Stimulation-Induced Reset of Hippocampal Theta in the Freely Performing Rat

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ABSTRACT: Previous research has suggested that visual and auditory stimuli in a working memory task have the ability to reset hippocampal theta, perhaps allowing an organism to encode the incoming information optimally. The present study examined two possible neural pathways involved in theta resetting. Rats were trained on a visual discrimination task in an operant chamber. At the beginning of a trial, a light appeared over a centrally located lever that the rat was required to press to receive a water reward. There was a 30-s intertrial interval before the next light stimulus appeared. After learning the task, all rats received surgical implantation of stimulating electrodes in both the fornix and the perforant path and recording electrodes, bilaterally in the hippocampus. After surgery, theta was recorded before and after the light stimulus to determine whether resetting to the visual stimulus occurred. During the intertrial interval, rats received single-pulse electrical stimulation of either the fornix or perforant path. Theta was recorded both before and after the electrical stimulation to determine whether resetting occurred. In this experiment, hippocampal theta was reset after all three stimulus conditions (light, perforant path, and fornix stimulation), with the greatest degree of reset occurring after the fornix stimulation. The results suggest that activation of the perforant path and fornix may underlie theta reset and provide a mechanism by which the hippocampus may enhance cognitive processing. *Hippocampus 2003;13:109–116.*

KEY WORDS: entorhinal; EEG; dentate; septum; hippocampus

INTRODUCTION

At approximately 1–2 mV, the 4–12-Hz hippocampal theta rhythm is one of the largest electroencephalographic (EEG) potentials in the mammalian brain (Bland, 1986). Although the functional significance of hippocampal (HPC) theta is still debated, there is considerable evidence that HPC theta plays an important role in information processing (Winson, 1978; Mizumori et al., 1990; Givens and Olton, 1990, 1994, 1995; Lisman and Idiart, 1995). However, despite the strong correlation between theta activity and mnemonic performance, the precise mechanism by which theta influences cognitive processing is not well characterized.

One proposed 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; Givens, 1996b; Vinogradova et al., 1996; Brankack et al., 1998). Theta reset follows the presentation of stimuli across different sensory modalities, suggesting that theta reset is a viable mechanism with which to enhance mnemonic processing across a variety of stimulus attributes (Adey, 1967; Givens, 1996b; Brankack et al., 1998).

The functional circuitry for HPC theta reset may involve signals from a sensory stimulus traveling to the hippocampus along two parallel pathways: (1) through the entorhinal cortex via the perforant path, and (2) from the medial septal area (MSA) via the septohippocampal pathway. According to this proposed model, the purpose of HPC reset is to ensure that the dentate granule cells are maximally responsive when relevant sensory information arrives from the entorhinal cortex, which receives converging sensory input from polymodal association areas in the cortex through its connections with the perirhinal cortex (Suzuki and Amaral, 1994a,b; Suzuki et al., 1997; Burwell and Amaral, 1998).

The MSA is most likely involved in the resetting of HPC theta, as resetting of single unit activity has been observed in MSA neurons after the presentation of a visual or auditory stimulus in a working memory task (Givens, 1996a). The MSA may prime the hippocampus to receive incoming sensory information from the entorhinal cortex, facilitating long-term potentiation, enabling synaptic plasticity (Larson and Lynch, 1986; Diamond and Rose, 1994), 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 burst was delivered 200 ms after

Grant sponsor: National Science Foundation; Grant number: IBN-97235143.
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Accepted for publication 4 February 2002
DOI 10.1002/hipo.10082

the initial bursting pattern (Larson and Lynch, 1986). Significantly, this 200-ms time interval corresponds to the general theta frequency. Similarly, stimulating the septum altered the size of the granule cell population spike evoked by perforant path stimulation in the HPC and removed the paired-pulse inhibition observed after electrical stimulation of the perforant path when the conditioning pulse to the MSA was timed to coincide (or nearly coincide) with the first evoked population spike (Bilkey and Goddard, 1985, 1987). These data support the hypothesis that theta reset may enhance encoding by depolarizing dentate granule cells at the time that sensory input arrives from the entorhinal cortex.

