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RAPID COMMUNICATION

Theta Reset Produces Optimal Conditions for Long-Term Potentiation

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ABSTRACT: Connections among theta rhythm, long-term potentiation (LTP) and memory in hippocampus are suggested by previous research, but definitive links are yet to be established. We investigated the hypothesis that resetting of local hippocampal theta to relevant stimuli in a working memory task produces optimal conditions for induction of LTP. The timings of the peak and trough of the first wave of reset theta were determined in initial sessions and used to time stimulation (4 pulses, 200 Hz) during subsequent performance. Stimulation on the peak of stimulus-reset theta produced LTP while stimulation on the trough did not. These results suggest that a memory-relevant stimulus produces a phase shift of ongoing theta rhythm that induces optimal conditions for the stimulus to undergo potentiation. © **2004 Wiley-Liss, Inc.**

KEY WORDS: hippocampus; long-term potentiation; memory; neuronal plasticity; rats; theta rhythm

INTRODUCTION

The relationship between long-term potentiation (LTP) and hippocampal theta rhythm has been well established experimentally, but how LTP and theta are coordinated in real time in an animal acquiring new information is unknown. LTP occurs optimally under conditions in which the high-frequency stimulation (HFS) is delivered at a frequency that mimics naturally occurring hippocampal theta (Larson et al., 1986; Diamond et al., 1988). Additionally, the induction of LTP is optimal when HFS is timed to occur at the peak of the theta rhythm (Pavlides et al., 1988; Huerta and Lisman, 1993; Orr et al., 2001). Inversely, when HFS occurs at the negative phase of ongoing theta rhythm, previously potentiated synapses can undergo depotentiation (Holscher et al., 1997). Furthermore, single hippocampal neurons in freely behaving rats fire normally at the peak of ongoing theta activity (Buszaki, 1986; Otto et al., 1991). The present study extends previous research by exploring LTP and theta in relation to the occurrence of natural stimuli in a working memory task.

LTP is theorized to strengthen the connectivity between neurons, thereby encoding stimuli along with their spatial and temporal contexts. Evidence for correlation between LTP and memory continues to accumulate in support of this model (Moser et al., 1993; Barnes et al., 1995; Rogan et al., 1997; Wilson and Tonegawa, 1997). A recent review supports the hypothesis that activity-dependent synaptic plasticity is both necessary and sufficient for memory formation (Martin et al., 2000).

There is considerable evidence that theta rhythm also plays a vital role in information processing and memory formation (Winson, 1972; Givens and Olton, 1994; O'Keefe and Burgess, 1999; Seager et al., 2002). A model has been proposed in which the two phases, the peak and trough of local theta, correspond to memory encoding and retrieval, respectively (Hasselmo et al., 2002). The dynamics of the synaptic mechanisms underlying theta rhythm and its modulation of hippocampal circuitry lead to the conclusion that theta-modulation likely has a significant role in hippocampal information processing (Vinogradova, 1995).

Although both LTP and theta rhythm appear to be integral to memory formation, the coordinated timing of LTP, theta, and the reception of information has not been fully explored. To link these physiological phenomena to information storage, we timed HFS with naturally occurring phases of theta in animals performing a working memory task. Using this method, we sought to determine whether the presentation of a relevant stimulus, which induces theta reset, primes the hippocampus for synaptic plasticity and optimal encoding of the stimulus.

Our previous research in rodents demonstrates that the onset of stimuli in a working memory task causes ongoing theta to reset such that it becomes phase-locked to the stimuli (Givens, 1996; Williams and Givens, 2003). Recent evidence indicates that ongoing oscillatory theta activity exists in the human hippocampus (Klimesch, 1999; Kahana et al., 2001). Interestingly, indwelling electrode recordings indicate that human hippocampal theta rhythm undergoes a very similar type of resetting to working memory stimuli (Rizzuto et al., 2003).

