Theta Rhythm of Hippocampal CA1 Neuron Activity: Gating by GABAergic Synaptic Depolarization

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INTRODUCTION

Synchronization of neural activity within mammalian brain structures, as occurs during hippocampal θ rhythm (O'Keefe and Recce 1993; Shen et al. 1997; Skaggs and McNaughton 1996), contributes to diverse forms of information coding (Draguhn et al. 1998; Rodriguez et al. 1999; Usher and Donnelly 1998). The frequency field oscillation, a major feature of the hippocampal electroencephalogram (EEG), for example, occurs during two specific behaviors, exploration and rapid-eye-movement (REM) sleep, and reflects synchronized synaptic potentials that entrain the discharge of neurons at frequencies between 4 and 12 Hz. The rhythm is believed by many to be a powerful way to selectively direct signal processing through the network.

METHODS

Chemicals

Agents were either injected into the recorded cells through the recording electrodes: benzolamide (gift from T. H. Maren, University of Texas at Austin). Benzolamide, a carbonic anhydrase inhibitor, also decreased place cell activity in vitro. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
of Florida, Gainesville; 0.1 mM; 0.5 nA, 500 ms at 50% on cycles for 10 min) and calecitin (260 ng/μl of cloned calecitin in 1 M K acetate, pH 7.4; ~2.0 nA, 700 ms at 33% on cycles for 15 min), or through the perfusion medium: kynurenic acid (Sigma), bicuculline methiodide (BIC; Sigma); carbachol (CHC; Sigma), acetazolamide (ACET; Sigma), and atropine sulfate (Sigma).

Hippocampal slice electrophysiology

CA1 field potentials were recorded with glass microelectrodes filled with an artificial cerebrospinal fluid solution (ACSF; see below). Male Sprague-Dawley rats (150–200 g) were decapitated, and the brains were removed and cooled rapidly in an ACSF solution (~4°C), bubbled continuously with 95% O₂-5% CO₂. Hippocampi were sliced (400 μm), placed in oxygenated ACSF (in mM: 124 NaCl, 3 KCl, 1.3 MgSO₄, 2.4 CaCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, and 10 glucose), and perfused (2 μl/min) with the oxygenated ACSF in an interface chamber at 30–31°C. Whole slices were used unless otherwise indicated. CA1 pyramidal cells were recorded intracellularly (Sun et al. 1999 for cell labeling) with sharp electrodes (3 M KAc; tip resistance: 60–120 MΩ; to prevent “run-down” of GABAergic responses in whole cell recordings due to wash out of intracellular factors). Stable GABAergic inhibitory postsynaptic response (IPSP) could thus be evoked for several hours without noticeable change in amplitudes. Signals were amplified with AxoClamp-2B amplifier, digitized, stored, and analyzed using DigiData 1200 and P-Clamp6 software (Axon Instruments). Frequency and amplitude values of oscillation were taken from an average of 5 consecutive traces, all triggered at the same level of the same phase. Capacitance was optimally adjusted during discontinuous current-clamp mode before and after cell penetration to neutralize capacitance and reduce overshoot/undershoot errors. Discontinuous single-electrode voltage-clamp mode was used for voltage-clamping, employing a sampling rate of 3.0–5.0 kHz (30% duty cycle). Gain was usually set at 6–8 nA mV⁻¹, slightly below the maximum value without causing overshoot or instability in the step response to a repetitive 10-mV step command. Bipolar stimulating electrodes (Teflon-insulated PtIr wire with 25 μm diam) were placed in s. pyramidale, within 200 μm from the recording electrode, for stimulation of interneurons (50 μA, 50 μA) in the pyramidale layer. In some cases, the position of the stimulating electrodes was slightly varied within the CA1 cell layer to obtain monophasic postsynaptic responses. Test stimuli were applied at 1 per minute (0.017 Hz). In some experiments, an additional stimulating electrode was placed in the stratum radiatum to stimulate the Schaffer collateral pathway (Sch). Experiments in which >20% variations in the evoked IPSP magnitudes occurred during the 10-min control period were discarded.

