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James P. Navarre

ENTITLED Extra Hippocampal Theta: Activity in the AV Thalamus and Cingulate Cortex (Area 29b) During Conditioned Avoidance Learning in Rabbits

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Extrahippocampal Theta: Activity in the AV Thalamus and Cingulate Cortex (Area 29b) During Conditioned Avoidance Learning of Rabbits.

By

James P. Navarre

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Models of the functional significance of theta based on the proposed location, generation, and the two types of theta, type I (movement-related) and type II (immobility) are based primarily on evidence concerning hippocampal theta. The present study is an initial step toward the incorporation of data about extrahippocampal theta with existing knowledge of hippocampal theta. Specifically, this study examined the relationship of theta-like neurons in thalamus and cingulate cortex to the acquisition of active avoidance behavior. Changes observed in the theta waveform as a function of learning indicate that theta activity represents neurological processes involved in the learning and memory of the conditioned avoidance task.
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As early as 1938, Jung and Kornmuller demonstrated that stimulation of peripheral nerves resulted in rhythmical slow wave activity (theta) in the hippocampus of rabbits. In 1954, Green and Arduini found changes in the frequency of gross electrical activity of the hippocampus of rabbits as a function of presented stimuli. Theta became defined as a rhythmic slow wave EEG (4-12 Hz) from research following two methods. The first approach attempted to relate theta with behavioral or psychological concepts. The second approach attempted to study the neural generation of theta. Various theories quickly developed concerning the functional significance of theta. In 1959, Grastyan and his associates reported that theta correlated with the orienting reflex and motivational processes. On the other hand, Vanderwolf (1969) argued that theta activity represents activity in the forebrain which occurs during voluntary motor acts. After this report, several other researchers related theta to movement. However, later data emerged which limited the generality of the hypothesis relating theta to voluntary movement. For example, it was found that theta did not accompany locomotion in cats and that theta could occur during alert immobility and during anesthesia. Other theories related theta to cognitive processes such as attention,
acquisition, and retrieval of stored information (reviewed by Bland, 1986).

**Type I vs Type II Theta**

Two types of theta have been distinguished on the basis of unique behavioral relations, pharmacology, and neural connections. Type I theta, or "movement-related" theta, occurs only during activities which involve voluntary movement. Thus, if the animal is involved in stereotypic motor behaviors, such as paw washing, type I theta is not observed. Researchers have found that the frequency of type I theta (7-12 Hz) is related to the vigor and/or force of motor output (e.g. Morris et al., 1976). In contrast, type II theta activity is present when the animal is not involved in motor behavior. Because type II theta does not occur with any behavioral action other than immobility, the behavioral significance of type II theta can only be inferred. Most researchers feel that type II theta (4-9 Hz) is related to attention processes. As evidence, type II theta can often be elicited by external stimuli such as a tone or the stroking of the animal's fur.

Type I theta appears to be pharmacologically independent of type II. In a study by Kramis et al. (1975)
the administration of atropine sulfate, a cholinergic (ACh) antagonist, abolished type II theta activity while having little effect on type I theta. Other ACh studies have demonstrated that this manipulation of ACh affects only type II theta activity (Vanderwolf, 1975; Robinson and Green, 1980). If ACh is not involved in type I theta activity, then what neurotransmitters are? Bland (1986) observed that lesions of the medial septum abolish type I theta. Therefore, it would appear that the medial septum plays an important role in the functioning of type I theta. Thus, researchers have looked for the presence of n-n-ACh neurons in the medial septum. Kohler et al. (1984) have conducted research which suggests that a large number of septal projections to the hippocampus utilize GABA as a neurotransmitter. A second neurotransmitter, serotonin (5-HT), has been implicated in type I theta activity. As evidence, Vanderwolf (1987) states that p-chlorophenylalanine (PCPA), which interferes with the synthesis of 5-HT, eliminates type I theta activity while leaving type II theta intact.

Although lesions of the medial septum abolish both types of theta, removal of cortical input to the hippocampus destroys only type I theta and leaves type II theta intact. Vanderwolf et al. (1985) proposed that type
I theta, not type II theta, is mediated by a diffuse pathway which traverses the hypothalamus, cingulate cortex, and neocortex before reaching the hippocampus via the entorhinal cortex.

