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HIPPOCAMPAL THETA RESET: A POSSIBLE ROLE IN WORKING MEMORY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
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2000

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ABSTRACT

Theta EEG activity has been found in a number of brain areas and has been associated with a number of cognitive parameters. One mechanism by which theta may influence cognitive processing is through theta reset in which an ongoing theta rhythm becomes phase-locked to an incoming sensory stimulus. Resetting may ensure that a brain area is in a maximal state of depolarization when cognitively relevant sensory information arrives, allowing the organism to better encode incoming information.

In Experiment 1, reset occurred in the entorhinal cortex (EC), hippocampus (HPC) and anterior cingulate (AC). EC theta reset to a visual stimulus occurred during the encoding, but not retrieval phase of a working memory task and was correlated with correct performance. HPC theta reset occurred during both the encoding and retrieval phases of the task, with theta resetting also being predictive of correct performance. Lastly, AC theta reset occurred during the retrieval, but not the encoding phase of the task and was not predictive of correct performance.

Experiments 2 and 3 examined the neuroanatomy responsible for HPC theta reset. Experiment 2 results indicated that both the perforant path, which originates in the EC and carries sensory information to the HPC, and the fornix, which carries fibers connecting the septum and the HPC, are involved in HPC theta reset. Experiment 3 results indicated that
reversible inactivation of the lateral septum significantly impaired working memory, but that this mnemonic impairment was not accompanied by decrements in the degree of theta reset.

Lastly, Experiment 4 examined whether electrical stimulation timed around task-relevant stimuli would affect cognitive performance. Trains of electrical stimulation, but not more discrete single pulse stimulations, centered around the sample light produced a slight impairment in working memory performance. Slight enhancements in performance followed single pulses delivered 200 msec before and after the sample light, which corresponds to the optimal timing patterns for LTP induction.

Overall, results indicated that theta reset is a viable mechanism to enhance working memory processes throughout the brain. However, follow-up studies will be necessary to fully understand the cognitive parameters and neuroanatomical connections involved in theta reset.
Dedicated to my family
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**FIELDS OF STUDY**

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CHAPTER 1
INTRODUCTION

At approximately 1-2 mV, the theta rhythm (which ranges in frequency from 4-12 Hz) is one of the largest electroencephalographic (EEG) potentials in the mammalian brain (Bland & Whishaw, 1976). Theta generators have been found in numerous brain areas, including the hippocampus, the entorhinal cortex and the anterior cingulate region of the prefrontal cortex (Bland, 1986; Chroback & Buzsaki, 1998; Demiralp, Basar-Eroglu, Rahn & Basar, 1994; Dickson, Trepel & Bland, 1994; Ishii, Ukai, Inouye, Ishihara, Yoshimine, Hirabuki, Asada, Kihara, Robinson & Tajeda, 1999; Sarnthein, Petsche, Rappelsberger, Shaw & von Stein, 1998). Of the three, the hippocampal (HPC) theta rhythm has been the most extensively studied, with theta generators being found in two different areas of the hippocampus: the stratum oriens of the CA1 field of Ammon’s horn and the stratum moleculare layer of the dentate gyrus (Bland, 1986). The HPC theta rhythm is dependent upon the integrity of the medial septal area (MSA), which provides cholinergic and GABAergic input to the hippocampus (Freund & Antal, 1988; Frotscher & Leranth, 1985). The MSA contains rhythmically bursting neurons which are thought to drive the hippocampus, as evidenced by the strong correlation between MSA rhythmic activity and HPC theta (Alonso, Gazeltu, Buno & Garcia-Ausst, 1987; Gazeltu & Buno,
Researchers have identified three distinct classes of MSA neurons (Alonso et al., 1987; Gazeltu & Buno, 1982). Type 1 neurons fire in a rhythmically bursting pattern and are phase-locked to a specific phase of the HPC theta rhythm. However, the phase at which Type 1 neurons fire can vary from cell to cell (Alonso et al., 1987). These type 1 MSA neurons can be further subdivided into three distinct classes depending upon the bursting patterns of the neurons. Type 1A neurons exhibit a regular bursting pattern with high firing rates within each burst. Type 1B neurons also exhibit a regular bursting pattern, but have shorter intraburst frequencies and longer between burst periods of inactivity. Type 1C neurons do not show as regular a bursting pattern as Type 1A and 1B neurons. For instance, Type 1C neurons have variable intraburst and interburst firing rates. However, all Type 1 neurons exhibit a clear phase-relationship with HPC theta. Type 2 neurons also phase-locked to a particular phase of the HPC theta rhythm, but do not show a regular bursting pattern, while Type 3 neurons show no clear phase relationship with HPC theta. Overall, these studies show that there is a clear relationship between the majority of MSA neuronal firing and the HPC theta rhythm. This conclusion is supported by lesions studies in which lesions to the MSA abolished HPC theta (Bland & Bland, 1986). Overall, there is no evidence of HPC theta persisting after complete lesions to the MSA (Vinogradova, 1996). For these reasons, the MSA is often referred to as the pacemaker of HPC theta.

Although early theories suggested that HPC theta was controlled primarily by MSA cholinergic neurons (Buzsaki, 1984; Buzsaki, Leung & Vanderwolf, 1983), more
recent studies have hypothesized that both cholinergic and GABAergic MSA projections to the HPC are involved in theta induction. More specifically, it is thought that the MSA cholinergic and GABAergic neurons project onto GABAergic HPC interneurons (although the cholinergic neurons also synapse with HPC pyramidal neurons), with rhythmic excitation and inhibition of HPC interneurons (by the cholinergic and GABAergic MSA neurons, respectively) being responsible for the HPC’s characteristic theta rhythm (Paulsen & Moser, 1998; Smythe, Colom & Bland, 1992; Stewart & Fox, 1990; Vinogradova, 1996). The HPC interneurons (theta cells) may be important for learning-induced synaptic changes in HPC granule and pyramidal cells (Bilkey & Goddard, 1985; 1987; Paulsen & Moser, 1998).

Researchers have identified two types of theta: Type 1 theta is 1) associated with voluntary movements, such as running, walking and lever pressing (Adey, Dunlop & Hendrix, 1960; Buno & Velutti, 1976; Rudell, Fox & Ranck, 1979; Sainsbury, 1998) 2) considered to be resistant to the cholinergic antagonist atropine (Bland, 1986; Heynen & Bilkey, 1994) and 3) abolished by lesions to the medial septal area or the entorhinal cortex (Stewart & Fox, 1990). Type 2 theta is 1) most often seen in anesthetized conditions, though it is also observed during times of alert immobility (Stewart & Fox, 1990) 2) abolished by atropine and 3) abolished by MSA, but not entorhinal cortex lesions. Some researchers have hypothesized that Type 2 theta may be more preferentially involved in learning, memory and attentional processes (Brazhnik & Vinogradova, 1987; Vinogradova, 1996). However, more studies explicitly addressing this issue need to be conducted to confirm this hypothesis.
Although a direct link between types of theta and cognitive processing has yet to be firmly established, there is considerable evidence that HPC theta plays a significant role in information processing. Manipulations which disrupt the HPC theta rhythm, such as inactivation of the medial septal area (MSA), significantly impair performance on working memory tasks (Givens & Olton, 1990; Mizumouri et al., 1990; Winson, 1978), with disruption of the HPC theta rhythm being correlated with working, but not reference, memory impairments (Givens & Olton, 1990; 1994; 1995). Intracranial infusions of tetracaine, muscimol and scopolamine into the MSA reveal a strong positive correlation (r = .78) between theta power and choice accuracy (Givens & Olton, 1990).

However, despite the strong correlation between theta activity and mnemonic performance, the precise mechanism by which theta may influence cognitive processing is not known. One proposed mechanism is a theta resetting - a phase-locking of an ongoing theta rhythm to an incoming sensory stimulus. Evidence strongly suggests that HPC theta is reset to incoming sensory stimuli (Adey, 1967; Brankack, Lui & Loannides, 1998; Givens, 1996b) during behavioral task performance (see Chapter 1 introduction for a more complete review). In addition, all three brain areas exhibiting theta (the EC, HPC and AC) have been linked to mnemonic processing (insert references). Thus, theta reset may be a mechanism to enhance the processing of cognitively relevant information within each of these structures. The neuroanatomical connections of the EC, HPC and AC are also consistent which such a hypothesis. Below is a brief overview of the neuroanatomical connections by which theta reset may occur in each of these structures.
Based upon its anatomical connections, the EC is well suited for a role in encoding processes, serving as a conduit for relaying sensory information from polymodal association areas to the hippocampus via the perforant path (Burwell & Amaral, 1998; Suzuki & Amaral, 1994b). Although there is evidence that sensory information from higher cortical centers can be conveyed to the HPC along a sparse cortical-entorhinal-hippocampal route (Burwell & Amaral, 1998; Suzuki & Amaral, 1994b), neuroanatomical connectivity patterns suggest that the EC receives the majority of its sensory cortical input from the perirhinal and parahippocampal cortices (Burwell & Amaral, 1998; Insausti, Amaral & Cowan, 1987; Naber, Caballero-Bleda, Jorritsma-Byham & Witter, 1997; Suzuki & Amaral, 1994b).

The perirhinal and parahippocampal cortices receive sensory information from cortical areas processing all sensory modalities, including unimodal and polymodal visual cortex, such as TE, TEO, V4 and retrosplenial cortex (Burwell & Amaral, 1998; Insausti et al., 1987; Suzuki & Amaral, 1994a), visuospatial areas in parietal cortex (Burwell & Amaral, 1998; Suzuki & Amaral, 1994a), auditory association areas in the temporal lobe (Burwell & Amaral, 1998; Suzuki & Amaral, 1994a) and olfactory cortex (Deacon, Eichenbaum, Rosenberg & Eckmann, 1983). The perirhinal and parahippocampal cortices convey this sensory input to the EC, with the perirhinal inputs projecting more preferentially to the lateral portion of the EC and the parahippocampal inputs projecting more preferentially to the medial EC (Naber et al., 1997). The EC, in turn conveys this sensory input to the HPC via the perforant path (Burwell & Amaral, 1998; Suzuki &
Amaral, 1994b). More specifically, projection neurons arise in layers II and III of the entorhinal cortex, with layer II neurons projecting to the dentate gyrus and CA₂ and CA₃ fields of Ammon's horn (Blackstad, 1958; Hjorth-Simonsen & Jeune, 1972; Tamamaki, 1997) and layer III neurons projecting to CA₁ of Ammon's horn (Tamamaki, 1997). Lastly, there is also evidence for a projection from the medial septal area to the EC (Irle & Markowitsch, 1984).

One possible neuroanatomical model to account for HPC theta reset is that a sensory stimulus may travel along two parallel pathways: 1) through the medial septal area via the brainstem and 2) through the perirhinal and entorhinal cortex. According to this proposed model, the purpose of HPC reset is to ensure that the hippocampus is maximally depolarized when relevant sensory information (e.g., the sample or choice light) arrives from the entorhinal cortex via the perforant path. Electrical stimulation of the septum prior to perforant path stimulation can alter the size of the granule cell population spike normally induced by perforant path stimulation (Bilkey & Goddard, 1985).

Although they may be involved in different aspects of a working memory task, the purpose of EC theta reset is hypothesized to be qualitatively similar to HPC theta reset. EC and HPC theta share many common characteristics, including the ability to be evoked by tail pinches, cholinergic manipulations and electrical stimulation of the posterior hypothalamus (Dickson et al., 1994). In addition, the integrity of the MSA is essential for the expression of both HPC and EC theta, as intracranial infusions of the local anesthetic procaine abolish theta in both areas (Dickson et al., 1994). Thus, it is possible that theta reset in each of these areas is due to a brief, stimulus-induced inhibition of MSA neuronal
One hypothesis is that the EC and HPC theta reset involve a progressive synchronization of neuronal firing as information moves through the hippocampal formation. The phase of theta rhythm in the hippocampal formation varies from structure to structure (Rudell, Fox & Ranck, 1986). Thus, theta reset may be a mechanism by which a structure is in a maximal state of depolarization at the time sensory input arrives.

**Neuroanatomy of anterior cingulate theta reset**

Although there are several neuroanatomical models which can explain the observed AC theta reset, these models can fall under two general classes: 1) models in which the AC theta reset is dependent upon direct input from the hippocampus and 2) models in which the AC theta is a “stand alone” mechanism that does not rely on connections with the entorhinal cortex and hippocampus.

In the first model, AC theta reset would result from direct input from HPC. Electrophysiological studies have shown that tetanic stimulation of hippocampal efferents to the prefrontal cortex (PFC) results in a long-term potentiation in the prefrontal cortex (Doyere, Burette, Del Negro & Laroche, 1990; Jay, Gurden & Yamaguchi, 1998; Laroche, Jay & Thierry, 1990), indicating that input from the HPC is capable of enhancing synaptic strength within the PFC and thus may enhance stimulus encoding, perhaps by affecting the degree of theta reset to a stimulus.

Neuroanatomical studies support the existence of a HPC to PFC pathway. Retrograde and anterograde tracing revealed a unilateral projection from AC to the CA1
field of Ammon's horn and a strong reciprocal connection between the AC and the subicular complex of the hippocampus and also to the entorhinal and perirhinal cortex (Arikuni, Sako & Murata, 1994; Jurgens, 1983; Vogt & Pandya, 1987). Thus, a subicular output pathway may be one route through which the HPC can affect AC theta reset. However, some have questioned the existence of a direct pathway from the subiculum to the AC, arguing instead that the HPC projects to the prelimbic, infralimbic and medial orbital cortices of the prefrontal cortex (Jay & Witter, 1991). Thus, the effects of HPC activity on AC theta reset may be indirect. For example, HPC theta output may first project to the prelimbic cortex, which in turn projects to the AC (Arikuni et al., 1994; Buchanan et al., 1994; Conde, Maire-Lepoivre, Audinat & Crepel, 1995; Sesack et al., 1989). However, it is possible that hippocampal output to the AC is not necessary for AC theta reset.

The "stand alone" model is based upon afferent input to the AC from either thalamic, basal forebrain, or brainstem areas. The anterior cingulate has mostly reciprocal connections with a number of thalamic nuclei, including the intralaminar (Buchanan et al., 1994), lateral (Buchanan et al., 1994), midline (Buchanan et al., 1994), ventrolateral (Sesack et al., 1989) ventromedial (Buchanan et al., 1994), ventral posterior (Buchanan et al., 1994) and especially the mediodorsal nuclei (Buchanan et al., 1994; Groenewegen et al., 1990; Guiguere & Goldman-Rakic, 1988; Sesack et al., 1989). In addition to the thalamic connections, several basal forebrain structures, including the medial septal area (Gaykema, Weeghel, Hersh & Luiten, 1991; Irle & Moscovitch, 1984; Uylings & van Eden, 1990), the nucleus basalis of Meynert (Conde et al., 1995; Uylings & van Eden,
1990) and the substantia innominata (Conde et al., 1995; Grove, 1988; Irle & Moscovitch, 1984), also form reciprocal connections with the AC. Lastly, several neurotransmitter specific brainstem areas also project to the AC, including the serotonergic-rich raphe nucleus, the noradrenergic-rich locus coeruleus, and the dopaminergic neurons of the ventral tegmental area (Buchanan et al., 1994).

Many of these structures, especially the MSA, have significant influences on hippocampal theta (Bland & Bland, 1985; Brazhnik et al., 1985; Gazeltu & Buno, 1982). It is possible that these structures may exert a direct influence on AC theta independent of hippocampal influence. For instance, the MSA may be directly responsible for both AC and HPC reset. A brief cessation of MSA rhythmic neuronal firing may lead to theta reset in both these areas. However, a systematic examination of the nature of AC theta has yet to be conducted, making it difficult to draw any specific conclusion about the dependence (or independence) of AC theta reset.

*Experimental goals and predictions*

Although the phenomenon of theta reset was first studied over three decades ago, little is known about the exact role of HPC theta reset in mnemonic processing. The present group of experiments was designed to examine the precise mechanism by which theta reset may influence the proper encoding of stimuli in working memory tasks. Experiment 1 examined the cognitive parameters of theta resetting by examining whether theta reset was more likely to occur during the encoding, retrieval or motor response
phase of a working memory task. Given the strong link between theta and motor movement, it was predicted that theta reset would occur following motor response. Also, given previous results in our laboratory, which showed that theta reset occurred to sensory stimuli in a working memory task (Givens, 1996b), it was predicted that theta would reset to sensory stimuli in the present experiment, with the degree of theta reset in each of these areas perhaps being dependent upon the stage of working memory in which the stimulus was encountered. In addition, Experiment 1 was designed to examine whether theta reset is predictive of correct task performance. The working hypothesis was that theta reset would enhance the encoding of incoming sensory stimuli and hence theta reset should be correlated with correct task performance. Experiments 2 and 3 were designed to identify the neuroanatomy of hippocampal theta reset, focusing on structures such as the medial septal area, the lateral septal area and the entorhinal cortex. Given the results of studies in anesthetized animals (Buno, Garcia-Sanchez & Garcia-Ausst, 1978; Gazeltu & Buno, 1982), it was predicted that all of these areas would be involved in HPC theta reset in the awake, freely behaving rat. Lastly, experiment 4 was designed to identify the timing patterns that could facilitate theta reset by delivering electrical stimulations centered at precise times during performance of a working memory task. It was predicted that discrete electrical stimulations centered around task-relevant sensory stimuli would either enhance or disrupt task performance, depending upon the timing of the stimulation.
CHAPTER 2
EXPERIMENT 1

One possible mechanism by which the HPC theta rhythm may enhance cognitive processing is through a resetting of the theta rhythm, in which ongoing theta becomes phase-locked to incoming sensory stimuli (Adey, 1967; Brankack, Liu & Loannides, 1998; Givens, 1996b; Vinogradova, Brazhnik, Kitchigina & Stafekhina, 1996). One hypothesis is that a stimulus (either a natural sensory or an exogenously applied electrical stimulus) causes a brief inhibition of MSA neuronal firing, which is followed by the re-starting of the normal rhythmic firing pattern, causing a resetting of the hippocampal theta rhythm (Buno, Garcia-Sanchez & Garcia-Austt, 1978). Support for this hypothesis comes from the fact that hippocampal theta reset (induced by mesencephalic reticular formation stimulation) was always preceded by a resetting of MSA neuronal activity (Gaztelu & Buno, 1982). In addition, a resetting of MSA single unit activity has been observed in MSA neurons following the presentation of a visual or auditory stimulus in a working memory task (Givens, 1996a).

The purpose of HPC theta resetting may be to ensure that the hippocampus is in a maximal state of depolarization at the time sensory input arrives in the hippocampus from the entorhinal cortex (EC). The EC receives converging sensory input from polymodal...
association areas in the cortex through its connections with the perirhinal cortex (Suzuki & Amaral, 1994; Suzuki, Miller & Desimone, 1997) and relays this information to the HPC via the perforant path. The MSA may prime the hippocampus to receive this incoming sensory information from the EC, thereby facilitating long-term potentiation (LTP), enabling synaptic plasticity (Diamond & Rose, 1994, Larson & Lynch, 1986) and ultimately enhancing the encoding of incoming information (Vinogradova et al., 1996).

For instance, when a priming burst of electrical stimuli was applied to one input of a HPC neuron in vitro, a prolonged potentiation to a second burst of electrical stimuli (applied to a second input of the same target neuron) was observed, if the second identical burst was delivered 200 msec after the initial bursting pattern (Larson & Lynch, 1986). Significantly, this 200 msec time interval corresponds to the general theta frequency. It is also important to note that potentiated responses were only observed to the second burst despite the fact that the electrical stimuli delivered in the first and second stimulations were identical. This suggests that the first electrical stimulation functioned as a primer, allowing an enhanced response to a subsequent sensory stimulus (Larson & Lynch, 1986).