Spatial maze tasks

Effects of reducing HCO₃⁻ formation in vivo on spatial memory were evaluated in rats with Morris watermaze task (Meiri et al. 1998). Male adult Wistar rats were housed in a temperature-controlled (20–24°C) room for 1 wk, allowed free access to food and water, and kept on a 12-h light/dark cycle. On the first day of experiments, all rats were randomly assigned to different groups (10 each) and swim for 2 min in a 1.5-m (diameter) × 0.6-m (depth) pool (22 ± 1°C). On the following day, rats were trained in a 4 trial/day task for 4 consecutive days. Each training trial lasted for up to 2 min, during which rats learned to escape from water by finding a hidden platform placed in a fixed location and submerged about 1 cm below the water surface. A quadrant test was performed after removing the platform 24 h after the last training trial. The route of rats’ swimming across the pool was recorded. The number of grid crossings on record paper in each quadrant was counted and used as arbitrary swimming distance units. A single dose of ACET (5 mg/0.5 ml saline/day, freshly prepared) was injected (intraperitoneal), about 65–70 min prior to the first trial or quadrant test. The control rats received the same volume (intraperitoneal) of saline.

Statistical analysis was performed using the Student’s t-test for paired or unpaired data or ANOVA whenever appropriate. The values are expressed as means ± SE of the mean, with n indicating number of the cells or rats. All animals used in these experiments were treated under National Institutes of Health guidelines for the welfare of laboratory animals.

RESULTS

CCH-induced θ field oscillation and intracellular θ

To simulate cholinergic septal activation and diffuse acetylcholine transmission (Descarries et al. 1997), we bath applied CCH (50 μM, 20 min), a cholinergic receptor agonist, to hippocampal slices from adult rats. CCH triggered a local θ field potential (Fig. 1A; peak amplitude: 0.75 ± 0.03 mV, mean ± SE, n = 12, P < 0.05 from background noise; at 7.8 ± 0.8 Hz; n = 12), lasting for the post-CCH recording period of ~3 h (Huerta and Lisman 1995; Pitler and Alger 1992). The θ activity varied in magnitude, indicating summation of different numbers of neurons discharging in each phase. The θ activity was blocked by bath atropine sulfate (1 μM, n = 6; not shown), a muscarinic antagonist, as reported by others (e.g., Huerta and Lisman 1995), and was generated in the CA1. The CCH-induced activity (0.73 ± 0.04 mV, n = 7, P < 0.05; at 7.7 ± 0.9 Hz; n = 7, P < 0.05) in CA1 minislices, after dissection away both CA3 and dentate gyrus, did not differ (P > 0.05; unpaired t-test) from that of the whole slices. The θ oscillation frequency did not change (n = 8, P > 0.05), although the oscillation magnitude was slightly reduced, in the presence of kynurenate, an N-methyl-D-aspartate (NMDA)- and non-NMDA receptor antagonist (Collingridge and Lester 1989). Kynurenate was applied extracellularly at 500 μM (20–30 min), a concentration at which it effectively abolished excitatory postsynaptic responses of CA1 pyramidal cells to stimulation of the Schaffer collateral pathway (Sun et al. 1999) or responses of other brain neurons to L-glutamate (Sun 1996). CCH induced θ oscillation of membrane potential (7.8 ± 1.1 mV; n = 20; P < 0.05) in CA1 pyramidal cells (intracellular θ; Fig. 1B), a response blocked by bath atropine sulfate (1 μM, n = 8, P < 0.05; not shown). At one-third to one-half of the maximum depolarizing phase, action potentials were triggered (Fig. 1, B and C). During a 5-min observing period, CCH induced an averaged discharge rate of 2.7 ± 0.3 spikes/s, significantly higher (n = 20, P < 0.05) than their pre-CCH rate (0.0 ± 0.0 spikes/s). These variations were consistent with those of the field θ magnitude recorded. The intracellular θ remained unchanged when the membrane potential of the cells was maintained at their pre-CCH levels.