In summary, type I theta (7-12 Hz) is associated with voluntary movement and type II theta (4-9 Hz) is associated with attention and immobility. It has been suggested that a pathway through cortical areas is important for type I theta and a pathway from the medial septum provides vital input to type II theta. In addition, the neurotransmitter serotonin may be involved in mediation of type I theta, while type II theta appears to be mediated by the neurotransmitter ACh. Finally, type II theta is believed to be involved in the sensory processing which primes motor responses.

A current viewpoint relates type II theta activity to sensory processing. It has also been suggested that the sensory processing function of type II theta is involved with priming motor responses (Bland, 1980). For this to be true, type II theta must always be present when type I theta (movement related) is active. Evidence has been obtained which supports the view that type II theta is always present when type I theta is activated. Dudar et al. (1979) found that ACh is released in the hippocampus
during both sensory stimulation and movement. This is significant, for ACh does not appear to be involved in the mediation of type I theta. Therefore, the ACh release can only be caused by the activation of type II theta.

As stated earlier, administration of atropine SO4 removed all type II theta activity (Bland et al., 1984). However, Bland also found that atropine SO4 reduced theta activity associated with type I behavior. From this evidence, Bland et al. suggest that type II theta is being subtracted by the atropine SO4, this subtraction leaves only type I theta. Thus, type II theta occurs at the same time as type I theta.

The final item of evidence is provided by a study of Vanderwolf et al. (1985), who postulates that type I theta is mediated by cortical pathways, based on observations that showed removal of cortical input to the hippocampus did not abolish all type I theta activity. However, subsequent administration of atropine SO4 destroyed all theta activity. Because atropine SO4 had not removed type I theta activity in previous non-lesion studies, Vanderwolf reasoned that the theta activity present after cortical lesion was type II theta. Thus type II theta is always present when type I theta is activated.

Bland et al. (1976) have suggested that hippocampal
theta (I and II) is the result of two closely coupled generators which are roughly 180 degrees out of phase. As evidence for this view, Bland demonstrated that theta rhythms have different amplitudes and wave shapes on either side of a phase reversal in the stratum radiatum cell layer of the hippocampus. In addition, Bland et al. (1976) found firing of hippocampal CA1 pyramidal cells and dentate granule cells to be rhythmically coupled with theta activity.

Feenstra and Holsheimer (1979) reported phase reversals in the stratum radiatum of the hippocampal CA1 field and in the molecular layer of the dorsal dentate gyrus. Based on this observation, Feenstra and Holsheimer suggested that the CA1 pyramidal cells and the dentate granular cell layers are the two sources of theta generation. However, Feenstra and Holsheimer obtained their data from anesthetized rats. Therefore they were only examining the generation of type II (immobility) theta because type I theta only occurs during voluntary motor behavior. Any theory which attempts to explain the generation of theta should take into account the two different types of theta.

Such a theory has been proposed by Leung (1984). According to Leung, type II theta is produced by a proximal cell body inhibition called Dipole I. Leung stated that
Dipole I has a null zone and phase reversal in the CA1 pyramidal cell layer. Type I theta is said to result from input from Dipole I phase shifted with distal dendritic excitation, producing Dipole II. Leung's model differs from the model presented by Feenstra and Holsheimer's in one important respect: Feenstra and Holsheimer believe that distal excitation accounts for type II theta, whereas as Leung feels that the type II theta profile is caused by proximal somatic inhibition. Leung proposes that the combination of type II theta, caused by somatic inhibition, and type I theta, produced by distal excitation, accounts for the gradual theta phase shift in the moving rat.

In summary, hippocampal theta appears to be generated in both the CA1 pyramidal cell layer and the dentate granular cell layer. The manner in which these cells produce theta has not been determined. More work examining the phase shifts found in the hippocampus is needed. These shifts signify a different source/sink or area of theta generation. In addition, by examining the profiles of the phase shifts in detail we might come to understand the pattern of cell activity which produces theta.
**Extrahippocampal Theta**

Initial reports of theta demonstrated the presence of theta only in the hippocampus. However, Holsheimer and Feenstra (1977) found theta occurring in areas of the neocortex. In addition, recent work by Gabriel et al. (1987) has shown that theta can be found in the AV thalamus and cingulate cortex (area 29b).