Numerous studies have confirmed that electrical stimulation of the MSA (Buño et al., 1978; Garcia-Sanchez et al., 1978) or afferent inputs to the MSA including the reticular formation (Gaztelu and Buño, 1982), medial forebrain bundle (Brazhnik and Vinogradova, 1988), the lateral septum (Brazhnik et al., 1985; Pedemonte et al., 1998), and the fornix (Buño et al., 1978; Garcia-Sanchez et al., 1978) all reset HPC theta. However, most of these studies primarily involved examining theta reset under anesthetized conditions. Thus, the present study used electrical stimulation to identify components of the neural circuitry of theta reset in awake, freely moving rats performing a cognitive task, with particular attention focused on the fornix and the perforant path. One specific goal of the present study was to quantify the degree to which the fornix and the perforant path are involved in resetting of the hippocampal theta rhythm and to determine whether there are quantitative differences between the fornix and perforant path stimulation-induced theta reset.

MATERIALS AND METHODS

Subjects

All experimental protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996). Eight male Long-Evans rats 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 the 85% ad libitum weight, with allowances made to account for the natural increase in weight with age.

Behavioral Apparatus

All behavioral studies were conducted in two types of operant chambers developed by Med Associates (St. Albans, VT): one used in the behavioral training and one adapted specifically for electrophysiological recording. Housed within a light and sound-attenuating shell ($64 \times 41 \times 41$ cm), the training chambers ($28 \times 21 \times 27$) 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 house light, 18 cm above the floor, was located centrally on the front panel, and a tone generator, 12 cm above the floor, was on the back panel. A water dispenser, 2 cm above the floor in the center of the back panel, delivered a 0.1-ml drop of water after rewarded lever presses. The electrophysiology chamber was identical to the 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 rat 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.

Procedure

Visual discrimination 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 s of illumination. After a lever press, or when 3 s elapsed, the light was extinguished and a 30-s ITI period began. The main purpose of the behavioral task was to ensure that rats were in an alert, active state throughout the recording session.

Surgery

After reaching criterion performance on the VD task (90% correct for 3 days, with fewer than 40% omissions), subjects underwent electrode implantation surgery. All surgeries were performed under ketamine/xylazine anesthesia under aseptic conditions. Supplemental injections of ketamine (0.1 ml) 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 (Harvard Apparatus, Holliston, MA).

During surgery, Teflon-coated, 125-μm stainless-steel recording electrodes (A-M Systems, WA) with a gold ITT/Cannon Centi-Lok pin attached to one end 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 online with a fast Fourier transformation (FFT). The purity and the power of the theta rhythm were used to locate the optimal recording position.

Teflon-coated 250-μm stainless steel stimulating electrodes with a gold pin at one end 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, 0.2-ms, 5-s 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.

In addition to the recording and stimulating electrodes, recording and stimulation reference wires (Teflon-coated 250-μm stainless steel, with a 1-mm uninsulated tip at one end and a gold pin at the other) were lowered ~1 mm 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 reference wires were securely fastened to the skull, the gold pins were inserted into an ITT/Cannon Insulator Strip that served as a connector for a preamplifier. After insertion into the insulator strip, the connector was secured to the skull with dental acrylic, and the animal was removed from the stereotactic 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 after surgery and were slowly returned to their normal daily water intake over the next 5 days. Body weight, posture, and locomotor activity were carefully monitored after surgery to ensure a healthy recovery.

Post-surgery Testing

After a 1-week recovery period, rats were retrained on the VD task. The purpose of this retraining period was (1) to ensure that the surgery did not affect task performance, and (2) to acclimate the rats to the recording protocol. During this retraining period, a preamplifier was attached to the headstage and cabled to a commutator that allowed the rats to move freely in the box. After performance on the VD task returned to pre-surgery levels, the post-surgical testing sessions began.

To collect the electrophysiological data, a preamplifier and cable were connected to the rat's head. The signal was passed to an amplifier (1,000 ×z)/filter (0.1–500 Hz) system (A-M Systems), 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 (Longmont, CO). Electrophysiological data were collected over 10 days (1 h/day; 5 days/week) from each animal. All electrical stimulation took place during the ITI period of the VD task. At 11 s after light offset, a single square wave electrical pulse (600 μA; 0.2 ms) was delivered to either the fornix or the perforant path. Two additional single stimulation pulses were delivered at 17 and 23 s after 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 same stimulation site was used on the subsequent trial and was then switched to the other site for the next two trials, and continued to alternate every other trial between the two stimulation sites. The stimulation intensity was determined after extensive pilot testing. During pilot surgeries, the electrical stimulation was gradually increased until a reliable theta reset occurred; 600 μA was the minimum stimulus intensity used to produce a consistent theta resetting after both fornix and perforant path stimulation; thus, the same stimulation parameters were used for both stimulation sites. Theta EEG recordings were acquired 1 s before and after electrical stimulation to determine whether resetting to the electrical stimulus occurred. Theta EEG recordings were also acquired 1 s before and after light onset to determine whether resetting to the light stimulus occurred.

