

# Restoring Theta-Like Rhythmicity in Rats Restores Initial Learning in the Morris Water Maze

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**ABSTRACT:** Neural activity often becomes rhythmic during mental processing. But there has been no direct proof that rhythmicity, *per se*, is important for mental function. We assessed this issue in relation to the contribution of hippocampal theta-frequency rhythmicity to learning in the Morris water maze by blocking theta (and other septal inputs to the hippocampus) and then using electrical stimulation to restore rhythmicity. We injected tetracaine into the medial septal area, and so blocked septal input to the hippocampus in rats throughout 16 consecutive trials in a Morris water maze. Rats with no hippocampal theta also showed no initial learning in the maze. Theta rhythmicity in the supramammillary area remained after septal blockade, and we used this to trigger electrical stimulation of the fornix superior. This substantially restored hippocampal theta-like rhythmicity throughout training at a normal frequency but with abnormal wave forms. This treatment applied throughout training substantially restored initial learning. Fixed frequency (7.7 Hz) stimulation produced rhythmic activity and a brief improvement in learning. Irregular stimulation with an average frequency of 7.7 Hz produced little rhythmicity and little improvement in learning. These results demonstrate that brain rhythmicity, *per se*, can be important for mental processing even when the detailed information originally carried by neurons is lost and when the reinstated pattern of population firing is not normal. The results suggest that the precise frequency of rhythmicity may be important for hippocampal function. Functional rhythmicity needs, therefore, to be included in neural models of cognitive processing. The success of our procedure also suggests that simple alterations of rhythmicity could be used to ameliorate deficits in learning and memory. © 2006 Wiley-Liss, Inc.

**KEY WORDS:** hippocampus; supramammillary area; septum; fornix; electrical stimulation

## INTRODUCTION

“Despite extensive work on the behavioral and physiological correlates of brain rhythms, it is still unresolved whether they have any important function in the mammalian cerebral cortex” (Sejnowski and Paulsen, 2006). A function for rhythmicity is suggested, but not proved, by the correlation of changes in rhythms with changes in task-related stimuli or responses. Likewise, neuronal blockade can alter rhythms and concurrently alter function. But such blockade affects not only rhythmicity

itself but also adjacent nonrhythmic neurons. Blockade of rhythmicity also eliminates the information contained in the spatial, and within-burst, pattern of firing of the population of rhythmic neurons. These additional changes produced by blockade could be the cause of the changed function. Critically, rhythmicity need not have a function, since synchronous oscillation may be “a continuum property of large fields of interconnected cells” (Robinson et al., 1998). Rhythmicity could, then, consistently accompany, and its parameters vary with, cognitive processing without necessarily contributing to it. However, in this article, we show that restoration of hippocampal theta-like rhythmicity using a simple “brain bypass”, which does not restore neural connectivity, restores at least some information processing by the hippocampus in a spatial task.

A very large literature exists that correlates the presence of hippocampal theta in animals, and rhythms such as frontal midline theta in humans, with one or more functions. But even when, for example, learning rate can be predicted from the EEG (Berry and Thompson, 1978), correlation alone cannot demonstrate cause with the observed theta being potentially the result of the associated functions rather than their cause or potentially being driven by some prior process that also results in the associated function. Careful monitoring of the EEG has allowed the demonstration that “if animals were given [learning] trials only when a computer analysis verified a predominance of slow-wave oscillation at theta frequencies, they learned in half as many trials as animals trained during nontheta hippocampal activity” in a simple eye blink classical conditioning task (Seager et al., 2002). But even in this case, there need be no causal link with theta. Theta production is generally associated with arousal and attention and these could mediate the improved learning.

Attempts to provide direct evidence for a functional role of theta have depended on the use of neuronal manipulations to alter theta and concomitant measures of changed function. For example, electrolytic lesions of the septum that abolish theta rhythm impair spatial learning, while similar sized lesions that do not abolish theta leave spatial learning intact (Winson, 1978). However, such theta-blocking lesions cause damage to a range of ascending systems (including the monoamine inputs to the hippocampus) and, in Winson’s experiment, the theta-sparing lesions, which were displaced from the midline, spared such systems unilaterally.

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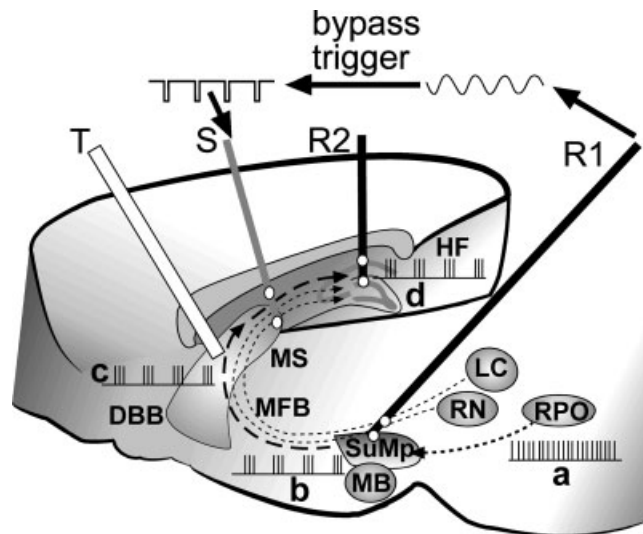
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There are problems even with more specific manipulations that block theta. Cytotoxic lesions of the septum can spare fibers but will affect neurons in addition to those solely involved in the control of rhythmicity. Injection of specific antagonists can impair theta and behavior and might possibly affect only neurons involved in the control of theta (Givens et al., 1992; Givens and Olton, 1994; Asaka et al., 2000). However, such manipulations will not only eliminate rhythmicity; they will eliminate any specific information being carried by the spatial pattern or rate of firing of the affected neurons. Elimination of the spatial pattern of neuronal firing is possibly not a problem when the frequency of theta is reduced by injection of a benzodiazepine receptor agonist into the supramammillary nucleus. This can alter both theta and behavior as much as systemic injection of the same drug (Woodnorth and McNaughton, 2002). However, even in this case, the anatomy of the area (Pan and McNaughton, 2004) leaves open the possibility that adjacent nontheta neurons were affected. Critically, even if the only neurons affected were those controlling theta and their spatial pattern of firing was intact, the reduction in theta frequency will have also reduced total impulse traffic. There is, then, no definitive evidence at present that changing theta rhythmicity, per se, alters cognitive function.