The present experiment uses visual stimuli during a delayed non-matching-to-position (DNMTP) task to reset the theta rhythm and time the induction of LTP naturally. This task is useful for examining the temporal properties of LTP and theta because there are multiple trials over which to average the physiological data and to investigate the timing and consequence of HFS. By establishing the occurrence of natural reset during the delay, we are able to deliver HFS at different points in the rhythm. Using this design, the present experiment investigates the hypothesis that the effect of HFS during working memory encoding is dependent upon the phase of theta during which HFS occurs.

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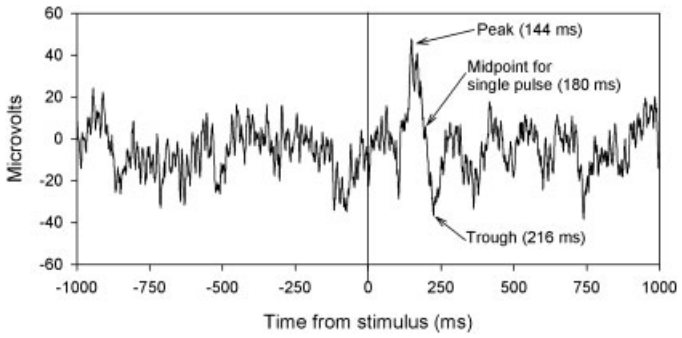

FIGURE 1. Average waveform around sample light onset was used to determine peak and trough timings. EEG was recorded 1 s after the sample light for each trial throughout the session. The average of these waveforms allowed us to observe the natural theta reset in response to the sample light. Each average was used to establish the timing of the peak and trough of each rat's reset theta rhythm for use in the stimulation protocol.

Eight male Long-Evans rats were singly housed and water restricted, to no less than 85% of their unrestricted weight, while being allowed ad libitum access to food. The rats were initially trained 5–6 days per week on the DNMTP task. This task requires rats to hold a discrete visual stimulus in working memory. Rats trained in successive approximations of the final task and met mastery criteria before advancing at each step (for a description, see Robinson et al., 2000).

Upon reaching criteria on the DNMTP task, the rats underwent surgery. A bipolar stimulating electrode, consisting of two pieces of Teflon-insulated twisted tungsten wire (each 150 μm in diameter), was lowered into the angular bundle (7.3 mm posterior and 4.4 mm lateral to bregma). A Teflon-coated stainless steel recording electrode (150 μm) was lowered into the dentate hilus (4.3 mm posterior and 3.0 mm lateral to bregma). The recording electrode was slowly lowered into the CA1 layer of the hippocampus and further advanced to the dentate hilus until a characteristic population excitatory postsynaptic potential (pEPSP) was observed (3.0–3.5 mm ventral to dura). A Teflon-coated, stainless steel electrode (250 μm) was implanted anterior to the hippocampus, to serve as an electrical ground. All wires and electrodes were secured in an insulator strip, which was then fixed to the skull with dental acrylic.

After 1 week of recovery, the rats were water restricted and training in the DNMTP task resumed. Once the animal reestablished criteria-level performance, electrophysiological recording began. Initially, each animal's theta rhythm was recorded for 1 s after each sample light over the course of three sessions. The resulting waveforms were averaged across trials and sessions for each rat. This established the position of the peak and trough of each rat's theta rhythm with respect to the onset of the stimulus (see Fig. 1). The first consistent peak and the immediately following trough were used for each animal. While in many cases there was an initial variable peak that occurred <200 ms following stimulus onset, the first consistent peak, and the one used for subsequent stimulation, on average occurred 377 ± 50.2 ms following stimulus onset and the trough occurred 89.9 ± 40.9 ms later. Although each peak and trough time differed among animals, the reset theta, and thus the timing of stimulation, was consistent within an animal across trials.

Each rat then completed 10 sessions in which the electrical stimulation alternated between peak and trough, with the order of sessions counterbalanced across animals. The pEPSP slope, population spike latency (PSL), and population spike amplitude (PSA) were recorded.