More direct support for the hypothesis that theta reset allows the HPC to be maximally responsive when sensory input arrives from EC comes from studies examining the effects of medial septal stimulation prior to perforant path stimulation (Bilkey & Goddard, 1985; 1987). Stimulation of the EC or perforant path can induce excitatory post-synaptic potentials (EPSPs) in the dentate gyrus. More interestingly, stimulating the septum can alter the size of the granule cell population spike evoked by perforant path stimulation in HPC and can remove the paired-pulse inhibition observed following
electrical stimulation of the perforant path if the conditioning pulse to the MSA was timed to coincide (or nearly coincide) with the first evoked population spike (Bilkey & Goddard, 1985; 1987). These data support the hypothesis that theta reset may act as a primer for the subsequent encoding of sensory information from the entorhinal cortex.

In addition to these purely electrophysiological studies, evidence from studies combining electrophysiological and behavioral methodology suggests a link between hippocampal theta reset and cognitive processing. The first known report of theta reset found that, in cats, CA1 hippocampal theta was reset to a visual stimulus in the early stages of learning a visual discrimination task, but disappeared once performance plateaued (Adey, 1967). More recent studies confirmed this finding in rats, showing that HPC theta was reset to sensory stimuli in the early stages of learning an oddball discrimination task. However, this reset disappeared once the task became over-learned (Brankack et al., 1998).

Although theta reset to a sensory stimulus disappears once a task becomes an over-learned, reference memory task, theta reset is maintained in working memory tasks. Reference memory processes the information independent of the context and includes such information as rule-based processing. Conversely, working memory is associated with context, including spatial, temporal or other trial-dependent characteristics (Olton, 1986). Working memory is believed to be HPC-dependent while reference memory is not.

A resetting of the hippocampal theta rhythm to mnemonically-relevant sensory stimuli was observed in rats performing a well-learned working, but not reference memory task (Givens, 1996b). In this study, rats were trained on an operant continuous
conditional discrimination (CCD) task that assessed working memory. At the beginning of each trial of the CCD task, a light or tone was presented. If the stimulus for the current trial was the same as the previous trial (i.e. light-light or tone-tone), the rat was required to press a designated “match” lever. If the stimulus for the current trial differed from the previous trial (i.e. light-tone or tone-light), the rat was required to press the designated “non-match” lever. Theta reset was observed to both the tone and the light in the working memory task, but not an analogous reference memory task which utilized the same sensory stimuli and required similar motor responses (Givens, 1996b). In addition, it is also important to note that theta reset followed the presentation of sensory stimuli across different sensory modalities, as theta reset was observed following presentation of both the auditory tone and the light. This suggests that theta reset is a viable mechanism to explain mnemonic processing across a variety of stimulus attributes.

Although the above studies (Adey, 1967; Brankack et al., 1998; Givens, 1996b) provide support for the hypothesis that theta reset is involved in cognitive processing, it is unclear whether theta reset is selectively involved in certain parameters of a working memory task. For instance, theta reset may be more selectively involved in the initial encoding (sample), the retrieval (choice) and/or the motor response stages of a cognitive task. The previous studies did not examine the precise cognitive parameters associated with theta reset. For example, in the CCD task, there was not a discrete sample and choice phase, as each stimulus presentation required the rat to simultaneously encode the new stimulus and make a response choice by comparing the new stimulus to the previous stimulus.
Experiment 1 addressed these issues by utilizing a delayed nonmatch-to-position (DNMTP) task that allowed a distinct comparison between visual stimuli encountered during the encoding versus retrieval phases of a working memory task. Given that the hippocampal theta rhythm has been associated with movement (Vanderwolf, 1980), Experiment 1 was also designed to examine whether theta reset was associated with motor lever pressing responses. In addition, if the resetting of the hippocampal theta rhythm is a viable mechanism to explain how sensory information is encoded in memory by the hippocampus, one would expect to see a strong correlation between resetting and correct performance. That is, a greater degree of theta reset should occur to either the sample light, the choice light or the motor responses on correct trials than incorrect trials. Lastly, it is important to note that structures other than the hippocampus, such as the entorhinal cortex (EC) and the anterior cingulate (AC) region of the prefrontal cortex, also display a prominent theta rhythm (Bland, 1986; Chroback & Buzsaki, 1998; Demiralp, Basar-Eroglu, Rahn & Basar, 1994; Dickson, Trepel & Bland, 1994; Ishii, Ukai, Inouye, Ishihara, Yoshimine, Hirabuki, Asada, Kihara, Robinson & Tajeda, 1999; Sarnthein, Petsche, Rappelsberger, Shaw & von Stein, 1998). Thus, Experiment 1 was also designed to determine whether the reset phenomenon applies not only to the hippocampal theta rhythm, but also to other areas which exhibit theta activity.

Method

Shaping procedures

Eight male Long-Evans rats were trained on a DNMTP working memory task.
Detailed descriptions of the subjects, shaping procedures and behavioral apparatus are provided in the general methods section (Chapter 6).

Sample phase: Light appears randomly over right or left lever

Delay period: 5-10" delay

Choice phase: Light over center lever signals start of choice phase. Rat must hit lever opposite to the sample light to get reward.

10" ITI period before next trial

Delayed Nonmatch-to-Position (DNMTP) procedure

The DNMTP task is a working memory task that is comprised of two components: a sample phase and a choice phase. At the beginning of each trial, a light appeared over either the left or right lever. Rats were required to press the lever beneath the light, at which time the light was extinguished and the rat received a 0.04 ml drop of water. This constituted the sample phase of the task, which can be considered the initial "encoding" phase of the task.

In the sample phase, the lever press was required to ensure that rats were cognizant of the position of the sample stimulus.

After the sample light, a five or ten second delay was imposed, after which a light over the center lever appeared. The center light signaled the start of the choice phase, which was analogous to the "retrieval" phase of the task. Rats were required to recall and press the lever opposite the sample light. For instance, if a light appeared over the right lever during the sample phase, then the rat was required to press the left lever during the choice phase. The choice phase continued until the rat made a response to either the right
or left lever. All correct choice phase responses were rewarded with a 0.08 ml drop of water. If the rat did not respond within five seconds, the trial continued until the rat pressed either the right or left lever, but the trial was recorded as a no response trial. A thirty second intertrial interval (ITI) followed each choice-phase lever response.

After reaching criterion performance on this final training step of the DNMTP task (over 75% correct for both long and short delayed response, and no response rate less than 40% for three days), subjects underwent electrode implantation surgery.

**Surgery**

All Experiment 1 surgeries implemented the surgical procedures outlined in the general methods section (Chapter 6). As such, only surgical aspects that are to unique to Experiment 1 will be described. In Experiment 1, two theta recording electrodes were placed bilaterally into the dentate hilus (4.0 mm posterior to bregma; ± 2.5 mm lateral to midline; 2.7-3.0 mm ventral to the dural surface) and unilaterally in the anterior cingulate region of the medial prefrontal cortex (0.7 mm anterior to bregma; 1.0 mm lateral to midline; 3.2-3.8 mm ventral to the dural surface at a 15° angle) and the entorhinal cortex (8.8 mm posterior to bregma; 4.5 mm lateral to midline; 4.7-5.5 mm ventral to the dural surface). The ventral coordinates for recording electrodes were determined for each rat during surgery by observing the theta signal on an oscilloscope and by examining theta power using a fast Fourier transformation (FFT) on-line. The purity and the power of the theta rhythm were used to locate the optimal recording position.
Post-surgical testing

After a one week recovery period from surgery, rats were retrained on the DNMTP task. During this re-training period, one end of the preamplifier was mounted to the connector on the rats’ heads, while the other end was attached to a cable which was in turn connected to a commutator. The commutator swivelled to allow the rats to move in the operant chamber without becoming entangled in the cable. The purpose of this re-training period was 1) to ensure that the surgery did not affect task performance and 2) to acclimate the rats to the recording protocol. After baseline performance returned to presurgery baseline levels, the post-surgical testing sessions commenced.

Electrophysiological data in Experiment 1 was collected for ten days (five days per week) from each animal.

In each of the ten recording sessions, theta EEG was acquired (at a sampling rate of 1200 Hz) one second before and after both sample light and choice light onset to determine if resetting occurred to light stimuli in the encoding and/or retrieval phases. In addition, theta was acquired one second before and after lever presses in the choice phase to determine if resetting occurred following motor responses. Markers were inserted by the acquisition software (Med-Associates, St. Albans, VT) to differentiate correct and incorrect lever presses for later analysis. Markers separating short and long delay trials were also inserted during behavioral performance. Recording sessions lasted one hour and fifteen minutes or until 150 trials were completed, whichever came first.
Analysis

The purpose of the post-surgical testing was to determine whether visual stimuli in the DNMTP task could reset theta and to determine the parameters for theta reset. These parameters included whether theta reset occurred preferentially during the sample (encoding), choice (retrieval) or motor response phase of the DNMTP task and whether there was a correlation between theta reset and correct/incorrect DNMTP performance. To address these issues, analyses were conducted to determine whether theta was reset to 1) the sample light 2) the choice light and 3) motor lever pressing. Trials were also broken down to assess whether theta reset to these three variables was predictive of correct task performance and whether theta reset to these variables was correlated with delay length. These analyses were applied to each of the four brain areas examined in this experiment (right dentate hilus, left dentate hilus, the anterior cingulate cortex and the entorhinal cortex) to determine whether there was a dissociation in the parameters of theta reset between different areas of the brain that exhibit a theta rhythm.

Results

Behavior

Post-surgically, rats took six to eleven days to return to baseline levels of performance and become acclimated to the electrophysiological recording procedures. Rats maintained a high level of DNMTP performance (79.97 ± 0.59 percent choice accuracy overall) throughout the electrophysiological recording sessions, with performance at the short delay being significantly higher than choice accuracy at the long...
delay (90.00 ± 0.59 versus 71.51 ± 0.82 percent choice accuracy, respectively). This delay dependent decrement in choice accuracy suggests that the DNMTP task was successful in measuring working memory processes. In addition, rats maintained a stable level of task motivation throughout testing, completing 116.26 ± 1.86 trials per testing session, with 30.40 ± 1.15 percent omissions.

A separate waveform averaging was performed upon the pre- and post-stimulus electrophysiology records for each testing session for each of the three brain areas examined in this experiment: the hippocampus (HPC), the entorhinal cortex (EC) and the anterior cingulate (AC). The stimuli of interest in this experiment were the sample and choice lights and motor lever responses. In addition, these stimuli were broken down in terms of correct, incorrect and no response trials based on choice phase performance. If theta was reset to a stimulus, a significant theta peak should have been evident in the final waveform average. Conversely, if no theta reset occurred, the final waveform average should have averaged to a relatively flat line with little theta activity evident in the final waveform average.

**Theta reset to sample light stimuli**

Paired sample t-tests comparing theta power in the final waveform average before and after sample light presentation were conducted to determine whether theta was reset to the sample light overall (i.e. regardless of whether the light occurred on correct, incorrect or no response trials). Overall, significant resets to the sample light were observed in the HPC [t (159) = 2.941; p<.01], but not in the EC [t (78) = .946; p>.05] or
AC regions \( t(69) = 1.919; p > .05 \). Next, the trials were broken down into correct, incorrect and no response trials to examine whether theta reset to the sample light was predictive of correct performance in the choice phase of the trial.

Paired sample t-tests revealed that in the HPC, a significant degree of theta reset was observed to the sample light on correct \( t(159) = 2.067; p < .05 \), but not incorrect \( t(159) = 1.198; p > .05 \) or no response trials \( t(159) = 0.225; p > .05 \), indicating that hippocampal theta resetting in the initial encoding phase of a working memory task may be predictive of correct performance (Figure 2.2). Similar results were observed in the EC. A significant degree of EC theta reset was observed to the sample light stimulus on correct trials \( t(78) = 2.614; p < .02 \), but not incorrect \( t(78) = 1.407; p > .05 \) or no response trials \( t(78) = 0.233; p > .05 \), suggesting that EC theta reset in the initial encoding phase of the DNMTP task is also important for later correct performance (Figure 2.2). In contrast to the results in the HPC and EC, no significant theta reset following sample light presentation was observed in the AC region of the prefrontal cortex in any of the three trial types, including correct \( t(68) = 1.151; p > .05 \), incorrect \( t(68) = 0.439; p > .05 \) or no response trials, suggesting that theta reset in the AC does not occur during the initial encoding phase of the DNMTP task (Figure 2.2).
Although it appears that there is a significant degree of AC theta reset following sample light presentation on correct trials, this non-significant trend is mostly due to four aberrant data points (out of seventy data points which went into the analysis). A Grub’s test for statistical outliers revealed that each of these points were indeed statistical outliers, with each of these four points exhibiting over a 1,000% increase in theta power in the final waveform average. This large increase is much higher than the 65.43% increase in theta power for the remaining sixty-six data points. If these four data points are removed from the final analysis (as justified by the Grub’s test for statistical outliers), this trend towards significance disappears [$t(64) = 0.342; p = .733$].

To determine whether the observed resets were the result of a phase-shifting of an ongoing theta rhythm or the evoking of a theta rhythm, an FFT was performed upon the

![Figure 2.2: A significant degree of theta reset is observed following sample light presentation in the HPC and EC on correct, but not incorrect trials. (* = p<.05)](image-url)

[Image description: The figure shows a bar graph illustrating the percentage change from baseline in theta reset to the sample light for correct trials, incorrect trials, and no response trials. The HPC, EC, and AC are represented on the x-axis, with bars indicating the percentage change for each category.]
raw pre- and post-stimulus records for the three stimulation conditions. These FFT analyses were performed upon the raw data records (as opposed to the previous FFT's which were performed upon the final waveform average). The FFT analyses of the raw data records revealed a strong theta peak centered at approximately $7.47 \pm .08$ Hz, indicating that there was a strong theta rhythm both before and after sample light presentation in the HPC, EC and AC. To quantify whether there was a change in theta power following stimulus presentation and/or motor responses, two-tailed paired sample t-tests were used to compare pre- and post stimulus theta power. Whether there was an increase (or decrease) in theta power following a stimulus or motor response varied by brain area. As such, each brain area will be discussed individually.

In the HPC, there were slight, but consistent increases in theta power following numerous stimulus conditions (Table 2.1). Theta power increased 16-18% following sample light presentation [$t (157) = 7.563; p<.001$]. Unlike the increase in theta power following sample light presentation in the final waveform average, the sample light-induced increase in theta power in the raw data files was not predictive of subsequent task performance, as increases in theta power were evident following the sample light on all trial types, including correct trials [$t (157) = 5.847; p<.001$], incorrect trials [$t (156) = 4.393; p<.001$] and no response trials [$t (157) = 4.181; p<.001$].

In contrast to the increase in hippocampal theta power in the FFT's of the raw data files, there was a much less pervasive theta power increase in the EC and AC following sample light presentation. In the EC, a slight, but significant 5% increase in theta power was evident in the FFT's of the raw data files following the sample light on no response
trials (p<.05). No significant increases in theta power were observed in the FFT’s of the raw data files following the sample light overall (regardless of trial type) or the sample light on correct or incorrect trials (p>.05). In the AC, an 18% increase in theta power in the FFT’s of the raw data files followed the sample light presentation on correct trials [t (61) = 2.168; p<.05; Table 2.1], but not following the sample light on incorrect [t (67) = 1.896; p>.05; Table 2.1] or no response trials [t (64) = 0.196; p>.05; Table 2.1] or following the sample light overall [t (61) = 1.081; p>.05; Table 2.1].
<table>
<thead>
<tr>
<th></th>
<th>HIPPOCAMPUS (DENTATE HILUS)</th>
<th>ENTORHINAL CORTEX</th>
<th>ANTERIOR CINGULATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORRECT LEVER PRESSES</td>
<td>-6.16 ± 5.25</td>
<td>2.55 ± 5.38</td>
<td>17.82 ± 9.75</td>
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<td>SAMPLE LIGHT OVERALL</td>
<td>15.82 ± 1.88</td>
<td>7.41 ± 2.49</td>
<td>7.14 ± 5.67</td>
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<td>SAMPLE LIGHT - CORRECT TRIALS</td>
<td>16.14 ± 3.35</td>
<td>3.79 ± 3.57</td>
<td>17.92 ± 6.49</td>
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<tr>
<td>SAMPLE LIGHT - INCORRECT TRIALS</td>
<td>17.84 ± 5.16</td>
<td>7.49 ± 6.51</td>
<td>30.77 ± 19.46</td>
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<tr>
<td>SAMPLE LIGHT - NO RESPONSE TRIALS</td>
<td>15.93 ± 3.40</td>
<td>4.95 ± 2.97</td>
<td>0.93 ± 8.72</td>
</tr>
<tr>
<td>CHOICE LIGHT OVERALL</td>
<td>10.46 ± 4.75</td>
<td>6.76 ± 2.25</td>
<td>9.67 ± 4.82</td>
</tr>
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<td>CHOICE LIGHT - CORRECT TRIALS</td>
<td>21.23 ± 2.57</td>
<td>11.22 ± 3.35</td>
<td>20.24 ± 7.65</td>
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<tr>
<td>CHOICE LIGHT - INCORRECT TRIALS</td>
<td>18.35 ± 6.59</td>
<td>11.61 ± 5.49</td>
<td>11.42 ± 19.82</td>
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<td>CHOICE LIGHT - NO RESPONSE TRIALS</td>
<td>7.87 ± 2.59</td>
<td>1.69 ± 3.83</td>
<td>-34.23 ± 28.29</td>
</tr>
</tbody>
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Table 2.1: The above numbers represent the % change in theta power between pre- and post-stimulus theta on the raw data records (not on the final waveform average). Shaded areas indicate a statistically significant increase or decrease in theta power (p<.05) following light onset or a motor response.
Frequency Shifts following sample light presentation

In addition to pre- and post-sample light changes in theta power, paired sample t-tests were performed to examine whether there were shifts in theta frequency in the final waveform average. In the HPC, there was no significant shift in theta frequency \( t (159) = 0.267; p > .05 \) to the sample light overall (i.e., regardless of whether the light occurred on correct, incorrect or no response trials). However, significant shifts in theta frequency were observed after breaking the sample light down according to trial types. There was a small 4-6% increase in theta frequency in the post-stimulus waveform average relative to the pre-stimulus waveform average to the sample light on correct \( t (159) = 3.044; p < .01 \) and incorrect trials \( t (155) = 2.230; p > .01 \), but no frequency shift to the sample light on no response trials \( t (159) = 0.194; p > .05 \).

In the EC, although there was no significant shift in theta frequency \( t (79) = 0.765; p > .05 \) to the sample light overall, a 7% increase in theta frequency in the post-stimulus waveform average following the sample light was observed on no response trials \( t (79) = 2.466; p < .02 \). No shifts in theta frequency were observed to the sample light on correct \( t (79) = 0.163; p > .05 \) or incorrect trials \( t (78) = 1.487; p > .05 \).

In the AC, although there was not a significant shift in theta frequency following the sample light overall in the final waveform average \( t (69) = 1.287; p > .05 \), small increases in theta frequency were evident in the final waveform average after breaking the sample light down according to trial type. However, this shift in theta frequency was not predictive of correct performance, as increases in theta frequency (6-9%) were seen to the sample light in correct \( t (68) = 2.597; p < .02 \), incorrect \( t (68) = 2.011; p < .05 \) and no
response \( t (69) = 2.980; p<.01 \).

To determine whether the frequency shifts observed in the final waveform averages were due to an overall shift in theta frequency, two-tailed paired sample t-tests were performed to examine whether there were shifts in theta frequency following sample light presentation in FFT’s of the raw data files (not just the final waveform average).

