tasks within a single day, and that hippocampal theta and gamma increase with maze running in a familiar situation, with no difference over trials on a simple-alternation task. Theta power is expected to show more of a decrease over trials during response than place learning.
INTRODUCTION

Background

The hippocampus is an important brain structure involved in learning and memory. One role the hippocampus may play is in the detection of novel stimuli; it is thought that successful encoding and retrieval of memory is contingent upon continuous comparison of the current environment with known representations (Lee et al., 2005). In the rodent hippocampus, local field potentials can be recorded that reflect different behavioral states. One prominent oscillation within the hippocampus is known as theta (4-12 Hz), and may be observed in the CA1, dentate gyrus and CA3 regions of the hippocampus (Buzsáki, 2002). Theta power and frequency depend on the rat's ongoing behavior—it is most consistently present during REM sleep and exploratory locomotor activities (Jouvet, 1969; Vanderwolf, 1969). It has been suggested that the hippocampus switches between encoding and retrieval states while processing information, and that theta may relate to the encoding of new information during exploratory behavior (Chrobak et al., 2000).

The gamma oscillation (25-140 Hz) is also found within the hippocampus, and may also be important in processing information. Montgomery and Buzsaki (2007) showed behavioral evidence that synchronous gamma activity in the CA3 and CA1 regions of the hippocampus support successful memory retrieval in a hippocampal-dependent task. Additionally, it has been shown that fast (greater than 65 Hz) and slow (less than 50 Hz) gamma in CA1 are discrete and tend to occur on different phases of the theta cycle (Colgin et al., 2009).

During aging, hippocampal functioning is impaired; specifically, aged humans, non-human primates, and rats show poorer performance on spatial tasks as compared to younger counterparts. An age-related reduction in the neurotransmitter acetylcholine has been postulated to underlie this impairment. Acetylcholine has been associated with theta; it has also been shown that disruptions to cholinergic transmission cause impairments in learning, while increasing cholinergic transmission can reverse these deficits. Thus, acetylcholine may regulate information processing in the hippocampus via its modulation of theta (Hasselmo, 2006).

A major challenge in my research is relating rhythmic oscillations to different aspects of cognitive function. We investigated how different bands of theta and gamma are
affected by aging, a change in the environment, task learning and cholinergic activation. Given that old rats show deficits in encoding new information, which may be linked to a degraded cholinergic system, both adult and old rats were examined.

In the following sections I will explore the ways in which various hippocampal subregions interact to facilitate the encoding and retrieval of spatial memories, how theta and gamma relate to information processing, how the cholinergic system modulates hippocampal activity, and how age-related neuronal degeneration affects all of the above. Furthermore, I will outline a paradigm that will be used by Dr. Etan Markus’ lab to investigate how various aspects of theta and gamma oscillations change over trials during one-day learning of a place or response task.

**Hippocampal anatomy**

The rodent hippocampus is an important component of the limbic system involved in the encoding and retrieval of spatial memory. Its gross structure may be visualized as two interlocking, curved sheets of cortex. The cytoarchitecture is organized vertically as well as horizontally; it is divided into multiple laminae of cortex, and contains three distinct zones: dentate gyrus, hippocampus proper (CA1, CA2 and CA3), and subiculum (Amaral & Witter, 1995).

The dentate gyrus (DG) consists of a molecular layer containing dendrites, and receives primarily sensory input from the entorhinal cortex (EC). The granule cell layer contains principle cells, as well as axons called “mossy fibers,” named for their myriad varicosities along their lengths. Finally, the hilus contains a polymorphic layer consisting of mostly interneurons.

The hippocampus proper exhibits more complex vertical organization as it contains five anatomically distinct laminae. Both CA1 and CA3 consist of the stratum oriens, the pyramidal cell layer, and the stratum radiatum and lacunosum. CA3 contains a distinct fifth layer, the stratum lucidium, which lies just beneath the pyramidal cell layer (Ishizuka et al., 1995). The subiculum is the main output of the hippocampus, lying between the entorhinal cortex and CA1 (Jackson et al., 2010).
**Figure 1: Hippocampal connectivity.** This is a schematic representation of the flow of information within the hippocampus. Notice how CA1 receives both intrinsic projections from CA3, and extrinsic projections from the entorhinal cortex. All synapses shown in this diagram are glutamatergic. Blue arrows represent inputs upstream of CA1, red arrows are outputs downstream of CA1.

The connectivity of the hippocampus has been the subject of extensive research for the past few decades. Andersen (1971) outlines a very basic, but useful model that focuses on unidirectional, largely glutamatergic projections that route the flow of information through various hippocampal and parahippocampal subregions (Fig. 1). In this model, the CA1 region receives both extrinsic and intrinsic projections, enabling both serial (the trisynaptic pathway: EC → DG → CA3 → CA1) and parallel (EC projections to all subfields) transfer of information. Neocortical inputs send information to layers II and III of entorhinal cortex. Layer II neurons project to the dentate gyrus and CA3 (the perforant pathway), while layer III neurons project to the subiculum and CA1 (the mossy pathway). Additionally, the dentate gyrus also projects to CA3, and CA3 projects to CA1 via the schaffer collaterals. CA1 then projects to layers V and VI of the entorhinal cortex, which then synapse back to layers II and III while also sending outputs to the neocortex.
In addition to these glutamatergic projections, it is also known that the hippocampus receives cholinergic and GABAergic inputs from the basal forebrain, including both the medial septum and the diagonal band of Broca (Nyakas et al., 1987). In CA1, cholinergic projections from both areas synapse onto pyramidal cells and GABAergic interneurons, while GABAergic projections only project to the GABAergic interneurons (Gúlyas et al., 1990; Leranth & Frotcher, 1985). These projections serve to modulate theta activity within the hippocampus and may degenerate with age (Sava & Markus, 2008).

**Information processing**

*Competition between brain systems*

The hippocampus may be thought of as one of the major parallel processors in the brain (Chang & Gold, 2003; Colombo et al., 2003; DeCouteau et al., 2003; Packard, 1999; Rich & Shapiro, 2009). Tolman (1946) postulated the existence of an internal cognitive map that animals use to navigate their environment based on his studies using an “ambiguous T-maze.” Animals were trained to always go to the same arm of a T-maze for food reward. Then, the orientation of the maze was flipped. Rats using spatial cues (“go there”) to solve the maze went to the correct arm, while those using the relying on a motor response strategy (“turn right”) to solve the puzzle ended up choosing the wrong arm on the reversal day.