Involvement of GABAergic postsynaptic depolarization in the CA1 θ activities

Bath-applied BIC (1 μM) eliminated the θ field oscillation (by 97.5 ± 4.2%, n = 8, P < 0.05; Fig. 1A) and CA1 intracellular θ activity (by 98.9 ± 3.4%, n = 10, P < 0.05; Fig. 1B). When applied before the CCH application, BIC did not produce obvious changes in the field potential (n = 6) or membrane potentials of CA1 pyramidal cells (n = 8), but prevented CCH effects on the θ activity induction. At 1 μM,
The intracellular currents altered by kynurenate (500 μM, 30 min) and bicuculline application (BIC, 1 μM, 30 min). D: membrane potential traces of recorded CA1 pyramidal cells: pre-CCH (control), during CCH application (50 μM, 30 min; the membrane was slightly depolarized and action potential truncated; 2nd trace from top) and with membrane potential maintained at pre-CCH level by passing negative current (3rd trace), and during application of BIC (1 μM, 30 min). C: examples of CCH-induced intracellular θ activity in 4 different cells, shown at low amplification and without action potentials truncated.

BIC did not produce any obvious excitation of the CA1 cells. Activation of the GABA_A receptors is thus necessary for CCH to elicit synchronous CA1 field events. Suppressing GABA_A receptor channels alone is insufficient to induce θ.

The GABAergic inputs were activated by microstimulation of s. pyramidale. The evoked IPSPs in CA1 pyramidal cells depended on the membrane potentials (e.g., Fig. 3). Thus the IPSPs were always monitored with values compared at their pretest control membrane potentials. The evoked IPSPs (Fig. 2A; peak response: −8.89 ± 0.29 mV, n = 89) were not altered by kynurenate (500 μM, n = 6), but abolished by BIC (1 μM; by 96.8 ± 3.7%; n = 8, P < 0.05), indicating GABA_A receptor mediation and an absence of contamination of any obvious excitatory component in the evoked IPSPs. Associated with the θ activity was a gradual reduction in the IPSPs (n = 25) and the ultimate production of an “excitatory” response (Fig. 2A, B, and C; from pre-CCH −9.0 ± 1.2 mV as compared with +5.1 ± 0.4 mV 30 min after the CCH application; n = 10, P < 0.05). This excitatory response was observed at the pre-CCH membrane potential maintained by intracellular injection of hyperpolarizing current. These voltage changes in the GABAergic responses corresponded to a gradual change of an outward current (0.18 ± 0.03 nA) toward an inward current (0.19 ± 0.05 nA; n = 5, P < 0.05) under voltage clamp (Fig. 2C). The intracellular θ activity became evident when the GABAergic responses became depolarizing (Fig. 2A). Measured when the θ activity became evident, the input resistance (79.2 ± 1.6 MΩ) of the cells did not significantly differ (n = 10; P > 0.05) from their pre-CCH value (80.5 ± 1.4 MΩ). Depressing GABA_A responses alone was insufficient to induce the θ activity since BIC did not induce the rhythmic activity (see last paragraph). The reversed excitatory response was also sensitive to BIC (Fig. 2B), indicating the involvement of the same type of receptor channel before and after the CCH administration.

