Median Raphe Stimulation Disrupts Hippocampal Theta Via Rapid Inhibition and State-Dependent Phase Reset of Theta-Related Neural Circuitry

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Jackson J, Dickson CT, Bland BH. Median raphe stimulation disrupts hippocampal theta via rapid inhibition and state-dependent phase reset of theta-related neural circuitry. J Neurophysiol 99: 3009 –3026, 2008. First published April 24, 2008; doi:10.1152/jn.00065.2008. Evidence has accumulated suggesting that the median raphe (MR) mediates hippocampal theta desynchronization. However, few studies have evaluated theta-related neural circuitry during MR manipulation. In urethane-anesthetized rats, we investigated the effects of MR stimulation on hippocampal field and cell activity using high-frequency (100 Hz), theta burst (TBS), and slow-frequency electrical stimulation (0.5 Hz). We demonstrated that high-frequency stimulation of the MR did not elicit deactuated patterns in the forebrain, but rather elicited low-voltage activity in the neocortex and small-amplitude irregular activity (SIA) in the hippocampus. Both hippocampal phasic theta-on and -off cells were inhibited by high-frequency MR stimulation, and -off cells exhibited a state-dependent modulation of cell firing. Subpopulations of phasic theta-on cells responded in either oscillatory or nonoscillatory patterns to MR pulses, depending on their intraburst interval. OFF cells exhibited a state-dependent modulation of cell firing occurring preferentially during nontheta. The magnitude of MR-induced reset varied as a function of the phase of the theta oscillation when the pulse was administered. Therefore high-frequency stimulation of the MR appears to disrupt hippocampal theta through a state-dependent, short-latency inhibition of rhythmic cell populations in the hippocampus functioning to switch theta oscillations to an activated SIA field state.

INTRODUCTION

The median raphe (MR) is hypothesized to function as a hippocampal theta desynchronizing nucleus (Vertes 2005; Vertes and Kocsis 1997; Vertes et al. 1981; Vinogradova et al. 1999), acting to antagonize the ascending brain stem hippocampal synchronizing system and inhibit the generation of hippocampal theta (Vertes et al. 2004; Vinogradova 2001; Vinogradova et al. 1999). These hypotheses are supported by experimental evidence demonstrating that high-frequency MR stimulation suppresses theta field oscillations (Assaf and Miller 1978; Peck and Vanderwolf 1990; Vertes 1981; Vinogradova et al. 1999), whereas MR lesion elicits theta recorded in the hippocampus (Kinney et al. 1994, 1995, 1996; Kitchigina et al. 1999; Li et al. 2005; Maru et al. 1979; Varga et al. 2002; Vertes et al. 1994). As proposed by Vertes et al. (2004), MR projections serve to overactivate medial septal GABAergic cells (Alreja 1996; Liu and Alreja 1997), which in turn inhibit GABAergic septohippocampal neurons and therefore decrease the production of hippocampal theta (Leranth and Vertes 1999).

Previous investigations of MR stimulation and hippocampal cell modulation by Segal (1975) demonstrated the inhibition of putative pyramidal neurons in the hippocampal CA1 region following MR stimulation. Since this early study only one other experiment has examined hippocampal cellular responses to MR stimulation (Kao et al. 1997). These authors determined that MR stimulation likely exerted its influence on the hippocampus via inhibition of interneurons in the dentate gyrus. However, neither of these prior studies focused on theta-related properties of hippocampal neurons and, furthermore, neither brain state nor network activity was examined.

In the current study we stimulated the MR and recorded from hippocampal neurons to determine whether the MR exerted its hippocampal influence via theta-related neural circuitry. Hippocampal field and unit activity were recorded to determine how MR stimulation modulated single cells and network activity. Hippocampal activity was measured during administration of high-frequency (100 Hz), theta burst (TBS), and low-frequency (0.5-Hz) stimulation. We found that the disruption of hippocampal theta by high-frequency stimulation of the MR stimulation occurred via a rapid (5-ms) inhibition of theta-related neural circuitry and phase reset of theta field activity. Theta-related (and not unrelated) hippocampal neurons were preferentially modulated by MR stimulation. These results demonstrate that MR projections have an important role in regulating hippocampal network activity and at least a portion of the MR circuitry may differentially modulate hippocampal activity during theta and nontheta states (large-amplitude irregular activity [LIA], slow oscillations, and sharp waves).

METHODS

Subjects/surgery

Sixty-five male Long-Evans rats (250–350 g) were obtained from the Life and Environmental Science Animal Research Council at the University of Calgary. All procedures adhered to guidelines set out by the Canadian Council of Animal Care.

Rats were first anesthetized with a mixture of halothane (MTC Pharmaceuticals, Cambridge, Ontario) and oxygen (1.5% minimum

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alveolar concentration). While under halothane anesthesia a small polyurethane tube attached to a 1.0-mL syringe was inserted into the jugular vein used to administer urethane (ethyl carbamate, 0.8 mg/mL) for the remainder of the experiment. Once stabilized under urethane, rats were secured in a stereotaxic apparatus and the skull was leveled between bregma and lambda. An uninsulated tungsten electrode (50 μm) was inserted into frontal cortex or cerebellum and served as a reference for extracellular field recordings. A tungsten electrode (0.1 mΩ) was inserted into the hippocampus; anterior–posterior (A/P): −3.8 mm; medial–lateral (M/L): 2.5 mm; dorsal–ventral (D/V): −2.2 to 2.5 mm from the dural surface. This electrode served to record field activity from the stratum lacunosum region of CA1 in the dorsal hippocampus. A bipolar stainless steel stimulating electrode (250 μm) attached to two miniature Winchester pins was slowly lowered into the MR at A/P: −7.9 mm; M/L: 0.00 mm; D/V: −7.0 to 8.0 mm from the dural surface. In these experiments only sites that exhibited hippocampal desynchronization following 100-Hz stimulation were used for analysis. An additional electrode was lowered into the contralateral hippocampus at coordinates identical to those described earlier and served to record local field activity and single units. Single units were isolated with tungsten (−1–2 mΩ) or glass microelectrodes (3–5 mΩ) lowered into the hippocampal CA1 region at a rate of 1–2 μm/s using a microdrive mounted into the stereotaxic apparatus.

High-frequency (100 Hz) stimulation and theta patterned stimulation

Once the hippocampal field activity was cycling spontaneously between theta and nontheta states, stimulation trials were administered for periods during both brain states. The protocol for high stimulation trials consisted of trains of stimulation lasting 2–5 s, delivered using a 100-Hz, 0.1-ms-duration square-wave pulse (0.1 to 0.9 mA), administered using a Grass S88 stimulator and a stimulus isolation unit. A series of recordings were taken with a theta burst stimulation (TBS) protocol, again using 100-Hz bursts lasting 80 ms, separated by 30–170 ms (giving 4–9 Hz). In another series of experiments, a slow-frequency 0.5-Hz (0.2–0.5-ms duration) pulse was administered to the MR using higher intensities (0.5–1.0 mA). Together, these stimulation protocols attempt to mimic the firing patterns of the majority of MR neurons under anesthesia (Viana de Prisco et al. 2002; with neurochemical identification: Kocsis et al. 2006) and in freely moving animals (Kocsis and Vertes 1992).