The hippocampus is thought to be the primary processor of spatial information (place strategy), and the striatum the seat of motor learning (response strategy). It has been observed that infusions of glutamate into either the hippocampus or striatum during training increased the likelihood that rats would use a place or response strategy, respectively; deactivation with lidocaine has the opposite effect (Packard, 1999; Packard & McGaugh, 1996). Furthermore, Chang and Gold (2003) found that hippocampal acetylcholine levels increase by as much as 60% following the first day of training on the cross maze, and remain stable at that level over all subsequent training sessions. In contrast, striatal acetylcholine levels increase slowly over many trials, reaching an asymptote of 30-40% increase. These changes in acetylcholine levels may determine who “wins” the competition between the brain systems. Early on, the hippocampus shows more of an increase in acetylcholine, and it is the predominant brain system used to solve the
maze in these earlier stages (place strategy). However, when acetylcholine levels increase in the striatum, this region “overpowers” the hippocampus and the animal begins to use the response strategy.

In DeCoteau et al. (2007), rats were trained on a modified T-maze where they had to choose to go to the left or the right arm depending on the frequency of a tone played just before the decision point. At the beginning of training, there was little coherence in the theta band between striatum and hippocampus. Coherence between the two oscillations gradually increased during training in the rats that learned the task, and was highest when the tone was being played. In the rats that did not learn the task, there was little change in coherence throughout training. This research suggests that there is a dynamic, task-dependent relationship between LFP oscillations in these two brain structures.

The hippocampus itself may also contain two functionally distinct processing units. Dorsal hippocampus is primarily concerned with processing spatial information, whereas ventral hippocampus is more sensitive to emotionally significant input from the amygdala and hypothalamus (Fanselow & Dong, 2010). Selective lesions to dorsal, but not ventral hippocampus result in learning deficits with respect to spatial tasks (Moser et al., 1995; Pothuizen et al., 2004). Furthermore, there is a higher density of place fields in dorsal than ventral hippocampus (Jung et al., 1994).

The role of CA1 in information processing

Different zones within the hippocampus are intricately connected (Fig. 1), forming a neural circuit capable of pattern separation and completion. Sensory input from the entorhinal cortex is first processed by the dentate gyrus and then passed on to CA3, where an autoassociative network of recurrent collaterals may form and store associations as ensembles of cells firing together (Amaral & Witter, 1995). This enables it to function as a pattern separator that can form, store and retrieve past associations of cells. Since CA1 receives input from both the entorhinal cortex and CA3, it is positioned to serve as a comparator between incoming sensory information and stored representations, detecting novelty based on a mismatch between these two major inputs (Hasselmo et al., 2000; Lee et al., 2005; Witter et al., 2000). CA1 neurons project to the subiculum, the primary output of the hippocampus, to the neocortex, and also back to the entorhinal cortex (Andersen, 1975;
Jackson et al., 2011). This connectivity may allow for the formation of cortical loops that are important for higher cognitive function.

The importance of theta and gamma oscillations

Within the hippocampus, distinct local field potentials (LFPs) can be recorded using implanted microelectrodes. The recorded signal is primarily generated by the summed electrical current flowing within a volume of tissue generated by nearby synaptic activity. Synaptic current flows across the resistance of the local extracellular space as large groups of cells fire in synchrony, producing a change in voltage that can be measured by the microelectrode. Since the activity of individual neurons is filtered out by use of a low-pass filter (250 Hz), the LFP reflects only these slower currents arising from the synchronized firing of inputs in the area around the electrode, rather than currents from individual actions potentials as spike data (Kajikawa & Schroeder, 2011).

The theta oscillation (4-12 Hz) is a type of LFP observed in the CA1, dentate gyrus and CA3 regions of the hippocampus, and is likely important for learning and memory (Buzsáki, 2002). The oscillation may be divided into two bands: lower frequency theta has been associated with arousal, attention and sensory processing mechanisms, while higher frequency theta has been associated with general movement (Bland, 1986). Jeewajee et al. (2008) found that low frequency theta is important for novelty detection; the peak frequency of theta increases as the environment becomes more familiar. The study also found that theta frequency shifts even lower than the initial exploration when the animal is presented with an unexpected change in a familiar environment, suggesting that theta acts as a signal for novelty detection.

A plethora of studies have shown that lesions to the medial septum cause memory impairments that are remarkably similar to those associated with hippocampal lesions (Gray & McNaughton, 1983). For example, Donovick (1968) found that septal lesions abolished all theta activity within the hippocampus and also induced impairments in both reversal learning and spatial alternation tasks. However, Colgin and Moser (2009) examines contrary findings that the hippocampus is capable of generating theta in an isolated hippocampus free of septal inputs, suggesting that the medial septum may play more of a modulatory role rather than acting as the primary generator of the theta
oscillation, as previously thought. These findings imply that the theta oscillation is dependent on the activity of hippocampal interneurons as modulated by cholinergic septal input.

The gamma oscillation is a higher-frequency, lower-amplitude LFP that co-exists with theta and has also been linked, to a degree, with learning and memory. Traditionally, the gamma oscillation has been defined as 25-140 Hz (Nyhus & Curran, 2010). Gamma power is modulated by theta phase, and is also increased during exploratory behaviors (Buzsaki & Chrobak, 1995; Chrobak & Buzsaki, 1998). Colgin et al. (2009) postulated the existence of two functionally distinct bands of activity within the gamma oscillation; slow (less than 50 Hz) gamma is coherent between CA1 and CA3, while fast (greater than 65Hz) gamma is synchronous between CA1 and entorhinal cortex. This difference in coupling may act to reinforce the theta-mediated segregation between encoding and retrieval states, as described in Hasselmo (2005).

It has been suggested that there are two distinct information-processing states within the hippocampus (Chrobak et al., 2000), and that theta may relate to the encoding of new information during exploratory behavior. Computer modeling based on physiological data suggests that performance in memory tasks may be modulated by rapid shifts between an encoding (theta trough) and a retrieval (theta peak) phase within each cycle of the theta rhythm. During the encoding phase, strong entorhinal input coupled with reduction in CA3 input facilitates accurate storage of new memories without interference from previously stored memories. During the retrieval phase, enhanced CA3 input promotes accurate retrieval of past representations (Hasselmo, 2005).