In the HPC, extremely small, but statistically significant 2-3% increases in theta frequency were evident following presentation of the sample light across all trial types \( p<.05 \). In both the EC and AC, 3-4% increases in theta frequency were observed following presentation of the sample light overall \( p<.01 \), but no significant increases were evident after breaking the sample light down into correct, incorrect and no response trials \( p>.05 \).

**Theta reset to choice light stimuli**

Paired sample t-tests were performed to determine if theta reset occurred following choice light presentation overall (i.e. regardless of whether the choice light occurred on correct, incorrect or no response trials). Overall, significant resets to the choice light were observed in the HPC \( t (159) = 3.040; p<.01 \) and AC \( t (69) = 3.264; p<.01 \), but not in the EC \( t (78) = 1.187; p>.05 \). Next, the trials were broken down into correct, incorrect and no response trials to examine whether theta reset to the choice light was predictive of correct performance in the retrieval phase of the trial.

In the HPC, the pattern of theta reset to the choice light was identical to that seen in the sample light analyses. Paired sample t-tests revealed a significant degree of theta
reset to the choice light on correct \( t (159) = 3.577; p < 0.001 \), but not incorrect \( t (157) = 0.019; p > 0.05 \) or no response trials \( t (159) = 0.088; p > 0.01 \), indicating that hippocampal theta resetting in the retrieval phase of a working memory task is also predictive of correct performance (Figure 2.3).

In the EC, unlike the sample phase analyses which revealed a significant degree of reset to the sample light on correct trials, no significant resets were observed following the choice light on correct \( t (78) = 1.014; p > 0.05 \) incorrect \( t (77) = 1.373; p > 0.05 \) or no response trials \( t (78) = 0.185; p > 0.05 \), indicating that a resetting of the EC theta rhythm is
associated more with the initial encoding phase of a working memory task rather than later retrieval aspects of the task (Figure 2.3).

In contrast to the lack of AC theta resetting to the sample light on any of the trials types, there was significant AC theta resetting to the choice light (Figure 2.3). However, the resetting to the choice light in the AC was not predictive of task performance, as a significant degree of theta reset was observed to the choice light on both correct \[ t(68) = 2.396; \ p < .02 \] and incorrect trials \[ t(67) = 2.502; \ p < .02 \]. No theta reset in the AC was observed following the choice light on no response trials \[ t(76) = 1.321; \ p > .05 \].

To determine whether the observed resets were the result of a phase-shifting of an ongoing theta rhythm or the evoking of a theta rhythm, an FFT was performed upon the raw pre- and post-stimulus records for the three stimulation conditions, as opposed to the previous FFT's which were performed upon the final waveform average. The FFT analyses of the raw data records revealed a strong theta peak centered at approximately 7.48 ± 0.07 Hz, indicating that there was a strong theta rhythm both before and after both choice light presentation in the HPC, EC and AC. To quantify whether there was a change in theta power following choice light presentation two-tailed paired sample t-tests were used to compare pre-and post stimulus theta power. Whether there was an increase (or decrease) in theta power following choice light presentation varied by brain area. As such, each brain area will be discussed individually.

In the HPC, there were slight, but consistent increases in theta power following numerous stimulus conditions (Table 2.1). Theta power increased 16-18% following choice light presentation \[ t(157) = 6.312; \ p < .001 \]. Unlike the increase in theta power
following choice light presentation in the final waveform average, the choice light-induced increase in theta power in the raw data files was not predictive of subsequent task performance, as increases in theta power were evident following the choice light on all trial types (Table 2.1), including correct \[ t (156) = 7.262; p < .001 \], incorrect \[ t (156) = 2.543; p < .02 \] and no response trials \[ t (154) = 2.101; p < .05 \].

In the EC, slight, but significant increases in theta power were evident in the FFT’s of the raw data files following the choice light overall [7% increase, \( t (77) = 2.028; p < .05 \); Table 2.1] and to the choice light on correct trials [11% increase; \( t (77) = 2.729; p < .01 \); Table 2.1]. No significant increases in theta power were observed in the FFT’s of the raw data files following the choice light on incorrect [\( t (77) = 0.273; p > .05 \); Table 2.1] or no response trials [\( t (76) = 0.243; p > .05 \); Table 2.1].

In the AC, significant increases in theta power in the FFT’s of the raw data files were observed following choice light presentation on correct [20% increase; \( t (63) = 2.815; p < .01 \); Table 2.1] and incorrect trials [11% increase; \( t (68) = 2.260; p < .05 \); Table 2.1]. No significant change in theta power in the FFT’s of the raw data files was observed to the choice light on no response trials [\( t (66) = 0.933; p > .05 \); Table 2.1] or to the choice light overall - though there was a strong trend towards significance towards a reset to the choice light overall [\( t (62) = 1.979; p = .052 \); Table 2.1].

**Frequency Shifts following choice light presentation**

In addition to pre- and post-choice light changes in theta power, paired sample t-tests were performed to examine whether there were shifts in theta frequency in the final
waveform average. Unlike the frequency shifts observed in the FFT's of the final waveform following sample light presentation, there were no significant shifts in theta frequency to the choice light across any trial type, including overall correct, incorrect or no response trials in any of the three brain areas (HPC, EC and AC) examined in this study (p>.05).

Despite the lack of observed frequency shifts in the final waveform averages, it was still possible that frequency shifts occurred not in the final waveform average, but in the individual raw data records. Therefore, two-tailed paired sample t-tests were performed to examine whether there were shifts in theta frequency following choice light presentation in FFT's of the raw data files (not just the final waveform average).

In the HPC, small, but significant 3% increases in theta frequency were observed following choice light presentation overall and choice light presentation on correct trials (p<.01). No significant increases in theta frequency were observed following choice light presentation on incorrect and no response trials (p>.05). In the EC, a 3% increase in theta frequency was observed following presentation of the sample light overall (p<.01), but no significant increases in theta frequency following sample light presentation were evident after breaking the choice light down into correct, incorrect and no response trials (p>.05). In the AC, a slight, but significant increase in theta frequency was observed following presentation of the choice light on correct (p<.01), but not incorrect or no response trials or the choice light overall, although there was a trend in that direction for the latter (p=.057).
Paired sample t-tests were performed to determine if theta reset occurred following motor lever presses on correct trials and on incorrect trials. All three brain areas examined revealed similar patterns of theta reset to motor responses. In the HPC, EC and AC (p<.0004; Figure 2.4), a significant degree of theta reset was observed following correct lever responses \[ t (158) = 5.350; \ p<.001; \ t (78) = 2.681; \ p<.01; \ t (67) = 3.702; \ p<.001, \text{ respectively} \], but not incorrect lever responses \[ t (155) = 0.183; \ p>.05; \ t (78) = 1.263; \ p>.05; \ t (68) = 1.742; \ p>.05, \text{ respectively; Figure 2.4} \], indicating that 1) motor
activity in all three theta-generating areas is capable of resetting the theta rhythm and 2) that this motor-induced resetting may be correlated with correct task performance.

To determine whether the observed resets were the result of a phase-shifting of an ongoing theta rhythm or the evoking of a theta rhythm, an FFT was performed upon the raw pre- and post-stimulus records for the three stimulation conditions. The FFT analyses of the raw data records revealed a strong theta peak centered at approximately 7.84 ± 0.07 Hz, indicating that there was a strong theta rhythm both before and after motor lever presses in the HPC, EC and AC. To quantify whether there was a change in theta power following motor lever presses two-tailed paired sample t-tests were used to compare pre- and post stimulus theta power. Whether there was an increase (or decrease) in theta power following lever presses varied by brain area. As such, each brain area will be discussed individually.

In the hippocampus, decreases in theta power were observed after both types of motor responses. A non-significant 12% decrease in HPC theta power was observed following correct lever responses [t (157) = 0.886; p > .05; Table 2.1]. In contrast, a significant 37% decrease in theta power was observed following incorrect lever responses [t (157) = 4.603; p < .001; Table 2.1]. In the entorhinal cortex, a small, but significant 3% increase in theta power was observed after correct lever presses [t (78) = 2.747; p < .01; Table 2.1]; whereas, a 16% decrease in theta power was observed after incorrect lever presses [t (76) = 2.066; p < .05; Table 2.1]. In contrast to the changes in theta power observed following lever responses in the HPC and EC, there was no significant change in
AC theta power following correct \( \text{[} \frac{t}{(67)} = 1.182; p>.05; \text{Table 2.1]} \) or incorrect \( \text{[} \frac{t}{(67)} = 0.411; p>.05; \text{Table 2.1]} \) lever presses.

**Frequency Shifts following motor lever responses**

In addition to pre- and post-choice light changes in theta power, paired sample t-tests were performed to examine whether there were shifts in theta frequency in the final waveform average. In the HPC and AC, a significant increase in theta frequency was observed in the final waveform average following correct \( \text{[} \frac{t}{(158)} = 2.637; p<.05; \frac{t}{(69)} = 2.267; p<.05; \text{respectively}] \), but not incorrect lever presses \( \text{[} \frac{t}{(155)} = 1.525; p>.05; \frac{t}{(68)} = 0.243; p>.05; \text{respectively}] \). In the EC, no significant changes in theta frequency were observed following either correct \( \text{[} \frac{t}{(79)} = 0.541; p>.05] \) or incorrect lever responses \( \text{[} \frac{t}{(79)} = 0.102; p>.05] \).

To determine whether the frequency shifts observed in the final waveform averages were due to an overall shift in theta frequency, two-tailed paired sample t-tests were performed to examine whether there were shifts in theta frequency following sample light presentation in FFT's of the raw data files (not just the final waveform average).

In the HPC and EC, no significant increases in theta frequency were observed following motor lever responses (\( p>.05 \)). In the AC, a small, but significant 3% increase in theta frequency was observed following correct lever responses (\( p<.006 \)). No significant increases in theta frequency in the AC were observed following incorrect motor responses (\( p>.05 \)).
Discussion

The results indicate that theta reset occurred in all three brain areas (the HPC, EC and AC) examined in this study, although the phases of the working memory task where theta reset was observed varied between brain areas. More specifically, in the EC, theta reset occurred following visual stimuli during the encoding, but not retrieval phase of the DNMTP task. HPC theta reset to a visual stimulus occurred during both the initial encoding and later retrieval phase of the DNMTP task and AC theta reset was observed to light stimuli only in the retrieval phase of the task. The EC theta reset during the encoding phase was correlated with accurate task performance, as theta resetting was observed during correct, but not incorrect trials. In the HPC, theta resetting was observed to visual stimuli during both the encoding and retrieval phases of the task, with theta resetting during both of these phases being predictive of correct task performance. Theta resetting in the AC occurred during the retrieval, but not the encoding phase of the task, but unlike the theta reset in the HPC and EC, the AC reset was not predictive of correct task performance, as resetting was observed to the visual stimulus on both correct and incorrect trials.

One possible model that can be derived from these results is a mechanism by which EC theta reset is primarily involved in the initial encoding of a stimulus that has to be remembered at a later period of time. The function of EC theta reset may be to enhance the initial encoding of a mnemonically relevant stimulus. The EC then would convey this sensory input to the HPC, which may integrate the initial encoding and retrieval phases of
the task. The function of HPC theta reset may be to enhance the initial encoding of the
information and maintain this information with hippocampal complex circuitry (perhaps
utilizing other EEG patterns such as 40-80 Hz gamma and 200 Hz frequencies) until the
retrieval phase commences. In the proposed model, the AC is hypothesized to be involved
in the executive planning aspects of the task, perhaps preparing the organism to make a
behavioral response.

Role of theta reset in the entorhinal cortex

A significant degree of theta resetting was observed in the entorhinal cortex (EC)
following sample, but not choice light presentation, indicating that EC theta reset may be
more involved in the initial encoding of a mnemonically relevant stimulus than in later
retrieval aspects of the DNMTP task. Also, EC theta reset to the sample light was
correlated with correct task performance, with theta resetting occurring on correct, but
not incorrect trials. These results are consistent with a hypothesized role for the EC in
working memory processes, especially in the initial encoding of sensory stimuli.

Lesions to the EC or the perforant pathway (which carries input from the
entorhinal cortex to the hippocampus) significantly impaired performance on a variety of
memory tasks, including contextual fear conditioning (Febinteanu et al., 1999), passive
avoidance (Baldi, Lorenzini, Sacchetti, Tassoni & Bucherelli, 1998), and spatial memory
tasks (Cho, Kesner & Brodale, 1995; Kirkby & Higgins, 1998; Ferbinteanu, Holsinger &
McDonald, 1999; Johnson & Kesner, 1994). These results are similar, though not
identical, to the effects of hippocampal lesions (Phillips & LeDoux, 1992; Markowska,
It is also important to note that the EC is not involved in all types of cognitive processing, but rather appears to be selectively engaged during performance of working memory tasks, as lesions to the EC did not impair performance on attentional tasks, such as the serial five-choice reaction time task (Kirkby & Higgins, 1998).

Electrophysiological studies also indicate that EC neuronal activity is associated with cognitive processing in a working memory task, especially in the initial encoding of mnemonically relevant stimuli. In an odor-based working memory task, Young, Otto, Fox & Eichenbaum (1997) found that approximately 90% of lateral EC neurons changed firing patterns in response to discrete trials events, such as sample presentation, trial initiation, delay period and reward approach. The majority (78%) of these task-related neurons underwent changes in firing activity during the initial encoding phases of the task (i.e., following sample odor presentation and during trial initiation). Only 22% of EC task-related neurons exhibited firing patterns related to the delay period and the reward aspects of the task. The pattern of results in this odor based memory task is congruent with the results of the present study, which found that EC theta reset was restricted solely to the initial encoding phase of the DNMTP task. The changes in EC neuronal activity patterns in both spatial and olfactory memory paradigms suggests that theta reset may not be restricted solely to one particular type of memory attribute. However, more explicit tests assessing a variety of mnemonic attributes will have to be conducted to determine whether this hypothesis is correct.

Another significant finding of the present study was that EC theta reset to the
sample light was predictive of the accuracy of subsequent performance in the choice phase of the DNMTP task. More specifically, theta reset was observed to the sample light on correct, but not incorrect or no response trials, suggesting that theta reset may be a mechanism to enhance the encoding of incoming, mnemonically relevant stimuli. Additional support for the hypothesis that changes in neuronal activity in the EC may be associated with enhanced encoding comes from human fMRI studies in which a slow, sustained activation in the entorhinal cortex following sample cue presentation was positively correlated with subsequent correct task performance (Fernandez, Brewer, Zhao, Glover & Gabrieli, 1999).

However, it is important to note that although EC theta reset appears to be preferentially involved in the initial encoding of information, results suggest that EC theta reset may be involved in some retrieval phase components of the DNMTP task. A significant degree of theta resetting was observed following motor lever pressing responses. It is possible that the theta reset to motor responses and to sensory stimuli are mediated by different mechanisms. For instance, the reset following motor responses may be a reset of Type 1 theta, which is associated with voluntary movements and is atropine-resistant (Bland, 1986; Heynen & Bilkey, 1994). In addition, the sensory stimulus-related reset may involve a resetting of Type 2 theta, which is observed during times of alert immobility (Stewart & Fox, 1990), is atropine-sensitive (Stewart & Fox, 1990) and has been hypothesized to be involved in memory and attentional processes (Brazhnik & Vinogradova, 1987; Vinogradova, 1996). However, this double dissociation is called into question by studies which have shown that lesions to the EC do not affect Type 2 theta.
(Stewart & Fox, 1990). Further studies will be necessary to further distinguish the contributions of Type 1 and Type 2 theta to the theta reset phenomenon in the three brain areas examined in this experiment.

In summary, the entorhinal cortex is an important component of working memory processes, especially during the initial encoding of information. Disruptions in EC theta reset to incoming visual stimuli during the encoding phase of the DNMTP task are correlated with incorrect responding in the subsequent choice phase. Of the brain structures examined in this experiment, this pattern of theta resetting solely to stimuli in the encoding phase of the task is unique to the EC. For instance, HPC theta reset appears to have a slightly different role in working memory performance than does the entorhinal cortex.

Role of theta reset in the hippocampus

In the present experiment, HPC theta reset occurred following the presentation of both the sample and choice phase stimulus, providing support to the hypothesis that the hippocampus is involved in both the encoding and the retrieval phases of the DNMTP task. This finding is consistent with the results of electrophysiological and positron emission tomography studies which showed that changes in hippocampal activation are not restricted to one phase of a working memory task, but rather represent task parameters throughout a trial (Deadwyler, Bunn & Hampson, 1996; see Desgranges, Baron & Eustache, 1998 for review; Hampson, Heyser & Deadwyler, 1993; Hasselmo, Wyble & Wallenstein, 1996; Olton, 1989). For instance, Hampson et al. (1993) found
that CA₁ and CA₃ neurons increased firing following sample responses, choice responses and/or reward delivery. In addition, a population of cells also showed increased activity during the delay period. It is important to note that changes in HPC neuronal firing patterns are associated with encoding and retrieval processes in working, but not reference memory tasks (Wible, Findling, Shapiro, Crane & Olton, 1986). Theta bursting activity (which is considered to be a part of the optimal parameters for expression of long-term potentiation) was time-locked to the more mnemonic components of an olfactory-based working memory task, with little phase-locking to non-mnemonic task events (Otto, Eichenbaum, Weiner & Wible, 1991).

Another significant finding from Experiment 1 regarding HPC theta reset was that the resetting to the sample light, choice light and motor response was predictive of correct performance in the choice phase of the DNMTP task. Improper encoding within the hippocampus has also been linked to subsequent incorrect responses in ensemble recording studies, in which errors in coding during the sample phase of a slightly different version of the DNMTP task used in the present experiment were correlated with incorrect responding in the choice phase (Deadwyler et al., 1996). Although improper encoding in the sample phase had little effect on short delay trials, correlations between improper encoding and impairments in performance increased as the delay interval was lengthened.

The results of Experiment 1 suggest that the hippocampus is actively involved in both the encoding and retrieval phases of a working memory task. However, the precise behaviors/cognitive processes resulting in theta reset is unclear from the present results. For example, theta resetting in the choice phase may be related to associating a reward to
a particular response or to motor planning. Further studies aimed at examining the behavioral and cognitive subcomponents of encoding and retrieval phases will be necessary to further elucidate the role of HPC theta reset in learning and memory.

Role of theta reset in the anterior cingulate

Unlike theta reset in the EC and HPC, anterior cingulate theta reset was only associated with the retrieval component of the WM task, with theta reset occurring following light presentation and motor responses in the choice phase of the task. This finding is consistent with the hypothesized role for the prefrontal cortex (including the anterior cingulate) in executive decision making processes (Baddeley & Della Sala, 1996; Bussey, Muir, Everitt & Robbins, 1997; Frith & Dolan, 1996; Muir, Everitt & Robbins, 1996; Robbins, 1996; Shallice & Burgess, 1996).