There is controversy concerning the generation of extrahippocampal theta. One prevalent belief is that the extrahippocampal theta is attributable to volume conduction of hippocampal theta (Bland, 1986). However, to date there is no clear explanation of theta transmission from the hippocampus to other structures. A second theory presented by Holsheimer (1982) proposes that theta found in the cingulate cortex is produced by local generators. As evidence, Holsheimer and Feenstra (1979) have reported a phase reversal in the cingulate cortex. Also, Holsheimer and Feenstra found increasing amplitude of theta when an electrode is moved away from the hippocampus. In addition, Holsheimer (1982) and Gabriel et al. (1987) have discovered cells in the cingulate cortex which fire in a rhythmic fashion and are phase-locked to the local theta activity. All these findings suggest that theta is generated outside
of the hippocampus in at least some instances.

**Function of Theta**

Although the phenomena of hippocampal and extrahippocampal theta have generated an extensive amount of research, the functional significance of theta is still not clear. Adey (1966) studied the relationship of theta activity to the acquisition and performance of visual discrimination and concluded that theta was involved in the processing, storage, and recall of information. However, subsequent research shifted from studying theta in the context of learning and memory to correlating theta activity with ongoing behavior. From this method of research Vanderwolf (1969) concluded that theta activity occurs during voluntary motor acts. This conclusion was refined and expanded upon by Bland (1986) when he proposed that theta functions in the capacity of providing voluntary motor systems updated feedback on their performance in relation to changing environmental conditions.

Recently, theta activity has again been associated with learning and memory. Lynch et al. (1987) have found that type II theta pattern electrical stimulation (high amplitude and 5 Hz stimulation) is optimal for induction of
LTP, a possible neurological basis for learning and memory. Also, Buzsaki (1989) has postulated that theta activity is essential for creating a memory trace. Thus, there is reason to believe that theta activity may be involved in learning and memory.

**Experimental Proposal**

The present study was conducted within the framework of a research program concerned with the development of a learning model for rabbits (Gabriel et al. 1988). In this research, activity is recorded from rabbits as they undergo training in a conditioned avoidance paradigm. Rabbits are trained to respond by running in a wheel, to a conditioned stimulus tone (CS+). If the rabbit does not perform the locomotion then it receives a mild footshock. The rabbit also hears a second tone (CS-) different in auditory frequency from the CS+ and not followed by a shock. The rabbit hears the two tones in a random order. Each tone is presented 60 times, resulting in a total of 120 tones in one daily session. The rabbits proceed through distinct learning stages, pretraining, acquisition, extinction, and reacquisition. During these daily sessions behavioral and neuronal activity are recorded. Neuronal data are obtained
through the use of chronically implanted recording electrodes placed in various areas of the limbic system. Gabriel et al. (1988) have discovered that over the stages of learning the neuronal activity differentiates between the CS+ and the CS-. The present model postulates the existence of two limbic systems, action and limiting, which interact at the cingulate cortex to enable the rabbit to make the appropriate response. (Gabriel et al. 1988).

**Specific Research Proposal**

This study examined type II hippocampal and extrahippocampal theta activity during the learning of a conditioned avoidance task. The purpose of this study was to initiate investigation of extrahippocampal theta activity and to determine if hippocampal and extrahippocampal type II theta activity exhibit associative change during avoidance learning in rabbits. Possible changes in EEG and multiple unit activity (MUA) related to training level and CS significance may provide insight into the functional relevance of theta.
METHODS

Subjects, Recording Electrodes, Target Areas, and Surgical Procedures

The subjects were 40 male New Zealand white rabbits, weighing 1.5-2.0 Kg at the time of their delivery to the laboratory. They were maintained on ad libitum water and food throughout the experiment.

Six recording electrodes were surgically implanted while the rabbits were under anesthesia (IV injection, 1mg/Kg of body weight of a solution containing 60mg/ml of ketamine HCl and 8 mg/ml of xylazine). Neuronal activity was monitored acoustically and with an oscilloscope during electrode descent, to supplement the standard stereotaxic criteria for electrode localization. The three recording electrodes used for data analysis were placed in the AV thalamus (AV) (AP=+2.0, L=2.3, DV=7.0-8.0), posterior cingulate cortex area 29b (AP=+7.0, L=0.6, DV=2.0-3.0), and dentate gyrus (AP=+5.0, L=-4.5, DV=3.2-3.5).