So, in the current experiments, we assessed the contribution to learning of hippocampal theta with a novel “brain bypass” technique (Fig. 1). Rhythmic cells in the medial septal (MS) area (Sailer and Stumpf, 1957; Petsche and Stumpf, 1960; Petsche et al., 1962) provide a “pacemaker” input for hippocampal theta. Our first step was to block the septohippocampal pathway in the MS, while still allowing stimulation of the fibers passing between the septum and the hippocampus in the fornix superior. The MS receives rhythmic input from the supramammillary area which converts increasing levels of diffuse nonphasic input from reticular areas into increasing frequencies of phasic theta-pattern output to the MS (Kirk and McNaughton, 1991; Kirk and McNaughton, 1993; Kocsis and Vertes, 1994; Kirk et al., 1996; Kocsis and Kaminski, 2006), probably as a result of simple recurrent inhibition (McNaughton et al., 1995). This provides a location from which theta rhythmicity can still be recorded after MS blockade of hippocampal theta. We fed such recordings into a trigger circuit controlling the stimulation of septohippocampal fibers. This bypassed the MS block and reinstated hippocampal rhythmicity. We also assessed the effects of stimulation at a fixed frequency in the middle of the normal theta range (and somewhat higher than that produced by the bypass) and controlled for any nonrhythmic effects of our electrical stimulation by the use of randomized stimulation of the same average frequency as the fixed stimulation.

Our procedure restored hippocampal rhythmicity but not the spatial or precise temporal distribution of the firing of the neurons. Our prediction was that, nonetheless, this would partially correct the hippocampal dysfunction produced by blocking rhythmicity and we tested this on learning in the Morris water maze (Morris, 1984), which is particularly sensitive to hippocampal lesions (Morris et al., 1982).



**FIGURE 1.** Schematic representation of the organization of the bypass. Injection of tetracaine (T) into MS blocks phasic (c) and other inputs (e.g., from LC and RN) to the hippocampal formation (HF), which would otherwise show phasic activity (d). Continuing theta activity (b) is recorded by electrode pair R1 from SuMp, which converts tonic input (a) from areas such as RPO into phasic output that is sent via MFB to MS and DBB. The wave peaks in the output from R1 are each converted (bypass trigger) to a pulse of stimulation of the inputs to HF through electrode pair S. Resultant activity in HF is recorded through electrode pair R2. Output from HF (not shown) can then produce phasic activity in MB. Abbreviations used: DBB, diagonal band of Broca; LC, locus coeruleus; MB, mamillary bodies; MFB, medial forebrain bundle; MS, medial septal nucleus; RN, raphe nuclei; RPO, nucleus reticularis pontis oralis; SuMp, parvicellular portion of the supramammillary area (Pan and McNaughton, 2004).

## METHODS

### Animals

Thirty-one male Sprague Dawley rats were included in the present experiment. Additional rats which did not show a sufficient tetracaine block of hippocampal theta or did not have clear supramammillary theta were used in a separate experiment, not reported here. They were obtained from the University of Otago Department of Laboratory Animal Sciences and allowed to acclimatize to the laboratory for at least 10 days. They were all anesthetized with ketamine (75 mg/kg, 100 mg/ml, Parnell Laboratories, New Zealand) and domitor (0.5 mg/kg, 1 mg/ml, Novartis Animal Health, Australia) and stereotactically implanted with bipolar twisted wire (70  $\mu$ m diameter) electrodes and a guide cannula (Plastics One, Roanoke, VA 24022), using aseptic surgical procedures. One bipolar recording electrode was aimed at the CA1/dentate layers of the hippocampus (AP  $-3.8$  mm, M-L  $-2.5$  mm, D  $-3.5$  mm, tip separation 1.0 mm) and a second (AP  $-4.8$  mm, M-L  $-0.9$  mm, D  $-9.4$  mm, tip separation 0.5 mm,  $6.0^\circ$  from vertical)