Executive processing involves “mechanisms by which performance is optimized in situations requiring the operation of a number of cognitive processes” (Baddeley, 1986). In rat, monkey and human studies, the prefrontal cortex has been shown to be involved in a number of executive processes including 1) combining concurrent cognitive tasks (Bussey, Muir, Everitt & Robbins, 1997), such as divided attention paradigms (Corbetta, Meizin, Dobmeyer, Shulman & Petersen, 1991; Esposita, Detre, Alsop, Shin, Atlas & Grossman, 1995; Okuda, Fujii, Yamadori, Kawashima, Tsukiura, Fukatsu, Suzuki, Ito & Fukudu, 1998), 2) enhancing the encoding of information being held in working memory (Goldman-Rakic, 1992; 1996; Okuda et al., 1998; Rainier, Rao & Miller, 1999), 3) modulating attentional resources by increasing attentional focus upon relevant stimuli and
attenuating the effects of distracting or interfering stimuli (Chao & Knight; Lecas, 1995; Mattochik, Zametkin, Cohen, Hauser & Weintraub, 1996; Ishii, et al., 1999; Williams & Givens, in press; Woods & Knight, 1985), 4) inhibiting inappropriate responses or strategies (Muir, Everitt & Robbins, 1996; Pardo, Pardo, Janer & Raichle, 1990; Peterson, Skudlarski, Gatenby, Zhang, Anderson & Gore, 1999; Robbins, 1996), 5) placing proper affective or motivational significance upon a behavioral outcome (Bussey, Everitt & Robbins, 1997; Bussey, Muir, et al., 1997; Devinsky, Morrell & Vogt, 1995; Gabriel, 1990; Lane, Fink, Chau & Dolan, 1997; Lane, Reiman, Axelrod, Yun, Holmes & Schwartz, 1998), and 6) preparing the organism to make an appropriate motor response (Devinsky et al., 1995; Gabriel, 1990).

In the present study, a clear resetting of the theta rhythm was observed to the choice light stimulus on correct and incorrect trials and to correct motor responses. Although no definitive conclusions can be drawn regarding the particular aspects of executive processing that are associated with AC theta resetting, given the timing of the theta reset (i.e. at the time of the choice light or choice phase motor response) and given what is known about the AC, the most likely functional significance of the AC theta reset is to either inhibit an inappropriate response (e.g., prevent premature lever responses), to associate emotional significance with a response (e.g., an action with an outcome or reward) or to prepare the rat to make a response choice. The most direct support from this study is for the last option.

Motor responses in the choice phase of the DNMTP task led to a resetting of the AC theta rhythm, with an overall 156% increase in theta power (regardless of trial type) in
the post-motor response final waveform average relative to the pre-response waveform average. However, it is not clear whether the reset following lever presses was due solely to the specific movement, to cognitive factors (e.g. anticipation of reward, response selection or motor planning) or to a combination of motor and cognitive factors.

Neurophysiological and neuroanatomical studies suggest that the anterior cingulate is involved in motor responding. The AC projects to several motor-related areas in the brain, including the primary and supplementary motor areas (Arikuni, Sako & Murata, 1994; Sesack, Deutch, Roth & Bunney, 1989; Uylings & van Eden, 1990) and the basal ganglia (Buchanan, Thompson, Maxwell & Powell, 1994; Groenewegen, Berendse, Wolters & Lohman, 1990). In addition, fMRI analysis revealed that significant changes occurred not only during more cognitive aspects of a Stroop interference task, but also following motor responses (Peterson et al., 1999).

However, although the AC may have components (especially more caudal regions of the AC) that respond specifically to motor acts (Peterson et al., 1999), the present results suggest that the AC theta reset following lever responses is not solely due to movement, as a dissociation in the degree of theta reset was observed between correct and incorrect trials, with theta reset only occurring following correct motor responses. If motor activity was the sole determinant of the lever press-induced theta reset, then an equal degree of reset should have been observed on correct and incorrect trials. Other studies have further concluded that the motor-related changes in AC activity occur in relation to motor responses in working, but not reference memory tasks (Petit, Courtney, Ungerleider & Haxby, 1998), indicating that the physical act of movement is not sufficient
to induce changes in AC activity.

A hypothesis that is more congruent with the present findings is that the AC is not involved in the initial encoding of task-relevant information (as AC theta reset was not observed following sample light presentation) or the physical act of motor responding, but rather the AC may reflect a "state of readiness" to make a behavioral response based on information being held in working memory during a delay period (Jung, Qin, McNaughton & Barnes, 1998; Petit et al., 1998). This hypothesis is best exemplified by studies indicating a role for the prefrontal cortex in prospective coding, which involves the anticipation of future events (Okuda et al., 1998; Rainer et al., 1999). During a delayed paired associate task and a delayed match-to-sample task, primate prefrontal neurons showed increased activity as the delay period progressed (Rainer et al., 1999). Other researchers have shown, using positron emission tomography, that the increased prefrontal activity associated with prospective-coding involves the AC (Okuda et al., 1998). Thus, AC theta reset following choice light presentation and correct lever presses may be a triggering mechanism by which the rat can switch from this period of prospective, anticipatory behaviors to the implementation of a response strategy.

Although the majority of studies, including the present study, suggest that the AC is involved in the planning of a motor response or response selection strategies during the retrieval aspects of a task (Henson, Rugg, Shallice, Josephs & Dolan, 1999; Jung et al., 1998; Okuda et al., 1998; Petit et al., 1998; Rainer et al., 1999), it does not rule out a role for the AC in either the initial encoding of information or the physical manifestation of the motor response. For instance the location of the recording electrode in the present study
may have influenced the experimental outcome. Peterson et al. (1999) found that different components of the Stroop interference task differentially activated specific areas of the AC, creating a rostral-caudal topographical map of task parameters. Stimulus encoding resulted in increased activity in the most rostral regions of the AC, followed along the rostral-caudal gradient by task update and monitoring, sensory tuning, premotor planning, vigilance, response selection and finally the actual motor response - which occupied the most caudal aspect of the AC (Peterson et al., 1999; Petit et al., 1998).

Although it is difficult to directly compare the precise locations of the rostral-caudal gradient observed in humans with a possible AC topographical map in rats, in general, the recording electrode in the current study was placed in the middle to caudal regions. Thus, the finding that the AC was more preferentially involved in the retrieval/response planning stages of the task as opposed to the initial encoding phase may be due to electrode placement. It is possible that electrophysiological recordings in the most rostral aspects of the AC might reveal a stronger theta resetting following sample light presentation. In addition, a placement in the most caudal regions of the AC might result in resetting to the physical motor response, not the planning of the response.

Lastly, in regards to electrode placement issues, laterality of the recording electrode might have influenced the finding that the AC theta reset was involved in the retrieval, but not encoding phase of the task. Many studies have found that activation of the left prefrontal cortex is associated with the encoding of new information; whereas, activation of the right PFC is associated with the retrieval of mnemonic information (Desgranges, Baron & Eustache, 1998; Shallice, Fletcher, Frith, Grasby, Frackowiak &
Dolan, 1994; Tulving, Kapur, Craik, Moscovitch & Houle, 1994). In the present study, all AC recording electrodes were placed in the right hemisphere, and thus it might be expected that theta reset might be restricted to retrieval phase parameters. However, a deeper examination of the literature reveals that while the posterior cingulate and other prefrontal regions show retrieval phase right hemisphere lateralization (Desgranges et al., 1998), no such lateralization was observed in the AC (Desgranges et al., 1998; Henson et al., 1999). In the present study, no distinct conclusions regarding laterality in the AC can be drawn and further investigation will be necessary to fully resolve this issue.
CHAPTER 3
EXPERIMENT 2

Results from Experiment 1 showed that a resetting of the HPC theta rhythm was associated with performance during both the encoding and retrieval phases of a working memory task. In addition, the degree of theta reset to incoming sensory stimuli was positively correlated with correct task performance. Thus, it is possible that deficits in mnemonic processing are at least partially due to a failure of the HPC theta rhythm to reset to incoming sensory stimuli. As such, it is important to determine the critical neural pathways underlying hippocampal theta reset, as disruptions of these pathways may lead to memory impairments. Two leading candidates for such a role include the perforant path from the EC to the HPC and the septo-hippocampal pathway which originates in the MSA and terminates in the HPC. The following sections will provide a brief overview of perforant path and septo-hippocampal connectivity. For a more complete neuroanatomical analysis, please see the Chapter 2 introduction section.

Entorhinal connectivity

The entorhinal cortex (EC) primarily receives converging sensory input of all modalities from polymodal association areas in the cortex via the perirhinal and
parahippocampal cortices (Burwell & Amaral, 1998; Insausti, Amaral & Cowan, 1987; Naber, Caballero-Bleda, Jorritsma-Byham & Witter, 1997; Suzuki & Amaral, 1994b) and relays this information to the HPC via the perforant path (Burwell & Amaral, 1998; Suzuki & Amaral, 1994b). More specifically, EC to HPC pathway originates with projection neurons in layers II and III of the entorhinal cortex, with layer II neurons projecting to the dentate gyrus and CA2 and CA3 fields of Ammon's horn (Blackstad, 1958; Hjorth-Simonsen & Jeune, 1972; Tamamaki, 1997) and layer III neurons projecting to CA1 of Ammon's horn (Tamamaki, 1997). These projections make the perforant path an ideal candidate for influencing theta reset, as generators of hippocampal theta are located prominently in the dentate gyrus and CA1 regions of the hippocampus proper (Bland, 1986; Bland & Wishaw, 1976).

**Septo-hippocampal connectivity**

In regards to MSA afferents, there are two general neural pathways from which the MSA could get inputs: an ascending pathway from the brainstem and a descending pathway from the temporal lobe via the lateral septum (LS), by which the MSA could get feedback from HPC. Electrical stimulation of various ascending afferent inputs to the MSA including the reticular formation (Gaztelu & Buno, 1982) and the medial forebrain bundle (Brazhnik & Vinogradova, 1988) cause a resetting of the hippocampal theta rhythm. Similarly, electrical stimulation of the lateral septum (Brazhnik et al., 1985; Pedemonte et al., 1998), medial septum (Buno et al., 1978; Garcia-Sanchez, et al., 1978), the fornix (Buno et al., 1978; Garcia-Sanchez, et al., 1978) and the horizontal limb of the
diagonal band of Broca (Brazhnik et al., 1985) also causes a reset of the hippocampal theta rhythm in an anesthetized preparation.

Stimulation of the EC or perforant path can induce excitatory post-synaptic potentials (EPSPs) in the dentate gyrus. More interestingly, some experiments have reported that stimulating the septum can alter the size of the granule cell population spike evoked by perforant path stimulation in the HPC (Alvarez-Leefmans, 1975; Bilkey & Goddard, 1985) and that the paired-pulse inhibition observed following electrical stimulation of the perforant path could be removed if a conditioning pulse to the MSA was timed to coincide (or nearly coincide) with the first evoked population spike. These data support the hypothesis that theta reset may enhance encoding by allowing the hippocampus to be in a maximal state of depolarization when sensory input arrives from the entorhinal cortex.

The present study utilized electrical stimulation to identify the neural circuitry in the rat that underlies the theta reset phenomenon, with particular attention focused on the neural components of the septo-hippocampal system, which includes such structures as the fornix and the entorhinal cortex. Although numerous studies have examined the neuroanatomical substrates for theta resetting, the majority of these studies involved non-rodent species (Brazhnik et al., 1985; Brazhnik & Vinogradova, 1988) or primarily involved examining theta reset in anesthetized conditions (Buno et al., 1978; Garcia-Sanchez, et al., 1978; Gaztelu & Buno, 1982; Pedemonte et al., 1998). One specific goal of the present study was to quantify the degree to which the fornix and the perforant path are involved in the resetting of the hippocampal theta rhythm in an awake, freely-moving
In Experiment 2, rats were run on a simple, hippocampally-independent visual discrimination (VD) task in which a light appeared over a centrally located lever. To obtain a water reward, rats were required to press the lever beneath the center light within three seconds of light onset. The purpose of the behavioral task was to prevent rats from sleeping during the acquisition of theta EEG records. Sleep disrupts hippocampal theta EEG and thus would prevent examination of the reset phenomenon. During the ITI period in the VD task, rats received electrical stimulation of the fornix and the perforant path, two important afferent inputs to the hippocampus. Anatomical, electrophysiological and behavioral studies (see introduction) suggest that both the fornix and the perforant path will be actively involved in the resetting of HPC theta, with the fornix stimulation perhaps resulting in a greater degree of HPC theta reset than perforant path stimulation, as numerous studies in anesthetized subjects have produced a robust HPC theta reset following fornix stimulation (Buno et al., 1978; Garcia-Sanchez, et al., 1978).

Overall, Experiment 2 was designed to examine the neural circuitry underlying the theta reset phenomenon, with particular attention to neural components of the septo-hippocampal system, which includes such structures as the fornix, the perforant path, the entorhinal cortex and the lateral septum.

Method

*Visual discrimination (VD) procedure*
**Shaping Step 1:**

Initially, rats were trained to press levers to receive a water reward. Left, right or center lever presses were rewarded with a drop of water. To prevent the development of a bias towards one lever, the total number of presses on one lever could not exceed those to any of the remaining levers by more than five. If this occurred, subsequent responding to the more frequently pressed lever were no longer rewarded until the imbalance was corrected. Rats were trained in this step daily for one hour sessions until they reached a criterion of 50 bar presses in one hour, after which they were moved to the visual discrimination task.

**Visual Discrimination (VD) task:**

The visual discrimination (VD) task was a simple behavioral task in which a light appeared over the center lever. In order to obtain a water reward, rats were required to press the lever beneath the center light within 3 seconds of illumination. Following a lever press or if three seconds had elapsed, the light was extinguished and a 30" ITI period began.

**Surgery**

After reaching criterion performance on the VD task (90% correct for 3 days, with a no response rate less than 40%), subjects underwent electrode implantation surgery as described in the general methods section of the proposal. During the surgery, two recording electrodes were placed bilaterally into the dentate hilus (4.0 mm posterior to
bregma; ± 2.5 mm lateral to midline; 2.7-3.0 mm ventral to the dural surface). The ventral coordinates were determined for each rat during surgery by observing the theta signal on an oscilloscope and by examining theta power on-line using a fast Fourier transformation (FFT). The purity and the power of the theta rhythm were used to locate the optimal recording position.

Stimulating electrodes were placed unilaterally into both the fornix (1.8 mm posterior to bregma; 2.0 mm lateral to midline; 3.0-3.7 mm ventral to the dural surface) and the perforant path (8.1 mm posterior to bregma; 3.0 mm lateral to midline; 2.8-3.5 mm ventral to the dural surface). The ventral coordinates for the stimulating sites were determined for each rat during surgery by delivering a series of single square wave electrical pulses (600 µA for 0.2 msec with a 5 second interpulse interval) via a Grass Instruments S8800 stimulator (Quincy, MA) to the fornix and the perforant path and observing an oscilloscope to locate the coordinates within each area that resulted in the maximal amount of electrically-elicited theta reset.

Post-surgery testing

After a one week recovery period from surgery, rats were re-trained on the VD task. During this re-training period, one end of the preamplifier was mounted to the connector on the rats' heads, while the other end was attached to a cable which was in turn connected to a commutator. The commutator swivelled to allow the rats to move in the box without becoming entangled in the cable. The purpose of this re-training period was 1) to ensure that the surgery did not effect task performance and 2) to acclimate the
rats to the recording protocol. After baseline performance on the VD task returned to pre-surgery baseline levels, the post-surgical testing sessions commenced.

Electrophysiological data in Experiment 2 was collected for ten days (five days per week) from each animal.

All electrical stimulation took place during the ITI period of the VD task. Eleven seconds following light offset, a single square wave electrical pulse (600 μA for 0.2 msec) was delivered via a Grass S8800 stimulator to either the fornix or the perforant path. Two additional single stimulation pulses were delivered at 17 and 24 seconds following light offset, for a total of three electrical stimulations per ITI period. The three electrical stimulations were delivered to only one brain structure during a given ITI period. The stimulation site was alternated every other trial between these two stimulation sites. Theta EEG recordings were acquired one second before and after electrical stimulation to determine if resetting to the electrical stimulus occurred. Theta EEG recordings were also acquired one second before and after light onset to determine if resetting to the light stimulus occurred.

**Analysis**

Histological verification of the placements of the recording and stimulation electrodes and the electrophysiological data analyses examining theta reset to fornix, perforant path and visual stimulation were analyzed according to the protocols described in the general methods section.

**Results**

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Behavior

Rats were trained on the visual discrimination (VD) task for approximately two weeks before reaching pre-surgery criterion levels. Choice accuracy remained consistent throughout post-surgery recording sessions (98.47% choice accuracy) and a high degree of responding (less than 25% omission rate) was maintained throughout recording sessions, indicating that the rats were awake and actively engaged in task performance.

Electrophysiology

Both dentate recording sites (i.e. those ipsalateral and contralateral to the stimulating electrodes) showed quantitatively similar ongoing theta activity as well as similar responses to stimulation. That is, there was no significant difference in theta frequency (p>.05), theta power (p>.05) or rate of resetting [F(1,48) = 0.004; p>.05] to the three stimulation conditions and thus data from both sites were combined for subsequent analyses.

A separate waveform average was performed upon the pre- and post-stimulus electrophysiology records for each testing session. If theta was phase-locked to the
Conversely, if no phase-locking occurred, the final waveform should have averaged to a relatively flat line with little theta evident in the final waveform. Figures 3.1, 3.2 and 3.3 illustrate representative examples of theta reset to the light stimulus, perforant path and fornix stimulation, respectively.

Visual inspection of the final waveform average revealed a strong evoked response.

Figure 3.1: Reset to the light stimulus. The main portion of the graph represents the final waveform average. The inserts represent FFT's performed upon the final waveform average.
Figure 3.2: Reset to the perforant path stimulation. The main portion of the graph represents the final waveform average. The inserts represent FFT’s performed upon the final waveform average.

Figure 3.3: Theta reset following fornix stimulation. The main portion of the figure represents the final waveform average. The inserts represent FFT’s performed upon the final waveform average.
following electrical stimulation of the perforant path and fornix. The duration of the evoked response varied between rats from 100–400 msec. Before quantifying the degree of reset, the evoked response was removed from the waveform average. A FFT was then performed upon the remaining waveform to determine whether theta reset occurred following stimulus presentation. Theta reset was defined as an increase in theta power in the final waveform average following a stimulus relative to theta power before a stimulus. A two-factor ANOVA with repeated measures was performed, using stimulus (light, perforant path and fornix stimulation) and time (pre- and post-stimulus) as repeated measures and maximum theta frequency and power at maximum theta frequency inherent in the final waveform average as dependent measures. The frequency analysis revealed a significant time effect, with theta shifting to a slightly higher frequency following the introduction of a light or electrical stimulus \( F(1,111) = 25.362; \ p < .01 \). Analyses of theta power in the final waveform average revealed significant stimulus \( F(2,222) = 15.219; \ p < .01 \) and time \( F(2,111) = 41.899; \ p < .01 \) effects, with post-stimulus theta power being greater in the final waveform average than

Figure 3.4: Theta reset seen following light, perforant path and fornix stimulation. * = \( p < .05 \)
pre-stimulus theta power, indicating that a significant degree of theta reset occurred following all stimulation conditions (Figure 3.4). Although a significant degree of theta reset was evident following all stimulus conditions, a significant stimulation condition X time interaction \[F(2,222) = 17.210; p<.01\] indicated differences in the degree of theta reset elicited by the stimulation conditions. A two-factor ANOVA with repeated measures using stimulus as a within factor and theta power in the pre-stimulus final waveform average and post-stimulus final waveform average as dependent variables revealed no significant differences in the pre-stimulus theta power between any of the stimulation conditions \[F(2,222) = .747; p>.05\]. Conversely, a significant stimulus effect was observed in post-stimulus theta power \[F(2,222) = 18.454; p<.01\]. Subsequent t-tests revealed more theta power in the final waveform average (i.e. a greater degree of theta reset) following fornix stimulation than following either light \([t (111) = 4.092; p<.0001; \text{Figure 3.4}]\) or perforant path stimulation \([t (111) = 3.733; p<.0001; \text{Figure 3.4}]\). There was no significant difference in the degree of theta reset produced by light and perforant path stimulation \([t (116) = 1.526; p>.05; \text{Figure 3.4}]\).