During each of the first 30 trials of a stimulation session, a single 0.1 ms test pulse was delivered via a stimulator (Grass Instruments, Quincy, MA) at the midpoint between the peak and trough of the reset theta, as estimated from the previously obtained average of theta reset, and the resultant evoked potential was recorded. During each of the next three trials, HFS (a train of 4 pulses at 200 Hz) was introduced at the time of peak or trough of reset theta for each animal. Stimulation then returned to single test pulses for 30 subsequent trials of the session. Sessions in which HFS was delivered at the peak were alternated with sessions in which HFS was delivered at the trough. Recording continued until five peak and five trough sessions were completed.

Once all the recording sessions were complete, rats were anesthetized with pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 0.9% heparinized (2,000 USP units/L) saline followed by 10% formalin. The brain was then sectioned (50 μm), mounted onto gelatin-coated slides, and stained with cresyl violet to verify the placements of the recording and stimulating electrodes. Histological analysis revealed a clustering of placements for the recording electrodes in the dentate hilus and a similar clustering of stimulating electrode placements in the angular bundle.

Single-pulse stimulation of the perforant path produced a characteristic pEPSP in the dentate gyrus. As expected, HFS led to potentiation of the evoked response. This potentiation was observed in the overall analysis as a main effect of HFS on PSA ($F(1,69) = 25.51$, $P < 0.001$), with stimulation leading to a larger population spike. The effect of HFS on slope ($F(1,69) = 2.45$, $P = 0.12$) and PSL ($F(1,69) = 2.26$, $P = 0.14$) failed to reach significance, due to the absence of potentiation on trough stimulation sessions. Figure 2 illustrates the potentiation immediately following HFS on a peak session, as opposed to a trough session.

The lack of potentiation on sessions with trough stimulation was confirmed by a highly significant interaction between HFS (pre vs. post) and stimulation timing (peak vs. trough) on the slope of the pEPSP ($F(1,69) = 19.41$, $P < 0.001$). As Figure 3 indicates, HFS produced a substantial difference in slope between peak and trough days, a pattern that repeated throughout the 10 sessions. The same pattern of results was observed in the population spike data with a highly significant interaction between stimulation timing and HFS on PSA ($F(1,69) = 20.38$, $P < 0.001$) and on PSL ($F(1,44) = 4.387$, $P < 0.04$; data not shown).

Analysis of performance accuracy revealed that performance in the task was highly dependent on the length of the delay ($F(1,54) = 23.16$, $P < 0.001$), but was not systematically affected by HFS ($F(1,54) = 2.22$, $P = 0.14$) or stimulation timing ($F(1,54) = 1.22$, $P = 0.27$). However, this delay-dependent decrement in accuracy was not observed throughout all conditions, as revealed by a significant interaction between HFS (pre vs. post) and length of the delay ($F(1,54) = 21.68$, $P < 0.001$). Figure 4