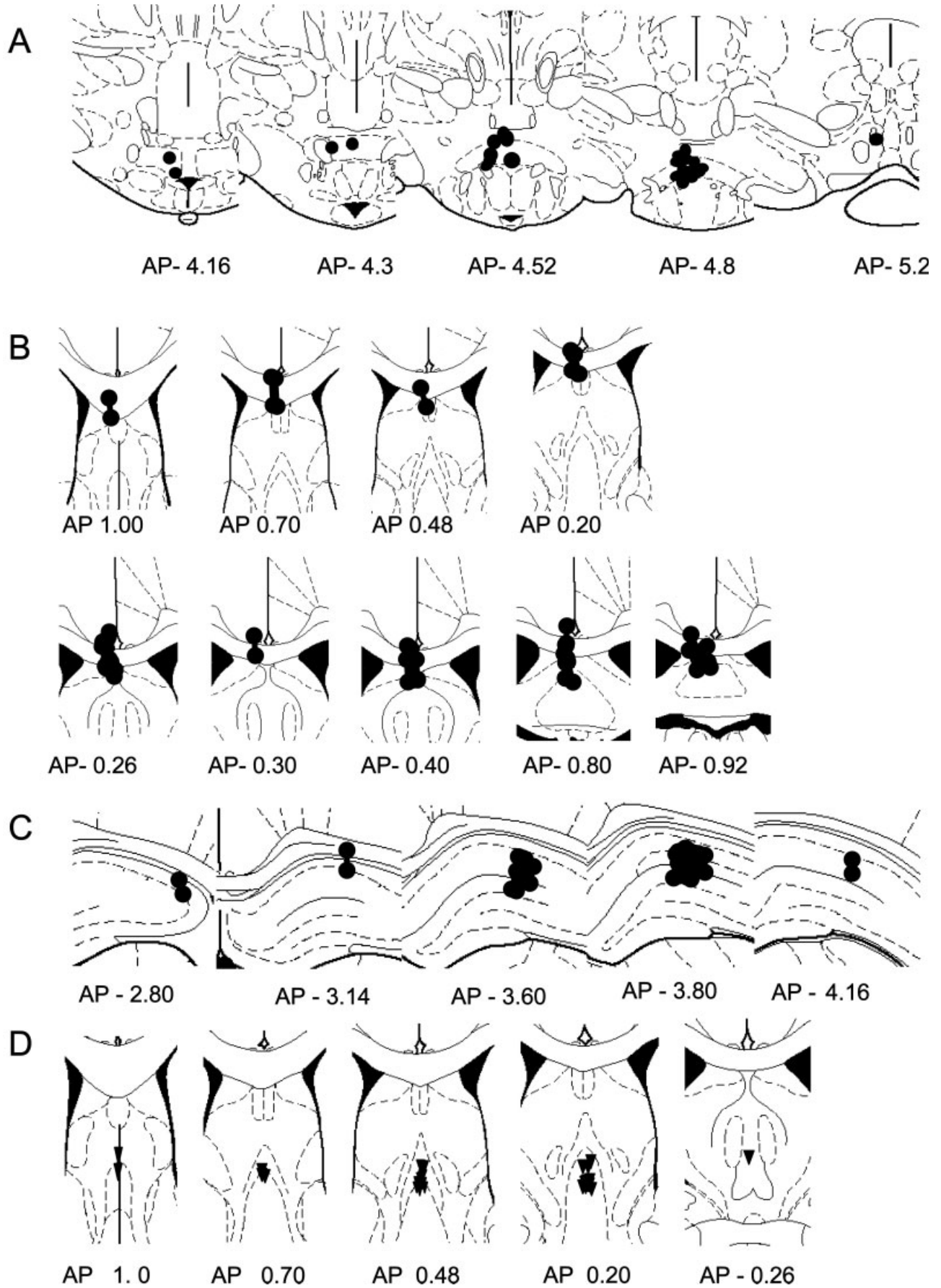


FIGURE 2.

at the parvicellular supramammillary nucleus (Pan and McNaughton, 2004). A stimulating electrode was aimed at the fornix superior (AP  $-1.0$  mm, M-L  $-1.0$  mm, D  $-4.00$  mm, tip separation  $0.5$  mm,  $8.0^\circ$  from vertical) and a 26-GA cannula guide at the MS area (AP  $+0.2$  mm, M-L  $-1.04$  mm, D  $-5.91$  mm,  $10.0^\circ$  from vertical). All coordinates were with reference to the skull surface, leveled between lambda and bregma, with electrode tracks approximately in the plane of section of the stereotaxic atlas of Paxinos and Watson (1986). A ground electrode consisting of a length of uninsulated silver wire ( $0.25$  mm diameter) was wound around a stainless steel skull screw. Five other skull screws acted as anchors. All electrodes were inserted via Amphenol gold pins into a McIntyre miniconnector that was secured to the skull and screws with dental cement. Antisedan ( $2.5$  mg/kg,  $5$  mg/ml, Novartis Animal Health, Australia) was administered to hasten recovery from anesthesia and this was followed by postoperative analgesic administration. The rats were then allowed to recover for at least 10 days before any electrophysiological or behavioral testing. The University of Otago Animal Ethics Committee approved these manipulations and the experimental procedures described below.

## Procedure

### Electrical recording

Two channels of gross electrical activity (EEG) were recorded from the bipolar electrodes located in the region of the supramammillary nucleus and in the hippocampus, respectively. Electrodes were connected via the McIntyre connector to a cable and a source follower, amplified (Grass P511K, 1–30 Hz band pass filter), and extracellular field activity was digitized at 100 Hz for subsequent analysis. Recording was started in advance of any other procedures and continued until after testing was completed and, in general, hippocampal electrical activity had completely recovered.