Voltage profile and current source density

In four rats, a neocortical electrode was also inserted into the frontal motor cortex (A/P: +2.0; M/L: 2.0; D/V: −0.5 mm from the dural surface). This electrode served as an additional indicator of brain state to assess the level of anesthesia and to observe spontaneous cycling between activated and deactivated states typical of urethane anesthesia (Clements et al. 2006). In these four rats, a 16-channel silicon linear probe (Neuronexus Technologies, Ann Arbor, MI) was positioned vertically through the dorsal hippocampus using coordinates described earlier. Contact points on the probe were spaced 100 μm in the dorsoventral plane and straddled both the pyramidal cell layer of CA1 and the granule cell layer of the dentate gyrus (DG). Thus the multiprobe provided simultaneous monitoring of activity in a 1.6-mm span through stratum oriens to the hilar region of the DG. Laminar voltage profiles and current source density (CSD) analysis of both spontaneous and evoked local field potential activity were performed as previously described (Wolansky et al. 2006). In brief, CSDs were computed using the second spatial derivative of the voltage measurement obtained across channels using the following formula: 

\[
\text{CSD} = \left[ (p_{i+1} - 2p_i + p_{i-1})/d^2 \right],
\]

where \( p \) is the signal from channel \( i \) and \( d \) is the distance between channels (0.1 mm).

Data acquisition and analysis

Signals from the silicon probe were referenced to stereotaxic ground and amplified at a final gain of 1,000 and wide-band filtered between 0.7 Hz and 10 kHz via a 16-channel head stage (unity gain) and amplifier system (Plexon, Dallas, TX). All signals were digitized with a Digidata 1322A A-D board connected to a Pentium PC running the AxoScope acquisition program ( Molecular Devices, Union City, CA). Signals were sampled at ±1 kHz and were digitized on-line after being low-pass filtered at 500 Hz (software controlled).

Field activity recorded from CA1 was amplified (×1,000) and filtered (1–100 Hz), using Grass P511 preamplifiers, and digitally stored using DataWave systems (Berthoud, CO) at sampling rate of 200 or 500 Hz. Analyses were performed in DataWave (Sciworks 4.1) or Matlab (MathWorks, Natick, MA), using scripts built in house. Hippocampal field potentials were quantified using the fast Fourier transform (FFT). FFTs were used to calculate power in different frequency bands (delta: 0.5–2.5 Hz; theta: 3–6 Hz; and beta: 15–30 Hz). Nontheta states are typically composed of large-amplitude irregular activity (LIA, broadband distribution of power over 1–25 Hz), slow oscillation (SO, 0.5–1.5 Hz), and sharp waves with ripples (80–200 Hz). For the interests of this study these states were collectively referred to as nontheta.

For analysis of field activity during single 0.5-Hz MR stimulation pulses, the data were analyzed for 1 s prestimulation and 1 s post-stimulation, referenced at time 0 to the MR pulses. FFTs were performed 1 s prior to and 1 s following all stimulation trials. This procedure assessed whether the MR pulse could change theta frequency or power instantaneously with one pulse. In addition, field data from 60 to 120 pulses were averaged using perievent time histogram (PETH) techniques, using the MR pulse as the trigger. This analysis assessed whether the MR evoked a consistent phase change in the signal arising from the fact that averaged activity in the prestimulus time should approach zero amplitude due to random phase fluctuations in the field activity over many pulses. Autocorrelations were performed on field activity to detect periodicities in the signal; regularly occurring peaks indicate an oscillatory fluctuation in neuronal activity with a period equal to the time between peaks. Theta activity was defined as constituting those epochs with a peak between 3 and 6 Hz and >50% of the signal (1–25 Hz) power in this region. Nontheta states typically maintained peak frequencies <3 Hz or a broad distribution of power across the 1- to 25-Hz frequency range. To quantify the field activity in both the neocortex and the hippocampus during MR stimulation, signal power was compared with the theta and nontheta states using three to six epochs (3–5 s in duration) in each condition. The power in each frequency band [delta (1–2.5 Hz), theta (3–6 Hz), and beta (15–30 Hz)] was normalized using the average power across all conditions within every animal. Field activity could then be expressed as a ratio, where a value of 1.0 indicates an epoch of data that expressed a power value equal to the overall averaged power within a given frequency band for that animal. Data were normalized within an animal and then averaged across all animals to achieve a grand mean. All data are therefore expressed as the grand normalized mean ± SE unless otherwise indicated. Statistical analyses were performed with a one-way ANOVA test where appropriate (Fig. 9). Otherwise, comparisons between conditions were conducted using planned pairwise comparisons.

Cell classification

Cells were initially classified according to the criterion of Colom and Bland (1987). Phasic theta-on cells maintained rhythmic autocorrelations, fired phase locked to hippocampal theta, and exhibited an elevated discharge rate during theta relative to nontheta; tonic theta-on cells also fired preferentially during theta, but were not rhythmic and did not display consistent phase relations with hippocampal theta. Phasic theta-off cells fired preferentially during nontheta, but also...
fired during low-frequency theta, maintaining a negative linear correlation between theta frequencies and firing rate. Phasic theta-off cells can also display a phase preference to theta, when discharging during lower frequencies. Tonic theta-off cells did not fire during theta and therefore fired nonrhythmically only during nontheta field activity. Nonrelated cells were those that did not display a brain-state-dependent firing rate and were not phase related to hippocampal theta.

Single-unit activity was amplified (>10,000), filtered (0.3–5 kHz), and digitally stored using SciWorks 4.1. Spikes were sorted off-line using identification of spike parameters such as spike height, width, peak time, and slope to differentiate between stimulus artifact and neuronal events. Firing rate, interspike interval (ISI), and phase relationship estimates were performed on data from both theta and nontheta brain states. Cross-correlations were performed by triggering on the trough of the extracellular field recording from stratum lacunosum of the contralateral hemisphere and binning spikes (5 ms) on either side of the trough (≥1 s). This process created a theta trough triggered-averaged histogram that assessed the phase relationship of the spike relative to the trough of hippocampal theta. The first-order ISI (0–500 ms) was measured with a 2-ms bin width. Autocorrelations were calculated on spike trains from both theta and nontheta brain states to assess periodicity in each state. Spike-train cross-correlations and autocorrelations were normalized to compare between cells and brain states and to eliminate differences in overall spike counts.

**PETH analysis**

PETHs of spike discharges were generated (5-ms bins) by triggering on each MR pulse and summing over all pulses (60–120). Analyses of latency and duration of inhibition or excitation were performed on raw PETHs in a manner similar to that reported by Hajos et al. (2003) and Puig et al. (2005). Prestimulation firing was estimated using the average spikes per bin in the 250 ms prior to stimulation onset. Stimulation effects were calculated by dividing the average number of spikes per bin in the 250 ms following the stimulation by the prestimulation firing rate. Using a 5-ms bin width the response pattern of each cell was classified (inhibition, excitation, biphasic, oscillatory), again using criteria similar to those used by Hajos et al. (2003) and Puig et al. (2005) (but modified for the inclusion of oscillatory responses). Inhibition was defined as a total cessation of spikes in four consecutive bins or a reduction to <25% of prestimulation firing rates. For the latency to inhibition, data were reanalyzed using a 1-ms bin width. Cells were considered to return to prestimulation rates when four consecutive 5-ms bins (or the mean of four bins) displayed spike counts equal to or greater than prestimulation levels. Excitatory responses were classified as four consecutive bins achieving a firing rate >2SDs above the prestimulation mean. Excitation offset occurred when spike count in four consecutive bins (or four-bin average) fell below the mean + 2 SD threshold. Biphasic responses required both inhibition and excitation within the first 250 ms poststimulation.