The relationship between the maximum responses of hippocampal CA1 pyramidal cells to stimulation of the GABAergic inputs and membrane potential at which the inputs were activated can be described with a straight line. BIC virtually abolished the GABAergic postsynaptic responses no matter whether the postsynaptic responses were evoked at membrane potentials positive or negative to the reversal potential (Fig. 2B). The reversal potential was −83.1 ± 2.6 mV; n = 6). This BIC effect contrasts with CCH-induced changes that were associated with a positive shift of the reversal potential (Fig. 2B; from −79.8 ± 3.2 to −68.4 ± 2.8 mV; n = 10, P < 0.05). Thus the CCH-induced changes in GABAergic responses are fundamentally distinct from a reduced response and could not result from a diminished GABAergic synaptic transmission (suppressed presynaptic release or postsynaptic response). Figure 3C illustrates an example in which the CCH-induced reversal potential appears to be above the threshold (approximately −57 mV) for generation of action potential. Thus single brief pulse of stimulation of the GABAergic inputs elicited action potential dur-
mimicked CCH in inducing the intracellular (Alkon et al. 1998; Sun et al. 1999), into CA1 pyramidal cells application of calexcitin, a memory-related signal protein involvement of intracellular carbonic anhydrase. Interestingly, $n_{12}$ to load Ca $^{2+}$ (Cobb et al. 1995; Paulsen and Moser 1998). How might been proposed to be a fundamental role of the interneurons for the conductance in an intracellular signaling cascade responsible CCH. These results indicate a critical role of HCO$_3$ $^{-}$ was also prevented by bath ACET (1 M) in six cells tested (Fig. 5C). These results indicate a critical role of HCO$_3$ conductance in an intracellular signaling cascade responsible for the $\theta$ rhythm.

Elimination of CA1 $\theta$ activities and GABA depolarization by carbonic anhydrase inhibitors

Bath ACET (1 $\mu$M), a carbonic anhydrase inhibitor, eliminated the CCH-induced changes in GABAergic postsynaptic responses (Fig. 4B). The evoked IPSP ($-7.7 \pm 1.0$ mV, $n = 12, P < 0.05$) in the presence of ACET and CCH did not differ ($n = 12, P > 0.05$) from their control values ($-7.8 \pm 1.1$ mV). Under such conditions, neither $\theta$ field oscillation ($n = 8$; Fig. 4A) nor intracellular $\theta$ activity ($n = 10$; Fig. 4B) was induced by CCH. Similarly, intracellular application of benzolamide, a membrane-impermeable carbonic anhydrase inhibitor, prevented the occurrence of CCH-induced reversed GABAergic responses and intracellular $\theta$ activity ($n = 6$), indicating an involvement of intracellular carbonic anhydrase. Interestingly, application of calexcitin, a memory-related signal protein (Alkon et al. 1998; Sun et al. 1999), into CA1 pyramidal cells mimicked CCH in inducing the intracellular $\theta$ activity (Fig. 5, A and B; $n = 12$), when associated with a depolarizing current to load Ca$^{2+}$. The calexcitin-induced intracellular $\theta$ activity was also prevented by bath ACET (1 $\mu$M) in six cells tested (Fig. 5C). These results indicate a critical role of HCO$_3$ conductance in an intracellular signaling cascade responsible for the $\theta$ rhythm.

Entraining CA1 pyramidal cells by GABAergic inputs

Entraining hippocampal pyramidal cells at $\theta$ frequency has been proposed to be a fundamental role of the interneurons (Cobb et al. 1995; Paulsen and Moser 1998). How might GABAergic interneurons entrain the pyramidal cells? The only mechanism that has been previously proposed is rebound action potential. However, rebound “depolarization” usually requires resting activity that was provided by constant current injection (Cobb et al. 1995), and hippocampal pyramidal cells normally do not show much spontaneous activity. In some cells (26 of 149 neurons in which effects of membrane potential changes on the GABAergic postsynaptic responses were examined), discharges lasted for a period of elicited depolarization and an evoked IPSP appeared to be able to delay subsequent spikes (Fig. 6A). The majority of cells (123 of 149), however, showed a rapid adaptation to depolarization (Fig. 3, A and B), resulting in a silent but depolarized state. At resting membrane potential, rebound depolarization requires very strong hyperpolarization, which naturally occurring IPSPs are unlikely to provide. No rebound action potential was observed with IPSPs of $-8.9 \pm 0.3$ mV evoked at resting membrane potentials ($-73.8 \pm 0.9$ mV, $n = 89$; Fig. 6B, trace 1). A train of pulses at 100 Hz was also ineffective (Fig. 6B, trace 2), suggesting that temporal summation of the unitary IPSPs is insufficient to evoke rebound depolarization. Furthermore, no significant rebound depolarization ($0.19 \pm 0.12$ mV, $n = 75, P > 0.05$) was evoked with intracellular negative pulses (up to 700 ms) sufficient to evoke $-10.8 \pm 1.4$ mV potential changes (Fig. 6C, trace 1) from the resting membrane potential ($-74.8 \pm 0.4$ mV). In addition, when evoked at depolarized membrane potentials, the occurrence and timing of individual “rebound” action potentials varied (Fig. 6D). Thus rebound action potentials, even when they occur, do not represent a precise control mechanism. On the other hand, in the presence of CCH, stimulation of GABAergic inputs elicited instantly phase-locked firing of pyramidal cells (Fig. 7, A and C; $n = 272$ M.-K. SUN, W.-Q. ZHAO, T. J. NELSON, AND D. L. ALKON