To determine whether the observed resets were the result of a phase-shifting of an ongoing theta rhythm or the evoking of a theta rhythm, an FFT was performed upon the raw pre- and post-stimulus records for the three stimulation conditions. In all cases evoked responses were removed from the data records before the FFT. The FFT analyses of the raw data records revealed a strong theta peak centered at approximately \(7.28 \pm .12\) Hz both before and after light, perforant path and fornix stimulation. A two factor ANOVA with repeated measures, using stimulus (light, perforant path stimulation and
fornix stimulation) and time (pre- and post-stimulus) as within factors and theta power as the dependent measure revealed a significant stimulus effect \[ F(2, 182) = 5.512 \], but no significant time or stimulus X time interaction (\( p > .05 \)). Due to the lack of a stimulus X time interaction, theta power before and after light, perforant path and fornix stimulation was collapsed across stimulation conditions. Subsequent t-tests revealed theta power was stronger during periods of perforant path \( t (111) = 1.987; p < .05; \text{Table 3.1} \) and fornix stimulation \( t (91) = 3.689; p < .0001; \text{Table 3.1} \) than periods of light stimulus presentation. There were no significant frequency differences in the pre versus post-stimulus records in any of the stimulus conditions (\( p > .05 \)) (Table 3.1).
<table>
<thead>
<tr>
<th>Stimulus Condition</th>
<th>Max. Frequency Pre-stimulus</th>
<th>Max. Frequency Post-stimulus</th>
<th>Power at Max. Frequency Pre-stimulus</th>
<th>Power at Max. Frequency Post-stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Stimulus</td>
<td>7.28 ± .09</td>
<td>7.32 ± .10</td>
<td>20,788 ± 2453</td>
<td>19,728 ± 1944</td>
</tr>
<tr>
<td>Perforant Path Electrical Stimulation</td>
<td>7.36 ± .10</td>
<td>7.33 ± .12</td>
<td>30,894 ± 4275</td>
<td>28,260 ± 2636</td>
</tr>
<tr>
<td>Fornix Electrical Stimulation</td>
<td>7.23 ± .14</td>
<td>7.18 ± .17</td>
<td>30,709 ± 3357</td>
<td>26,917 ± 2992</td>
</tr>
</tbody>
</table>

Table 3.1: FFT’s performed upon each individual data record (i.e. not just the final waveform average) reveal no significant differences, indicating that there was a similar theta rhythm (in terms of both theta frequency and power) before and after light and electrical stimulation.
No significant differences in overall theta power were observed between electrical stimulation of the fornix and perforant path (p>.05). The lack of a significant time effect when comparing theta power before light, perforant path and fornix stimulation with theta power after light, perforant path and fornix stimulation respectively indicated a strong ongoing theta rhythm throughout the testing session, suggesting that the observed resetting was not the result of evoking a theta rhythm, but rather phase-shifting the ongoing theta rhythm.

Discussion

The results demonstrate that electrical stimulation of the fornix and, to a lesser degree, the perforant path are capable of resetting the hippocampal theta rhythm. These results also indicate that both of these structures may contribute to the naturally occurring theta reset following light onset in the memory task. As shown in Fig. 3.5, the major inputs to MSA are an ascending pathway from the brainstem and a descending pathway (either directly from the hippocampus itself or via the lateral septum or both) carrying feedback information from the HPC. The fact that electrical stimulation of the fornix can reset the hippocampal theta supports the previous findings that MSA is one of the pacemakers of the hippocampal theta rhythm. The fact that electrical stimulation of the perforant path can

![Figure 3.5: Schematic diagram of proposed reset circuitry](image)
reset HPC theta indicates that MSA might receive feedback information from a descending pathway (via the LS) that subsequently sends output to reset the hippocampal theta rhythm. However, these results do not rule out a role for the ascending pathways. Ascending and descending pathways may be active within different time windows. Ascending input could occur immediately after stimulus onset prior to the time when the HPC gets its input from the EC, but then descending inputs are active later when the HPC gets its input from the EC. Thus, ascending and descending pathway may be co-active in different time windows and have different functions in information encoding.

Results suggest that electrical stimulation does not merely evoke theta, but rather resets ongoing theta (i.e. the ongoing theta rhythm becomes phase-locked to the electrical stimulation.). As a pacemaker of the HPC theta rhythm, the MSA is doing more in regulating ongoing theta rhythm than merely generating them. Previous studies have shown that in order to have an augmenting effect, input to the HPC from the MSA has to stay within a 2 msec time window with input to the HPC from the EC (Alvarez-Leefmans, 1975). It should be noted that the resetting of the hippocampal theta rhythm may not be the only EEG rhythm involved in information processing. Besides the theta rhythm, there are other higher frequency components (40-100 Hz gamma rhythms and a 200 Hz rhythm) in the hippocampus that are coupled to the slower theta rhythm (Chroback & Buzsaki, 1998). Although their functions are not well understood, these high frequency rhythms may play a significant role in regulating information flow to the HPC. For instance, Lisman and Idiart (1995) have proposed that the coupling of theta and gamma activity may account for the 7 ± 2 limited storage capacity of working memory. More specifically,
each of these 7 ± 2 memories may be represented in a gamma frequency oscillation that is
refreshed by theta-frequency oscillations. Hence, HPC theta reset may be a mechanism to
enhance the coupling of gamma and theta EEG frequencies. However, more specific
studies will have to be conducted to confirm this hypothesis. Future analyses will
specifically examine the relationship between theta and gamma EEG patterns and will
determine whether gamma EEG activity is reset by incoming sensory stimuli.

Given the results of previous studies, it was surprising to see a significant degree
of reset following presentation of a light stimulus in this reference memory task. Previous
studies have indicated that theta was reset in a working, but not reference memory task
(Givens, 1996). In addition, experiments have shown that in the early stages of an oddball
discrimination task - when an animal is still learning a task - there exists theta resetting to
a visual stimulus. However, once an animal is over-trained, the resetting to the visual
stimulus disappears (Brankack, et al., 1998). Given that rats had received extensive
training on the VD task, it was not expected that theta reset would be seen following light
presentation in this task. One possible explanation is that theta reset to the light stimulus
in the current reference memory task was due to the electrical stimulation. One significant
difference between the current RM task and the previous RM task in our laboratory is that
electrical stimulation occurred during the ITI of the current RM task. It is possible that the
electrical stimulation sensitized the system, leading to a reset of hippocampal theta rhythm
following the reference memory light stimulus.

In summary, the results support the previous finding from this lab that the
hippocampal theta rhythm resets following the onset of a relevant stimulus, suggesting that
theta reset may be a neural mechanism by which an organism can optimally encode mnemonically relevant, naturally occurring stimuli. The fornix and the perforant path appear to be two important components of this neural system.
CHAPTER 4
EXPERIMENT 3

Results from Experiment 2 indicate that electrical stimulation of the fornix is capable of resetting HPC theta. Several researchers (Brazhnik, Vinogradova & Karanov, 1985; Gallagher, Zheng, Hasuo & Shinnick-Gallagher, 1995) have suggested that this fornix-induced resetting may involve the lateral septum (LS), through a feedback loop involving the MSA, HPC, and LS. Although these three structures are the primary focus of Experiment 3, it should be noted that ascending brainstem and hypothalamic inputs may also contribute to the proposed feedback model. Evidence supporting a role for the lateral septum in a potential feedback loop can be found in both neuroanatomical studies concerning the afferent and efferent connections of the LS and electrophysiological studies examining the relationship between the lateral septum and HPC theta.

Neuroanatomical connections of the lateral septum

Although numerous terminologies have been used to describe the neuroanatomy of the LS, for purposes of clarity, this neuroanatomical overview will adopt the most widely accepted nomenclature, which divides the LS into three distinct components: the dorsolateral, intermediate and ventral subdivisions (Swanson & Cowan, 1979). Although
more recent papers have redefined the subdivisions into rostral, caudal and ventral areas (Risold and Swanson, 1996; 1997a; 1997b), this newer nomenclature has yet to be widely incorporated into the lateral septum literature. As such, all neuroanatomical discussions will utilize the older nomenclature and all references to the newer nomenclature will be translated into the older terminology using the anatomical comparisons described in Risold and Swanson (1997a).

Afferent inputs

The main afferent inputs to the LS are descending hippocampal inputs which arise from the CA1 and CA3 fields of Ammon's horn and the subiculum and travel to the lateral septum via the fornix, with CA3 cells projecting more to the dorsal regions of the LS and CA1 cells projecting primarily to the intermediate and ventral LS (Risold & Swanson, 1996; 1997b). More specifically, pyramidal hippocampal neurons terminate on GABAergic (and other unidentified) LS neurons (Leranth et al., 1992). In general, fibers from the rostro-dorsal portions of Ammon's horn and the subiculum project to the dorsal subdivision of the lateral septum (LSd), whereas more caudo-ventral parts of Ammon's horn and the subiculum terminate in progressively more ventral regions of the lateral septum (Swanson & Cowan, 1979). In addition to these hippocampal afferents, the lateral septum also appears to receive input from another important component of the septo-hippocampal system: the medial septal area (MSA). Double immunostaining studies reveal that collaterals of the GABAergic, but not cholinergic, hippocampally-projecting MSA neurons terminate on LS neurons, forming an inhibitory pathway from the MSA to
The lateral septum also receives several ascending neurotransmitter-specific inputs from the brainstem, including noradrenergic fibers originating in the locus coeruleus, dopaminergic fibers arising from the ventral tegmental area, and serotonergic fibers originating from the raphe nucleus (Swanson & Cowan, 1979). In addition, numerous hypothalamic nuclei also ascend via the medial forebrain bundle to all LS subdivisions (see Risold & Swanson, 1997b and Swanson & Cowan, 1979 for a more complete description). Some of the stronger hypothalamic inputs to the lateral septum include 1) the ventromedial hypothalamus, which projects to the ventral portion of the lateral septal nucleus (Swanson & Cowan, 1979); 2) the medial preoptic area (Risold & Swanson, 1997b) and 3) the supramammillary nucleus (Vertes, 1988; 1992) and the posterior hypothalamic nuclei, which have both been implicated in the generation of hippocampal theta (Bland, Oddie, Colom & Vertes, 1994; Oddie, Bland, Colom & Vertes, 1994; Oddie, Stefanek, Kirk & Bland, 1996) and project to all three subdivisions (LSa, LSs, and LSj) of the lateral septum (Vertes et al, 1995). Lastly, the lateral septum may also receive afferent input from the amygdala, with the majority of amygdaloid projections traveling to the intermediate LS (Risold & Swanson, 1997b).

Efferent projections

In both rats (Phelan et al., 1996; Swanson & Cowan, 1979) and guinea pigs (Staiger & Nurnberger, 1991a), efferent projections from the LSa course ventrally through the intermediate portion of the lateral septum (LSj) and terminate heavily in the nucleus of
the diagonal band of Broca (DB). The largest proportion of lateral septal projections to the diagonal band arise from the LSd, although significant DB projections also arise from the intermediate and ventral LS (Staiger & Nurnberger, 1991a). However, it should be noted that the existence of the lateral septum to diagonal band projection has been debated, with some researchers failing to find evidence corroborating this hypothesized pathway (Leranth et al., 1992). Although the existence of the lateral septum to diagonal band projection exists is unclear, most researchers agree that direct projections from the LSd to the medial septal area (MSA) are extremely sparse (Leranth et al., 1992; Phelan et al., 1996; Staiger & Nurnberger, 1991a; Swanson & Cowan, 1979). Lastly, some LSd projections also innervate the lateral hypothalamus (Swanson & Cowan, 1979).

Efferent fibers from the intermediate portion of the lateral septum (LSi) project ventrally through the medial forebrain bundle, traveling to both the MSA and the medial aspects of the diagonal band of Broca (Risold & Swanson, 1997b; Swanson & Cowan, 1979). Other LSi efferent fibers travel via the medial forebrain bundle to both the medial and lateral preoptic areas (Leranth et al., 1992; Risold & Swanson, 1997b; Swanson & Cowan, 1979) and either 1) terminate in the dorsomedial hypothalamus or 2) pass through the lateral hypothalamic nucleus, eventually terminating in the capsule surrounding the lateral mammillary nucleus (Risold & Swanson, 1997b; Swanson & Cowan, 1979). In addition, although the results were not discussed in relation to the three subdivisions of the lateral septum, strong LS-hypothalamus projections were also observed in studies involving 1) injections of the anterograde tracer biotinylated dextran-amine (BDA) into the rat mediolateral LS, which corresponds primarily to the medial aspects of the
intermediate LS, but also includes portions of the dorsolateral and ventral LS, using the classic nomenclature (Varoqueaux & Poulain, 1999) and 2) injections of the Phaseolus vulgaris-leucoagglutinin (PHA-L) anterograde tracer into the guinea pig LS (Staiger & Nurnberger, 1991b; Staiger & Wouterlood, 1990). Interestingly, there is also evidence for a weak ascending projection from the LS; to the HPC via the fimbria, with fibers terminating in the ventral regions of Ammon’s horn and the ventral subiculum (Risold & Swanson, 1997b). This LS; to HPC projection (although very sparse) may relay brainstem/hypothalamic information to the HPC or provide direct feedback to the HPC following HPC input to the LS.

Lastly, while the ventral portion of the lateral septum (LSv) projects heavily to the MSA and the entire diagonal band of Broca (Swanson & Cowan, 1979), the majority of LSv efferents project to the medial preoptic area (Leranth et al., 1992; Risold & Swanson, 1997b; Swanson & Cowan, 1979). Efferent fibers also pass through the dorsomedial and lateral hypothalamic nuclei, terminating in either the supramammillary region (Risold & Swanson, 1997b; Swanson & Cowan, 1979) or the capsule surrounding the lateral mamillary nucleus (Risold & Swanson, 1997b; Swanson & Cowan, 1979; Varoqueaux & Poulain, 1999). The LSv also projects to a lesser degree to the bed nuclei of the stria terminalis (Risold & Swanson, 1997b), the anterior hypothalamic nucleus (Risold & Swanson, 1997b; Swanson & Cowan, 1979; Varoqueaux & Poulain, 1999), the amygdala (Risold & Swanson, 1997b), the ventral tegmental area (Staiger & Nurnberger, 1991b; Swanson & Cowan, 1979), the median raphe nuclei (Staiger & Nurnberger, 1991b) and the locus coeruleus (Staiger & Nurnberger, 1991b), although some of these LS
projections may not be entirely restricted to LS, efferents. As with the LS,, there is also
evidence for a weak ascending projection from the LS, to the HPC via the fimbria, with
fibers terminating in the ventral regions of Ammon's horn and the ventral subiculum
(Risold & Swanson, 1997b).

Electrophysiological evidence

As a part of the septo-hippocampal system, the lateral septum (LS) is thought to
have an important influence over the hippocampal theta rhythm. However, the exact
relationship between HPC theta and the LS is not well characterized, with conflicting
results or methodological issues sometimes clouding the issue. For instance, one study
found that ibotenic acid lesions of the lateral septal area attenuated HPC theta by
approximately 50% (Leung, Martin & Stewart, 1994); whereas another study utilizing
electrolytic lesions of the lateral septum failed to observe changes in HPC theta (Bland &
Bland, 1985). Another study showed that infusions of the cholinergic agonist carbachol
into the LS induced a clear HPC theta rhythm during times, such as during relaxed
immobility, when no theta rhythm is normally present. This carbachol-induced HPC theta
rhythm was reversed if the cholinergic antagonist atropine was infused into the LS several
minutes after carbachol (Monmaur, Ayadi & Breton, 1993; Monmaur & Breton, 1990).
However, in this study, the infusion site was very close to the medial septal/diagonal band
area and thus it is unclear whether the observed results were due to medial or lateral septal
manipulations.

For the most part, however, the literature indicates a strong relationship between
the lateral septum and HPC theta. Intracellular and extracellular recordings of LS neurons revealed that the majority of LS neurons were HPC theta-dependent - that is, the level of firing in 68% of LS neurons depended upon the presence or absence of HPC theta. 76% of these HPC theta-dependent LS neurons showed a clear phase-relationship with HPC theta, with the majority of HPC theta-dependent LS neurons firing during the negative peak of the HPC theta wave (Pedemonte, Barrenchea, Nunez, Gambini and Garcia-Austt, 1998).

Additional support for a link between the lateral septum and HPC theta comes from studies utilizing electrical stimulation. Pedemonte et al. (1998) found that the majority of LS neurons changed their firing rate following electrical stimulation of the reticularis pontine oralis (which has been shown to induce or enhance HPC theta). Also, electrical stimulation of the LS led to a resetting of the HPC theta rhythm (Brazhnik, Vinogradova & Karanov, 1985; Pedemonte et al., 1998).

Overall, although the electrophysiological data concerning the relationship between the lateral septum and HPC theta does not always concur, the literature does provide strong evidence that neuronal activity in the LS is an important component for either the maintenance or modulation of the HPC theta rhythm. The results of these electrophysiological studies, combined with the extensive morphological studies which have characterized the neuroanatomical connections of the LS, support the notion that the LS may be a part of a feedback loop from the HPC back to the septum.

_Hippocampal feedback loop_
The proposed hippocampal feedback loop (Figure 4.1) consists of a tri-synaptic connection between the HPC, the LS and the MSA (Gallagher et al., 1995). Anatomical studies suggest that descending fibers from the HPC connect to the LS and that the LS, in turn, has direct projections to the MSA (Risold & Swanson, 1996; 1997a; 1997b). The MSA completes the loop by projecting to the HPC via the fornix. In this feedback model, descending input from the hippocampus arrives in the dorsolateral septum via the fornix (Gallagher et al., 1995). The neurons in the dorsolateral septum critical for this feedback loop are hypothesized to be GABAergic neurons that receive glutamatergic input from HPC afferents (Gallagher & Hasuo, 1989; Joels & Urban, 1984; Leranth et al., 1992; Stevens & Cotman, 1986; 1991), although other neurotransmitters may also play a role (see Gallagher et al., 1995 for review).

The excitation of the dorsolateral septal neurons following input from the hippocampus is thought to be very brief. Single pulse stimulation of the fimbria, which carries hippocampal output to the LS, results in a brief excitation of LS neurons lasting approximately 4-18 msec followed by a lengthy inhibition period lasting 50-800 msec (McLennan & Miller, 1974; 1976), suggesting that the LS neurons may receive inhibitory recurrent collateral feedback. Interestingly, stimulating the fimbria in the 7-12 Hz theta frequency results in a decrease or complete reduction in the time of this inhibitory period.
During the brief 4-18 msec period of excitation, the GABAergic dorsolateral septum neurons are hypothesized to cause a brief inhibition of MSA neuronal firing. This cessation of MSA firing is followed by the re-starting of the normal MSA rhythmic firing pattern, causing a resetting of the HPC theta rhythm (Buno et al., 1978; Gazeltu & Buno, 1982). This hypothesis is supported by the finding that a brief inhibition of MSA neuronal firing is observed prior to a resetting of the hippocampal theta rhythm (Buno et al., 1978) and following electrical stimulation of the fimbria (McLennan & Miller, 1974; 1976), although it is not clear whether this latter result is due to LS feedback or antidromic responses to the fimbria stimulation.