Oscillatory and state-specific responses were determined by first smoothing the raw PETH with a 50-ms sliding window, creating a low-pass filtered (<20 Hz) waveform representing the relative height of the poststimulus bins for the 1 s of data following the 0.5-Hz stimulation. The resulting waveform was then zero mean normalized and subjected to autocorrelation and FFT analysis to identify periodicities in the poststimulus period. The frequency of the dominant peak in the FFT, achieving a peak height >4SD from the mean power in the spectrum, was considered to be oscillatory. In addition, the state dependence of responses was assessed using cross-correlation (near zero lag, Pearson’s r value) between the PETH waveform generated in theta compared with that created during nontheta conditions. A state-independent response required that the cell response type was identical in both states (i.e., excitatory, inhibitory, biphasic) and that a significant correlation existed between theta and nontheta PETHs.

**Histology**

Following termination of the experiment, animals were given an overdose of urethane and perfused transcardially with saline (0.9%) and then with a 10% formalin solution. Brains were postfixed in sucrose and sectioned 40 μm at a temperature of −20°C using a cryostat. Sections were mounted on gelatin-coated slides and stained with cresyl violet for confirmation of electrode placements.

**RESULTS**

Electrode placements eliciting the stereotyped low-amplitude fast activity in response to 100-Hz stimulation were located in the MR (Fig. 1A, left). Stimulation locations slightly outside the MR elicited hippocampal theta rhythmicity. The location of hippocampal field electrodes was confirmed within the stratum lacunosum region (Fig. 1A, right). Single cells recorded were located in the same anterior–posterior plane and were verified using reconstruction as well as field activity profiles during recording. No effect of location was found in the single-unit studies. Multiprobe tracks were all in a plane that traversed the CA1 pyramidal cell layer, through the hippocampal fissure and the DG. The termination of probe tracks was typically in stratum granulosum or in the hilar region of the DG just ventral to the granule cell layer. The position of individual contact sites was estimated from the position of the histological tract in combination with comparisons with the distribution of spontaneous theta and SO activities (Wolansky et al. 2006).

**MR modulation of brain state**

Neocortical and hippocampal field activity were recorded during deactivated state (Fig. 1B, left), activated state (Fig. 1B, middle), and high-frequency (100-Hz) stimulation of the MR (Fig. 1B, right). MR stimulation elicited low-amplitude activity in the hippocampus, inhibiting the presence of hippocampal theta as described previously (Vertes 1981). However, neocortical activity appeared similar to that of the activated state. Autocorrelation analysis revealed that the neocortical field activity during the activated state was similar to that during MR stimulation (Fig. 1C, top). Similar results were attained using FFT analysis (Fig. 1D, top). Analysis of delta, theta, and beta frequency bands also indicated that MR stimulation resulted in power values similar to those observed during the activated brain state (Fig. 1E, top). However, both delta ([0.28 ± 0.19 vs. 2.50 ± 0.24, t(3) = 5.26, P = 0.013] and theta band power [0.37 ± 0.12 vs. 2.11 ± 0.19, t(3) = 5.76, P = 0.01] during MR stimulation were significantly reduced relative to that measured during the deactivated state, indicating that MR stimulation more closely approximated the activated, rather than the deactivated, brain state in terms of neocortical activity (Fig. 1E, top). In the hippocampus, MR stimulation abolished hippocampal theta as indicated by the autocorrelation function (Fig. 1C, bottom) and the FFT analysis (Fig. 1D, bottom). In the hippocampus, MR stimulation also induced a low-amplitude activity with significantly less hippocampal delta power than that in the deactivated state (P < 0.01), and with less theta power than that in the theta state (P < 0.01), thereby indicating that in the hippocampus, MR stimulation elicited field activity that did not resemble either theta or the typically defined nontheta conditions (i.e., LIA, slow oscillations).
tions, or sharp waves) (Fig. 1E, bottom). Under urethane anesthesia, neocortical and hippocampal activity spontaneously oscillated between activated and deactivated states (Clement et al. 2006). MR stimulation did not affect the ability of the animal to cycle between brain states. The brain state following MR stimulation was determined by the global cyclic brain state spontaneously cycling between activated and deactivated states (data not shown). Furthermore, 5–10 stimulation periods (within 1–2 min) did not affect spontaneous state transitions under urethane.

Levels of MR stimulation and hippocampal field activity

Eleven animals were tested with 100-Hz MR stimulation at three different intensities (low, moderate, and high), determined relative to the intensity that proved to be the most effective at reducing hippocampal power. The low- and moderate-intensity levels were defined, respectively, as 50 and 75% of the high-intensity stimulation. The minimum intensity used to elicit desynchronization was found to be 0.25 mA and the maximum was 0.80 mA (Fig. 2A). The reduction in power was stimulation intensity dependent (Fig. 2B). High-, moderate-, and low-intensity MR stimulation reduced hippocampal theta to 19 ± 4, 49 ± 8, and 67 ± 11% of the power in prestimulation theta conditions, respectively. High-intensity stimulation produced less power than the moderate [t(_10) = 3.30, P = 0.004] and low [t(_10) = 4.13, P < 0.001] stimulation intensities. High intensities (>0.6 mA) produced a near flattening of the electroencephalogram. Again, as emphasized earlier, the spectral content of hippocampal activity during 100-Hz MR stimulation did not resemble either theta or the presently defined nontheta field conditions. Instead, MR stimulation elicited low-amplitude activity, occasionally with a small theta component (see Fig. 1D, bottom) and broad distribution of power across the 1- to 25-Hz region. The effect of 100-Hz MR stimulation on hippocampal and neocortical activity was independent of the brain state within which the stimulation was administered. However, as shown in Fig. 2, higher-intensity stimulation was required for a complete loss of rhythmicity, whereas at lower-intensity levels, only a lack of power was noted. Therefore the loss in theta range frequency results only secondarily to a loss in hippocampal theta power.

MR stimulation of 100 Hz and hippocampal theta-on cells

Forty phasic theta-on cells were tested with 100-Hz MR stimulation. Two representative phasic theta-on cells are shown (cell 1, top panel; cell 2, bottom panels) during theta (Fig. 3Ai) and nontheta (Fig. 3Aii) conditions and during 100-Hz MR stimulation (Fig. 3Aiii), respectively. Autocorre-
HIPPOCAMPAL THETA DISRUPTION BY MR STIMULATION


FIG. 2. Modulation of hippocampal field activity by 100-Hz MR stimulation. A: field activity is suppressed in an intensity-dependent manner. B: histogram showing relative theta power during high-, moderate-, and low-intensity MR stimulation conditions.