FIG. 2. Carbachol (CCH)-induced $\theta$ oscillations of hippocampal CA1 field potential and of membrane potential of CA1 pyramidal cells are associated with GABAergic postsynaptic depolarization. A: single pulse stimulation (50 $\mu$A, 50 $\mu$s) of the GABAergic inputs from interneurons evoked inhibitory postsynaptic potentials (IPSPs), which were gradually reduced and reversed to depolarizing responses during CCH application (50 $\mu$M; CCH), associated with increased amplitude values of $\theta$ activity. The averaged maximum IPSP values of each cell during 10-min stable recording period were defined as 100% baseline PSP. A minus sign was added to indicate its inhibitory nature. For clarity, only every other data point is shown with insets (calibration bars: 50 ms and 5 mV; dashed horizontal lines indicate potential level of $-70$ mV) showing representing traces at approximate time pointed by broken arrows. B: $3$ of the traces are placed together for comparison. The depolarizing response was blocked by BIC (1 $\mu$M, 30 min; BIC + CCH). Membrane potential was maintained at the pre-CCH level by passing current. C: under voltage clamp at $-74$ mV, the evoked GABAergic response was an outward current (Control), which was reversed to inward during CCH application (CCH, 50 $\mu$M, 20 min). Arrowheads indicate the time of the stimulation.
The postsynaptic GABAergic response to the first stimulation pulse usually did not reach action potential threshold (Fig. 7, A and C). The postsynaptic GABAergic responses were sensitive to BIC, indicating the involvement of the same receptor channels (Fig. 7A). In eight cells, single pulse stimulation of Sch (10–30 μA, 50 μs) evoked an excitatory postsynaptic potential of 7.5 ± 1.2 mV, which was about 50% below the threshold. Before the CCH administration, co-stimulation of Sch at the set intensity (50% below the threshold) and GABAergic inputs (50 μA, 50 μs) largely abolished the Sch stimulation-induced excitatory potential (by 89.5 ± 4.3%, n = 8, P < 0.05; Fig. 7C). The single-pulse Sch stimulation-evoked excitatory postsynaptic potential was not altered (P > 0.05) by CCH (not shown). Action potentials, however, were evoked by the threshold. Before the CCH administration, co-stimulation of Sch at the set intensity (50% below the threshold) and GABAergic inputs (50 μA, 50 μs) largely abolished the Sch stimulation-induced excitatory potential (by 89.5 ± 4.3%, n = 8, P < 0.05; Fig. 7C). The single-pulse Sch stimulation-evoked excitatory postsynaptic potential was not altered (P > 0.05) by CCH (not shown). Action potentials, however, were evoked by

![Image](https://i.imgur.com/3.png)