### Blockade of theta activity

All rats, except the controls, received injections of  $0.5 \mu\text{l}$  2% tetracaine hydrochloride (Sigma) over 1 min through a 33-GA internal cannula inserted into the guide cannula and connected to a Hamilton microsyringe with an electronic drive (Razel Sci-

entific Instruments, Stamford, CT). The effects of injection on hippocampal activity were monitored and electrical stimulation was tested only once the hippocampal record had no observable rhythmic activity.

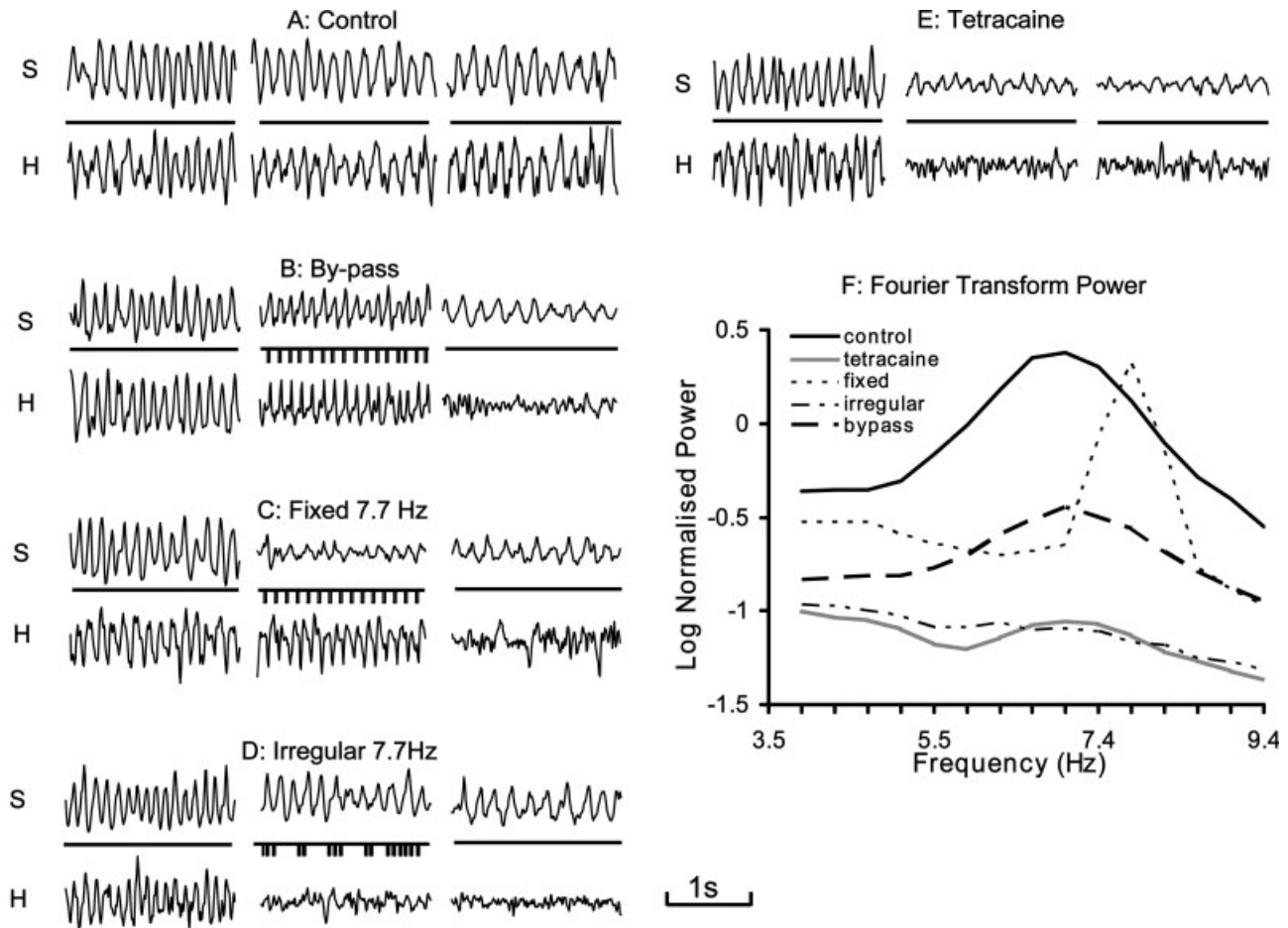
### Electrical stimulation

The threshold stimulating voltage for driving theta (James et al., 1977) at 7.7 Hz (one 0.5-ms pulse, 2–10 V, per cycle) was then determined for the fixed and irregular groups and for driving via the bypass circuit for the bypass group. The “tetracaine” group received no stimulation. For irregular stimulation, a computer used a random number generator to generate trains of pulses with an average frequency of 7.7 Hz (see trigger pulses in Fig. 3D). For the bypass circuit, the already amplified EEG signal from the supramammillary electrode was fed not only into the computer but also, in parallel, into a low gain amplifier that allowed additional low pass filtering of the signal and adjustment of the DC offset. The output from this amplifier was fed into a trigger circuit controlling the stimulator and the amplification and DC offset adjusted so that trigger output occurred close to the peak of each supramammillary wave. This second stage of amplification (which only affected input to the trigger circuit) needed to be adjusted since, in some animals, the supramammillary signal was attenuated by tetracaine injection (Fig. 3C). Once clear triggering was established, stimulation was switched on and the strength (one stimulation pulse per trigger pulse, as for regular and irregular stimulation) was adjusted until the waveforms, monitored on an oscilloscope that was triggered in parallel with the stimulator, showed phase locking or “theta driving” (James et al., 1977). In some animals, the waveform obtained was more typical of an evoked potential than of a driven theta wave. For stimulated animals, the stimulation was allowed to run continuously during behavioral testing with the form of the evoked waveforms monitored on an oscilloscope. Occasional small reductions in stimulation voltage were made if large evoked potentials appeared and small increases in stimulation voltage were made if the size of the initially evoked waveforms decreased or if driving ceased. Failure of driving was more common than the occurrence of large potentials as we wanted to ensure that seizures were not generated.

### Water maze

Water maze testing was conducted while continuing electrical recording. It followed the single day protocol of Pan and McNaughton (1997). The water maze consisted of a rigid black plastic pool (150 cm in diameter, 35 cm high) and was placed in a testing room surrounded by various extramaze visual cues, such as a door, tables, lamps, and instruments. The pool was filled to a 25 cm depth with  $26^\circ\text{C}$  ( $\pm 2^\circ\text{C}$ ) water. A black plastic platform (15-cm square) was placed 1.5 cm beneath the water surface in the center of the southeast quadrant. The rat was placed in the pool facing the wall and allowed 60 s to find the escape platform. If it found the platform within the given time, it remained there for 15 s, otherwise it was guided to it