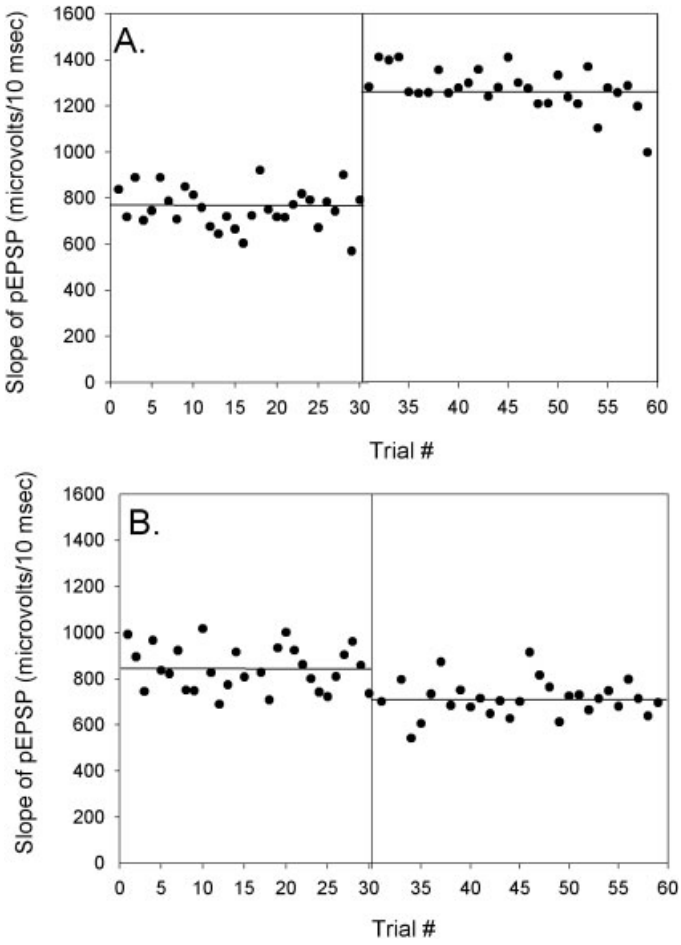

FIGURE 2. Slopes of population excitatory postsynaptic potential (pEPSP) for a single animal across one session as a function of trial number. Horizontal lines represent the mean slope before and after high-frequency stimulation (HFS). A: Representative peak stimulation session illustrates the dramatic increase in pEPSP slope immediately following HFS (represented by vertical lines). B: In contrast, a trough stimulation session demonstrates no increase in pEPSP slope.

shows that the delay-dependence in accuracy was observed in pre-HFS trials but not after HFS, with a significant decrease occurring at the 5 but not the 10 s delay. None of the other interactions were significant for the accuracy data ($P > 0.05$).

The rats vigorously perform the DNMTP task with <10% omission. However, as we have observed previously, there are more missed trials after a short delay than a long one due to reward consumption and grooming (main effect of delay on omission: $F(1,56) = 42.00$, $P < 0.001$). Animals also omitted more trials after HFS ($F(1,56) = 71.4$, $P < 0.001$), a result that may simply reflect an increase in omission later in the session due to satiation. However, there was an interaction between delay and HFS ($F(1,56) = 45.12$, $P < 0.001$), such that the delay-dependence in omission observed for pre-HFS trials was not seen for trials following HFS.

As expected, the latency to respond to the sample stimulus slowed down during the later trials of the task. Thus, there was a main effect of HFS on latency to respond to both the sample stimulus ($F(1,53) = 16.08$, $P < 0.001$) and the choice stimulus ($F(1,53) = 4.545$, $P < 0.04$). Interestingly, the slowing of the

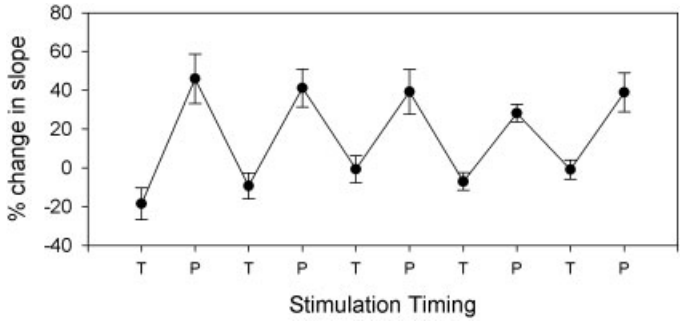

FIGURE 3. Percentage change in slope and population spike amplitude (PSA) of population excitatory postsynaptic potential (pEPSP) following high-frequency stimulation (HFS) as a function of session. Timing of HFS (peak or trough) had a significant main effect on pEPSP slope and PSA. Magnitude of the effect varied between animals; however, the direction of the relationship for both slope and PSA remained constant across animals. The percentage increase in slope and PSA following HFS was significantly greater on peak sessions than on trough sessions. Vertical bars indicate ± SEM.

response to the sample light was moderated by stimulation timing. The animals responded significantly slower to the sample light on post-HFS trials during peak stimulation sessions than during trough sessions ($F(1,53) = 14.47$, $P < 0.001$). No interaction was observed for the latency to respond to the choice stimulus.