**FIG. 3.** Carbachol shifts reversal potentials of GABAergic postsynaptic responses in hippocampal CA1 pyramidal cells. A: responses of CA1 pyramidal cells to activation of GABAergic inputs at different membrane potentials before (Control) and in the presence of bath 1 μM BIC (middle). The relationship between the maximum postsynaptic responses and membrane potential can be described with a straight line, determined with the least sum squares criterion and was flattened by BIC without changing the reversal potential (bottom). B: responses of CA1 pyramidal cells to activation of GABAergic inputs at different membrane potentials before (Control) and during CCH application (50 μM; middle). Membrane potential was maintained at the pre-CCH level by passing current. Arrowheads indicate the stimulation. C: left shows an example of CCH-induced reversal of GABAergic response that was above threshold for generation of action potentials. The postsynaptic response exhibits a similar relationship between the maximum responses and membrane potential. For clarity, only 2 traces are shown. The same intensity of stimulation of the GABAergic inputs triggered an action potential in the cells post-CCH (CCH) as compared with IPSP pre-CCH (Control; right). Inset shows the initial segment at ×3 magnification with action potential truncated. Arrowheads indicate the time when brief pulse of stimulation was delivered.

![Image](https://i.imgur.com/4.png)

**FIG. 4.** Carbachol (CCH)-induced θ oscillations of hippocampal CA1 field potential and of membrane potential of CA1 pyramidal cells depend on HCO₃⁻ formation. A: an example of recorded field potentials in hippocampal CA1: pre-CCH control (top), during CCH (50 μM, 30 min) and acetazolamide (ACET) application (1 μM, 30 min). B: membrane potential traces of recorded CA1 pyramidal cells: pre-CCH (control), during CCH application (50 μM, 30 min) in the presence of ACET (1 μM, 30 min). In the presence of 1 μM ACET, single pulse stimulation (50 μA, 50 μs) of the GABAergic inputs evoked an IPSP (Control), which was not altered by CCH application (50 μM, 30 min).
Spatial memory deficits by ACET administration in vivo

We asked whether reducing HCO$_3^-$ formation with a carbonic anhydrase inhibitor that can pass through the blood brain barrier could affect rat spatial memory. In rats, an intraperitoneal (ip) dose of ACET produces a peak concentration in the blood within 1 h and is cleared by 2 h (Cassin et al. 1963; Sone et al. 1998). Effects of ACET on spatial learning (Meiri et al. 1998) were determined during this short period. A single dose of ACET (14–18 mg/kg, sufficient to reduce the EEG $\theta$ power by about 50% at maximum during rat REM sleep) (Sone et al. 1998) was sufficient to produce memory impairment (Fig. 8A). The ACET group showed a strikingly smaller reduction ($F_{1,18} = 34.79, P < 0.0001$) in escape latency during training trials than the saline group did. The memory impairment became more significant as the training days progressed and was particularly evident in the first trial (65–70 min after the injection) of each successive day (Fig. 8, A and B). The latter might reflect a relatively normal short-term (vs. long-term) learning after ACET or more likely influence of a rapid clearance of the drug (Cassin et al. 1963; Sone et al. 1998).

DISCUSSION

In vitro $\theta$ rhythm and cholinergic involvement

The CCH-induced $\theta$ in our study is consistent with the in vitro $\theta$ previously reported by many other groups (Golebiewski et al. 1998) was sufficient to produce memory impairment (Fig. 8C). Thus reversed synaptic responses re-shaped the GABAergic inhibitory function into amplification (Sun et al. 1999) and reconfigures the operations of hippocampal networks into patterns of activity associated with GABAergic inputs (Fig. 7B).