The theta rhythm is thought to play an important role in synchronizing the activity of pyramidal cells within the rodent hippocampus. Ensembles of place cells in the CA1 region of the hippocampus fire selectively as a rat navigates through specific locations in the environment (termed “place fields”). The firing of these place cells is phase-locked to the theta frequency: as a rat traverses through a place field, place cells fire in progressively earlier phases relative to the theta oscillation (Skaggs et al., 1996). This phenomenon is known as phase precession, and is thought to aid in synchronizing the firing of ensembles of cells across time (Maurer et al., 2006). Inhibitory CA1 interneurons also exhibit activity
similar to place cells in that they are also phase-locked to the theta rhythm and modulated by location within a place field (Ego-Stengel & Wilson, 2007).

Furthermore, Jezek et al. (2011) found that the theta rhythm paces the alternation between the firing of two distinct ensembles of place cells during a “teleportation task.” Rats were trained in two nearly identical environments distinguishable only by the arrangement of lights around the maze. When the rats were placed in a third environment containing both sets of lights, it was observed that changing the lights from one environment to the other caused the two distinct ensembles of place cells to fire in competition, with only one population dominating each theta cycle. Over time, the representation that reflects the current environment “wins out.” In the context of the model described in Hasselmo (2005), this paper provides a clear experimental demonstration of theta’s role in organizing the processing of spatial information.

**The cholinergic system and aging**

As described previously, the rodent hippocampus receives cholinergic inputs from the basal forebrain area, including the medial septum (MS) and the diagonal band of Broca (Nyakas et al., 1987). Hippocampal theta, acetylcholine (ACh) levels and learning are intimately linked, as demonstrated by a variety of studies involving septal lesions and cholinergic manipulations (Gray & McNaughton, 1983). A positive relationship between ACh release and theta power within the hippocampus has been observed in vivo (Keita et al., 2000). Lee et al. (1994) found that hippocampal theta power gradually decreased following septal lesions with a selective cholinergic toxin (192 immunoglobulin G). Treatment of normal and lesioned rats with physostigmine, an acetylcholine esterase inhibitor, shifted the theta peak to a lower frequency in normal rats, but not in lesioned animals. Movement-associated higher frequency theta was unaffected by lesion or drug administration. Further pharmacological studies have confirmed that administration of physostigmine shifts the peak frequency of theta to lower frequencies without changing the amplitude, and have also found that co-administration of scopolamine (a muscarinic antagonist) suppresses this effect (Podol’skii et al., 2001).

There is evidence from both human and rodent studies to suggest that the cholinergic inputs to the hippocampus degenerate with age as well as in Alzheimer’s
disease. This degeneration may be related to changes in the way that the hippocampus processes information (Schliebs & Arendt, 2011). The hippocampi of both adult and old rats contain place cells that create spatial representations of the environment (Oler & Markus, 2000; Sava & Markus, 2008). Place fields generally remain in the same location across repeated exposures to a familiar setting. When the environment is altered significantly, it has been shown previously that place cells can remap in normal adult rats (Frank et al., 2006). However, old rats tend to retain old representations, even when exposed to a novel situation (Tanila et al., 1997a, b). Sava and Markus (2008) found that in a familiar setting, septal activation with carbachol (a cholinergic agonist) impaired retrieval of stored place cell representations irrespective of age. However, in a novel setting, septal activation facilitated encoding in aged rats, but impaired it in adult rats. These data support the idea that the MS plays a role in controlling hippocampal retrieval and encoding states via its modulation of theta, and that decreased cholinergic inputs to the hippocampus from this brain region provide a plausible explanation for age-related cognitive impairment.

While much is known about age-related changes in place-cell remapping, differences in oscillatory activity between adult and aged rats as they explore novel environments has not been adequately addressed. The current study consists of two distinct, but related experiments. The first experiment (Part I) explores how the relationship between theta and gamma might change with age, novelty or cholinergic stimulation using a radial arm maze task. Local field potentials were recorded in both young adult and old rats during exploration of both a familiar and novel maze trajectory, after administration of either saline (control) or physostigmine. Given that old rats show deficits in encoding new information and the possible link to a degraded cholinergic system, the first experiment assessed how several measures of oscillatory activity were affected by aging, a change in the environment, and cholinergic activation (see Radial Arm Maze Methods/Results).

Tolman (1946) established that rats use either a place (“go there”) or a response (“turn right”) strategy when navigating a T-maze for food reward. Packard and McGaugh (1996) used a similar place-response paradigm to show that the hippocampus mediates spatial learning while the striatum mediates motor-response learning. The second experiment (Part II), still underway, measured changes in hippocampal theta and gamma
oscillations during place or response learning in adult rats using a plus maze task. Rats were trained on either a place or a response task in a novel environment over a single recording session, and then re-tested following a runway recording. It was expected that hippocampal theta will initially increase across all conditions, however this increase will be larger in the novel place and response recordings. Local field potentials were analyzed for each trial, and examined for possible changes as the animal explored the environment, learned the task and performed the task following mastery. Theta and gamma power, frequency and coherence are expected to show change differently over trials between place and response days (see Plus Maze Methods/Results).
METHODS

Part I: Radial Arm Maze Recordings

Subjects
Data was collected from 7 adult (12.2 ± 0.15 months) and 10 old (23.3 ± 0.32 months) Fisher 344 male rats (Harlan, IN and Taconic, NY). Rats were individually housed in clear Plexiglass cages and maintained on a 12 hr light:dark cycle in a temperature and humidity controlled room. Animals were allowed ad libitum access to water. All procedures were performed in accordance with the University of Connecticut’s Institutional Animal Care and Use Committee.

Pre-training procedure
All animals were food restricted to 85% of their ad libitum weight and acclimated to chocolate sprinkle reinforcement. Rats were initially trained to alternate on a U-shaped maze for 20 minutes a day until they reached a criterion of 50 trials a day for 3 consecutive days. After reaching criteria, rats underwent surgery to permanently implant electrode arrays into the hippocampus.