Although the proposed hippocampal feedback model does not incorporate ascending influences from the brainstem or hypothalamus, these inputs may influence theta reset. Numerous studies have shown that electrical stimulation of various ascending afferent inputs to the septal regions, including the reticular formation (Alonso, Gazeltu, Buno & Garcia-Austt, 1987; Gaztelu & Buno, 1982), the supramammillary body (Gaztelu & Buno, 1982) and the medial forebrain bundle (Brazhnik & Vinogradova, 1988) can reset HPC theta. In fact, some researchers have hypothesized that the lateral septum should not be included in this feedback loop due to the debated LS to MS pathway, arguing instead that brainstem and hypothalamic influences are more likely to directly affect MSA neuronal discharges (Leranth, et al., 1992). The present experiment was designed to assess whether the lateral septum is indeed a critical structure for the resetting of the HPC theta rhythm following incoming sensory stimuli.
The working hypothesis is that the LS is a critical structure in this theta reset feedback loop and inactivation of the LS with kynurenate, a glutamatergic antagonist, will block the resetting of hippocampal theta to incoming sensory stimuli. The results of Experiment 1 indicate that proper resetting of the HPC theta rhythm was associated with correct DNMTP task performance. If theta reset is a neural mechanism leading to optimal encoding of incoming stimuli then manipulations which disrupt HPC theta reset to sensory stimuli should be correlated with impaired choice accuracy on a working memory task. Experiment 3 was designed to 1) assess the effects of lateral septal inactivation on theta reset and 2) determine whether the predicted kynurenate-induced blockage of theta reset is correlated with mnemonic deficits in the DNMTP task. It was predicted that infusions of kynurenate into the LS would decrease both choice accuracy and the degree of theta reset.

Method

Behavioral Procedure

Rats were trained on the DNMTP task according to the DNMTP protocols outlined in Experiment 1. After reaching criterion performance (75% correct performance at both delays over a 3 day span, with less than 40% no response rate) on the final training step, the rats underwent surgery.

Surgery

Rats underwent surgery according to the main protocols detailed in the general methods sections. During the surgery, a recording electrode was placed unilaterally into
the dentate hilus (4.0 mm posterior to bregma; ± 2.5 mm lateral to midline; 2.7-3.0 mm ventral to the dural surface). Data from Experiments 1 and 2 revealed no significant hemispheric differences in the degree of theta reset in dentate recording sites. Therefore, only a unilateral recording electrode was utilized in this surgery. The use of a unilateral recording electrode was employed to significantly decrease the time of surgery and to decrease the amount of ketamine supplements necessary to keep the rats anesthetized. Previous surgeries in this laboratory suggest a negative correlation between surgery length and quality of theta (unpublished observations).

As in Experiments 1 & 2, the ventral coordinate for the recording electrode was determined for each rat during surgery by observing the theta signal on an oscilloscope and by examining theta power using an on-line fast Fourier transformation (FFT). The purity and the power of the theta rhythm was used to locate the optimal recording position.

In addition to implantation of the unilateral dentate recording electrode, two guide cannulae (26 gauge stainless steel tubing; 18mm in length) were placed bilaterally into the lateral septal area (0.5 mm anterior to bregma; 1.6 mm lateral to midline; 2.1 mm ventral to the dural surface; at a 15° angle) and secured to the skull with dental acrylic to allow for infusion of the glutamatergic antagonist kynurenate. Following surgery, a stylet was placed into each guide cannula to prevent clogging. Following a one week surgical recovery period, post-surgical testing began.

Post-surgical testing
Experiment 3 was designed to assess the effects of intracranial infusions of kynurenate into the lateral septum on theta reset to mnemonically relevant visual stimuli and thus required different post-surgical testing protocols than those described for Experiments 1 and 2, which each involved ten identical post-surgical recording sessions. In Experiment 3, ten post-surgical recording sessions were not feasible due to tissue damage caused by repeated intracranial infusions. Previous studies in our laboratory suggest that behavioral results become unreliable after approximately eight intracranial infusions; therefore, we restricted the number of infusions to six in all cases.

After the one week recovery period from surgery, rats were retrained on the DNMTP task procedures. During this re-training period, one end of the preamplifier was mounted to the connector on the rats’ heads, while the other end was attached to a cable which was in turn connected to a commutator. The commutator swivelled to allow the rats to move in the box without becoming entangled in the cable. The purpose of this re-training period was 1) to ensure that the surgery did not effect task performance and 2) to acclimate the rats to the recording protocol.

After baseline performance on the DNMTP task returned to pre-surgery baseline levels, rats were given a sham infusion in which an injector was inserted into each of the guide cannulae. However, the injector did not extend into the brain and no substance was infused into the lateral septum. The purpose of the sham infusion was to acclimate the rat to the infusion procedure. Five minutes after the sham infusion procedure, rats were placed into the operant chamber and the DNMTP task commenced. If the sham infusion procedure caused a decrement in task performance, then the sham procedure was repeated.
the following day(s) until the infusion procedures no longer affected task performance. If
the rats performed at pre-surgery levels following the sham infusion, then post-surgical
assessment of the effects of lateral septal inactivation on theta reset and behavioral
performance was begun on the following testing session.

The following three testing sessions involved the infusion of either saline or one of
two doses of kynurenate (2.5 and 5 μg) into the dorsolateral septal area. Experiment 3
was designed as a within-subjects study, with each rat getting all three drug conditions
(one on each testing day). The order of the infusions was counterbalanced across rats.
On infusion days, each rat was brought to the testing room in its home cage. The stylets
were removed and a sterile injector was placed into each guide cannula. Each injector was
connected to a 10 μl glass Hamilton syringe with polythene tubing. The Hamilton syringe
was then placed into a microinfusion syringe pump (Harvard Apparatus, Holliston, MA)
which was programmed to deliver fluid at a rate of .25 μl/min over a one minute span.
After the infusion was completed, the injectors were slowly removed from the guide
cannulae. Five minutes after the intracranial infusion, rats were placed into the
electrophysiology chamber, the headmount was connected to the preamplifier/commutator
system to allow for electrophysiological recording and the DNMTP task began. Testing
concluded after 150 trials or 1 hour elapsed, whichever came first. At the conclusion of
the task, rats were removed from the operant chamber, stylets were placed in the guide
cannula to prevent blockage of the guide cannulae and rats were returned to their home
cages. Three days separated all infusions. Rats were run on the DNMTP task between
infusion days to prevent any inactivity-induced decrements in performance and to ensure
that the intracranial infusions did not have any permanent effects on DNMTP performance.

Following the completion of intracranial testing, animals were anesthetized with ketamine/xylazine (1 mg/kg and 6 mg/kg doses respectively). Once anesthetized, .25 μl of ibotenic acid was infused (at a rate of .25 μl/minute) into the lateral septal area via the same guide cannulae used for the infusions of kynurenate and saline. The purpose of the ibotenic acid lesions was 1) to assess the general spread of kynurenate in the lateral septal area to ensure that the infusions did not encroach upon areas such as the medial septal area which are also involved in hippocampal theta and 2) to determine if there were any differences between temporary and permanent inactivation of the dorsolateral septal area. To assess the effects of a permanent lesion to the dorsolateral septal area on DNMTP performance and theta reset, rats were run on the DNMTP task (while theta was recorded) on Days 2-10 following the ibotenic acid lesion.

Analysis

The main analyses ascertained whether lateral septal inactivation affected DNMTP task performance and whether any possible decrements in performance were associated with decrements in the degree of theta reset. To determine the behavioral effects of lateral septal inactivation (both reversible and permanent) two-way ANOVA’s with repeated measures and t-tests were performed, comparing performance following saline, kynurenate (both low and high doses) and ibotenic acid infusions into the dorsolateral septal area. The most important comparisons were overall percent accuracy, percent accuracy at the
short delay, percent accuracy at the long delay, percent omissions and the number of trials completed. The first three measures provided information on the effects of lateral septal inactivation on memory, whereas the latter two measures assessed any potential motivational deficits.

Histological verification of the placements of the guide cannulae and the recording electrode and analysis of the electrophysiological records to determine whether theta reset was affected by lateral septal manipulations followed the protocols described in the general methods section.

Results

Histology

Histological analysis of cannula placements revealed that all guide cannulae were located in the dorsolateral septum, with the majority (N=5) of injector tips located towards the more ventral aspects of the dorsolateral septum. The remaining two injector tips were located more centrally in the dorsolateral septum. To determine the general extent of the infusion area, ibotenic acid lesions (in a volume equal to the kynurenate infusions) were made using the same guide cannulae utilized for the kynurenate infusions. Following the ibotenic acid lesions, there was a significant amount of cell loss in the more central and ventral aspects of the dorsolateral septum. However, damage to the dorsolateral septum was not complete, as there was still a significant amount of cell bodies remaining in the dorsolateral septum, especially in the more dorsal regions of the dorsolateral septum. There was also a significant amount of cell loss in the more dorsal
aspects of the intermediate zone of the lateral septum in five of the seven cases. Lastly, the infusion did not appear to extend into either the ventrolateral septum of the medial septal area.

**Behavior**

**Trials to criterion:**

One rat was unable to complete testing due to a blockage of the right lateral septal guide cannula. All subsequent analyses pertain to the remaining seven rats. Post-surgically, rats were re-trained on the DNMTP task for an average of 12 days before intracranial infusions were begun. (This re-training period included the time needed for the rats to acclimate to the electrophysiological recording and intracranial sham infusion procedures). During this re-training period, the length of the long delay period between sample light offset and choice light presentation was adjusted to maintain a delay-dependent decrement in performance between short and long delays. The short delay was always maintained at 5 seconds, while the long delay was varied from 10sec (N=2), 12sec (N=3), 15sec (N=1), and 20sec" (N=1).

**Effects of Intracranial Infusion:**
A two-factor ANOVA, using delay (short and long) and infusion (saline, low kynurenate, high kynurenate) as within factors and choice accuracy as the dependent measure, was performed to determine the effects of intracranial infusions into the lateral septal area on DNMTP task performance. Overall, choice accuracy was significantly better at the short delay than the long delay \[F(1,6) = 19.244; p<.006\], with performance being approximately ten percentage points higher in the short delay condition, suggesting that the DNMTP task was assessing working memory. Analysis of the effects of infusion upon choice accuracy revealed a significant overall infusion effect \[F(2,12) = 9.335; p<.005\]. However, there was no significant delay X infusion interaction \[F(2,12) = 0.259; p>.05\], indicating that the intracranial infusions affected both delays equally. Therefore, the behavioral data was collapsed across delays for all subsequent analyses. An one-factor

![Figure 4.2: Infusions of kynurenate into the lateral septum significantly impair DNMTP choice accuracy. * = p<.01.](image)

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ANOVA, using infusion as the within factor and choice accuracy (collapsed across delays) as the dependent measure, revealed a significant overall infusion effect. Subsequent t-tests revealed that infusions of both the low [t (13) = 3.807; p<.001; Figure 4.2] and high [t (13) = 4.893; p<.001; Figure 4.2] doses of kynurenate significantly impaired DNMTP performance relative to saline infusions. There was no significant difference on choice accuracy between high and low dose kynurenate infusions [t (13) = 1.701; p>.05; Figure 4.2].

The decrements in choice accuracy did not appear to be due to a decreased motivation to perform the DNMTP task.

An one-factor ANOVA, using infusion as the within factor and percent omissions as the dependent measure, revealed no significant overall infusion effect [F(2,12) = 2.368; p>.05; Figure 4.3]. Analysis of the effect of infusion on the number of trials completed did reveal a significant overall infusion effect [F(2,12) = 2.368; p>.05]. However, kynurenate infusions did not decrease task motivation, but rather high doses of kynurenate significantly increased the number of trials completed relative to saline infusions [t (6) = 2.969; p<.05; Figure 4.4]. Although there was a trend towards significance, there was no statistical difference between high and low kynurenate infusions on the number of trials completed [t (6) = 2.223; p=.068; Figure 4.4].
Effects of kynurenate infusions on theta

A repeated measures ANOVA, using infusion as the within-factor and percent change in theta power in the final waveform average following stimulus presentation as the dependent measure revealed that infusions of kynurenate into the lateral septum did not impair theta reset to either the sample \( [F(2,14) = .771, p>.05] \) or choice light \( [F(2,14) = .02; p>.05] \) relative to saline controls. In addition, paired sample t-tests comparing pre- and post-stimulus theta power in the final waveform average within each infusion condition failed to reveal theta resetting to either the sample or choice light in any of the infusion conditions, including the saline condition.

Although kynurenate did not decrease the degree of theta reset relative to saline controls, the drug did have an overall affect on overall theta power. A repeated measures
ANOVA, using infusion condition as the within-factor and overall theta power throughout the testing session as the dependent variable, revealed a significant main effect of infusion. \[F(2,12) = 5.619; p<.05\]. Subsequent paired sample t-tests revealed that infusions of the

![Bar chart](image)

Figure 4.5: Infusions of the high dose of kynurenate decrease overall theta power relative to saline infusions. (*=p<.05)

high dose of kynurenate significantly decreased theta power relative to infusions of saline \[t (6) = 2.691; p<.05; \text{Figure 4.4}\]. Lastly, a Pearson product-moment correlation coefficient was calculated and subsequently converted into a t value to determine whether there was a correlation between overall theta power and correct task performance. Results revealed a moderate positive correlation between overall theta power and correct task performance \((r=.524; p<.05; \text{Figure 4.6})\).
Figure 4.6: Changes in overall theta power following LS infusions are positively correlated with correct task performance ($p<.05$). ○ = saline; △ = low dose of kynurenate; □ = high dose of kynurenate.

**Ibotenic acid lesion effects**

Rats were tested for nine days following ibotenic acid lesions of the lateral septum. To determine the effects of ibotenic acid lesions on DNMTP performance, behavioral data was grouped into three blocks, with each block consisting of three testing sessions. A two-factor repeated measures ANOVA, using delay and block as within factors and choice accuracy as the dependent variable, revealed a main effect of delay [$F(1,6) = 14.937; p<.01$], due to a delay-dependent decrement in performance, with decreased choice accuracy at the longer delay. In addition to the main effect of delay, there was also a significant main effect of block [$F(2,12) = 20.361; p<.01$] and a significant delay X block
interaction, due to decreased choice accuracy in the first block of data, which consisted of the first three testing sessions immediately following surgical testing. Further post-hoc analysis revealed that, at the short delay, choice accuracy during the first block was significantly lower than choice accuracy in the second and third blocks. At the longer delay, choice accuracy during the first block was significantly lower than choice accuracy in the third block. However, by the beginning of the second testing session, DNMTP task performance returned to pre-lesion baseline levels \( t (6) = 0.721; p > 0.05 \); Figure 4.7], indicating that there was no long-term decrement in choice accuracy following ibotenic acid lesions of the lateral septum. The decrement in choice accuracy observed during the first block was not due to a decreased motivation to perform, as there were no significant differences in the number of trials completed \( F(2,40) = 1.194; p > 0.05 \) or the percent

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![Figure 4.7: Choice accuracy is impaired in the first three testing sessions (Block 1) following surgery (* = p<.05), but quickly returns to pre-lesion performance levels.](image)
omissions \[ F(2, 40) = 1.320; p > 0.05 \]

The ibotenic acid lesion also had very little effect on hippocampal theta. There were no significant differences in either the degree of theta reset to either the sample light \[ t(53) = 1.479; p > 0.05 \] or the choice light \[ t(53) = 0.914; p > 0.05 \], overall theta power \[ t(105) = 1.131; p > 0.05 \] or theta frequency \[ t(104) = 0.459; p > 0.05 \] between the ibotenic acid-lesioned rats and control rats (from Experiment 1).

Discussion

The results of the Experiment 3 revealed that infusions of kynurenate significantly impaired performance on the DNMTP task and that this kynurenate-induced working memory deficit was positively correlated with a decrease in overall theta power. The finding that lateral septal manipulations can decrease HPC theta power is consistent with the results of Leung et al. (1992) who found that ibotenic acid lesions decreased HPC theta power by 50%.

Contrary to the pre-experiment predictions, impairments in working memory following kynurenate infusions into the LS were not accompanied by decreased theta reset. There was no significant difference between the degree in theta reset following saline infusions and kynurenate infusions. This result calls into question whether the feedback loop from the hippocampus to the lateral septum to medial septum is actively involved in theta resetting. However, it should be noted that, unlike the results of Experiment 1, there was no significant reset to either the sample or choice light following
saline infusions, making it difficult to assess whether kynurenate truly decreased the degree of theta reset. This discrepancy between the results of Experiments 1 and 3 may be the result of differences in the number of testing sessions that went into the final analysis. Due to the invasive nature of the intracranial infusion technique, only a limited number of infusions could be given before extensive tissue damage occurred. Therefore, the number of testing sessions available to assess the effects of intracranial infusions upon theta reset was limited. More specifically, 70-80 testing sessions were analyzed in Experiments 1 and 2 to determine the parameters and neuroanatomy behind theta reset; whereas in the present experiment, only seven testing sessions were included in each analysis. Thus, Experiment 3 results were subject to a greater degree of variability which could have affected the experimental results.

At this point, it is important to note that theta reset is not observed in every testing session. Rather, the trend of theta reset emerges as a pattern over numerous testing sessions. Thus, by decreasing the number of observations, there is an increased chance of making a Type II statistical error. The reasons for the variability between days in the observance of theta reset is unclear, but may have to do the task design. Theta EEG records were recorded one second before or after light presentation. However, there was no mechanism in place to ensure that the rats were actually attending to the stimulus when it was initially presented. Therefore, the EEG records being collected may not always have reflected the exact moment when the animal was actively encoding the incoming stimulus. Studies planned for the future in our laboratory will videotape behavior during task performance to determine whether the rats’ precise movements are associated with
theta reset.

It is also possible that the inability of kynurenate lesions to affect theta reset was due to guide cannula placement. As stated earlier, the extent of the infusions was localized mostly to the lower half of the dorsolateral septum and the uppermost portion of the intermediate lateral septum. However, the infusion site did not extensively involve the more dorsal aspects of the dorsolateral septum, as evidenced by a significant degree of neuronal sparing in this region following ibotenic acid lesions. Importantly, neurons in this region receive considerable glutamatergic inputs from the hippocampus (Gallagher et al., 1995). Thus, it is possible that lesions which include this region of the dorsolateral septum or which include the ventrolateral septum, which projects heavily to the MSA/DB complex (Swanson & Cowan, 1979), could disrupt theta reset. Follow-up studies will be necessary to determine whether this hypothesis is correct.

Overall, given the decrease in HPC theta following kynurenate infusions and the positive correlation between task performance and the decrease in HPC theta following LS infusions, it appears likely that the LS plays a significant role in HPC theta modulation. However, based on the results of Experiment 3, it is not clear whether the LS is actively involved in HPC theta reset.
CHAPTER 5
EXPERIMENT 4

Introduction

A strong relationship exists between theta periodicity and long-term potentiation (LTP), a proposed mechanism for memory that involves a long-lasting change in synaptic efficacy following presentation of trains of high frequency stimulations (Bliss & Collinridge, 1993; Gustafsson & Wigstrom, 1988). LTP was first described by Bliss & Lomo (1973) who observed LTP after stimulating the perforant path with high frequency trains. LTP is generally considered to be a synaptic potentiation that is NMDA-receptor dependent and lasts for more than one hour (Bliss & Collingridge, 1993) - though it can last several weeks (Bliss & Gardner-Medwin).

Since its discovery, LTP has been considered a strong model for identifying the synaptic changes associated with new learning. Blockade of LTP by substances such as the NMDA receptor antagonist AP5 impaired acquisition of new information (Morris, 1989; Morris, Anderson, Lynch & Baudry, 1986). In addition, enhancing the LTP response has lead to facilitation of contextual fear condition (Maren, DeCola, Swain, Fanselow & Thompson, 1994).

One conceptually problematic issue in trying to relate LTP to normal, everyday
learning and memory mechanisms was that the early methods used to induce LTP (e.g., trains of 50-400 stimuli, delivered at frequencies of 100-400 Hz) did not occur naturally in the brain (see Rose and Dunwiddie, 1986 for review). However, more recent examinations of the parameters capable of inducing LTP have attempted to resolve this issue by using more physiologically relevant patterns of stimulation (Capocchi, Zampolini & Larson, 1992; Greenstein et al., 1988; Larson et al., 1985; Rose & Dunwiddie, 1986). Interestingly, although the induction of LTP involves the exogenous application of electrical stimuli to an organism, there does appear to be a natural mechanism related to this phenomenon.