**FIGURE 2.** Placement of electrodes and cannulae according to the atlas of Paxinos and Watson (1986). **A:** Placement of supramammillary electrodes. Each rat had a bipolar electrode aimed at SuM with one pole acting as the active electrode and the other pole as the passive reference electrode. For each rat, the tip closest to the supramammillary area is shown. The second pole of the pair is omitted for clarity. **B:** Placement of medial septal cannulae. **C:** Placement of fornix stimulating electrodes. Each rat had a bipolar electrode (dots joined by straight lines) aimed at the fornix superior and polarity of stimulation was selected to optimize driven theta. **D:** Placement of hippocampal recording electrodes. Most of the bipolar pairs (shown with dots joined by straight lines) have one pole in area CA1 and one pole in the DG.



**FIGURE 3.** Electrical activity recorded during the experiment. A–E: Samples of typical raw records from the supramammillary (S) and hippocampal (H) areas. Column 1 shows activity in the baseline period immediately before tetracaine injection (individually scaled to match each other; later traces from the same electrode are the same scale as the first trace). Column 3 shows activity after water maze testing and without stimulation—showing the posttetracaine EEG that would have been produced during training in the absence of stimulation. Column 2 shows activity during water maze testing, demonstrating the effects of electrical stimulation. The occurrence of stimulation pulses is shown in the top trace of each set. F: Fourier power from hippocampal recording electrodes, averaged over the entire period of testing for the first four trials of the water maze. Power was normalized for each rat to an average over all frequencies of 1.0 for its baseline recording period immediately before the administration of tetracaine and was log transformed prior to ANOVA. A log power value of zero for any frequency in the graph therefore is equivalent to the average power across all frequencies in the range before treatment. For the control data plotted in the graph, the average log normalized power across all frequencies was  $-0.10382$  and so overall power

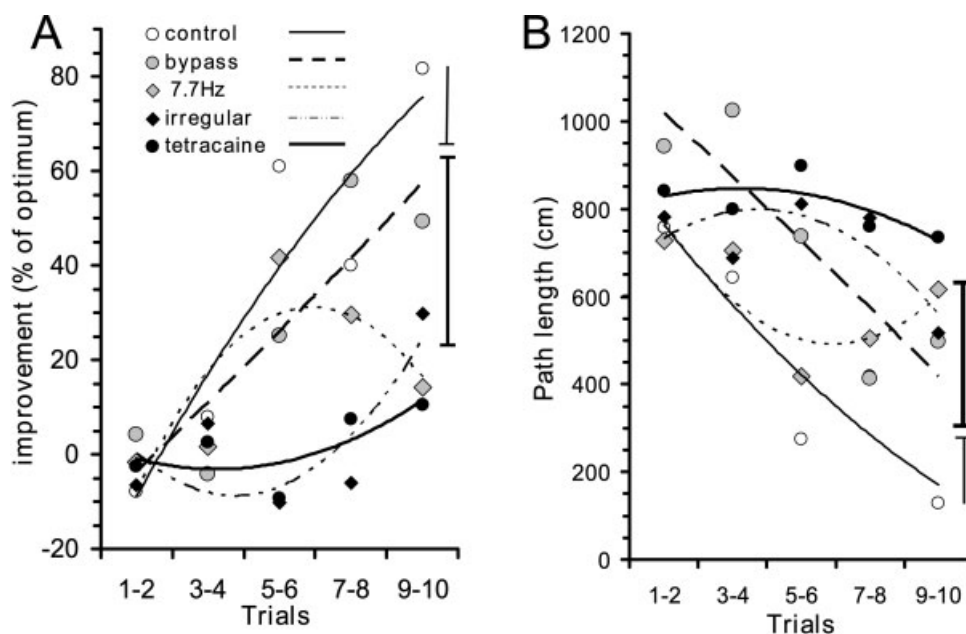
(ignoring frequency) during swimming was 79% of overall power of the same rats, when they were showing exploratory behavior prior to water maze testing. A value of  $-1.0$  represents a power that is 10% of this frequency-independent average before treatment; a value of  $0.5$  represents a power that is 320% of the average power before treatment and the peak power for the controls (obtained at 7.1 Hz) was 240% of the average power across all frequencies in the range before treatment. *Control:* rats ( $N = 5$ ) implanted with a cannula and electrodes but not receiving tetracaine or electrical stimulation. All other groups received tetracaine injections that blocked hippocampal theta. *Tetracaine:* rats ( $N = 7$ ) receiving no electrical stimulation. *Bypass:* rats ( $N = 7$ ) with supramammillary recording passed through a trigger circuit to stimulate the fornix superior and reinstate hippocampal rhythmicity; note the similar form of the spectrum to that in controls. *Fixed:* rats ( $N = 6$ ) receiving stimulation at 7.7 Hz with a fixed regular stimulation interval; note the very sharp spectral peak resulting from the one-to-one correspondence of stimulus pulse to driven wave or evoked potential. *Irregular:* rats ( $N = 6$ ) receiving stimulation at an average of 7.7 Hz in an irregular pattern.