Surgery and Re-training
Animals were anesthetized with isoflurane (1.5-2.5%) and placed in a stereotaxic apparatus (ASI Instruments, Warren, MI). Once anesthetized, the rats were given Metacam (.06-.1 mg/kg S.C.) and Penicillin/Streptomycin (.1mL), the scalp was shaved, aseptically cleaned, and ophthalmic ointment applied to the eyes. A midline incision was made and several small anchor screws were fastened to the skull. Electrode arrays (four 50μm tungsten wires, CA Fine Wire Co., Grover Beach, CA) arranged and spaced using fused silica tubing (Polymicro Tubing, Phoenix, AZ), were implanted into dorsal hippocampus (AP -3.5, ML 2.5 from bregma, and DV 2.5 from the skull and AP -4.5, ML 3 from bregma, and DV 3.3 from the skull). Each array was cut on a small diagonal and targeted to straddle the CA1 layer. Two old rats had custom made headstages with 7 tetrodes and 2 electrodes (50 μm tungsten wires, CA Fine Wire Co.) (unit data not presented here). Electrodes were targeted ventral to the stratum pyramidale and dorsal to the hippocampal fissure (AP -4.5, ML 3 from bregma, and DV 3.3 from the skull). Two stainless steel screws above the cerebellum
served as reference and ground. Dental acrylic then secured the arrays and anchors. After surgery animals were placed in a clean cage with a heating pad and monitored until ambulatory.

Animals were allowed to recover for one week before re-training commenced. Training took place on an 8-position maze atop a table 60cm off the ground, two Plexiglas runways (19cm in height by 61cm in length) radiated out from a center platform (24cm in diameter). The configuration of the maze for re-training then became the familiar maze trajectory during testing. Recordings began once animals were again well trained and alternating.

![Schematic of radial arm maze trajectories and procedures](image)

**Figure 2: Schematic of radial arm maze trajectories and procedures.** Recording sessions started as the rats sat in their home cage outside the maze room. The first maze epoch was always the familiar trajectory, followed by an injection of saline or physostigmine (.1mg/kg). The second maze epoch was either the same familiar trajectory or a novel trajectory. The four conditions of maze 2 were familiar saline, familiar physostigmine, novel saline, or novel physostigmine.

**Recording procedure**

Recordings were performed while sitting in the home cage and during running on the maze both before and after saline or drug injections (Fig. 2).

**Familiar maze trajectory:** Data was collected while rats sat quietly in the home cage and then while running the familiar trajectory. After 5 minutes on the maze, animals were taken off and given an injection of saline or physostigmine (.1 mg/kg S.C.) and then allowed to rest for 15 minutes while the drug took effect. Animals were then placed back on the
same maze trajectory and recorded another 5 minutes, followed by an additional 2 minutes back in the home cage.

**Novel maze trajectory:** The procedure pre-injection was the same as during the familiar trajectory; data was collected in the home cage and then while running the familiar trajectory. Following injections (either saline or physostigmine) and rest, animals were recorded in a novel trajectory. One maze arm remained stationary while the second arm was rotate to another position at least 90° away from the familiar position (Sava and Markus, 2008).

**Time course of physostigmine**

In addition, after all maze recordings were completed animals underwent 4 sessions of long-term recordings while they remained in their home cage outside the testing room. A 3-minute baseline recording was done before injection of either saline (2 sessions) or physostigmine (.1mg/kg S.C.) (2 sessions). Following injections, recordings were conducted for 2 hours, 3 minute recordings were taken every 10 minutes (minutes 8-11, 18-21, etc.).

**Histology**

Following testing, rats were euthanized with CO₂ and perfused intracardially with saline followed by 10% phosphate buffered formalin solution. Brains were extracted and further fixed in formalin, cryoprotected in 30% sucrose, then sliced into 40μm sections, stained with .25% Thionin, coverslipped, cleaned, and examined for electrode placements.

**Data Analysis**

Wide-band electrical activity was recorded (1-2000 Hz, 3787 samples/sec) using Neuralynx Data Acquisition System (Bozeman, Montana). Light-emitting diodes attached to the headstage were tracked with an overhead camera. Data was selected and analyzed off-line. All data was initially inspected visually (Neuraview, Neuralynx, Bozeman, MT) to remove any segments of bad signal (e.g. due to loose connection, bumping head) then downsampled to 473.4 samples/s (Neuralynx, Bozeman, MT). Data was then segmented
using Neuralynx Video Tracker File Playback and Event Session Splitter (Bozeman, MT) to exclude data during food consumption and turning at the end of each arm. All signal analysis was conducted using custom-written programs in MatLab (Mathworks, Natick, MA). Statistical analysis was then carried out in SPSS or Excel.

![Figure 3: Spectral analyses.](image)

**Figure 3: Spectral analyses.** (A) Example power spectral density plot from a young adult rat while running a familiar trajectory. Note the increase in theta power (“theta peak”) between sitting in the holder and running on the maze. (B) Example filtered traces of theta (4-12 Hz) and gamma (25-55, 65-140 Hz).

**Spectral Indices**

Power spectral density estimates were obtained in Matlab using Welch’s averaged modified periodogram method (Welch, 1967; **Fig. 3A**). Each epoch was then blocked, and power estimates obtained for each running segment (trial). Velocity for each trial was calculated
as the positional difference between successive tracking samples and then low-pass filtered (cutoff = 0.25 Hz) in order to minimize the contribution of head movements and movement artifacts to the overall speed.

Theta frequency was obtained for each trial using the Hilbert transform of the band pass-filtered signal (4.5-12Hz) and calculating the change in phase divided by the change in time between each sample. Each segment of running within different behavioral epochs was concatenated into a single continuous string of data using a cross fading procedure where the first and last 100 msec of each data segment is ramped or faded respectively with a smooth B-spline window with continuous second-order derivatees (Roark and Escabi, 1999). Adjacent start and end blocks from subsequent segments were then overlapped and morphed by adding the signals overlapping the ramp and fade regions. Power estimates were obtained for separate theta and gamma bands based on the upper and lower ranges of previous studies (Dimpfel, 2005; Manns et al., 2007, Colgin et al., 2009; Sullivan et al., 2011): theta band (4.5-12 Hz), low gamma band (25-55 Hz) and high gamma band (65-140 Hz) and represented as decibels (dB) relative to 1μV (Fig. 3B).
Part II: Plus Maze Recordings

In order to assess changes in hippocampal theta and gamma over trials during learning, a single-day place/response paradigm was designed.