Condition, ISI, and cross-correlation analyses (Fig. 3, A, B, and C, left panels, respectively) for the theta field condition all verified the rhythmic cell discharge pattern, whereas the equivalent analyses for the nontheta condition (Fig. 3, A, B, and C, right panels) verified the nonrhythmic discharge pattern. Figure 3E shows that phasic theta-on cells maintained a significantly higher firing rate during theta (8.5 ± 0.6 Hz) than that during nontheta (3.3 ± 0.4 Hz) [t(30) = 11.6, P < 0.001]. The cells exhibited total inhibition (16/40, 40%), a reduction in firing rate, and loss of rhythmicity (21/40, 53%) or simply a loss of rhythmicity (3/40, 7%) during 100-Hz MR stimulation applied during theta field activity, when compared with firing rate during spontaneously occurring hippocampal theta field activity. Averaged across cells where spikes could be extracted from the artifact, the firing rate during stimulation using high-intensity levels was also significantly reduced compared with spontaneously occurring nontheta field activity [3.3 ± 0.4 Hz (nontheta) vs. 1.8 ± 0.4 Hz (MR stimulation); t(30) = 3.73, P < 0.001, Fig. 3E (indicated by inset black line)]. Eighteen cells were tested at two or more intensity levels and were inhibited in an intensity-dependent manner (Fig. 3F) similar to the reduction in hippocampal theta power. High-intensity stimulation results in a cell firing rate (3.25 ± 0.8 Hz) significantly less than the firing rate when intensity levels were reduced to moderate [6.3 ± 0.9 Hz; t(15) = 5.4, P < 0.001] or low levels [8.4 ± 1.0 Hz; t(16) = 4.96, P < 0.001]. Figure 3G shows that a negative linear relationship was maintained between the interburst interval and the firing rate with MR stimulation. (r² = −0.241, P = 0.003).

Tonic theta-on cells (n = 19) fired at a greater rate during theta field activity than that during nontheta field activity [7.8 ± 1.5 vs. 3.5 ± 0.8 Hz; t(18) = 5.70, P < 0.001]. A representative theta-on cell is shown during spontaneously occurring theta (Fig. 4Ai) and nontheta (Fig. 4Aii). Autocorrelation and cross-correlation analyses during theta field activity (Fig. 4, B and C, left panels) and nontheta field activity (Fig. 4, B and C, right panels) verified the nonrhythmic discharge patterns during both these field activities. Figure 4Aiii shows this cell responding with inhibition during 100-Hz MR stimulation. Analysis of the group data revealed that MR stimulation at 100 Hz reduced the tonic theta-on cell firing rate (relative to theta) to 5.65 Hz [t(18) = 2.83, P = 0.011, Fig. 4D]. Despite overall inhibition of firing (68%, 13/19), some tonic theta-on cells were excited (16%, 3/19) or remained unaltered (16%, 3/19) by stimulation. Figure 4Aiiv shows an example of a tonic theta-on cell responding with excitation during 100-Hz MR stimulation. In addition, no intensity-dependent relation was observed between the MR stimulation and the firing rate of tonic theta-on cells (Fig. 4E) in contrast to phasic theta-on cells.

Six theta-off cells were tested in response to 100-Hz stimulation of the MR (five phasic theta-off, one tonic theta-off). A representative phasic theta-off cell is shown (Fig. 5A, cell 1, top) during theta (left) and nontheta conditions (middle) and during 100-Hz MR stimulation (right). Autocorrelation, ISI, and cross-correlation analyses (Fig. 5, A, C, and D, left panels, respectively) for a representative off cell during the theta field condition all verify the rhythmic cell discharge pattern, whereas the equivalent analyses for the nontheta condition (Fig. 5, A, C, and D, right panels) verify the nonrhythmic discharge pattern. Figure 5E illustrates how in some cells the spike discharges could be distinguished from the 100-Hz MR stimulation artifacts using wave shape and spike amplitude. All off cells responded with reduced firing rates to 100-Hz MR stimulation, ranging from partial (for an example see Fig. 5Aiii, top right) to complete inhibition. Figure 5F shows the group data for theta-off cells, showing that they fired at a significantly higher rate during nontheta than during periods of theta activity [7.3 ± 1.2 vs. 3.5 ± 0.8 Hz; t(19) = 3.06, P = 0.028]. MR stimulation reduced the firing rate of off cells during stimulation relative to nontheta field activity [0.89 ± 0.4 Hz; t(5) = 4.21, P = 0.008] and marginally reduced rates relative to theta [t(5) = 2.96, P = 0.03]. Interestingly, in the period following the stimulation (2–8 s), cells often fired robustly (4.3 ± 1.13 Hz) despite the occurrence of a period of rebound theta activity. This brief period of increased firing likely arises from the fact that the stimulations were typically administered during nontheta (given this was the state of maximum cell discharges from off cells) and, although “rebound” theta activity occurred following the stimulation, the field activity quickly returned to nontheta field activity. As previously demonstrated, off cells tend to fire robustly in this situation since they appear to signal a state transition back to nontheta field activity.

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The firing rate of hippocampal neurons classified as nonrelated (n = 16) was not significantly altered in response to 100-Hz stimulation of the MR. A representative nonrelated cell is shown during spontaneously occurring theta (Fig. 6A, left) and nontheta (Fig. 6A, middle). Autocorrelation and cross-correlation analyses during theta field activity (Fig. 6, B and C, left panels) and nontheta field activity (Fig. 6, B and C, right panels) verified the nonrhythmic discharge patterns during both these field activities. Figure 6A (right) shows the cell did not alter its firing rate during 100-Hz MR stimulation. Analyses shown in Fig. 6D revealed nonsignificant differences between firing rates during nontheta (6.7 ± 2.0 Hz), theta (6.2 ± 1.9 Hz), and MR stimulation conditions (6.3 ± 1.9 Hz).
**Effect of TBS on hippocampal field activity**

Six animals received theta burst stimulation (TBS) at stimulation intensities identical to those necessary to elicit hippocampal desynchronization. TBS (100 Hz, 0.1- to 0.2-ms duration, 0.3–0.6 mA) was administered at frequencies ranging from 3 to 9 Hz and intensities identical to those used for 100-Hz experiments. The effect of TBS on hippocampal field activity was compared with spontaneously occurring theta field activity and to the effect of MR stimulation at 100 Hz administered during spontaneously occurring theta. For analysis, spectral power was averaged across all burst frequencies ranging from 3 to 9 Hz. Figure 7A shows an example of spontaneous theta (top), the autocorrelation analysis (middle), and the spectral power analysis of the sample (bottom). Spontaneously occurring theta under urethane typically occurred between 3 and 4 Hz. As shown previously, 100-Hz MR stimulation disrupted this oscillatory activity, producing small-amplitude activity (Fig. 7B, top), a nonperiodic autocorrelation function (Fig. 7B, middle), while abolishing theta rhythmicity (Fig. 7B, bottom). However, TBS induced theta activity at the frequency of the stimulation; for example, a 5- or 9-Hz burst pattern produced theta activity with a rhythmic autocorrelation (Fig. 7, C and D, middle panels) and a peak in the power spectrum at 5 and 9 Hz, respectively (Fig. 7, C and D, bottom panels). Therefore the greater the interburst frequency, the closer the stimulation mimicked the original 100-Hz high-frequency stimulation (compare FFT from Fig. 7, C and D). TBS significantly increased signal power [1.02 ± 0.16 vs. 0.33 ± 0.05, t(5) = 4.15, P < 0.001], theta power [1.0 ± 0.18 vs. 0.22 ± 0.06, t(5) = 4.22, P < 0.001], and peak power [1.56 ± 0.24 vs. 0.18 ± 0.05, t(5) = 5.66, P = 0.001] relative to 100-Hz MR stimulation (Fig. 7E). Figure 7F shows the modulation of power as a function of burst frequency. In this plot, all data across all animals were pooled. The most effective stimulation frequency—in terms of theta power (black) and peak value of the power spectrum (gray)—was found to be 5 Hz, with higher frequencies generally producing lower power values. The quadratic trend was not analyzed due to a variable number of data points at each frequency value, and with only one value at 3 Hz. Sufficient data were not collected to determine the effect of TBS on single hippocampal neurons.