and it stayed there for 15 s. The next trial then started with no intertrial interval as in previous single day and multiday tests in our laboratory (Pan and McNaughton, 1997, 2002). All rats were released from the north location on the first trial and then followed a counterbalanced sequence over 16 consecutive trials (NSWE ENSW WENS SWEN). The swim path was recorded and the swim distance, speed, and escape times were analyzed

later by a computer image analysis system (HVS IMAGE, UK).

### Overall testing procedure

In days prior to behavioral testing, the rats were screened for electrical recording. This involved handling them and placing



**FIGURE 4.** Effects of the bypass and other treatments on learning in the Morris water maze. The curves show the linear plus quadratic orthogonal polynomial functions extracted by ANOVA and which provide the basis for assessing statistical significance in the text. The thinner single ended error bar represents one standard error for within group comparisons of individual means. The thicker double ended error bar represents two standard errors for between group comparisons of individual means. Note that the implied *t*-tests, while based on the relevant residuals of the

ANOVA, represent tests of lower power than the polynomial analyses reported. Groups (and rat numbers) are the same as for Figure 3. **A:** Improvement as percent of maximum; a zero score represents no change from the initial path length taken; 100% represents the shortest possible (i.e., straight line) path. This measure takes into account variation in initial swimming strategy between rats and the difference in path length for the different starting positions. **B:** Actual path lengths for comparison.

them in a metal rubbish bin with wood shavings in the bottom. Otherwise they were experimentally naïve at the start of the current experiment. Each animal was tested only once for the present experiment. They were brought into the testing room, connected to the electrical equipment, placed in the rubbish bin in which they had been pretested, recording parameters were adjusted, and about 5 min of baseline EEG were obtained while the animal was allowed to explore. Except for control rats, tetracaine was then injected (see above). Once this was effective, electrical stimulation was adjusted (see above) and, once effective stimulation was established, the animal was removed from the rubbish bin and tested in the water maze for 16 consecutive trials with the stimulation continuously switched on throughout testing (except for the control and tetracaine groups). As noted above, stimulation strength had to be adjusted during testing to maintain a normal level of rhythmicity. On completion of 16 trials in the water maze, the stimulation was switched off and the animal dried with a towel. Electrical recording continued for at least 5 min and until the hippocampal EEG had recovered from the effects of tetracaine.

## Data Analysis

### Electrophysiology

All hippocampal electrical activity from the start of the first water maze trial to the end of the fourth water maze trial was broken into multiple 1-s epochs (i.e., there were 60 such

epochs for each minute of testing) and was subjected to Fourier power transform. The resultant transforms were averaged for each rat to produce a single average power spectrum in the range of 3.91–9.38 Hz. The longest available continuous period of hippocampal theta was then selected from the pre-tetracaine baseline period for each animal and similarly subjected to Fourier power transform in 1-s epochs. The baseline spectra were then averaged over both epochs and frequency to produce a single value for overall average power. With this procedure a broad peak of moderate height and a narrow peak of much greater height would be equivalent, with the single value obtained, reflecting average power in the theta range ignoring its precise frequency composition. The power value at each frequency in the averaged power spectra for the period covering the first four trials for each rat was divided by the single power value (averaged across frequency) calculated for the baseline period for that particular rat to produce a normalized transform. Note that the power averaged across all frequencies of the resultant transform would be 100% if EEG in the water maze (during swimming) were the same as EEG in the baseline period (during exploration). The highest power value at any single frequency would therefore be well above 100%. An analysis of variance (ANOVA) was calculated in which each animal was represented by a single normalized spectrum. The power values were first log transformed to homogenize error variance and make them appropriate for parametric analysis.