Electrophysiological Analysis

All electrophysiological data were analyzed using software developed by DataWave Systems. Each individual data record was examined for noise and all data records containing non-neural signals (artifacts) were removed before being further examined for theta reset. 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. Visual inspection of the final waveform average revealed a strong evoked response after electrical stimulation of the perforant path and fornix. The size of the evoked response varied from animal to animal but always occurred during the first 400 ms after stimulus onset. To avoid confounding of the evoked response, a FFT analysis was performed on the 600 ms of the final waveform average after the evoked response to determine whether theta reset occurred. The dominant frequency located within the 6–10-Hz range and the power at that frequency was recorded. The FFT results were then subjected to two-factor analysis of variance (ANOVA) and paired sample *t*-tests to determine whether there was a significant difference between pre- and post-stimulus theta frequency and 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. If theta was phase-locked to the stimulus, a significant theta EEG pattern should have been evident in the post-stimulus final waveform average. Conversely, if no phase-locking occurred, the post-stimulus final waveform should have averaged to a relatively flat line, showing little or no theta activity in the final waveform average.

To determine whether the results represented a phase-locking of an on-going theta rhythm to the stimulus or alternatively the stimulus evoked a theta rhythm, FFT analysis was performed on each of the raw pre- and post-stimulus records (i.e., not just the final waveform average). The dominant frequency located within the 6–10-Hz range and the power at that frequency was recorded for further statistical analysis. As described above, two-factor ANOVA and paired sample *t*-tests were also used to quantify whether there was an increase in overall theta power after light presentation.

Histology

After completion of behavioral testing, rats were deeply anesthetized with sodium pentobarbitol and an electrical current (20 μA for 40 s) was passed through the electrode to produce a lesion at the tip of the electrode to verify the accuracy of the recording and stimulating electrode placements. After the electrolytic lesions were completed, each rat was perfused transcardially with saline

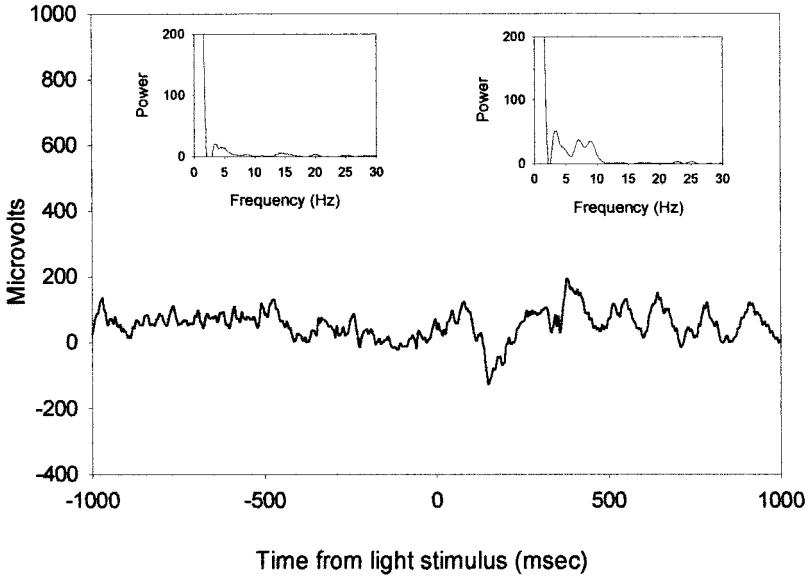

FIGURE 1. Example of hippocampal theta reset after the onset of a light stimulus. The data represent the average of 86 light presentations. Insets represent FFTs performed on the final waveform average before (left) and after (right) light onset. Power expressed as arbitrary DataWave units.