The electrodes were fabricated from #00 stainless-steel insect pins insulated with epoxylite. Recording surfaces were created by removing insulation from the tip of the pins (range of tip-length: 15-50 microns; range of
tip-width: 20-35 microns; impedance: 500 kilohms-2 megohms).

**Behavioral Training**

Following a minimum recovery period of one week, each rabbit underwent avoidance conditioning in a wheel apparatus with a grid floor through which a footshock (US) could be delivered. The wheel was contained within a shielded chamber in which an exhaust fan and a speaker produced a masking noise of 70dB. In different experiments two of three possible types of auditory stimuli were used; 1KHz, 8KHz, and a white noise whose, intensity was 85 dB re 20 N/m (duration: 500 msec, risetime: 3 msec). Onset of one of the three tones, the positive CS (CS+), was followed after five seconds by the unconditioned stimulus (US). Both CS+ and US could be terminated by a response, defined as a 2-degree (or greater) turn of the wheel. A response prior to US onset prevented US delivery. A second tone (CS-), of different frequency than the CS+, was not followed by footshock. Each trial ended after the five second period following CS(+/-) onset or when a response occurred. The intertrial interval was 8, 13, 18, or 23 seconds, these values occurring in an irregular sequence.
The rabbits received daily sessions of training (60 trials with each CS) to a criterion (CRIT) requiring that the percentage of responses to the CS+ exceed the percentages of responses to the CS- by 60% or more, for any 60 trial block, in two consecutive sessions. After CRIT, three additional sessions (overtraining) were given, followed by extinction training (CS(+/−)) presented without US and reacquisition sessions. The extinction criterion required that the percentage of responses to the CS+ and CS− was not more than 10% in two consecutive sessions. The criterion used for reacquisition was the same criterion as that used for acquisition.

Prior to training, each rabbit received two pretraining (PT) sessions in which the two CS tones were presented 60 times each, with the same timing and ordering as in training. The first pretraining session involved presentation of the tones only, and the second involved presentation of the tones with the US interspersed on an explicitly unpaired schedule. The PT sessions provided baseline data for detecting associative neural and behavioral changes brought by pairing the CS+ with the US.

Collection of Neural Data
During training, neural records were fed into field effect transistors (FET) located 2.0-3.0 cm from the recording sites in the brain. The FET outputs were fed via a shielded cable to single-ended preamplifiers (gain: 8000, bandwidth: 0.1-10,000 Hz). The preamplifier outputs entered an active band-pass filter (1/2 amplitude cutoffs: 600 and 10,000 Hz). Outputs of the band-pass filters were fed to Schmitt triggers, which were adjusted automatically to yield a mean rate of output pulses within limits of 110-190 per second. With this criterion, typically the largest three or four spikes per record were sampled. In addition, the high pass filter outputs were full-wave rectified and integrated. The time constants for the rise and fall of the integrators were 15 and 75 msec respectively. The spike frequency data provided an index of the discharge frequency of the largest spikes, whereas the integrated unit activity measured the energy fluctuations of the entire record, including activity below the triggering thresholds.

The Schmitt trigger pulses were counted and the integrator signals digitized on each conditioning trial for 1.0 sec, 0.3 sec before CS onset and 0.7 sec after CS onset. A digital value was stored for each index and
electrode, every 10 msec during the sampling interval. The individual trial data were subsequently averaged and analyzed statistically on a Harris computer.

**Data Analysis**

The data analysis focused on behaviorally defined stages of training. Each stage was constituted by the data of a single training session. The stages analyzed were: 1. Pretraining (PT) with the two CS(+/−) tones and noncontingent footshock; 2. The first exposure (FE) to conditioning; 3. The first significant (FS) behavioral discrimination; 4. The second criterial (CRIT) session. The criterion for the first significant behavioral discrimination required that the percentage of avoidance responses exceed the percentage of responses to the CS- by 25% or more; The criterion of learning required required that the rabbit obtain 60% greater responding to the CS+ than the CS- for two consecutive training sessions.