![Image of plus maze and runway recording procedures](image)

**Figure 4: Schematic of plus maze and runway recording procedures.** The rat is first placed in a holder and baseline LFP activity recorded for 5 minutes. The rat is then recorded while learning to perform either a place (A) or a response (B) task for 50 trials on the plus maze. Between place and response days, the rat performs a simple alternation task on the runway (C) for 50 trials. Grey dots indicate potential start locations, red dots indicate goal locations, and dashed lines indicate a blocked arm.
Recording procedure

Once pre-trained, adult rats were surgically implanted with electrodes bilaterally into the CA1, CA3, and dentate gyrus regions of the dorsal and ventral areas of the hippocampus, as described (see Surgery and Re-training). Local field potentials were recorded as rats rested in their home cage, and then as they learned the place or response task (Fig. 4). During the place task, rats were trained to go to one arm of the plus maze in Room A for a food reward (Fig. 4A). In the response task, rats were be trained in Room B to make a right-hand turn for a food reward, regardless of their start location (Fig. 4B). The animals also performed a familiar simple alternation task on the runway in Room C between each recording day (Fig. 4C). Each animal completed 50 trials per 2-hour session, with a minimum of two place and two response days.

Data analysis

Power spectral density estimates were again obtained in Matlab using Welch’s averaged modified periodogram method (Fig. 3). Each epoch was then blocked, and power estimates obtained for each trial. Velocity for each trial was calculated as the positional difference between successive tracking samples and then low-pass filtered (cutoff = 0.25 Hz) in order to minimize the contribution of head movements and movement artifacts to the overall speed. Power and frequency estimates in the theta and gamma bands were calculated for the first and last ten trials of each recording session.
**RESULTS**

*Part I: Radial Arm Maze Recordings*

**Electrode placement**

An electrode located within the stratum radiatum layer of dorsal CA1 in each rat was used for all power and frequency analyses (Fig. 5). While robust theta and gamma oscillations were observed in both populations of animals, in old rats a lower frequency of theta activity and a decrease in variability of gamma amplitude were seen across the theta cycle (Fig. 5D).

![Electrode placements and sample LFP trace](image)

**Figure 5: Electrode placements and sample LFP trace.** (A) Schematic of electrode array implanted into dorsal hippocampus. (B) Photomicrographs of electrode locations. A single electrode was chosen from each rat from the stratum radiatum layer (arrow). (C) Electrode locations of all rats; placements in young rats are represented in black squares, old rats are represented in gray circles. (D) Unfiltered traces from the selected electrodes during maze running show prominent theta activity in both young and old rats. Note the slower frequency in the trace from the old rat, and the difference in modulation of gamma amplitude across the theta cycle.

**Time course of physostigmine**

In order to assess the time course of physostigmine, LFPs were recorded as rats rested in their home cage. Previous studies have suggested that there are two distinct types of theta activity, and that theta sensitive to cholinergic modulation is lower in frequency than theta related to movement (Lawson & Bland, 1993). Therefore, the effects of physostigmine
treatment on theta power were examined within the lower range of theta (low theta = 4.5-8 Hz) and the higher range of theta (high theta = 8-12 Hz) separately (Fig. 6).

Figure 6: Time course of physostigmine. The change in power from baseline (pre-injection) at each 10min interval (top) spanning 2 hours after saline (solid line) or physostigmine (dashed line) for both young (black) and old (gray) rats. (A) In the low theta band, there was no difference for young rats between saline and physostigmine. Old rats had increased low theta power after physostigmine. (B) In the high theta band there was a decrease in power from physostigmine in young rats. (C) Low gamma and (D) High gamma power did not differ between physostigmine and saline treatment in either young or old rats. (* p< 0.01).

Rats were injected with saline or physostigmine (.1 mg/kg SC) following a baseline recording. Then, 3-minute long recordings were taken every 10 minutes over the course of a 2-hour recording session following injection. The change in power from baseline at each 10-minute interval was calculated, negative values indicate a decrease in power during that interval, and positive values indicate an increase in power (Fig. 6). Repeated measures
ANOVA between physostigmine and saline was carried out for each group at each frequency band. Saline treatment did not differ from baseline in any band (all $p > 0.1$). In the low theta band, old rats had increased power in the physostigmine condition ($F_{1,154} = 9.0, p < 0.01$), while adult rats showed no change in either condition ($p > 0.1$) (Fig. 6A). In the high theta band, adult rats showed decreased power from pre-injection ($F_{1,66} = 42.18, p < 0.001$). The same trend was present in old rats ($F_{1,154} = 3.8, p = 0.07$) (Fig. 6B). Physostigmine did not change either the low or high gamma band (all $p > 0.1$) (Fig. 6C,D).

**Power and velocity in a familiar or novel environment**

Power in both theta and gamma frequency bands increased when running on the maze in both adult and old animals (one sample t-test: difference from zero, all $p < .001$). The change in power was calculated as animals transitioned from sitting in their home cage to running on the maze for each session (Fig. 7A); old rats showed less of an increase than the adult rats in the theta range ($t_{222} = 2.54, p < 0.05$) and the high gamma range ($t_{222} = 3.26, p < 0.001$). The same trend was present in the low gamma range ($t_{222} = 1.82, p = 0.07$) (Fig. 7B).

![Figure 7: Change in power from sitting to running. (A) Baseline recordings were taken in the holder before running on the maze. (B) In all frequency bands, young and old showed increased power during running compared to sitting in their home cage. Young rats increased more in all but the low theta band. (* $p < 0.01$; ṭ $p=0.07$).](image)

In addition, the average velocity was computed from the mean of all trials for each recording condition (Table 1). Old rats ran slower than adult rats during the maze 1 epoch ($t_{173} = 10.57, p < 0.01$). To determine effects of the novel trajectory, a 2-way-ANOVA (age * environment) was conducted. During the second epoch, old rats ran slower than
adult rats \((F_{1,261} = 74.69, p < 0.001)\), though for both groups velocity decreased on the novel trajectory \((F_{1,261} = 17.69, p < 0.001)\). No other significant interactions were observed \((all \ p > 0.1)\).

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<td>Adult</td>
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<td>Old</td>
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**Table 1: Mean (±SEM) velocity during each maze epoch.** All values are in centimeters/second. Old rats ran slower than adult in all conditions. Both the novel maze trajectory and treatment with physostigmine decreased running speed.