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et al. 1996; Huerta and Lisman 1993, 1995; Konopacki and Golebiowski 1993; Pitzer and Alger 1992; Vertes and Kocsis 1997; Williams and Kauer 1997) and appears to be fundamentally identical to the θ rhythm in vivo for its sensitivity to muscarinic receptor antagonists, dependence on GABAergic interneurons, and independence of glutamatergic inputs. Acetylcholine’s activation of muscarinic receptors on pyramidal cells is considered to be modulatory and much too slow to generate rhythmic θ directly (Dutar et al. 1995; Vertes and Kocsis 1997). The ineffectiveness of blocking glutamatergic inputs on the θ is also consistent with the evidence that during θ oscillations in vivo CA3 neurons rarely reach action potential threshold (Bland and Wishaw 1976; Fox and Ranck 1981) and excitatory inputs from CA3 are unlikely to contribute to CA1 θ (Soltesz and Deschenes 1993; Thompson and Best 1989; Ylinen et al. 1995). The effectiveness of the specific GABAB receptor antagonist BIC in eliminating the postsynaptic response and θ activity strongly suggests that GABAB receptor activation did not contribute significantly to the responses. The CA1 θ activity does, however, appear to be distinct from the activity oscillations that were BIC insensitive, involved epileptiform bursting, and were generated by CA3 neurons in one report (William and Kauer 1997). This difference may depend on the preparations or age of the animals. In their study, slices were obtained from younger animals so that cells may have a high intracellular Cl− concentration, due to the lack of a developmentally expressed Cl−-extruding K+/Cl− co-transporter in early age (Rivera et al. 1999).

Involvement of muscarinic receptors in hippocampal θ induction has been well established. Low-frequency MS/Dbv stimulation activates cholinergic inputs to the hippocampus and drives θ in vivo (Descarries et al. 1997). Microinusions of CCH or eserine into areas including CA1 induce an atropine-sensitive hippocampal θ activity in vivo (Rowntree and Bland 1986). Atropine administration has been found to eliminate hippocampal θ in vivo (Brazhnik and Vinogradova 1986; Vertes and Kocsis 1997). The effectiveness of muscarinic antagonists does not mean, however, that there is only one form of θ. In anesthetized rats, atropine eliminates θ (Stewart and Fox 1989). Under such conditions, an unconventional
small “residual θ” was described, that, in the absence of θ, could be shown by using the activity of the MS/Dev neurons that discharged rhythmically to trigger hippocampal EEG in analysis (Stewart and Fox 1989). The involvement of serotonergic transmission has been proposed (Vanderwolf et al. 1987). The particular role and importance of such an atropine-resistant component in memory remains to be established. Furthermore, intracellular activity of pyramidal cells has been claimed to result from depolarizing or hyperpolarizing membrane potential oscillations (Vertes and Kocsis 1997). The activities induced in our study were most likely evoked by muscarinic receptor activation, given their sensitivity to atropine. We do not, however, rule out the possibility that multiple cell targets might be required for CCH to induce the θ. Nor can we rule out the involvement of the nicotinic receptors entirely. However, it has been shown that the interneurons in or near the stratum pyramidale and with axonal projections within and around this layer exhibit no nicotinic response (McQuiston and Madison 1999).

**HCO₃⁻-mediated GABAergic synaptic depolarization**

Encoding experiences into lasting memory may involve a qualitative diversity of synaptic plasticity (Brenowitz et al. 1998; Kornhauser and Greenberg 1997; Otis et al. 1996; Paulsen and Moser 1998), including changing operations of preexisting synapses and growing new ones. GABAergic postsynaptic depolarizing responses have been observed by several groups (Alkon et al. 1992; Kaila et al. 1993; Michelson and Wong 1991; Rivera et al. 1999; Siklós et al. 1995; Staley et al. 1995). The depolarization induced in the present study differs from that reported by Kaila et al. (1997), who applied a high-frequency train of pulses to the stratum radiatum to induce depolarizing responses that showed a slow time course but lasted for several seconds. Nevertheless, our results are consistent with the evidence that GABAergic depolarization can be induced by enhancing HCO₃⁻ conductance through GABA_A receptor channels in adult hippocampal cells, a response sensitive to carbonic anhydrase inhibitors (Kaila et al. 1993; Staley et al. 1995). Carbonic anhydrase exists in pyramidal cells (Pasternack et al. 1993). Indeed, the θ activity and the reversed GABAergic postsynaptic responses were largely abolished by carbonic anhydrase inhibitors. The effectiveness of intracellular benzolamide, a membrane-impermeable carbonic anhydrase inhibitor, indicates that the response depends on activity of an intracellular enzyme. Supporting the functional importance of carbonic anhydrase activity in synaptic plasticity is also the result that a partial blockade of the enzyme activity in vivo markedly impaired retention of rat watermaze learning. HCO₃⁻ has a reversal potential about −212 mV (Staley et al. 1995). With an increased HCO₃⁻/Cl⁻ permeability ratio, outward HCO₃⁻ flux would depolarize the membrane at the resting membrane potential. Alteration in HCO₃⁻ conductance and/or transmembrane concentrations (thus the driving force) would be expected to dramatically alter the synaptic response.