The neuronal data from the three recording areas (AV thalamus, cingulate cortex 29b, and dentate gyrus) for each behavioral stage were submitted in z-score form to analysis by a Pascal computer program. The program analyzed the
standardized rhythmic waveform by delineating peaks and valleys matched with their respective latencies after CS onset. The algorithm for determining a peak consisted of comparing the average values of 5 data points around N with 5 points around N-1 and N+1 (N being a given data point on the waveform). If the averaged value for data set N was larger than the averaged values for data set N-1 and N+1, then N was stored as a peak. A similar algorithm was used to locate valleys, except in this case if the averaged value for data set N was smaller than the averaged values for data set N-1 and N+1, then N was stored as a valley.

The first three corresponding CS+ and CS- EEG and multiple unit activity (MUA) peaks which occurred 10 ms after tone onset were submitted to repeated measures ANOVA. The amplitude of the three peaks was analyzed by ANOVA for discrimination between the two tones. The time intervals between peaks one and two, two and three, and one and three divided by two were used to calculate the frequency for a given wave. The wave frequency, after averaging the three time intervals and converting to hertz, was then analyzed by ANOVA for discrimination between the two tones. Because the subjects did not show rhythmic EEG and MUA activity across all of their respective learning stages, the amplitude and frequency ANOVA's were performed
independently for each stage. Phase differences between the CS+ and CS- peaks were determined by subtracting the latency of each CS- peak from the corresponding CS+ peak. Thus, a negative number indicates that the CS+ peak is phase shifted forward in comparison to the CS-. The phase differences between the three peaks were averaged to determine the mean phase shift for a given rhythmic wave. For EEG data, ANOVA was performed on a subset of records, which demonstrated rhythmicity across all stages, to determine if a change in phase relationship occurred as a function of the training stages. A subset of records demonstrating rhythmic MUA activity across all stages was not found. Although ANOVA could not be performed, mean values for phase shift at each learning stage were calculated.

The MUA data from the dentate gyrus were not submitted to ANOVA due to a small sample size.

**Histology**

Following testing, all rabbits were deeply anesthetized with an overdose of pentobarbital sodium, and perfused with normal saline followed by 10% formalin-saline solution. Sections of the brain were made at 40
microns after they had been frozen. After slicing, the sections were photographed while still wet.

Results

AV Thalamus

The EEG and MUA activity in the AV thalamus showed significant changes in discrimination between CS+ and CS- across training for the theta parameters of amplitude, frequency, and phase (see figure 1). The mean CS+ and CS- EEG amplitudes were 5.67 and 8.10, respectively during pretraining (PT). Across the learning sessions the mean CS+ amplitude increased dramatically while the mean CS- amplitude only showed a slight increase. This resulted in a significant EEG amplitude discrimination between CS+ and CS- by the final stage of training (CRIT) (see figure 1a) (CS+ MEAN=11.65, CS- MEAN=8.60, F(1/6)=57.47, P<0.001). The amplitude of the bursts in the AV thalamus also showed increased discrimination between CS+ and CS- after the
rabbit learned the correct response (see figure 1b). The mean CS+ and CS-amplitudes were 6.61 and 5.00, respectively during pretraining. For criterion, the CS+ amplitude was greatly increased to 8.38 and the CS-amplitude was lowered to 4.00. Thus, a significant amplitude discrimination between the two tones was observed during criterion ($F(1/3) = 9.17, P = .05$).

The frequency of the EEG activity in the AV thalamus showed changes in discrimination between CS+ and CS- across the learning period, resulting in a significant discrimination between the two tones during criterion (see figure 1c). The mean CS+ and CS- frequency was 7.24hz and 6.94hz respectively during pretraining. The CS+ frequency increased to a mean of 7.87hz, while the CS- showed a slight increase to 7.42hz during criterion ($F(1/6) = 27.78, P < .002$). In addition, the frequency of the MUA activity in the AV thalamus exhibited a similar trend with increased discrimination between the two tones upon completion of training (see figure 1d). During pretraining, the mean CS+ and CS- frequency was 7.94hz and 8.14hz. The mean CS+ frequency increased to 8.20hz during criterion while the mean CS- frequency decreased to 7.00hz.