**Effects of novelty on power**

A within-animal comparison was used to assess how running on the novel trajectory affected the power in each frequency band. The change in power from the baseline maze was calculated for each session \((Fig\ 2, 2^{nd}\ maze – 1^{st}\ maze epoch)\) and averaged for either the familiar or novel condition \((Fig\ 8)\). Both adult and old rats showed decreased velocity on the novel trajectory \((F_{1,88} = 12.35 \ p < 0.001)\), the effect was stronger in the adult rats (main effect of age \(F_{1,88} = 8.621, p < 0.01\) and age by environment interaction \(F_{1,88} = 7.14, p < 0.01\)). On the novel trajectory, an independent t-test assessed the change in velocity between adult and old rats; velocity decreased more in adult than in old rats \((t_{29} = 3.46, p < 0.001)\).
Despite the decreased velocity on the novel trajectory, theta power increased (Table 1; Fig. 8; $F_{1,91} = 30.82, p < 0.001$) for both adult and old rats, and there was a trend for an interaction ($F_{1,91} = 3.57, p = 0.06$). On the novel trajectory, the extent of the increase was greater in the adult rats ($t_{30} = 2.53, p < 0.01$).

The change in low gamma power across environments revealed a main effect of both age ($F_{1,91} = 7.02, p < 0.01$) and environment ($F_{1,91} = 4.77, p < 0.05$) and again a trend for an interaction (Fig. 9A; $F_{1,91} = 2.86, p = 0.09$). Within the novel trajectory there was a trend for an age difference ($t_{30} = 1.88, p = 0.07$), adult rats had increased low gamma power on the novel trajectory.

Similar results were found for the change in high gamma power across environments (Fig. 9B). Initially, a 2-way ANOVA showed a main effect of age ($F_{1,91} = 4.07, p < 0.05$) and an age * environment interaction ($F_{1,91} = 5.06, p < 0.05$). On the novel
trajectory, there was trend that the adult rats had increased high gamma power while old did not ($t_{30} = 1.87, p = 0.07$).

Figure 9: Change in gamma power on maze 2. For both low (A) and high (B) gamma power, physisostigmine decreased power in the young but did not change the power in old rats.

Effects of physisostigmine on velocity, power and coupling

Prior to recording the maze 2 epoch, animals received an injection of either saline or physisostigmine (Fig. 2). Physisostigmine decreased running speed for both young adult and old rats (Table 1). The effects of physisostigmine on theta and gamma power were examined by comparing the saline and physisostigmine conditions in both novel and familiar environments (Fig. 8; Fig. 9). There was a main effect of age on the change in theta power in the familiar environment ($F_{1,122} = 2.10, p < 0.05$); adult rats showed a greater decrease in power on the familiar trajectory (Fig. 8). No effects or interactions of physisostigmine on theta power were observed in the familiar environment (all $p > 0.1$). In addition, physisostigmine did not affect low gamma power (all $p > 0.1$) (Fig. 9A), and decreased high gamma power for both adult and old rats ($F_{1,122} = 7.19, p < 0.01$; Fig 9B). No age effects or interactions were observed (all $p > 0.1$).
On the novel trajectory, no main effect of age or physostigmine on theta power was observed (both $p > 0.1$), though a 2-way ANOVA (age * physostigmine) shows that physostigmine decreased theta power in the adult rats, but increased it in the old rats ($F_{1,122} = 17.94, p < 0.001$; Fig. 8). There was a main effect of physostigmine ($F_{1,122} = 3.98, p < 0.05$) but not age ($p > 0.1$) on low gamma power, and a trend for an age * physostigmine interaction ($F_{1,122} = 3.35, p = 0.07$). Physostigmine decreased low gamma power in adult, but not in old rats (Fig. 9A). Similar results for high gamma power showed a trend for an
age * physostigmine interaction \((F_{1,122} = 3.35, p = 0.07)\), where physostigmine selectively decreased high gamma power in adult rats (Fig. 9B).

\textbf{Dynamics of low and high theta power after physostigmine}

Low and high theta power were differentially affected by physostigmine administration, increasing low theta while decreasing high theta (Fig. 10). An example power spectrum density plot shows the power distribution across all frequencies (Fig. 10A). Notably, increased power in the lower range (leftward-shift) was observed following physostigmine administration (Fig. 10B). To better examine the dynamics within the entire theta range and determine how power changed between maze 1 and maze 2 epochs, a ratio of low (4.5-8 Hz) and high (8-12 Hz) power was calculated \([\text{low power}/(\text{low power} + \text{high power})]\) (Fig. 10C).

For all conditions (MANOVA: maze 1 ratio and maze 2 ratio * age, environment, physostigmine), old rats had a higher ratio of low theta power on both maze epochs corresponding to the lower peak frequency (maze 1, \(F_{1,157} = 86.59, p < 0.001\); maze 2, \(F_{1,157} = 64.30, p < 0.001\); Fig. 10C). Paired t-tests between ratios were computed for each group and condition to examine shifts of the ratio from maze 1 (before treatment) to maze 2 (after treatment). Following saline injections (Fig. 10C, top row), there no change in the ratio of the low to high theta power was observed for either age group in either the familiar or novel environment (paired t-test, \(all p > 0.1\)). However, following physostigmine administration (Fig. 10C, bottom row), power shifted to the lower theta band in both adult \((t_{22} = 4.15, p < 0.001)\) and old \((t_{34} = 3.27, p < 0.01)\) animals when the second maze was again familiar. When the second maze was novel, the same shift for old rats \((t_{10} = 2.594, p < 0.05)\) and a trend for the adult rats were observed \((t_{7} = 2.22, p = 0.06)\).
Part II: Plus Maze Recordings

In order to assess the feasibility of the plus maze recording paradigm, one animal ran a simple alternation task on the runway over seven recording sessions. Recordings took place in a familiar environment over 50 trials.

Figure 11: Changes in power in dorsal hippocampus. Theta, low and high gamma power increased between holder and maze on a simple alternation task. No statistically significant changes in power were observed between the first and last ten trials, with the exception of high gamma power. Student’s t-test. *: p < .05, **: p < .01, ***: p < .001, NS: p > .05 (n = 7).