(200 ml) and then with a 10% formalin solution (200 ml). The brains were then removed and postfixed for 24 h in 10% formalin solution and transferred to a 25% sucrose/phosphate-buffered solution. The brains were sectioned (40 μm), mounted on gelatin-coated slides and stained with cresyl violet. Lesion sites were then examined with a light microscope to determine placement accuracy.

RESULTS

Histology

Eight animals were trained on the visual discrimination task. One rat was unable to complete the electrophysiological recording sessions and was removed from the study. The following results pertain to the remaining seven rats. Histological analysis confirmed the proper placement of bilateral recording electrodes in the hippocampus and unilateral stimulating electrodes in both the fornix and the perforant path.

The hippocampal recording electrodes were located mainly in the dentate hilus, with 13 of the 14 recording electrodes falling in this region. The remaining recording electrode was located in the CA1 region of the hippocampus. For this one rat, there was no significant difference between the degree of theta reset between the CA1 and contralateral dentate recording electrodes and thus the CA1 results were included in the analysis. Overall, the final placements of the hippocampal recording electrodes ranged from 3.3 mm to 3.8 mm posterior to bregma. Histological analysis also confirmed that all fornix stimulating electrodes were located in the fornix, with placement sites ranging from 1.4 mm to 2.1 mm posterior to bregma. Similarly, all perforant path stimulating electrodes showed accurate placements, with locations ranging from 7.3 mm to 8.0 mm posterior to bregma.

Behavior

Rats were trained on the VD task for approximately 2 weeks before reaching pre-surgery criterion levels. Choice accuracy remained consistent throughout post-surgery recording sessions (98.5% choice accuracy) and a consistent degree of responding (23.5% 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 ipsilateral 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 between the sites in theta frequency ($P > 0.05$), theta power ($P > 0.05$) or the degree of theta resetting (as measured by theta power in the post-stimulus final waveform average) under the three stimulation conditions [$F(1,48) = 0.004$; $P > 0.05$]; thus, data from both dentate sites were combined for subsequent analyses.

A separate waveform average was performed on the pre- and post-stimulus EEG records for each testing session. If theta reset occurred after a stimulus, a significant theta peak should have been evident in the post-stimulus final waveform average. Conversely, if no reset occurred, the post-stimulus final waveform should have averaged to a relatively flat line. Figures 1, 2, and 3 illustrate examples of theta reset to the light stimulus, perforant path, and fornix stimulation, respectively.

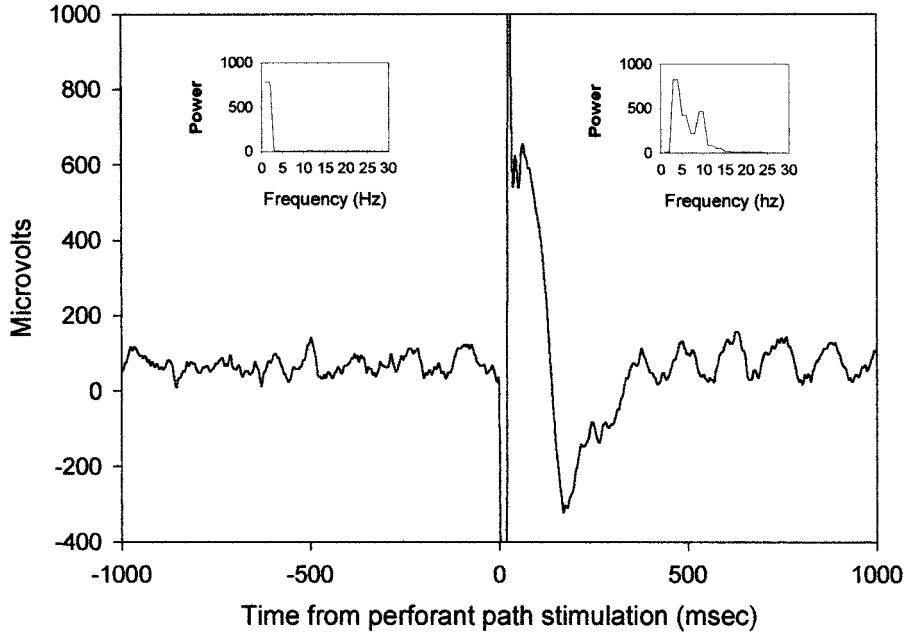

FIGURE 2. Example of hippocampal theta reset after electrical stimulation of the perforant path. The data represent the average of approximately 130 stimulations of the perforant path. The insets represent FFTs performed on the final waveform average before (left) and after (right) perforant path stimulation. Power expressed as arbitrary DataWave units.