## Behavior

The minimum straight-line distance from the start to the platform (which was long with North and West starting positions and short with East and South) was subtracted from that swum on each trial, so that a zero score on any trial equated with perfect (straight line) performance, independent of starting position. For each rat, the average distance swum on the first two trials was taken as a measure of chance performance to eliminate the effect of variations in swimming pattern from rat to rat. Data analysis was then carried out on percent improvement scores calculated as the difference between a particular path length and the chance path length for that rat as a percentage of the difference between chance and perfect performance. The data were submitted to ANOVA with extraction of orthogonal polynomial components.  $P < 0.05$  was considered significant. Initial significance in overall ANOVA was followed by pair-wise, post hoc, ANOVA comparisons of groups and the results of the latter are reported in the text. An analysis of raw path length gave similar statistical results (Fig. 4B).

## RESULTS

### Histology

Hypothalamic recording electrodes (Fig. 2A) all had one tip in or near the supramammillary nucleus. Rats excluded from the current experiment because of loss of hypothalamic theta after tetracaine administration usually had deeper placements in the mammillary bodies. Some included rats showed modest reductions in the amplitude of hypothalamic theta (Fig. 3).

Septal cannulae (Fig. 2B) all had tips in or fractionally above the upper part of the MS, except for one posterior placement that is likely to have been in the rostral part of the fornix superior in which fibers controlling theta travel. Rats in which tetracaine did not block hippocampal theta were excluded from the current experiment and were used in a separate study.

Fornix stimulating electrodes (Fig. 2C) mostly had their lower tip in the fornix superior and the upper tip in the corpus callosum with a few electrodes having their upper tip in the fornix superior and their lower tip in the fornix proper.

Hippocampal recording electrodes almost all had their electrodes placed so as to capture differentially the activity of the CA1 and dentate gyrus (DG) generators. The most anterior electrode appeared to be recording theta from area CA3, but this could have been the result of volume conduction from CA1/dentate.

### Electrophysiology

Control rats showed clear hippocampal theta while swimming (for a typical example see Fig. 3A). Their Fourier transform (Fig. 3F) (averaged successive epochs over the entire testing period of the first four trials) showed a clear but fairly broad (5–

9 Hz) peak (maximum power in the 7.1-Hz bin) in the theta range. Bypass occasionally produced similar activity but more often (for example Fig. 3B) produced waves with faster rise times. Partly because of this wave narrowing, and partly because variations in stimulation threshold during the experiment resulted in incomplete restoration of theta, bypass theta power was considerably less than control (Fig. 3F; control vs. bypass  $\times$  frequency, quadratic  $F = 18.1$ ,  $df = 1/140$ ,  $P < 0.001$ ). Fixed frequency stimulation at 7.7 Hz also tended to produce sharp waveforms (Fig. 3C) but was somewhat more effective and, of course, because of the fixed relation between each stimulation pulse and the resultant driven wave, had its power in a very narrow band with the main power in the 7.8 Hz and adjacent bins (Fig. 3F; bypass vs. fixed  $\times$  frequency, cubic  $F = 22.0$ ,  $df = 1/154$ ,  $P < 0.001$ ; quartic  $F = 14.4$ ,  $df = 1/154$ ,  $P < 0.001$ ). Tetracaine generally eliminated obvious rhythmic activity (for example Fig. 3E) and, while the power spectrum may have contained a slight peak (in the region of the 7.1-Hz bin) in the theta range, this was substantially smaller than in the bypass group (Fig. 3F; bypass vs. tetracaine  $\times$  frequency, linear  $F = 17.8$ ,  $df = 1/168$ ,  $P < 0.001$ ; quadratic  $F = 19.42$ ,  $df = 1/168$ ,  $P < 0.001$ ). Irregular stimulation seemed superficially no different from tetracaine (for example Fig. 3D). However, the power spectrum suggested that the superimposition of the irregular stimulation was eliminating such slight rhythmicity as remained in the tetracaine group (Irregular vs. tetracaine  $\times$  cubic  $F = 5.83$ ,  $df = 1/154$ ,  $P < 0.02$ ).

### Behavior

In the Morris water maze, the control rats showed steady learning that was close to optimal performance (Fig. 4A), by the 10th trial. Consistent with previous reports of hippocampal dysfunction, rats with a tetracaine block of the septum (but no electrical stimulation) showed little learning with only a fractional improvement by the tenth trial (control vs. tetracaine, linear  $F = 10.46$ ,  $df = 1/40$ ,  $P = 0.002$ ). Bypass rats showed surprisingly good learning relative to tetracaine rats (bypass vs. tetracaine  $F = 9.84$ ,  $df = 1/12$ ,  $P < 0.01$ ), given the incomplete restoration of rhythmicity (both over the period of learning and in terms of the conformation and average power of the waveform). However, by the end of 10 trials, they appeared to be less efficient than controls (control vs. bypass  $\times$  trials, cubic  $F = 2.91$ ,  $df = 1/40$ ,  $P < 0.1$  NS) but were somewhat better than unstimulated tetracaine rats (bypass vs. tetracaine  $\times$  trials, linear  $F = 5.1$ ,  $df = 1/48$ ,  $P < 0.05$ ).

The two final groups of rats were both injected with tetracaine and stimulated at an average of 7.7 Hz, which is in the range associated with good learning in normal rats (Pan and McNaughton, 1997). Those with regular stimulation (and restored theta-like rhythmicity) showed reasonable initial learning but by trials 7–10 became similar to those with irregular stimulation (regular vs. irregular  $\times$  trials, quadratic  $F = 4.46$ ,  $df = 1/32$ ,  $P < 0.05$ ). Rats with irregular stimulation were, in general, no better than unstimulated tetracaine-treated rats (all  $P > 0.3$ ).