Changes in power with maze running

Theta power increased in dorsal and ventral hippocampus as the animal went from sitting in the home cage to running on the maze (t7 = 3.38, p < .05; t7 = 4.535, p < .01). Low gamma power increased on the maze in dorsal and ventral hippocampus (Fig. 10; t7 = 4.09, p < .01; Fig. 11; t7 = 3.52, p < .05). Finally, high gamma power also increased on the maze in both dorsal and ventral hippocampus (Fig. 10; t7 = 9.94, p < .001; Fig. 11; t7 = 5.593, p < .01).
Figure 12: Changes in power in ventral hippocampus. Theta, low and high gamma power increased between holder and maze on a simple alternation task. No statistically significant changes in power were observed between the first and last ten trials. Student’s t-test. *: p < .05, **: p < .01, NS: p > .05 (n = 7).

Stability of power over trials in a familiar environment

As expected, theta power did not change between the first and last ten trials on the runway in dorsal or ventral hippocampus (Fig. 10; t7 = 1.34, p = .204; Fig. 11; t7 = 1.44, p = .18). Similarly, low gamma power did not change over trials in dorsal or ventral hippocampus (Fig. 10; t7 = .94, p = .37; Fig. 11; t7 = .395, p = .70). An increase in high gamma power was observed between the first and last ten trials in dorsal, but not ventral hippocampus (Fig. 10; t7 = 2.42, p < .05; Fig. 11; t7 = 1.79, p = .11).
DISCUSSION

These results support the hypothesis that rhythmic oscillations such as theta and gamma play an important role in information processing within the hippocampus. Hasselmo (2005) postulated that the hippocampus rapidly switches between competing encoding and retrieval states as organized by the theta rhythm. Jezek et al. (2011) confirmed that the theta rhythm segregates the firing of competing environmental representations when the environment changes suddenly. Furthermore, Colgin et al. (2009) found evidence that low gamma may be related to the flow of information between CA1 and CA3 in the trisynaptic pathway, while high gamma may synchronize CA1 and entorhinal cortex in parallel. This combination of inputs to CA1 may allow it to serve as comparator between past representations and current sensory input, providing a signal of novelty that induces the encoding of new spatial memories (Lee et al., 2005).

The current study examined how various aspects of hippocampal theta and gamma oscillations change with age, novelty, cholinergic stimulation and task learning. On the radial arm maze task (see Part I), the animals either navigated a familiar (retrieval task) or partially novel (encoding task) maze configuration. Previous studies have shown that theta power increases in response to novelty (Buzsáki, 2002). While it is thought that hippocampal theta is primarily generated by an interaction between excitatory pyramidal cells and inhibitory interneurons, there is significant evidence that its frequency is modulated by cholinergic inputs from the medial septum (Colgin & Moser, 2009). These inputs degenerate with age; this degeneration may be linked to a reduction in theta power (Schliebs & Arendt, 2011; Gray & McNaughton, 1983). Since previous studies have shown that the ability to encode new memories is impaired in aging (Sava & Markus, 2008), it was hypothesized that aged animals would show reduced theta in a novel environment as compared to their younger counterparts. The effects of cholinergic stimulation with physostigmine were examined as the animals traversed both familiar and novel environments. Finally, in order to further examine the importance of these oscillations in learning and memory, a place/response paradigm was designed where the animals learned each task over a single, continuous recording session (see Part II).
**Part I: Aging, novelty and cholinergic stimulation**

*Changes in theta power on the radial arm maze task*

Theta power increased as animals transitioned from sitting in their home cage to running on the familiar maze configuration. While both age groups showed an increase across all measured frequency bands, the younger rats showed more of an increase in high theta power than old rats. High theta power has been associated with movement; increased velocity in the adult rats on the Maze 1 epoch may explain this disparity between age groups (Bland, 1986).

When the animals explored a familiar environment following saline injection, velocity and theta power decreased for both age groups. However, when the maze was changed to a novel configuration, a paradoxical increase in theta power was seen, despite reduced velocity for both age groups. Adult rats showed a greater increase in theta power, yet they also had a larger reduction in velocity compared to the old rats. This decoupling of theta power and velocity suggests that, in the novel situation, cognitive factors (i.e., the encoding of new spatial information) are having a larger effect on theta power than movement (Wyble *et al.*, 2004). The smaller increase in theta power seen in old rats suggests impairment in the process of encoding the novel setting.

The effects of physostigmine were first quantified while the animals rested in their home cage over a period of two hours. Physostigmine had different effects between age groups. Old rats alone showed increased low theta power following drug administration. Surprisingly, high theta power was reduced in young rats following physostigmine injection.

Across all maze conditions, old rats had a higher ratio of low to high theta power than their younger counterparts. Physostigmine administration significantly reduced velocity in both groups, shifting power to the low theta range during the Maze 2 epoch. Treatment with physostigmine further increased low theta power in old rats in both familiar and novel environments. Notably, physostigmine administration increased low theta power in old rats to the same level as an untreated adult animal in the novel environment.
Changes in gamma power on the radial arm maze task

Unlike theta, it is unclear to what degree gamma oscillations change with task demands, however it is thought that the gamma oscillation may function to synchronize local and global activity between brain structures and across hippocampal subregions (Colgin et al., 2009). Some behavioral studies have linked gamma oscillations in CA1 to cognitive processing; Montgomery and Buzsaki (2007) found that gamma power selectively increased during the decision portion of the maze. However, others have failed to find any clear-cut link to behavior or cognition (Shirvalkar et al., 2010).

The current study found that absolute gamma power did not differ between adult and old rats as they traversed a familiar environment, although old rats showed less of an increase than adult rats with maze running. No significant changes in gamma power were seen when animals explored the familiar maze again during the second recording epoch. Interestingly, adult, but not old rats showed an increase in gamma power on the novel maze trajectory. These findings are in line with studies that suggest the fast GABAergic interneuron system that is responsible for generating hippocampal gamma may be deregulated in aged animals (Stanley et al., 2011), and could contribute to age-related memory deficits. Furthermore, this suggests that, like theta, gamma may be an important physiological signal that is relevant to the encoding of new information within the hippocampus.

Treatment with physostigmine did not affect gamma power while animals were sitting in their home cage. Gamma power decreased on the maze with physostigmine administration in adult, but not old rats. Further investigation will be required to determine the significance of this disparity.