A two-factor ANOVA was performed on the final waveform average, using STIMULUS (light, perforant path, and fornix stimulation) and TIME (pre- and post-stimulus) as repeated measures and theta frequency and theta power as dependent measures. The frequency analysis demonstrated a significant TIME effect, with theta shifting to a slightly higher frequency after the introduction of a light or electrical stimulus [$F(1,111) = 25.362$; $P < 0.01$].

In addition to increasing theta frequency, the stimuli caused resetting of the HPC theta rhythm, in which the ongoing theta rhythm became phase-locked to the incoming stimulus. Analyses of theta power in the final waveform average revealed significant stimulus [$F(2,222) = 15.219$; $P < 0.01$] and time [$F(2,111) = 41.899$; $P < 0.01$] effects, with post-stimulus theta power greater in the final waveform average than pre-stimulus theta power, indicating that a significant degree of theta reset occurred after light, perforant path, and fornix stimulation (Fig. 4).

In addition, a significant stimulus $\times$ time interaction [$F(2,222) = 17.210$; $P < 0.01$] indicated that theta reset was

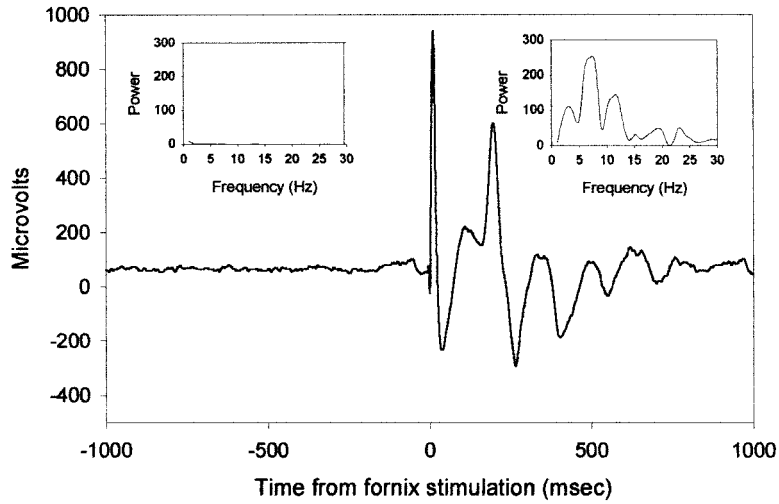

FIGURE 3. Example of hippocampal theta reset after electrical stimulation of the fornix. The data represent the average of approximately 130 stimulations of the fornix. The insets represent FFTs performed on the final waveform average before (left) and after (right) fornix stimulation. Power expressed as arbitrary DataWave units.

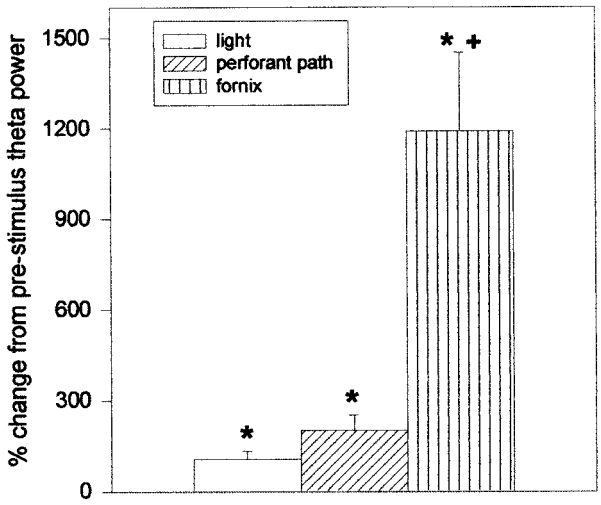

FIGURE 4. Hippocampal theta reset (averaged across all rats) after light, perforant path, and fornix stimulation. The data represent the post-stimulus change in theta power in the final waveform average relative to pre-stimulus theta power. *, significantly different than pre-stimulus power ($P < 0.01$). +, significantly greater reset as compared with light and perforant path stimulation ($P < 0.01$).