A significant change in the phase relationship between the AV thalamus EEG CS+ and CS- peaks was observed across
the learning stages \((F(3/12)=3.48, P=.05)\) (see figure 1e). During pretraining the average CS+ waveform was phase shifted forward by 4.7 msec. In contrast, after the rabbit reached criterion, the average CS+ waveform was phase forward by a 20.0 msec. A similar change in the phase relationship between the AV thalamus MUA CS+ and CS-peaks was observed across the learning stages (see figure 1f). During pretraining, a mean phase shift between the two tones was not observed for MUA. In contrast, for criterion, the average CS+ waveform was shifted forward by 16.64 msec.

**Cingulate Cortex Area 29b**

The EEG and MUA activity in cingulate cortex area 29b showed changes in discrimination between the two tones across training similar to the associative changes found in the AV thalamus (see figure 2). For example, the amplitude of the EEG activity in the cingulate cortex (29b)

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Insert figure 2 about here

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demonstrated changes in discrimination across training (see figure 2a). The mean CS+ and CS- amplitude was 7.04 and
6.52 respectively during pretraining. The area 29b CS+ amplitude increased substantially and the CS- amplitude decreased during training. The mean CS+ amplitude was 11.31 and the mean CS- amplitude was 4.78 during criterion (F(1/3) = 4.01, P = .14). The amplitude of the bursty activity in cingulate cortex area 29b demonstrated a similar trend as the EEG amplitude with changes approaching significance across the training stages (see figure 2b). The mean CS+ and CS- amplitudes were 9.38 and 8.60 in pretraining. Across the behavioral training, the CS+ amplitude retained a similar value while the CS- amplitude decreased. This resulted in a mean CS+ amplitude of 9.14 and CS- amplitude of 6.36 during criterion (F(1/4) = 3.13, P = .15).

The frequency of the EEG activity in area 29b showed an increase in discrimination between CS+ and CS- over the learning stages (see figure 2c). For pretraining, the CS+ had a mean Hz of 7.10 and the mean CS- hertz was 7.20. The CS+ frequency increased and the CS- frequency decreased resulting in a discrimination approaching statistical significance during criterion (CS+ MEAN=7.40, CS-MEAN=6.85, F(1/3) = 4.56, p = .12). The frequency of the MUA activity in cingulate cortex area 29b demonstrated a similar change in discrimination between the two tones across the learning stages, resulting in a significant discrimination between
the two tones during criterion (see figure 2d). The mean area 29b CS+ and CS- frequency was 6.75hz and 8.10hz respectively during pretraining. For criterion, the CS+ frequency increased to 7.80hz and the CS- frequency decreased to 6.92hz ($F(1/4)= 10.32$, $P=.03$).

The phase relationship in cingulate cortex area 29b EEG demonstrated a change across stages approaching significance ($F(3/6)=3.91$, $P=.07$) (see figure 2e). During pretraining, the average CS- waveform was phase shifted forward by 1.1 msec. In contrast, for criterion the average CS+ waveform was shifted forward by 23.3 msec. A similar trend was found in cingulate cortex area 29b MUA (see figure 2f). During pretraining, the average waveform for CS- was shifted forward by 16.70 msec. But for criterion, the average CS+ waveform was phase shifted forward by 17.34 msec.

**Dentate Gyrus**

The EEG activity recorded from the dentate gyrus did not demonstrate the amount of discrimination between the two tones as recorded from the two extrahippocampal structures (see figure 3). The amplitude of the EEG
activity in the dentate gyrus (DG) demonstrated changes approaching significance across the training stages (see figure 3a). The mean dentate gyrus CS+ and CS- amplitudes were 8.73 and 7.91, respectively during pretraining. Across the learning stages the mean CS+ amplitude increased while the mean CS- amplitude decreased. Thus, for criterion the dentate gyrus CS+ and CS- mean amplitudes were 9.11 and 6.63 respectively (F(1/7)=3.34, P=.11).

The frequency of the EEG activity in the dentate gyrus showed did not obtain a significant discrimination between CS+ and CS- after the rabbit learned the correct response (see figure 3b). The mean CS+ frequency and CS- frequency was 8.14hz and 7.30hz during pretraining. The mean frequency for the CS+ was similar during criterion, while the CS- frequency increased (CS+ MEAN=8.10hz, CS- Mean = 7.70hz).