General conclusions

The current study found that, in a novel environment, velocity decreased while theta power increased in both adult and old rats, however, the degree of this power increase was less in the aged population of animals. In addition, adult (but not old) rats showed increased gamma power on the novel configuration. Furthermore, physostigmine administration selectively boosted low theta power in old rats. Taken together, these
findings support the idea that hippocampal theta and gamma are both important signals involved in processing new spatial information.

These findings also help explain why systemic cholinomimetics (such as physostigmine) have only a modest clinical effect in the treatment of Alzheimer’s disease (Eagger et al., 1991). Physostigmine administration boosts theta, but not gamma activity in aged animals. In addition, this drug appears to be most effective in restoring the ability of the hippocampus to encode novel environments; in a familiar setting, physostigmine has less of an effect in old animals, and actually reduces theta and gamma activity in normal adult animals. Since individuals are bombarded every day with novel and familiar stimuli, the effect of simply boosting cholinergic transmission regardless of cognitive demands will likely never successfully restore complete function to the aged hippocampus.

**Part II: Changes in theta power with learning on a plus-maze task**

*Behavioral paradigm*

In order to determine how hippocampal dependent and independent learning affect theta and gamma, a pilot study was conducted where four animals were implanted and run through the new plus maze paradigm. The study seeks to quantify how theta and gamma power and frequency change over trials as the animal is learning to do a place task over a single recording session. Animals are subjected to at least two place recordings (Room A) and two response recordings (Room B) on different plus-mazes.

The first day in each room represents a novel experience for the animal; exploratory behaviors such as sniffing and looking around are common at first. In order to make it easier for the animals to learn the task, the arm across from the start was blocked, so the animal was forced to choose to turn either left or right. Early data showed that the animals were capable, but not sufficiently motivated to learn and run the tasks for 50 trials over multiple consecutive recording days. Adding a runway recording (Room C) between each plus-maze day solved this problem in subsequent animals. Running each task on the second day would show that the animal has mastered it; the expectation is that they will make at least 45 correct choices out of 50 trials.

This study is powerful because it contains several built-in controls. The place task, as a hippocampal-dependent, spatial learning task is the experimental condition that will
be compared against the response and runway tasks. The response task represents motor learning (although the hippocampus is likely still active, see Schmidt et al., 2011), and the runway task, as well as the place and response “day 2” tasks, take place in familiar environments with no learning. It is also different from other LFP studies about place/response learning, such as DeCoteau et al. (2007), in that it shows the learning process within one recording: exploring the new environment, making random choices, beginning to learn the pattern and finally mastering the task are all captured in one recording.

**Stability of theta and gamma power on a simple alternation task**

Pilot data were analyzed for one animal over seven recording sessions on the runway. Based on our findings with the radial arm maze task, we expected that power would increase in all frequency bands with maze running, even in a familiar environment. Unsurprisingly, it was found that theta, low and high gamma power significantly increased on the runway. Analyzing just the first and last ten trials showed that theta, low and high gamma did not change over trials in either dorsal or ventral hippocampus, with the exception of high gamma in dorsal hippocampus. Since all of these data are based on just one animal, it is impossible to make any larger claims about learning and memory. It suggests that the runway task may be an effective control, but nothing more can be inferred.

**Predictions**

It is expected that hippocampal theta power will initially decrease on the novel plus-maze recording days over trials as the animal becomes habituated to the environment in both the place and response tasks. Following task mastery (criteria), the degree of the decrease in theta power will be greater in the response than the place task, as the response strategy is less hippocampal-dependent than the place strategy. Theta power is not predicted to change over trials as the animal performs the alternation task, or during the second day of either the place or response task (**Fig. 13**, first row).
Figure 13: Hypothesized changes in theta power and frequency in dorsal hippocampus. The first row of plots shows how theta power might change over trials compared to sitting in the home cage. The black curve represents the first day of recording, and the red line indicates the trial where the animal meets criteria. The blue line represents the second day of recording, where the maze is familiar and the task has been mastered. The second row shows how peak theta frequency may shift over trials. The black peak represents theta frequency over the first ten trials, the red represents the last ten trials.

Peak theta frequency is expected to shift to the right over trials in both place and response task. Low theta frequencies have been associated with cognition during spatial tasks, while higher theta frequency is generally associated with movement (Bland, 1986). While the animal is exploring the environment and learning the task, low theta will be more prominent. After task mastery, velocity will increase, boosting high theta simultaneously. However, it is expected that this rightward shift in frequency will be larger on response than place days, as the animal is not using a hippocampal-dependent strategy to solve the response task (Fig. 13, bottom row).

Alternative predictions

It is also possible that theta power and frequency may not differ between place and response days. While the striatum is thought to be the seat of response learning, the hippocampus is still active even during striatal-dependent tasks, with coherent theta
activity between the two brain structures (DeCoteau et al., 2007). In addition, Hinman et al. (2011) found that theta power decreases over time with continuous maze running. Taking both of these findings into account, the shapes of the curves could change (Fig. 14).

![Figure 14: Alternative model of changes in theta power in dorsal hippocampus.](image)

This would be an interesting finding as it implies that the hippocampus is processing information at a steady rate, regardless of task demands or degree of learning. The only thing that drives change in theta power in this model is the degree of novelty, and it would simply decline regularly over trials.

Another question that has yet to be answered is whether or not dorsal and ventral theta will differ from each other as the animal learns the task. Dorsal hippocampus is primarily concerned with processing spatial information, while ventral hippocampus is concerned with the emotional context (Fanselow & Dong, 2010). Even if the dorsal region responds in the ways that have been outlined so far, it is possible that the ventral region could be processing information in a different way. For example, the dorsal hippocampus could be more active while the animal is exploring the maze, while ventral hippocampus could become more active when the animal is mastering the task and is being (or not being) rewarded for its choices.

Whatever the case, it is clear that this experiment provides an exciting and powerful tool to study how local field potentials relate to the learning process in normal adult animals. The single-day learning paradigm captures the entire learning process on a trial-
by-trial basis, rather than studying animals that have already mastered the task. Future studies could also explore age-related differences in how animals learn the place/response task, and how it correlates with theta power, frequency and coherence. Perhaps old rats would struggle with encoding this task due to their reduced capacity to generate theta in response to novelty (see Fig. 8). Once it has been established how the hippocampus functions in a normal animal during task learning, it may be informative to examine how this changes with age.
REFERENCES