greater under certain stimulation conditions. Before stimulus onset, there was no significant difference in theta power in the final waveform average of the three stimulus types. 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 showed no significant differences in the pre-stimulus theta power between any of the stimulation conditions [$F(2,222) = 0.747$; $P > 0.05$]. Conversely, a significant stimulus effect was observed in post-stimulus theta power [$F(2,222) = 18.454$; $P < 0.01$], with greater theta power in the final waveform average (i.e., a greater degree of theta reset) after fornix stimulation than after either light, [$t(111) = 4.092$; $P < 0.01$; Fig. 4] or perforant path stimulation, [$t(111) = 3.733$; $P < 0.01$; Fig. 4]. There was no significant difference in the degree of theta reset produced by light and perforant path stimulation [$t(116) = 1.526$; $P > 0.05$; Fig. 4].

Two possible explanations for why theta power was greater in the post-stimulus waveform average than the pre-stimulus waveform average are that (1) an ongoing theta rhythm (prevalent throughout both the pre- and post-stimulus periods) momentarily stopped and reset to the incoming stimulus; or (2) there was no or little theta EEG activity during the pre-stimulus period and the stimulus merely evoked a theta rhythm. To examine these possibilities, a FFT analysis was performed on each of the raw pre- and post-stimulus records for the three stimulation conditions (instead of the final waveform average). FFT analyses of the raw data records showed a strong theta peak centered at $\sim 7.28 \pm 0.12$ Hz both before and after light, perforant path, and fornix stimulation (Table 1). Subsequent analysis indicated there was no difference in the pre- and post-stimulus theta frequency or power in any of the three conditions ($P > 0.05$), suggesting that the observed resets were not due to the stimulus eliciting theta EEG activity, but rather a phase-shifting of an 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, suggesting that these structures may contribute to the naturally occurring theta reset after light onset in the behavioral task. Although both perforant path and fornix stimulation are capable of resetting HPC theta, they may involve different mechanisms or functions. For instance, theta resetting induced by perforant path stimulation, although using the same parameters as fornix stimulation, produced a degree of reset that was more similar to the reset observed after light onset, indicating that the natural signal responsible for stimulus-induced theta resetting may involve activation of the entorhinal cortex. Thus, the initiation of theta reset may involve activation of the medial temporal lobe by sensory stimuli, that in turn activates the MSA, where the rhythmically active neurons responsible for driving the HPC theta rhythm are located. However, a more extensive

TABLE 1.

FFT Analysis of Individual Theta EEG Records

Stimulus condition	Theta frequency pre-stimulus[a]	Theta frequency post-stimulus[a]	Power at theta frequency pre-stimulus[b]	Power at theta frequency post-stimulus[b]
Light stimulus	7.28 ± 0.09	7.32 ± 0.10	207.88 ± 24.53	197.28 ± 19.44
Perforant path electrical stimulation	7.36 ± 0.10	7.33 ± 0.12	308.94 ± 42.75	282.60 ± 26.36
Fornix electrical stimulation	7.23 ± 0.14	7.18 ± 0.17	307.09 ± 33.57	269.17 ± 29.92

FFT, fast Fourier transformation; EEG, electroencephalographic.

FFTs performed on each individual data record (i.e., not just the final waveform average) show no significant differences, indicating that there was a similar theta rhythm (in terms of both theta frequency and power) both before and after light and electrical stimulation.

[a]Frequency units in hertz (Hz).

[b]Power in arbitrary DataWave units.

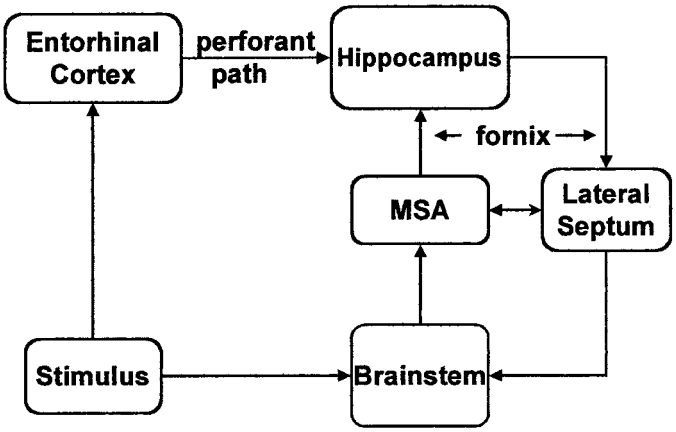

FIGURE 5. Schematic diagram of proposed reset circuitry.