**Effect of slow-frequency (0.5-Hz) stimulation on HPC field activity**

Slow-frequency stimulation was performed in the same experiments and at the same anatomical locations used for 100-Hz and TBS protocols. Hippocampal field and cell activity were evaluated during slow-frequency MR pulse stimulation (square wave, 0.2–0.5 ms, 0.5–1.0 mA). By superimposing different trials of stimulation at a wide variety of random phases of ongoing theta activity, a prominent alignment of the subsequent theta activity could be observed across stimulation trials for a variable period of time following the stimulus (Fig. 8A, top). Indeed, the average field responses generated from 32 to 120 pulses exhibited significant theta rhythmicity just poststimulation, indicated by the presence of a 3- to 4-Hz component in the average signal in the absence of rhythmicity prestimulation (Fig. 8A, second trace). This poststimulus alignment of theta rhythm (and, indeed, any rhythmicity) was observed only when stimulation occurred during ongoing theta. The decay of rhythmicity apparent in the average trace in the poststimulus period was not especially due to fluctuation of frequency between trials, as is shown in the third trace in Fig. 8A, where the same 32 trials were aligned according to the ongoing phase of theta just prior to the MR pulse (using negative to positive zero crossings). The fact that there is a significant theta component prior to the stimulation indicates that prestimulus frequency was relatively stable. We confirmed this finding over a greater extent of time by using continuous theta traces from the same experiment in which no stimulation was undertaken. Individual segments of 3-s duration were aligned by phase as described earlier and the average was computed (Fig. 8A, bottom trace). The decay of rhythmicity in the average trace, even 2 s following the triggered alignment, is observed to be quite small.

The phase reset evoked by MR stimulation could be evaluated systematically by comparing autocorrelation functions and spectra of the average trace for 1-s periods of time prior to and following stimulation (Fig. 8, B and C). Prominent rhythmicity in this reset phenomenon was observed only in the period just poststimulation. As described earlier, this reset phenomenon was transient and appeared to dissipate following the initial second after stimulation. Indeed, the autocorrelation function and spectrum of the subsequent 1-s period following stimulation were both similar to those computed for the period just prior to stimulation.

Spectral analysis (FFT) was performed on individual 1-s epochs for the time pre- and post-MR pulse. Neither theta power nor peak frequency was changed by the pulse during theta or nontheta (Fig. 8D, top). However, as expected, there was a greater theta power and peak frequency in the theta versus nontheta state. Average waveforms were generated for each animal and consistently showed a greater theta component in the poststimulus 1 s relative to the prestimulus 1 s, indicating a significant reset effect. Both theta power and peak frequency were stronger in the theta...
state compared with those in the nontheta state. These data show that on an individual trial basis, theta frequency and power are unaltered by MR stimulation, but that the timing of the pulse has a significant effect on subsequent theta phase since the average waveform shows a net component of the reset rhythm.

To evaluate whether this rhythmicity expressed post-MR stimulation showed a similar laminar voltage and CSD profile to spontaneous theta, we conducted similar experiments using the multiprobe. As previously shown (Buzsáki et al. 1986; Wolansky et al. 2006), depth profiles through CA1 and the molecular layer of the dentate gyrus ($n = 4$; Fig. 8E) during spontaneous theta demonstrated a voltage maximum near the level of the hippocampal fissure. This zone was also the location of the largest sink-source alternations within the CSD (Fig. 8F). Plotting the average laminar profile post-MR stimulation revealed a voltage and CSD profile identical to those of spontaneous theta (Fig. 8G). The large sink in the hilar region represents the transient-evoked potential. This may be indicative of the massive innervation of the hilar interneurons from the MR (Vertes et al. 1999), although further study is needed to determine this conclusively.
Phase dependence of hippocampal theta field reset

Even within the theta brain state, variability in cellular response following MR pulse was noted. To gain insight into the possible mechanism underlying this response “jitter” we quantified the network response to MR pulse across the 360° theta phase angle. The magnitude of the reset activity was determined in six 60° bins according to the phase when the MR pulse was delivered. To determine instantaneous phase, the data were band-pass filtered (2–10 Hz) and subjected to a Hilbert transform (Matlab). Five animals were used for this analysis and data were averaged across all animals and plotted as a function of the phase when the MR pulse was administered. Figure 9, A and B demonstrates that when MR pulses arrived at certain phases of the oscillation, reset was strong, whereas other phases were followed by less predictable phase values and a weaker reset. Overall, as shown in Fig. 9, C and D, when the pulse occurred near the peak of theta recorded from the lacunosum molecular layer of CA1, reset was weaker than when it

FIG. 5. Hippocampal theta-OFF cells are inhibited with high-frequency MR stimulation. A: phasic theta-OFF cells fire at reduced rates during theta compared with nontheta. Stimulation of the MR during nontheta, when the cell was firing at its highest rate, produced a decrease in discharge rate, followed by poststimulation rebound excitation. Scale bar is 1 s. The period of MR stimulation is indicated below with the black bar and visualized by the appearance of the 100-Hz stimulation artifact. B: autocorrelation function for an OFF cell is shown during theta (left) and nontheta (right). Although the cell fires at reduced rates during theta, it fires rhythmically at 3.5 Hz, whereas during nontheta states no rhythmicity is noted. Not shown is the autocorrelation at higher frequencies of spontaneous theta, where the cell was for the most part inhibited. C: the ISI histogram for a phasic theta-OFF cell shows a bimodal distribution of spikes, indicating rhythmic firing at theta frequencies (at 250 ms) and another cluster of spikes that fired at 60 ms, representing the intraburst spikes. D: cross-correlation between a phasic theta-OFF cell and the trough of the local field potential recorded from stratum lacunosum of CA1 during theta (left) and nontheta (right). This cell fires preferentially on the falling phase of the theta wave just prior to the trough. During nontheta no relationship is exhibited despite the increase in firing rate. E: example of raw data during MR stimulation from the cell in A, which shows how cells could be extracted based on the shape of the spike. F: grand means of cell discharge rates for nontheta (NT), theta (T), and MR stimulation at 100 Hz (MR) for all OFF cells tested. The firing rate during nontheta was significantly higher than that during theta, following MR stimulation, or during MR stimulation (black line) (*P < 0.05).
occurred progressively closer to the trough \[ F(5,24) = 4.76, P = 0.003 \]. Therefore theta phase (in addition to brain state) modulated the response to MR stimulation.