In addition, a significant change in phase relationship was not observed in the dentate gyrus during the learning period (see figure 3c).
Discussion

The purpose of this study was to examine hippocampal and extrahippocampal theta activity in the context of a conditioned avoidance task to see if the theta activity demonstrated associative changes across learning. It was suggested that if associative changes were observed in the theta activity, these changes might provide insight into the functional role of theta. A secondary purpose was to initiate investigation of extrahippocampal theta.

As the results indicate, both extrahippocampal and hippocampal theta activity demonstrated changes after the rabbit had learned the correct response (see figure 4).

Insert figure 4 about here.

However the, extrahippocampal theta activity was more plastic than the hippocampal theta. The extrahippocampal theta demonstrated significant discrimination between the two tones in amplitude, frequency, and phase. In contrast, the hippocampal activity demonstrated a distinction between CS+ and CS- approaching significance for only amplitude data. Thus, the extrahippocampal theta appears to be more involved in the associative nature of the task than the
hippocampal theta.

Furthermore, the extrahippocampal parameters of amplitude, frequency, and phase may represent neural encoding of the relevant association between CS+ and shock. These three parameters demonstrated a significant discrimination between the two tones only after the animal had learned the relevant association.

Based on this study, it would appear that theta activity, especially extrahippocampal theta, is involved in the neurological representation of a learned association. This would suggest that theta is critically involved in CS-US association and/or retrieval of that association during CS presentation.

It is interesting to note that the distinction which the literature makes between type I (movement) theta and type II (immobility) theta maps on to the distinction between the action and limiting system made by the theoretical model of Gabriel et al. (1988).

The action system and type I theta are both postulated to be involved with movement. In contrast, the limiting system and type II theta are associated with attention to a stimulus and suppression of motor activity.

The limiting system operates by firing of hippocampal neurons. The hippocampal activity exerts a suppressive
influence on the AV thalamus by closing a gate in the cingulate cortex. The closing of this cortical gate prevents the expression of movement by the action system. Thus, the type II theta recorded in this experiment might be a neurological representation of the hippocampus suppressing the action system. In addition, type I theta may represent disinhibition of the action system by the hippocampus.

Future research should examine the relationship between hippocampal and extrahippocampal theta activity. The present data indicate that the theta activity recorded in the two extrahippocampal structures may be independent of the theta activity recorded in the hippocampus. A lesion of the hippocampus could be made and the subsequent effect on extrahippocampal activity could be observed. Preliminary data indicate that the theta activity in the AV thalamus remains and the theta activity in cingulate cortex area 29b is disrupted by such a lesion. However, the parameters of amplitude, frequency, and phase must be analyzed to determine if the associative changes which occur extrahippocampally are affected by removal of hippocampal input.

In conclusion, the present data point to a significant role for hippocampal and extrahippocampal theta activity in
learning and memory. Further research is needed to explicate the role of theta.
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Figure Captions

Figure 1

Changes in discrimination between the two tones for EEG and MUA activity from the AV thalamus when comparing across learning. (A) EEG amplitude. (B) MUA amplitude. (C) EEG frequency. (D) MUA frequency. (E) EEG phase. (F) MUA phase.

Figure 2

Changes in discrimination between the two tones for EEG and MUA activity from cingulate cortex area 29b when comparing across learning. (A) EEG amplitude. (B) MUA amplitude. (C) EEG frequency. (D) MUA frequency. (E) EEG phase. (F) MUA phase.

Figure 3

Changes in discrimination between the two tones for EEG activity from dentate gyrus when comparing across learning. (A) EEG amplitude. (B) EEG frequency. (C) EEG phase.

Figure 4

Summary of results.
### Summary of Results

<table>
<thead>
<tr>
<th>Extrahippocampal</th>
<th>Amplitude</th>
<th>Frequency</th>
<th>Phase</th>
</tr>
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<tbody>
<tr>
<td>AV thalamus</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>cingulate cortex area 29b</td>
<td>=</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hippocampal</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>dentate gyrus</td>
<td>=</td>
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</tbody>
</table>

+ statistical significant discrimination between two tones after the rabbit has learned the correct response.

= discrimination between the two tones approaching significance after the rabbit has learned the correct response.