examination of stimulation intensities, fiber thresholds, and electrode placements is necessary to rule out alternate explanations. Ultimately, however, the mechanism responsible for theta reset is likely to involve septal inputs of either cortical or subcortical origin which trigger a brief inhibition and subsequent restarting of MSA rhythmic neuronal firing, causing a resetting of the hippocampal theta rhythm (Buño et al., 1978; Gaztelu and Buño, 1982).

As shown in Figure 5, one major input to the MSA is a descending pathway from the hippocampus. Although fornix stimulation affects both ascending and descending fibers to and from the hippocampus, the ability of fornix stimulation to reset HPC theta is most likely mediated by descending fibers affecting the rhythmically bursting neurons of the MSA, which serves as the pacemaker of the HPC theta rhythm. Stimulation of the fornix produces a GABA-dependent inhibition of MSA neurons and resets their rhythmic bursting properties at stimulation currents below those required to produce antidromic activation (Givens and Breese, 1990). Theta reset induced by perforant path stimulation may likewise involve feedback from a descending pathway to the MSA that subsequently resets the rhythmic output that drives the hippocampal theta rhythm.

Although these descending pathways appear to be important for theta reset, the present results do not rule out a role for the ascending pathways from the brainstem to the MSA (Fig. 5), which are known to influence HPC theta (Vertes et al., 1993; Kinney et al., 1994; Oddie et al., 1994). In fact, electrical stimulation of brainstem inputs to the MSA are capable of resetting HPC theta (Gaztelu and Buño, 1982). Ascending and descending pathways to the MSA may be active within different time frames, with the ascending inputs occurring immediately after stimulus onset and the descending inputs becoming active after the HPC receives input from the entorhinal cortex. Thus, ascending and descending pathways may be sequentially activated with the brainstem input initially triggering a reset that is later reinforced and amplified by the descending input. Further studies will be necessary to delineate the roles of ascending and descending pathways in resetting HPC theta.

The results also suggest that electrical or light stimulation does not merely evoke theta, but rather resets ongoing theta, supporting previous findings (Givens, 1996b). The introduction of a stimulus did not increase theta power per se, but rather caused the ongoing theta rhythm to become phase-locked to the stimulus. Thus, as a pacemaker of the HPC theta rhythm, the MSA is not merely generating theta rhythm, but is actively shifting its phase relation to significant environmental events. The MSA may regulate HPC theta to ensure that the HPC is in a maximally receptive state when sensory input arrives from the entorhinal cortex. In order to have an augmenting effect, input to the HPC from the MSA must closely synchronize with input to the HPC from the entorhinal cortex, indicating that the ability of the MSA to facilitate learning and memory processes is tightly coupled to entorhinal input to the HPC (Alvarez-Leefmans and Gardner-Medwin, 1975).

The theta reset observed after the presentation of a light stimulus in this reference memory task was not predicted from our previous study that reported theta reset in a working, but not reference memory task (Givens, 1996b). There are several possible explanations for the discrepancy. First, the previous study revealed a large distinction between working and reference memory on theta, but did not focus on the possibility of a subtle reset with the reference memory group. Second, the reset to the light stimulus in the present reference memory task was significantly less than the reset observed in the previous working memory task and may reflect a residual behavioral component, such as occasional orienting or task-related motor responses, that does not significantly contribute to the more "cognitively-driven" reset observed in the working memory task. Hippocampal theta has been associated with motor activity in a number of studies (Whishaw and Vanderwolf, 1973; Oddie and Bland, 1998) and may be a major underlying factor in the theta reset observed in the present study. However, it should be noted that theta reset is not an entirely motor-related phenomenon. Greater theta is observed during working memory performance than reference memory performance despite similar motor requirements (Givens, 1996b). In addition, although theta reset occurs after motor responses during spatial working memory performance, theta reset has also been linked to visual stimuli even after motor influences have been accounted for (Williams et al., 1999).

In summary, the results support the previous finding from this lab that the hippocampal theta rhythm resets after 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.

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