**Effect of slow-frequency (0.5-Hz) stimulation on HPC theta-related cellular activity**

Thirty-eight cells in the hippocampal formation, classified as phasic theta-on, were tested in response to 0.5-Hz MR stimulation. Twenty-two of these cells were also tested during 100-Hz stimulation of the MR and were included in the previous analysis. Of the 24 phasic theta-on cells tested with 0.5-Hz stimulation, 23 (94%) responded with a short-latency (5.2 ± 1.8 ms) inhibition lasting 68.3 ± 9.0 ms (Fig. 10A). Further analysis revealed that despite the common response of short-latency inhibition, some of the cells showed phase reset as demonstrated by oscillatory responses to stimulation (oscillatory responders, red throughout Fig. 10), whereas others did not (nonoscillatory responders, blue throughout Fig. 10). This phase reset was found to be state dependent (Fig. 10A). Figure 10A shows PETHs and dot raster displays for one example of a nonoscillatory responder (top) and two examples of oscillatory responders (middle and bottom) during theta (Fig. 10Al) and nontheta (Fig. 10Aii), respectively. All cells were initially inhibited, but nonoscillatory responders did not exhibit the reset in the phase of oscillation. The autocorrelations of PETHs for oscillatory responders indicated that reset occurred only during the theta state (Fig. 10Aiii). FFTs of the PETH-generated autocorrelations demonstrated a significant theta peak, indicating a rhythmic process (Fig. 10Av). The horizontal gray line in the FFTs indicates the \( P < 0.05 \) significance level and was used to assess whether a cell was a nonoscillatory or oscillatory responder. The superimposed gray line in Fig. 10Av is the FFT generated for the nontheta PETH.

Oscillatory responses were observed in 14 cells, biphasic responses were observed in 4 cells, 5 cells responded with pure inhibition, and one cell with excitation. Therefore phase reset occurred in 14/23 cells tested. We analyzed whether the firing rate, phase of firing, or interspike interval (ISI) could determine which cells would respond in an oscillatory versus nonoscillatory pattern. Figure 10B shows an example of the discharge patterns of phasic theta-on cells during 0.5-Hz MR stimulation, superimposed on hippocampal theta field activity. The overall firing rate of nonoscillatory cells was not changed during the MR single pulse during theta (6.7 ± 1.0 vs. 6.1 ± 1.3 Hz) or nontheta (1.9 ± 0.5 vs. 1.8 ± 0.4 Hz, Fig. 10C) relative to spontaneous (SP) conditions. Likewise, the firing rate of the oscillatory responding cells was also not changed with the MR pulse during theta (8.6 ± 0.8 vs. 9.0 ± 0.8 Hz) or nontheta (2.8 ± 0.5 vs. 3.7 ± 1.3 Hz, Fig. 10C). Furthermore, there was a nonsignificant increase in the firing rate of oscillatory responders relative to nonoscillatory responders. The phase re-

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**FIG. 6.** A: a representative nonrelated hippocampal cell firing during theta, nontheta, and during an epoch of high-frequency MR stimulation. Note the lack of an effect on the cell’s discharge rate during either stimulation or poststimulation. Scale bar is 1 s. The period of MR stimulation is indicated below with the black bar and visualized by the appearance of the 100-Hz stimulation artifact. B: autocorrelations for a nonrelated cell during theta (left) and nontheta (right) are shown, demonstrating a lack of periodicity in the firing pattern during both states. C: cross-correlations between a nonrelated hippocampal cell and the trough of the local field potential recorded from stratum lacunosum of CA1 during theta (left) and nontheta (right) demonstrate the lack of the consistent phase relationship in either state. D: histogram of group data for all nonrelated cells showing the discharge rates for the 3 states of nontheta, theta, and 100-Hz MR stimulation.
relationship and ISI during spontaneous theta were calculated and compared between those cells exhibiting oscillatory and nonoscillatory response patterns. Nonoscillating responding cells ($n = 9$) formed a homogeneous group in terms of spontaneous theta phase relation, suggesting they constituted a unique cell population. However, oscillatory responders could fire prior to ($n = 5$) or following trough ($n = 9$, Fig. 10D), demonstrating that phase of firing did not entirely predict the MR response. The ISI was found to more accurately predict the frequency of the stimulus burst. As the frequency increased, a reduction in power was noted due to the fact that the stimulus train began to approximate a 100-Hz pattern (D). E: quantification of the difference in hippocampal response between 100-Hz stimulation and TBS, which restores overall signal power, theta power, and peak power back to baseline values. F: the power of hippocampal field activity with TBS changes as a function of burst frequencies, with maximal power occurring between 4 and 6 Hz, whereas higher frequencies reduce power to sub-baseline values. Both theta power (dark line) and peak power value (light line) show similar trends. The broken line indicates the normalized value of spontaneous hippocampal theta power. *$P < 0.05$.

Tonic theta-on cells ($n = 7$) did not significantly alter their firing rate during the MR pulse (0.5 Hz) compared with spontaneous (SP) theta or nontheta conditions (Fig. 11Ai). In addition, these cells did not exhibit a stereotyped response to 0.5-Hz MR stimulation. One cell was not altered whereas another two displayed inhibition, three displayed a biphasic response, and one exhibited excitation during theta activity. The five cells exhibiting inhibition did so with a 6.5 ± 1.7-ms latency and 79 ± 9 ms duration. Figure 11Aii shows an example of a biphasic cell response and an inhibitory cell response to 0.5-Hz stimulation. Raster plots and PETHs for the biphasic responding tonic theta-on cell and the inhibitory responding tonic theta-on cell during 0.5-Hz MR stimulation for the theta and nontheta states are shown in the top and bottom panels of Fig. 11Aiii, respectively. Spikes were accumulated in 5-ms bins for 250 ms before the stimulation (time 0) and for 100 ms following the stimulation. However, state-independent responses were typical of these cells ($n = 5/7$, $r = 0.51 ± 0.07$ between theta and nontheta PETHs). One cell changed from an inhibitory response to a biphasic response and another from no response (during theta) to inhibition during nontheta. Despite increases in spikes in the poststimulus time period during nontheta in six of seven cases, the overall response magnitude was not significantly different between theta and nontheta states (93 ± 6 vs. 137 ± 25%, $P > 0.05$).

Six phasic theta-off cells were tested with 0.5-Hz stimulation of the MR during nontheta, five of which were also tested during theta. Overall firing rates were not changed by

![Figure 7](https://example.com/figure7.png)

**FIG. 7.** Theta burst stimulation (TBS) of the MR elicits hippocampal field activity phase locked to the phase of stimulation. A: spontaneous theta occurs with a frequency of 4 Hz and an obvious rhythmic process (shown in the autocorrelation, middle) and a theta peak in the power spectrum (bottom). B: high-frequency MR stimulation (100 Hz) reduces power in the low-frequency (delta and theta) bands and eliminates oscillatory activity. C and D: changing the timing of stimulation pulses to a theta burst pattern using identical stimulation intensities reinstates the rhythmic activity, with the resulting peak frequency matching the frequency of the stimulus burst. As the frequency increased, a reduction in power was noted due to the fact that the stimulus train began to approximate a 100-Hz pattern (D). E: quantification of the difference in hippocampal response between 100-Hz stimulation and TBS, which restores overall signal power, theta power, and peak power back to baseline values. F: the power of hippocampal field activity with TBS changes as a function of burst frequencies, with maximal power occurring between 4 and 6 Hz, whereas higher frequencies reduce power to sub-baseline values. Both theta power (dark line) and peak power value (light line) show similar trends. The broken line indicates the normalized value of spontaneous hippocampal theta power. *$P < 0.05$.
the 0.5-Hz pulse relative to control spontaneous (SP) conditions, in either state (Fig. 11Bi). Two cells were inhibited, three were excited, and one exhibited a biphasic response during nontheta. The latency to inhibition was 7.3 ± 3.4 ms and lasted 92 ± 22 ms. The response magnitude during nontheta was 139 ± 27%, indicating the slight increase in cell response following stimulation, whereas the response magnitude in theta was found to be 65 ± 24% (P > 0.05). Excitations occurred at 39 ± 13 ms in four cells and were usually short in duration (59 ± 18 ms). Figure 11Bii shows an example of an inhibitory response (top) and an excitatory response (bottom). Raster plots and PETHs for a cell responding with inhibition during nontheta only is shown in Fig. 11Biii, top, and a cell responding with a biphasic response during nontheta only (Fig. 11Biii, bottom). Of the three cells that were tested under both states, one maintained a state-independent response (inhibition) and two maintained a state-dependent response. Because cFR cells did not fire at high rates during theta, two cells did not generate enough spikes for a PETH during the theta state and therefore were considered state dependent.