## DISCUSSION

Our results show that reinstatement of a degree of synchronous low frequency phasic firing, without complete reinstatement of normal neuronal firing patterns, largely reinstates initial learning in the Morris water maze. This shows, for the first time, that phasic activity, per se, is important for cognitive processing. This would be consistent with theta activity (and perhaps other forms of cortical rhythmicity) functioning to time the passage of packets of information around cortical loops (Miller, 1991) or perform a range of more complex functions (Worden, 1992; Carpenter and Grossberg, 1993; Hasselmo and Wyble, 1997; Jensen and Lisman, 1998; Chrobak et al., 2000; Lisman and Otmakhova, 2002; Hasselmo, 2005; Lisman, 2005).

Both the bypass and 7.7-Hz stimulation reinstated hippocampal rhythmicity. But they may also have activated noradrenergic, serotonergic, and other fibers. The monoamine fibers, in particular, travel in the medial forebrain bundle and pass adjacent to the MS and so will have been blocked by MS tetracaine. Likewise, they are likely to have been activated by stimulation of the fornix. However, irregular stimulation, delivering the same overall impulse traffic as 7.7-Hz stimulation and likely greater impulse traffic than in the bypass case, did not affect initial learning. So, simple activation of the input fibers to the hippocampus, independent of the stimulation pattern, cannot account for the effects of the rhythmic stimulation. The improvements in learning produced by our bypass procedure must result from synchronous rhythmicity. It is likely that at least some of the previous reports (Gray, 1972; Deupree et al., 1982; Williams et al., 1989; Williams and Gray, 1996) of the effects of theta-driving septal stimulation on behavior (which did not use tetracaine blockade of theta) are the result of changed rhythmicity relative to controls.

In rats not subjected to interference with the control of theta, learning of the Morris water maze is normally better when theta frequency is above, rather than below, about 6.5 Hz (Pan and McNaughton, 2004). The rats receiving 7.7-Hz stimulation showed similar learning to the bypass rats over the first six trials. But they appeared to show poorer learning later. Because of the cooling effects of the water (Pan and McNaughton, 1997), the frequency of supramammillary theta was lower at the end of training than at the beginning (e.g., compare the initial and final columns of Fig. 3). By the end of training, the 7.7-Hz stimulation was, therefore, producing activity of a substantially different frequency to normal. It is possible, then, that hippocampal activity may need to be not only phasic but also of a similar frequency to the activity of other structures to be effective. Further experiments would be needed to confirm this speculation. Another possible reason why the bypass pattern was more effective than the fixed frequency stimulation is that its irregularities may have contained breaks linked to the ongoing processing of stimuli that, on a small scale, could match the phase reset of theta seen in explicit working memory experiments (Givens, 1996).

In some rats, rhythmic septal stimulation produced, as in previous experiments (James et al., 1977), driven waves indistinguish-

able from natural theta except in that they were phase-locked to the stimulus. But, even in these, the underlying cellular activity is likely to have been abnormal (Scarlett et al., 2004). In other rats, the waveforms were abnormal (e.g., Fig. 3B and to a lesser extent 3C), and so single cell activity patterns must also have been abnormal. The improvement in learning in these animals is, thus, powerful evidence for the functional importance of synchrony and rhythmicity, per se, in initial learning. The precise set of pacemaker neurons controlling phasic firing appears less important.

In a sensory pathway, particularly in the visual system, we would not expect such bypass stimulation to work. In these pathways, critical information is carried more by which particular neurons are firing at any one time than it is carried by the precise pattern of firing of any single neuron. Indeed the firing of an individual neuron is often characterized experimentally simply by its rate. The pattern of interspike intervals is frequently ignored. This is reasonable since neurons often integrate, and hence temporally blur, conductances over periods of tens or hundreds of milliseconds.

However, the phasic aspect of hippocampal theta activity is controlled by the firing of small numbers of interneurons that inhibit very large numbers of the principal neurons in the target structures (Buzsaki and Chrobak, 1995). So, it is quite likely that, at the level of the MS pacemaker, it is not crucial which particular neurons fire at any one time, provided enough fire to synchronize target neurons. Nonphasic inputs to those target hippocampal neurons, likely from the cortex, would then carry the critical information to be processed. Correct processing of information by the hippocampus would then depend on both the timing of non-specific (information poor, modulatory) release from MS inhibition and the specific (information rich) nature of excitatory inputs from areas other than MS, such as the neocortex.

Low frequency rhythmic "theta" activity occurs in the hippocampus of all species so far investigated, from pigeons to rats to monkeys to humans (Vanderwolf, 1969; Sano et al., 1970; Crowne et al., 1972; Kahana et al., 1999; O'Keefe and Burgess, 1999; Siegel et al., 2000; Tesche and Karhu, 2000; Basar-Eroglu and Demiralp, 2001; Stewart and Fox, 2001; Cantero et al., 2003; Ekstrom et al., 2005). It is therefore an ancient common feature of hippocampal operation and, at a computational level, the function that we have demonstrated for it is likely to be homologous across those species that show it. Our demonstration of a function for hippocampal rhythmicity, per se, raises the possibility that other rhythmic activity, particular frontal midline theta, may also be important for cognitive function.

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