Optimal LTP induction occurs when the pattern of electrical stimulation (5-10 Hz) closely mirrors natural hippocampal theta frequencies (Capocchi, Zampolini & Larson, 1992; Larson et al., 1985; Greenstein et al., 1988; Rose & Dunwiddie, 1986). For instance, delivery of trains of electrical stimuli consisting of five pulses at 100 Hz failed to induce LTP. However, if one of the five bursts was given separately (approximately 200 msec before the other 4 pulses), LTP was observed (Cappochi et al., 1992; Rose & Dunwiddie, 1986). This 200 msec lag between a priming pulse and subsequent sensory stimuli is interesting because it falls into the range of naturally occurring theta. Peaks of activity every 200 msec correspond to the 5 Hz (5 cycles per second) theta frequency.

Studies have also found that the natural firing patterns of certain behaviorally-relevant single CA1 pyramidal neurons closely align with the optimal LTP induction parameters (Otto et al., 1991). These similarities include: 1) firing in high frequency bursting patterns and 2) bursting patterns that are phase-locked to hippocampal theta
rhythms (Otto et al., 1991).

One clear conclusion that can be drawn from the above studies is that the timing of the electrical stimulation is an important factor in optimizing the degree of long-term potentiation and ultimately the encoding of natural sensory stimuli. Given that HPC theta reset may work by a similar mechanism as LTP (i.e. both involve an enhancement of a response to an incoming sensory stimulus arriving in the HPC from the entorhinal cortex), it is important to understand the timing mechanisms behind theta reset.

Experiment 4 examined the effects of electrical stimulations capable of resetting HPC theta on performance on the DNMTP task. More specifically, a discrete electrical stimulation was delivered at a set time surrounding either the sample light or choice light. The main purpose of Experiment 4 was to determine whether the timing of the theta reset (as manipulated by varying the time of electrical stimulation) is critical for proper encoding of the stimulus. The underlying hypothesis of Experiment 4 was that a properly timed stimulus would either facilitate or disrupt the ability of the ongoing theta rhythm to reset to an incoming stimulus, thus resulting in either an enhancement or impairment of DNMTP task performance.

**METHOD**

*Procedure*

Rats were trained on the DNMTP task according to the DNMTP protocols outlined in Experiment 1 and the General Methods. When criterion performance (75% correct performance at both delays over a 3 day span, with less than 40% no response
rate) was achieved on the final training step, rats underwent surgery.

_Surgery_

Rats underwent surgery according to the main protocols detailed in the general methods sections. During the surgery, a recording electrode was placed unilaterally into the dentate hilus (4.0 mm posterior to bregma; ± 2.5 mm lateral to midline; 2.7-3.0 mm ventral to the dural surface). Because Experiments 1 and 2 revealed no significant hemispheric differences in the degree of theta reset in dentate recording sites, unilateral recording was utilized in Experiment 4, as in Experiment 3. (For more details, see surgical methods in Chapter 4). As in Experiments 1-3, the ventral coordinate for the recording electrode was determined for each rat during surgery by observing the theta signal on an oscilloscope and by examining theta power using an on-line Fast Fourier Transformation (FFT). The purity and the power of the theta rhythm was used to locate the optimal recording position.

In addition to the recording electrode, a stimulating electrode was placed unilaterally into the fornix (1.8 mm posterior to bregma; 2.0 mm lateral to midline; 3.0-3.7 mm ventral to the dural surface), ipsalateral to the recording electrode. The ventral coordinates for the stimulating sites were determined for each rat during surgery by observing an oscilloscope to locate the site with the maximal amount of electrically elicited theta reset.

_Post-surgical testing_
One week after surgery, rats were retrained on the DNMT task (see Chapter 2 methods for more complete re-training protocols). After performance returned to pre-surgery levels, post-surgical testing commenced. There were eighteen post-surgical testing sessions. On fourteen of the first sixteen testing days, rats received an electrical stimulation of the fornix (a single 600 μA square wave pulse for 0.2 msec) centered around the onset of the sample or choice light for every trial of the testing session. The remaining two sessions were baseline sessions in which no electrical stimulation was delivered. These no stimulation sessions were randomly interspersed among the electrical stimulation testing sessions. Electrical stimulation occurred either simultaneously with the sample or choice light or 100, 200 or 500 msec before or after the sample or choice light (See figure 5.1). Experiment 4 was a within-subjects design, with all rats receiving each timing condition. The order of the electrical stimulations was determined in a pseudo-random order with the following constraints: 1) Within a daily testing session, all electrical stimulations occurred at the same exact time during a trial (e.g. on Day 1, a rat might have received an electrical stimulation at 500 msec before the choice stimulus on every single trial); 2) The order of the timing of the electrical stimulation was counterbalanced across testing sessions; 3) within a testing session, electrical stimulation was centered around either the sample or choice light, but not around both and 4) all electrical stimulations...
centered around the sample light and all electrical stimulations centered around the choice light were grouped together. For example, for Days 1-7, a rat might have had all electrical stimulations centered around the sample light and on days 8-14 the rat would have received electrical stimulations centered around the choice light. The determination of whether Days 1-7 were sample or choice light-centered were counterbalanced across rats. A representative diagram for one rat’s testing order is illustrated below in Table 5.1.

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stimulation Time</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500 msec before sample</td>
<td>100 msec after sample</td>
<td>same time as sample</td>
<td>200 msec before sample</td>
<td>100 msec before sample</td>
<td>No stim</td>
<td>200 msec after sample</td>
<td>500 msec after sample</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Stimulation Time</td>
<td>same time as choice</td>
<td>No stim</td>
<td>200 msec after choice</td>
<td>500 msec after choice</td>
<td>500 msec before choice</td>
<td>100 msec after choice</td>
<td>200 msec before sample</td>
</tr>
</tbody>
</table>

Table 5.1: Representative diagram of testing sessions 1-16 for Experiment 4.

Following these sixteen testing sessions, two final testing days were initiated, in which a train of electrical pulses (0.2 msec, 400\(\mu\)A square wave pulses every 50 msec) were delivered to the fornix from 500 msec before until 500 msec after the sample and choice light. One testing session consisted of trains of electrical stimulation around the sample light and the other consisted of trains around the choice light, with the order of these testing sessions counterbalanced between rats.
Analysis

The main analyses concerned whether the timing of electrical stimulations affected performance on the DNMTP task. Two-way ANOVA’s with repeated measures were performed, comparing performance at each of the stimulation time points. The most important comparisons were overall percent accuracy; percent accuracy at the short delay, percent accuracy at the long delay, percent omissions and the number of trials completed. The first three measures provided information on the effects of the timing of electrical stimulations on memory, whereas the latter two measures assessed any potential motivational deficits.

Results

Post-surgically, rats were re-trained on the DNMTP task for an average of 11 days before electrical stimulation testing sessions commenced. (This re-training period included the time needed for the rats to acclimate to the electrophysiological recording procedures). During this re-training period, the length of the long delay period between sample light offset and choice light presentation was adjusted to maintain a delay-dependent decrement in performance between short and long delays. The short delay was always maintained at 5 seconds, while the long delay varied from 10-20 seconds.

To assess the effects of electrical stimulations centered around the sample light, a two-factor ANOVA with delay and stimulation time as within factors and percent correct as the dependent measure was performed. Results revealed a significant main effect of
Figure 5.2: Trains of electrical stimulation decrease choice accuracy in the DNMTP task relative to the no stimulation condition. (* = p<.05)

delay \( [F(8,56) = 2.427; p<.01] \), with performance at the short delay being approximately 15% better than performance at the longer delay, suggesting that the DNMTP task was an appropriate paradigm for assessing working memory deficits. Results also revealed a main effect of stimulation time \( [F(8,56) = 2.427; p<.03] \), indicating that electrical stimulations of the fornix centered around sample light presentation significantly affected task performance. However, no significant delay \( \times \) stimulation time interaction was observed \( [F(8,56) = .599; p>.05] \). Thus, for all subsequent analyses, choice accuracy was collapsed across delays.

In order to determine whether stimulation at a particular timepoint around the sample light could disrupt or enhance choice accuracy, two-tailed, paired-sample t-tests were performed comparing each of the time points with the no stimulation condition. Results indicated that the significant main stimulation effect (as revealed by the overall ANOVA) was due to decreased performance when the train of electrical stimuli was delivered during sample light presentation \( [t (15) = 2.910; p<.02; \text{Figure 5.2}] \). The more
discrete single pulses centered at one particular time point around the sample light (i.e. -500, -200, -100, 0, +100, +200 and +500 msec from sample light) did not impair or enhance performance relative to the no stimulation condition \([t (15) = 1.633; p>.05; t (15) = 0.482; p>.05; t (15) = 1.117; p>.05; t (15) = 0.357; p>.05; t (15) = 2.043; p>.05; t (15) = 1.130; p>.05; and t (15) = 1.581; p>.05\), respectively; Figure 5.2]. However, there were slight enhancements in performance when an electrical stimulus was delivered 200 msec before or after the sample light onset relative to other electrical stimulation points. Electrical stimulations delivered 200 msec before the sample light slightly enhanced performance relative to the trains of stimuli \([t (15) = 2.970; p<.05; Figure 5.2]\); and stimulation 500 msec before the sample light \([t (15) = 2.437; p<.05; Figure 5.2]\). In addition, electrical stimulations delivered 200 msec after the sample light enhanced performance relative to trains of stimuli \([t (15) = 2.619; p<.05; Figure 5.2]\) and stimuli delivered either 500 msec after \([t (15) = 2.540; p<.05; Figure 5.2]\) or 100 msec after \([t (15) = 2.390; p<.05; Figure 5.2]\) the sample light.

The electrical stimulations centered around the sample light did not appear to affect motivation to perform the DNMTP task. There were no significant main effects of stimulation time on either percent omissions \([F(8,56) = 1.049; p>.05; Figure 5.3]\) or the number of trials completed \([F(8,56) = 1.283; p>.05; Figure 5.4]\).
Figure 5.3: Electrical stimulations centered around the sample light had no affect on % omissions on the DNMTP task. (p>.05)

Figure 5.4: Trains of electrical stimuli centered around the sample light did not affect the number of DNMTP trials completed. (p>.05)
In contrast to the results of electrical stimulation centered around the sample light, similarly-timed electrical stimulations around the choice light failed to affect task performance (Figure 5.5). Although a significant overall delay effect (with performance at the short delay being significantly better than performance at the long) was revealed by a two-factor ANOVA \([F(1,7) = 24.726; \ p<.01]\) with delay and stimulation time as within factors and percent correct as the dependent measure, there was no overall main effect of stimulation \([F(8,56) = 1.358; \ p>.05]\) and no significant delay \(\times\) stimulation time interaction \([F(8,56) = 1.841; \ p>.05]\). In addition, electrical stimulations centered around the choice light did not appear to affect motivation to perform the DNMT task, as there...
Figure 5.6: Electrical stimulations centered around the choice light did not affect % omissions (left) or the number of trials completed (right) in the DNMTP task. (p>.05)

were no significant effects of stimulation time on either the number of trials completed $[F(8,56) = 0.259; p>.05; \text{Figure 5.6}]$ or percent omissions $[F(8,56) = 0.276; p>.05; \text{Figure 5.6}]$.

Discussion

Overall, electrical stimulation centered at different time points around the sample or choice light had minimal effect on choice accuracy on the DNMTP task. None of the more discrete, single, square wave pulse stimulation time points significantly enhanced or
impaired choice accuracy relative to the baseline no stimulation condition. The only significant stimulation effect was a 9% decrease in choice accuracy when a train of electrical stimulation was delivered from 500 msec before until 500 msec after the sample light.

One possible explanation of this result is that the impairment in choice accuracy does not reflect mnemonic deficits per se, but rather a motivational deficit due to the possible aversive effects of a constant train of electrical stimuli. However, it does not appear that the electrical stimulations affected task motivation, as there were no significant differences on the number of trials completed or percent omissions relative to control no stimulation days. In addition, if the train of electrical stimulation was having an aversive affect on the rat, there should have been a similar decrement in performance following delivery of a train of stimuli around the choice light. However, no such decrement in task performance was observed. Overall, the deficits in DNMTP performance following the train of electrical stimulation appears to be due to deficits associated with an inability to properly process mnemonically relevant stimuli during the initial encoding, but not retrieval phase of the task. Trains of electrical stimulation centered around the sample light impaired performance, while stimulation around the choice light did not affect task performance.

Although there were no significant enhancements of task performance following electrical stimulation as compared to the no stimulation condition, there did appear to be a slight enhancement of task performance following electrical stimulation delivered 200 msec before and after sample light presentation (Figure 5.2). These approximately 4-5%
enhancements were relative to other electrical stimulation time points.

Electrical stimulations delivered 200 msec before the sample light slightly enhanced performance relative to the trains of stimuli and stimulation 500 msec before the sample light. In addition, electrical stimulations delivered 200 msec after the sample light enhanced performance relative to trains of stimuli and stimuli delivered either 500 or 100 msec after the sample light (Figure 5.2). No other enhancements of task performance were observed following electrical stimulation at any of the other time points. Although some of these enhancements may be more accurately described as an impairment in performance following trains of electrical stimuli, this observation does not totally account for the observed enhancements, as there were also slight enhancements following stimulations 200 msec before and after the sample light relative to other discrete single pulse stimulation times.

Overall, this pattern of enhancements is consistent with studies showing that optimal LTP induction occurs when a priming stimulus is delivered 200 msec before a subsequent tetanic stimulation (Capocchi, Zampolini & Larson, 1992; Larson et al., 1985; Greenstein et al., 1988; Rose & Dunwiddie, 1986).

However, as mentioned before, the enhancements observed were slight and did not occur in relation to the no stimulation control condition. The failure to find a more robust enhancement could be due to several factors. For instance, the rats may have reached a ceiling in terms of behavioral performance, thus making it difficult to readily observe enhancements in choice accuracy. The present study was designed to examine possible bi-directional effects of electrical stimulations. Therefore, performance had to be maintained
at a level at which both decrements and enhancements of choice accuracy could be observed. It is possible that the level of performance was too high in the baseline conditions to adequately assess cognitive enhancement. In future studies, it might be fruitful to focus on a more uni-directional approach and to adjust the delay periods to maintain baseline performance at higher task performance when interested in examining decrements in performance and to maintain baseline performance at slightly lower levels when interesting in cognitive enhancement.

One other possible factor that could explain the relative ineffectiveness of the electrical stimulations to produce impairments or improvements in performance is the pattern of electrical stimulation applied to the fornix. In the present study, a single 600 μA square wave pulse lasting 0.2 msec in duration was chosen for fornix stimulation. This stimulation level was chosen based on the results of experiment 2, which used this stimulation pattern to elicit HPC reset following both fornix and perforant path stimulation. However, it is possible that the utilization of a different stimulation pattern, such as the patterns used to induce LTP (Capocchi et al., 1992; Larson & Lynch, 1989; Rose & Dunwiddie, 1986) or the patterns used to measure excitatory post-synaptic potentials following perforant path stimulation (Bilkey & Goddard, 1987), could result in a greater level of enhancement than currently evidenced in the present study.

Overall, the results of Experiment 4, though not robust, are highly consistent with the literature concerning timing mechanisms associated with encoding new information.
More specifically, the data suggests that priming stimuli delivered within the parameters of naturally occurring theta frequencies can enhance the encoding of new stimuli, perhaps by an LTP-like mechanism.
CHAPTER 6

GENERAL DISCUSSION

Although theta reset was first described in the late 1960's by Adey (1967), the phenomenon has yet to be well-characterized. The present study was aimed at examining theta reset on a variety of different levels including 1) the functional significance of theta reset, especially as it relates to cognitive processes such as encoding and retrieval 2) the neuroanatomy behind theta reset and 3) the timing mechanisms behind theta reset and its relationship to other EEG oscillatory patterns in the brain.

Although theta reset has mostly been discussed in relation to the hippocampus, Experiment 1 provided evidence that theta reset applies to other areas exhibiting a prominent theta rhythm, including the entorhinal (EC) and anterior cingulate (AC) cortices. Results indicate that although theta reset in all of these areas is related to working memory processes, all three areas are involved in different aspects of working memory. Theta reset in the EC is more involved in the initial encoding of information. HPC theta reset is involved in both the initial encoding and the subsequent retrieval phases of the task and theta reset in the AC is associated with the later retrieval aspects of the task.

Although it might be a little too speculative at this point, given the relative lack of
research regarding the phenomenon of theta reset, to have a complete model of the
neuroanatomical/neurophysiological mechanisms governing theta reset, this discussion will
attempt to pull together information from the rich history of entorhinal, hippocampal, and
prefrontal literature to develop a cohesive model that can serve as a starting point for
discussions regarding theta reset.

Although a number of structures may be involved in a theta reset pathway
(especially brainstem and hypothalamic areas), this proposed model will primarily restrict
itself to the areas studied explicitly in this group of experiments. The first portion of the
model concerns the entorhinal cortex. Given the finding that the EC appears to be
preferentially involved in the initial encoding of sensory stimuli, as opposed to the later
retrieval aspects of a working memory task, it is predicted that theta reset will first occur
in the EC. The mechanism behind this theta reset in the EC is unclear, but the medial
septal area (MSA) is a likely candidate to play a role in EC reset.

Neuroanatomically, the MSA sends efferent fibers to the EC (Dickson et al., 1994;
Irle & Markowitsch, 1984). In addition, reversible medial septal lesions have been shown
to abolish EC theta (Dickson et al., 1994) or change the firing patterns of EC neurons
(Mizumouri, Ward & Lavoie, 1992). In this proposed model, EC theta reset is thought to
involve a mechanism similar to the hypothesized cause of HPC theta reset. Buno et al.
(1978) proposed that HPC theta resetting occurs when MSA cells are briefly inhibited,
most likely following ascending brainstem/hypothalamic inputs (Alonso et al., 1987;
example, electrical stimulation of the mesencephalic reticular formation inputs to the MSA
caused a resetting of both MSA neuronal firing and HPC theta. These inputs caused the MSA neurons to briefly stop firing and then to quickly begin firing again, causing a resetting of the HPC theta rhythm (Gazeltu & Buno, 1982). Support for this theory comes from the fact that HPC theta reset was always preceded by an inhibition of MSA neuronal firing (Gazeltu & Buno, 1982).

A similar mechanism is proposed to apply to the entorhinal cortex. Although there is no direct evidence to suggest that EC theta resetting is always preceded by an inhibition of MSA firing, given the anatomical and electrophysiological evidence supporting a strong link between the MSA and the EC, this portion of the model remains a viable hypothesis.

According to this proposed model, MSA input to EC will allow the EC to be in a maximal state of depolarization when mnemonically relevant sensory stimuli arrives from the perirhinal cortex, thus allowing the EC to better encode the stimulus before sending the information on the HPC. Evidence from Experiment 1 suggests that theta reset in the EC may allow the organism to better encode the stimulus, as the occurrence of theta reset was correlated with correct task performance.

Perhaps the best supported portion of the theta reset model concerns the neurobiological mechanisms behind HPC theta reset. As stated earlier, theta resetting is most likely caused by a brainstem/hypothalamus mediated inhibition of medial septal firing (see Gazeltu & Buno, 1982; Vertes, 1981; Vertes et al., 1995; Vinogradova, 1995 for more complete details regarding these inputs to MSA). Although it has been hypothesized that a lateral septum-MSA-HPC feedback system may be involved in HPC theta reset, the present results do not support such a conclusion. Infusions of kynurenate into the lateral
septum failed to affect theta reset relative to saline controls. Following this brief inhibition, the MSA neurons begin to fire again, causing a resetting of the HPC rhythm. The purpose of the HPC theta resetting is to allow the hippocampus to be maximally activated when sensory input arrives from the entorhinal cortex via the perforant pathway, thereby enhancing synaptic activity and facilitating the encoding of incoming task-relevant stimuli. The integrity of the perforant path is an important component of the transfer of task-relevant information to the hippocampus. Lesions to the perforant path impair performance on place learning and contextual fear conditioning tasks, which are traditionally considered to be hippocampally-dependent tasks (Ferbinteanu, Holsinger & McDonald, 1999).