DISCUSSION

The data presented here demonstrate that high-frequency stimulation of the MR does not elicit hippocampal field activity typical of the nontheta (LIA, sharp wave, slow oscillation) state in the hippocampus, but rather produces a brain state consisting of an activated neocortex and small-amplitude irregular hippocampal activity. Therefore although the neocortex appears to be in the activated state with 100-Hz MR stimulation, the hippocampus does not; thus this activity is most similar to small-amplitude irregular activity (SIA) described by Vanderwolf (1972), Whishaw (1972), Vanderwolf et al. (1975), Leung et al. (1982), and more recently by Jarosiewicz et al. (2002) and Jarosiewicz and Skaggs (2004a,b). As described by Jarosiewicz and Skaggs (2004a), SIA represents a brain state consisting of an activated neocortex and small-amplitude irregular hippocampal activity. Therefore although the neocortex appears to be in the activated state with 100-Hz MR stimulation, the hippocampus does not; thus this activity is most similar to small-amplitude irregular activity (SIA) described by Vanderwolf (1972), Whishaw (1972), Vanderwolf et al. (1975), Leung et al. (1982), and more recently by Jarosiewicz et al. (2002) and Jarosiewicz and Skaggs (2004a,b). As described by Jarosiewicz and Skaggs (2004a), SIA represents a brain state consisting of an activated neocortex and small-amplitude irregular hippocampal activity. Therefore although the neocortex appears to be in the activated state with 100-Hz MR stimulation, the hippocampus does not; thus this activity is most similar to small-amplitude irregular activity (SIA) described by Vanderwolf (1972), Whishaw (1972), Vanderwolf et al. (1975), Leung et al. (1982), and more recently by Jarosiewicz et al. (2002) and Jarosiewicz and Skaggs (2004a,b). As described by Jarosiewicz and Skaggs (2004a), SIA represents a brain state consisting of an activated neocortex and small-amplitude irregular hippocampal activity. Therefore although the neocortex appears to be in the activated state with 100-Hz MR stimulation, the hippocampus does not; thus this activity is most similar to small-amplitude irregular activity (SIA) described by Vanderwolf (1972), Whishaw (1972), Vanderwolf et al. (1975), Leung et al. (1982), and more recently by Jarosiewicz et al. (2002) and Jarosiewicz and Skaggs (2004a,b). As described by Jarosiewicz and Skaggs (2004a), SIA represents a brain state consisting of an activated neocortex and small-amplitude irregular hippocampal activity. Therefore although the neocortex appears to be in the activated state with 100-Hz MR stimulation, the hippocampus does not; thus this activity is most similar to small-amplitude irregular activity (SIA) described by Vanderwolf (1972), Whishaw (1972), Vanderwolf et al. (1975), Leung et al. (1982), and more recently by Jarosiewicz et al. (2002) and Jarosiewicz and Skaggs (2004a,b).
here, the MR may have a role in the generation of these transient brain states.

In agreement with previous authors (Peck and Vanderwolf 1990; Vertes 1981; Vinogradova et al. 1999) we also demonstrate that high-frequency MR stimulation inhibits or disrupts theta. We investigated the cellular mechanisms responsible for this theta suppression. The predicted decrease in theta-related cell firing was confirmed with MR stimulation. However, off cell firing is also generally reduced with high-frequency MR stimulation, further supporting the argument that MR stimulation elicits a hippocampal state dissimilar to both theta and the nontheta state of LIA. However, off cells are more likely to respond with excitation during nontheta, indicating that different theta-related cells maintained state-dependent firing properties with MR stimulation. Further differences are found between rhythmic theta-on cells and tonic theta-on cells in that only the phasic theta-on cells are inhibited in an intensity-dependent manner. Also, the data support the conclusion that the MR modulated its effect on the hippocampus by acting specifically on theta-related neuronal circuitry, given that non-related cells are not affected by high-frequency MR stimulation. When using theta-burst stimulation, hippocampal field activity becomes locked to the burst frequency. This stimulation pattern is more physiologically relevant, given that a subpopulation of MR neurons fire in this manner during theta in vivo (Kocsis and Vertes 1992; Kocsis et al. 2006; Viana di Prisco et al. 2002) and suggests that certain cells in the MR could play a role in synchronizing hippocampal circuitry at
theta frequencies. The higher the burst frequency, the closer the stimulation approximates the high-frequency 100-Hz stimulation response (see Fig. 7). These data resemble the response of hippocampal field activity to high-frequency stimulation of the medial septum (Kramis and Routtenburg 1977) and the dentate gyrus (Bland and Vanderwolf 1972). In like manner to medial septum stimulation, it appears that the effect of high-frequency stimulation of the MR functions to reset hippocampal theta activity on a fast timescale, such that the cycle of theta oscillations is driven beyond its duty cycle and consequently inhibited. Although it has been found that high-frequency stimulation can actually inhibit local cell populations in other midbrain sites, pharmacological lesion of the MR produces hippocampal theta (Kitchigina et al. 1999; Vertes et al. 1994), suggesting that in this case MR cells are excited by this stimulation protocol. In conclusion, the utility of high-frequency stimulation (although effective for identifying structural connections between neurons) is not ideal for examining the temporal dynamics of these complex systems. It is difficult to determine whether MR stimulation exerts its theta-suppressing effect directly or whether the actions on medial septal (MS) neurons are responsible. However, single-pulse stimulation similar to that used in this study did not affect rhythmic theta cells in the MS (Assaf and Miller 1978). In the