The present study establishes a critical time point, after the presentation of memory-relevant stimuli, when the dentate gyrus can undergo potentiation by HFS of the perforant path. Identical HFS delivered ~70 ms later fails to produce potentiation. The difference in these two time points corresponds to the phase of theta rhythm at the time of HFS delivery. HFS delivered at the peak of reset theta consistently induced LTP, while HFS delivered at the trough of reset theta had no effect. These results are consis-

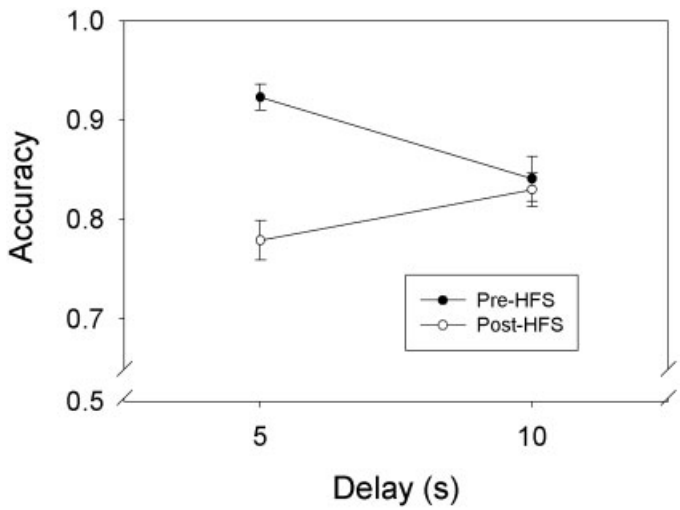

FIGURE 4. Expected delay-dependent deficit was only present for pre- high-frequency stimulation (HFS) trials. A significant interaction between HFS (pre vs. post) and the length of the delay indicated that the accuracy on trials with a 5-s delay was significantly greater before HFS than following HFS. However, this disparity was not observed for 10-s delay trials.

tent with findings that LTP is optimally induced when HFS is timed to the peak of theta (Pavlides et al., 1988; Huerta and Lisman, 1993; Holscher et al., 1997). However, this research extends those findings and connects hippocampal potentiation to memory formation by demonstrating this effect with regard to theta that is reset by stimuli to be encoded into working memory.

As in previous work, memory-relevant stimuli produced a reset, or phase shift, in theta (Givens, 1996). The data support the hypothesis that the function of theta reset is to align theta activity with the arrival of relevant stimulus representations so that those representations can undergo potentiation and be encoded into memory. Although HFS was delivered at the peak phase of reset theta, phase alignment continued for 4–6 cycles, which could allow for encoding or retrieval of sequences of information through the precession of neural activity across theta cycles (O'Keefe and Burgess, 1999; Hasselmo et al., 2002). Further studies are needed to investigate whether later cycles of reset theta are also points of enhanced plasticity.

The present study was designed primarily to investigate theta and LTP; however, some interesting behavioral results emerged. Although the expected decrease in accuracy for trials with longer delays was observed, this effect only occurred on trials prior to HFS. After HFS was delivered, regardless of stimulation timing, performance was delay-independent. One possible explanation for this finding is that HFS disrupted the circuits needed to accurately encode and retrieve stimulus information in the task. If the circuits needed for stimulus encoding were disrupted, the animals might have drawn on alternate memory strategies to solve the task (Poldrack and Packard, 2003). Note that the type of encoding needed to solve a working memory task is clearly different from the type of encoding modeled by LTP. Accordingly, theta reset may be related not only to long-term memory formation but also to short-term forms of encoding and retrieval.

Our previous research indicates that the presentation of relevant sensory stimuli is accompanied by theta reset (Givens, 1996). The current study demonstrates that LTP can be induced with HFS when delivered at the peak of reset theta but not at the trough. The results are consistent with our hypothesis that theta reset may be a mechanism by which hippocampal circuits facilitate the potentiation of relevant stimuli. By this mechanism, theta reset can synchronize incoming sensory information with hippocampal circuitry in order to produce optimal conditions for the encoding of stimuli into memory.

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