Additional support for the hypothesis that a priming input from the MSA may reset HPC theta and thus enhance encoding of sensory input from the perforant path comes from the work of Bilkey & Goddard (1987) who demonstrated that electrical stimulation of the perforant path resulted in excitatory post synaptic responses (EPSP) in the granule cells of the dentate gyrus, which is one of the important theta generators in the hippocampus proper - the other being located in CA1 (Bland, 1986; Bland & Whishaw, 1976). This granule cell EPSP has a significant negative going component which is referred to as a population spike. The height of the population spike is thought to be positively correlated with increased granule cell activation (Bilkey & Goddard, 1987). Importantly, delivering a priming pulse to the MSA prior to electrical stimulation of the perforant path significantly increased the size of the granule cell population spike, indicating that the conditioning pulse may be allowing the dentate to be in a maximal state.
of depolarization when input arrives from the entorhinal cortex.

Bilkey and Goddard (1985) further proposed a mechanism to explain how this depolarization might occur. According to their theory, stimulation of the perforant path (either by natural sensory stimuli or by electrical stimulation) excites dentate granule cells. However, the granules cells have collaterals that project to GABAergic neurons which serve to inhibit that same granule soon after the cell fires (Bilkey & Goddard, 1985; Paulsen & Moser, 1998). The role of the MSA in this model is to prevent this inhibition. More specifically, GABAergic neurons in the MSA project to the GABAergic interneurons in the dentate which serve to inhibit the dentate neuronal firing. Hence, the MSA inhibits this inhibitory input, allowing the granule cells to fire for a longer period of time and perhaps facilitating encoding of incoming stimuli and improving task performance. The ability of HPC theta reset to enhance the encoding of hippocampally-dependent information from the perforant path is supported by the results of Experiment 1, which showed that HPC theta reset is associated with correct task performance.

Although the proposed model discussed in this section on the role of HPC theta reset has thus far been concerned with the initial encoding of information, it is important to note that HPC theta reset does not only occur to the sample light in the initial encoding phase of the DNMTP task, but also to the choice light in the retrieval phase of the task. Similar results have been found in other studies (Deadwyler, Bunn & Hampson, 1996; see Desgranges, Baron & Eustache, 1998 for review; Hampson, Heyser & Deadwyler, 1993; Hasselmo, Wyble & Wallenstein, 1996; Olton, 1989). Thus, it is important for models of theta reset to account for temporally distinct stages of information processing within the
hippocampus.

One such model that is consistent with the findings from this group of experiments is a "read-in/read-out" model of hippocampal functioning (Paulsen & Moser, 1998). In this model, the read-in mode is analogous to the initial encoding of mnemonic information; whereas the read-out mode refers more to the later retrieval phases of cognitive processing. Although some read-in/read-out models have suggested that read-in and read-out processes are governed by different areas of the HPC formation (Buzsaki, 1984), Paulsen and Moser (1998) argue that the individual neurons are capable of modulating both read-in and read-out processes. The results of experiment 1 support this latter conclusion. Although no specific conclusions regarding the role of individual neurons in read-in versus read-out processes can be made from the present results (since single unit activity was not examined in Experiment 1), it is clear that theta reset was observed in both the sample and choice phases of the DNMTP task by the group of neurons recorded by one theta recording electrode. Thus, cells within the small, spatially defined area of the recording electrode are capable of both read-in and read-out functions. However, the precise mechanisms behind these read-in and read-out processes are not well understood and more research will be needed to further identify the neural mechanisms involved in these processes.

Inherent in the proposed model (that the HPC is involved in both the encoding and retrieval phases of a mnemonic task) is the notion that there should be a mechanism by which the HPC can store or maintain sample phase information until an appropriate behavioral response can be made. One potential mechanism, proposed by Lisman & Idiart
(1995) suggests that incoming task-relevant information increases neuronal firing and, in the presence of neuromodulators such as acetylcholine and serotonin, this firing results in a brief afterdepolarization, which is "too brief to account for the duration of short-term memory, but is long enough to store information between cycles of oscillations in the theta-alpha range (5-12 Hz)." Thus, repeating theta oscillations may enable the HPC to maintain information until the time of retrieval when a behavioral response can be made.

Theta resetting in the anterior cingulate is proposed to be one mechanism by which a behavioral response might occur. The results of experiment 1 support this conclusion in that AC theta reset occurs only during the retrieval aspects of the DNMTP task. Little is known about theta reset in the anterior cingulate. However, as with the EC and the HPC, the medial septal area is likely to play a significant role in theta reset. The MSA projects directly to the AC and thus may play a strong role in AC theta reset. The role of the MSA might be to ensure that the AC is in a maximal state of depolarization when sensory input arrives from structures such as the hippocampus and the amygdala, which has strong interconnections with the AC (Price, Russchen & Amaral, 1987). The enhancement of sensory input from the amygdala, which processes affective information (Iwata et al., 1986; Kesner & Williams, 1995) might be especially important at the retrieval stage of the task in terms of associating affective information with a behavioral response. This association between affect and behavioral response is thought to be one of the executive processes governed by the prefrontal cortex (Bussey, Everitt & Robbins, 1997; Bussey, Muir, et al., 1997 Devinsky, Morrell & Vogt, 1995; Gabriel, 1990; Lane, Fink, Chau & Dolan, 1997; Lane, Reiman, Axelrod, Yun, Holmes & Schwartz, 1998). However, there
is no evidence to conclusively suggest that the MSA is directly responsible for theta reset.

Another possibility to explain AC theta reset is through a direct projection from HPC to AC. Tetanic stimuli applied to the hippocampal efferents to the AC result in long-term potentiation in the AC. Given the potential relationship between HPC reset and the optimal induction parameters of LTP, it seems likely that hippocampal output may affect AC reset. However, more detailed studies will have to be conducted to provide more definitive evidence for the proposed model.

Overall, although the role of theta reset is still relatively novel, the present results provide strong support for a role of theta reset in working memory processes. Theta reset 1) occurs in many different brain structures (such as the entorhinal cortex, hippocampus and anterior cingulate) which have been implicated in working memory; 2) has been associated with precise stages of cognitive processing, such as encoding, retrieval and motor response; and 3) is correlated with correct task performance.
CHAPTER 7

GENERAL METHODS

Subjects

All experimental protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996). Eight male Long-Evans rats were used in each of the four main experiments comprising this dissertation. An additional four rats were used in a pilot experiment, for a total of 36 rats. Protocols using eight rats per experiment have been extensively used within our laboratory throughout numerous studies and have been proven sufficient for obtaining meaningful experimental results. Animals were housed in a colony room maintained at a constant temperature on a 12:12 light/dark cycle. At the start of behavioral training, the body weight of each rat was decreased to approximately 85% of the rat’s ad libitum weight by restricting the rat’s water intake. During training, rats were given an unlimited amount of food and a sufficient amount of water to maintain a restricted body weight.

Behavioral apparatus

All behavioral studies were conducted in two types of operant chambers developed
by Med Associates (St. Albans, VT): one was utilized in the initial behavioral training and
one was adapted specifically for electrophysiological recording. Housed within a light and
sound-attenuating shell (64 X 41 X 41 cm), the initial training chambers
(28 X 21 X 27) consisted of two
side walls of 0.5 cm clear plexiglass,
front and back panels of stainless
steel and a floor consisting of
parallel stainless steel rods, 1 cm
apart. The front panel of the
chamber contained three levers, 7.5 cm above the floor. A circular light, 2.5 cm in
diameter and 12 cm above the floor, was located above each lever. A central houselight,
18 cm above the floor, was located centrally on the front panel and a tone generator, 12
cm above the floor, is on the back panel. A water dispenser, 2 cm above the floor in the
center of the back panel, delivered a drop of water following rewarded lever presses. The
electrophysiology chamber was identical to the initial training chambers except for higher
side panels (42 cm) and a larger water port to accommodate the preamplifier/cable system
that was attached to the rats’ headmount during recording sessions. Both types of operant
chambers were interfaced to a personal computer that controlled all behavioral acquisition
and analysis with software developed by Med Associates (East Fairfield, VT).

Figure 6.1 Schematic representation of the
electrophysiological recording chamber

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Shaping steps for DNMTP task

Shaping Step 1:

Initially, rats were trained to press levers to receive a water reward. Left, right or center lever presses were rewarded with a drop of water. To prevent the development of a bias towards one lever, the total number of presses on one lever could not exceed those to any of the remaining levers by more than five. If this occurred, subsequent responding to the more frequently pressed lever was no longer rewarded until the imbalance was corrected. Rats were trained in this step daily for one hour sessions until they reached a criterion of 50 bar presses in one hour, after which they were moved to Shaping Step 2.

Shaping Step 2:

In this shaping procedure, rats were trained to respond to the illumination of lights on the front panel of the operant chamber. At the beginning of each trial, a light appeared randomly over either the left or right lever. The rat was required to press the lever beneath the illuminated light for a water reward. The light remained illuminated until the rat pressed either the right or left lever. Rats received 150 trials of this procedure per day, 5-6 days per week. A 10 second intertrial interval (ITI) separated each trial. Rats were shaped in this procedure until they reached a criterion of 75% correct over three consecutive sessions, at which point they were moved to Shaping Step 3.

Shaping Step 3:

Rats were trained on a delayed non-match to procedure (DNMTP) task utilizing a 5 second delay. The DNMTP task is a WM task that is comprised of two components: a sample phase and a choice phase. At the beginning of each trial, a light appeared over
either left or right lever. Rats were required to press the lever beneath the light, at which
time the light was extinguished and the rat received a 0.04 ml drop of water. This
constituted the sample phase of the task, which can be considered the initial “encoding”
phase of the task. In the sample phase, the lever press was required to ensure that rats
were cognizant of the position of the sample stimulus. After the sample light, a 5 second
delay was imposed, after which a light over the center lever appeared. The center light
signaled the start of the choice phase, which is analogous to the “retrieval” phase of the
task. Rats were required to recall and press the lever opposite the sample light. For
instance, if a light appeared over the right lever during the sample phase, then the rat was
required to press the left lever during the choice phase. The choice phase continued until
the rat made a response to either the right or left lever. All correct choice phase responses
were rewarded with a 0.08 ml drop of water. If an incorrect response was made, a
correction trial was initiated in which the same trial was repeated again. The correction
trial was repeated until the rat made the correct response or until 5 correction trials had
elapsed. A 30 second intertrial interval (ITI) followed each choice-phase lever response.
Rats were shaped in this procedure until they reached a criterion of 75% correct over
three consecutive days (correction trials were not included in determination of criterion
performance), at which point they were moved to Shaping Step 4.

**Shaping Step 4:**

Shaping Step 4 utilized the same training protocols described in Shaping Step 3
with a few key exceptions. In Shaping Step 4, two different delay periods were used:
either a short (5 seconds) or long (10 seconds) delay separated the sample and choice
phases. In addition, rats were required to make a choice phase response within five
seconds of choice light presentation. If the rat did not respond within five seconds, the
trial continued until the rat pressed either the right or left lever, but the trial was recorded
as a no response trial. All correct choice phase responses were rewarded with a 0.08 ml
drop of water. A 30 second intertrial interval (ITI) followed a choice-phase lever
response. Rats were shaped in this procedure until they reached a criterion of 75%
correct over three consecutive days (% correct on correction and no response trials were
not included in determination of criterion performance) with less than 40% omissions, at
which point they were moved to the final shaping step, which is described in detail in the
Chapter 2 methods section.

Surgeries

All surgeries were performed under ketamine/xylazine anesthesia under aseptic
conditions according to the Guide for the Care and Use of Laboratory Animals (National
Academy Press, Washington, D.C., 1996). Supplemental injections of ketamine (0.1cc)
were administered if corneal, hindlimb or tail reflexes or if rapid respiratory rates were
present. During the surgery, the body temperature of each rat was monitored and kept
constant at approximately 36°C with a homeothermic blanket control unit (Harvard
Apparatus, Holliston, MA).

During the surgery, Teflon coated, 125 μm stainless steel wire recording
electrodes (A-M Systems, Inc., WA) with a gold ITT/Cannon Centi-Lok pin (Time
Electronics, IL) attached to one end were placed bilaterally into the areas of interest. For
select surgeries (see individual experiments for more details) stimulating electrodes (Teflon coated, 250 μm stainless steel wire, with a gold ITT/Cannon Centi-Lok pin at the end) were placed into areas of interest. More detailed information regarding sites and coordinates for recording and stimulating electrodes is presented in later sections.

In addition to the recording and stimulating electrodes, recording and, for select experiments, stimulation ground wires (Teflon coated, 250 μm stainless steel wire, with a 1 mm uninsulated tip at one end and a gold ITT/Cannon Centi-Lok pin at the other) were lowered approximately 1mm into the cortex. The electrodes were secured to the skull with dental acrylic. Before applying the dental acrylic, 5-8 small screws were inserted into the skull to provide extra bonding surfaces for the dental acrylic. Once the recording, stimulation and ground wires were securely fastened to the skull, the wire ends with the gold ITT/Cannon Centi-Lok pins were inserted into an ITT/Cannon Insulator Strip that served as a connector for a preamplifier. Following insertion into the insulator strip, the connector was secured with dental acrylic and the animal was removed from the stereotaxic equipment.

After surgery, Mycitracin Plus (a local antibiotic/anesthetic) was applied to the edges of the dental acrylic to prevent infection and minimize discomfort. Animals were kept warm under a heating lamp until recovery from the anesthesia was complete. Animals had free access to food and water following surgery and were slowly returned to their normal daily water intake. Body weight, posture and locomotor activity were carefully monitored following surgery to ensure complete recovery and a veterinarian was available for consultation in the event of emergencies. Testing resumed one week after surgery.
The surgical methods allowed for on-line monitoring to locate the optimal electrode placement for recording theta rhythm during surgery and to locate a stimulation site capable of resetting the hippocampal theta rhythm. Previous studies from our laboratory showed that ketamine/xylazine is an appropriate anesthetic for the proposed surgeries. Although theta power theta was reduced under ketamine/xylazine, a clear theta rhythm was still present under the anesthesia. Following recovery from surgery, the hippocampal theta rhythm was still present and the ability of the stimulating electrodes to reset the hippocampal theta rhythm was still intact (unpublished observations).

Electrophysiological recordings

To collect the electrophysiological data, a preamplifier was mounted to the connector mounted on each rat’s head. The signal was passed via a cable from the preamplifier to an amplifier(1000×)/filter(0.1-500Hz) system (A-M Systems, Inc.) which in turn sent the signal to an A-D board for digitization. The digitized signal was sent to data acquisition software developed by DataWave Systems, Inc. (DataWave Systems, Inc., Longmont, CO).

Analysis

All electrophysiological data was analyzed using software developed by DataWave Systems, Inc. Each individual data record was examined for noise. All data records containing non-neural signals (artifacts) were removed before examining whether theta reset occurred. To determine whether theta reset occurred, a quantitative analysis was
applied to the electrophysiology records. First, a waveform averaging was performed on the pre-stimulus records and the post-stimulus records and the final waveform average was saved for further analysis. Next, a FFT analysis was performed upon the output of the final waveform average to determine whether theta reset occurred. The dominant frequency located within the 6 to 10 Hz range and the power at that frequency was recorded. If there was no obvious peak within theta range, the value at the frequency where theta was seen before (or after) the stimulation was recorded. If a reset occurred, when examining the final waveform output, theta power following stimulus onset should have been greater than theta power prior to stimulus onset. If the ongoing theta rhythm did not become phase-locked to the onset of the stimulus, both pre- and post-stimulus records should have averaged to a flat line after the waveform averaging.

To provide a quantifiable measure of theta reset, FFT analysis was performed upon the pre-stimulus waveform average and the post-stimulus waveform average. The frequency in the 6-10 Hz theta range with maximal power and the power at that theta frequency were recorded for subsequent analysis. More specifically, the FFT results were subjected to paired sample t-tests to determine if there was a significant difference between pre- and post-stimulus theta power in the final waveform averages. Theta reset was defined as an increase in theta power in the post-stimulus final waveform average relative to the theta power evident in the pre-stimulus waveform average. Overall, the stimuli that resulted in theta reset varied between the three brain areas.

One limitation of the proposed analyses is the use of multiple t-tests which may increase the chances of a Type 1 statistical error (rejecting the null hypothesis when it is
true). Although this was (and still is) a major concern in interpreting Experiment 1 results (which rely almost exclusively on multiple t-tests to statistically analyze the data, the predominant statistical alternatives are equally problematic. For instance, the adoption of the Bonferroni correction procedure would lead to an excessive degree of Type II statistical errors (accepting the null hypothesis when it is false). Given the large number of t-tests, in order for a result to be considered statistically significant, a t-test would have to yield a p-value less than .0002, thereby potentially masking significant results. This dilemma is a growing problem in the field of electrophysiological recording studies and has been the subject of numerous panel discussions at conferences within this field, especially as increasing technology allows for simultaneous recording in a large number of brain sites and allows correlation with an increasing number of unrelated or indirectly related variables. The results of these panel discussions have led to multi-million dollar studies examining this issue, but at this time, no ideal solution for this statistical problem has been adopted by researchers in the field of EEG recording (John Ernst, personal communication, 1999). However, given the limitation of the use of multiple t-test, results of Experiment 1 must be interpreted with this in mind, especially in interpreting significant results with relatively smaller t-values.

Lastly, to determine whether the results actually represented a phase-locking of an on-going theta rhythm to the stimulus or alternatively whether the stimulus merely evoked a theta rhythm, a FFT analysis was performed on the entire set of raw pre- and post-stimulus records (i.e. not just the final waveform average). The dominant frequency located within 6-10 Hz range and the power at that frequency was recorded for further
statistical analysis. As described above, multiple t-tests were also used to quantify whether there was an increase in overall theta power following light presentation or motor response.

**Histology**

Following completion of behavioral testing, histology was performed. To verify the accuracy of the recording and stimulating electrode placements, rats were deeply anesthetized with sodium pentobarbital and an electrical current (20 µA for forty seconds) was passed through the electrode to produce a lesion at the tip of the electrode. In Experiment 3, to gauge the accuracy of guide cannulae placements and to approximate the spread of the infused substances through the guide cannulae, ibotenic acid (in a volume (.25 µL) equal to the volume of substances infused during behavioral testing) was infused via an injector placed through the guide cannulae to lesion the tissue surrounding the injector tip.

After the electrolytic and/or ibotenic acid lesions were completed, each rat was first perfused transcardially with saline (200 ml) and then with a 10% formalin solution (200 ml). The brains were then removed, sectioned (40 µm), mounted on gelatin-coated slides and stained with cresyl violet. Lesion sites were then examined to determine placement accuracy.
References


Blackstad, T. W. (1958). On the termination of some afferent to the hippocampus and fascia


Ferbinteanu, J., Holsinger, R. M. D., & McDonald, R. J. (1999). Lesions of the medial or lateral perforant path have different effects on hippocampal contributions to place learning and on fear conditioning to context. *Behavioural Brain Research, 101,* 65 - 84.


Cognitive Neuroscience, 10(4), 525 - 535.


encoding/retrieval asymmetry in episodic memory: positron emission tomography findings. The Proceedings of the National Academy of Science USA, 91, 2016-2020.