**FIG. 10.** Cells are grouped as those that exhibited phase reset (oscillatory responders, red) and those that did not exhibit reset (nonoscillatory responders, blue). A: raster plots and peri-event time histograms (PETHs) showing the responsive to 0.5-Hz MR stimulation (at time 0) during theta (i) and nontheta (ii). All cells are initially inhibited, but nonoscillatory responders do not exhibit the reset in the phase of oscillation. The response is brain state independent in the nonoscillatory neuron and state-dependent in the oscillatory neurons. Autocorrelations of the PETH generated during theta (iii) and nontheta (iv) and FFTs from the autocorrelations (v) confirmed the presence of the oscillatory activity in the PETH of oscillatory responding cells only. The gray line in the FFTs indicates the $P < 0.05$ significance level. Physiological differences in phasic theta-ON cells determined the response to MR stimulation at 0.5 Hz. B: 2 representative spike trains superimposed on theta field activity. Top trace with blue spikes is a representative nonoscillatory responding cell and the bottom trace represents an oscillatory responding cell. Note both cells are correlated with theta phase. C: histograms of firing rates of nonoscillatory (blue) and oscillatory (red) responders during theta and nontheta conditions, showing that 0.5-Hz stimulation (MR) did not significantly change firing rates compared with spontaneous (SP) firing rates during nontheta and theta field conditions. D: nonoscillatory responders (blue) consistently fire following the trough of theta field activity ($n = 9$, top), whereas oscillatory responders (red) formed 2 groups: those firing following the trough theta ($n = 9$, middle) and those firing before the trough of theta oscillation recorded from the lacunosum molecular layer (SLM) ($n = 5$, bottom). These cell cross-correlations are averaged across all cells in each group (colored lines: the mean; black lines: the SE). E: probability density functions for ISIs for nonoscillatory and oscillatory responders in both theta (top) and nontheta states (bottom). F: median ISI is significantly different between oscillatory and nonoscillatory neurons. G: mean correlation between PETHs generated during theta and nontheta for nonoscillatory and oscillatory neurons. Nonoscillatory cells have a higher between-state correlation in the PETHs, indicating a greater state-independent response.
context of our results, it seems rhythmic hippocampal neurons are more affected by the MR stimulation than their GABAergic counterparts in the MS, suggesting differences between the MR–HPC and MR–MS pathways. In addition, we also show that the level of inhibition of hippocampal theta field activity with MR stimulation corresponds to the degree of inhibition of phasic theta on cells, suggesting a direct action on hippocampal neurons is responsible for theta disruption. Although inhibition of rhythmic activity in the MS is less prominent than that in the hippocampus, MS neurons nonetheless become arrhythmic with high-frequency MR stimulation (Assaf and Miller 1978) and therefore the MR–MS pathway may also be partly responsible for actions on hippocampal rhythmicity.

Possibly the most intriguing finding of this study is that MR stimulation resets hippocampal theta with a single pulse, indicating a rapid and precise action of MR input throughout the entire hippocampal formation, as predicted by the earlier findings of Winson (1980) and Kao et al. (1997). Certain populations of hippocampal phasic theta-on cells respond to MR perturbation by resetting their theta oscillation, whereas other cells do not. The difference between these two populations was in the ISI; oscillatory responding cells had a shorter ISI than that of nonoscillatory cells, suggesting two distinct classes of neurons. Given that ISI has been shown to differentiate certain classes of hippocampal neurons (Klausberger et al. 2003), these two subclasses likely maintain functionally different
roles in the theta-generating network. State-dependent responses of rhythmic cells (both ON and OFF) suggest that membrane potential at the time of stimulation determines the response to MR pulse. Although inhibition is usually present in both states, cell firing recovers more quickly in the cell’s preferred state (defined by the state of higher firing rate).

Theta reset has been proposed as a mechanism of stimulus processing in hippocampal networks (Givens 1996; Rizzuto et al. 2003; Williams and Givens 2003). Our findings complement the data from Vinogradova et al. (1999) who found that sensory responses and theta phase reset of hippocampal neurons to acoustic stimulation were lost following MR lesions with procaine. Medial septal lesions did not abolish this sensory stimulation-induced inhibition. These earlier studies as well as the data presented here suggest that the MR projection to the hippocampus is involved in resetting hippocampal theta oscillation via inhibition of theta-generating neural circuitry. Without intracellular recordings and labeling we cannot determine the precise cell type and synaptic action responsible for this inhibition, given that both pyramidal and interneurons maintain phase relations with hippocampal theta under urethane (Bland et al. 2002; Klausberger et al. 2003, 2004, 2005). However, it has been shown that inhibition-based reset is the likely candidate for theta reset, rather than excitation (Cobb et al. 1995). The wiring of hippocampal networks places interneurons in an ideal position to influence entire cell ensembles simultaneously via inhibition (Buzsáki 2002; Freund and Buzsáki 1996). MR afferents selectively innervate cholecystokinin (CCK)-containing interneurons while avoiding those containing parvalbumin (PV) (Freund et al. 1990; Halasy et al. 1992; Miettinen and Freund 1992), the activation of which could transiently silence the entire theta-generating network causing theta phase reset. Given the discrete localization of 5-HT$_3$ receptors on CCK-positive interneurons (Freund 2003; Morales and Bloom 1997), serotonin (or some other neurotransmitter) could excite these cells (Staubli and Xu 1995), causing inhibition of both PV and principal cell subtypes (Gulyas et al. 1996). In the current experiments we are unable to discern whether the cells recorded contained CCK or PV because juxtaglomerular labeling was not performed. Many experiments remain to determine the primary pathway, neurotransmitter, and synaptic contacts used by MR afferents to reset the hippocampal theta rhythm.

Pharmacological evidence presented by Kinney et al. (1995, 1996) indicate that the MR functions to inhibit theta activity. Serotonergic MR lesion or glutamatergic antagonism (within the MR) produce theta at fast latencies. GABAergic MR manipulation within the MR produces hippocampal theta at various latencies and frequencies depending on the receptor subtype that is inhibited (Li et al. 2005). Although complete lesion of the MR produces theta for extended time periods, hippocampal theta following MR lesion is fundamentally different from normally occurring theta (Maru et al. 1979), indicating that different cell populations within the MR may serve divergent roles in regulating hippocampal theta activity. In the current experiments the fast conduction velocities (latency to inhibition ranging from 2 to 7 ms) suggest either an effect mediated by the 5-HT$_3$ receptor or by a nonserotonergic mechanism (Srebro et al. 1982; Winson 1980). Preliminary data from our lab (unpublished results) and others (Varga et al. 2007) indicate a nonserotonergic component mediates this MR-induced inhibition. Crunelli and Segal (1985) described putative nonserotonergic MR projections to both the medial septum and hippocampus from fast-firing MR neurons. Viana de Prisco et al. (2002) also show that 12% of MR neurons are fast-firing neurons and are phase-locked to theta, although it remains to be determined whether these cells function as local raphe circuits or hippocampal projection cells. Kiss et al. (2002) described a glutamatergic pathway from the MR to the supramammillary nucleus (SUM). Given the role the SUM is thought to maintain in determining theta frequency (Pan and McNaughton 2004), this pathway may prove to be an important component in the MR modulation of hippocampal theta rhythm. Our data also suggest that the MR may predominantly influence hippocampal networks in the type 2 theta frequency band (see Fig. 7) because TBS preferentially drove hippocampal theta at lower theta frequencies. Experiments in freely moving animals will assist in testing this hypothesis.

In conclusion, we provide new insights into the role of the MR in regulating single-cell and network activity in the hippocampus. High-frequency stimulation disrupts hippocampal theta via a short-latency inhibition of theta-related neural circuitry, most specifically phasic-ON cells. This brief inhibition is most likely accomplished by CCK and CB-positive interneurons that could reset entire hippocampal networks to a common phase of activity. The MR therefore exerts an important input to the hippocampus, possibly facilitating information processing. MR-induced theta reset may function as a mechanism of significance signaling by the hippocampus used to process behaviorally relevant sensory stimuli. The response to stimulation is dependent on brain state, cell type, and theta phase, which highlights the complexity of median raphe–hippocampal circuitry. Future work should focus on temporal dynamics of MR-mediated inhibition between subclasses of hippocampal cells in freely moving rats and determine the pharmacological mechanism mediating theta phase reset.

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