Regulation of carbonic anhydrase activity is not an entirely new concept. The existence of physiological regulators of
carbonic anhydrase has been proposed, including those that activate the anhydrase by facilitating its membrane association (Parkes and Coleman 1989). Activation of cholinergic receptors is well known to increase cytosolic $\text{Ca}^{2+}$ concentrations, at least partially through $\text{Ca}^{2+}$ release from intracellular stores (Seymour-Laurent and Barish 1995). Enhancing effects by $\text{Ca}^{2+}$ on the anhydrase activity was suggested by the calexcitin and ryanodine receptor results (Sun et al. 1999), although the identity of the intracellular signaling intermediate(s) remains to be determined. In molluscan neurons, the carbonic anhydrase-HCO$_3^-$ system has been found to be the most potent regulatory factor in intracellular pH regulation. Depolarized snail neurons, for example, were associated with increased proton conductance (e.g., Thomas and Meech 1982). Changes in intracellular pH could also alter ion channel function as well as metabolic activity. It remains to be determined whether intracellular pH is significantly altered or plays a role in the CCH-induced $\theta$ and/or regulation of memory behavior.

Phase relationship of $\theta$ activities of CA1 pyramidal cells and interneurons

Two major classes of hippocampal CA1 neurons are the $\theta$ cells and the “place cells” (Paulsen and Moser 1998). The GABAergic interneurons, including basket cells and axo-axonic cells, have been called $\theta$ cells (Paulsen and Moser 1998). The basket interneurons are particularly active and express strongest rhythmic discharges (Cscsvari et al. 1999) when hippocampal EEG is dominated by $\theta$ rhythm. One basket interneuron selectively and perisomatically innervates approximately 1,000 pyramidal cells (Cobb et al. 1995) and thus can entrain a large population. The pyramidal neurons, on the other hand, are largely quiescent during the $\theta$ rhythm associated with exploration, but a subpopulation shows strong firing that is highly correlated with specific locations in space (Dutar et al. 1995; Paulsen and Moser 1998; Vertes and Kocsis 1997). These “place” cells fire at all phases of the $\theta$ rhythm (O’Keefe and Recce 1993). Our results show that during $\theta$ oscillation, the GABAergic postsynaptic responses are altered. Gating through a postsynaptic mechanism, as described in the present study, could explain why some pyramidal cells become active while the vast majority of others remain silent during $\theta$ EEG, even if they are innervated by the same interneuron.

Every pyramidal cell is innervated by 10–12 GABAergic interneurons, preferentially making synapses on cell bodies, proximal dendrites, and axon initial segments of CA1 pyramidal cells (Buhl et al. 1994; also Paulsen and Moser 1998 for review). If pyramidal cells were activated by rebound excitation from GABAergic inhibition, one would expect that pyramidal cells should discharge when interneurons become silent. This may be the case in anesthetized states. The intracellular $\theta$ activity of CA1 pyramidal cells when recorded under anesthesia have often been reported to fire out-of-phase, delayed about a half cycle (Soltesz and Deschenes 1993; Ylinen et al. 1995). In behaving animals or during REM sleep, however, the earlier discharge peaks of these interneurons precede peaks of population activity of pyramidal cells during $\theta$ activity, and both pyramidal cell firing and interneuronal discharge occur within the same $\theta$ phase period (Cscsvari et al. 1999). Anesthesia is known to attenuate a large peak of $\theta$, revealing rhythmic hyperpolarization of pyramidal cells from basket interneurons (Ylinen et al. 1995). Thus the $\theta$ activity during exploration or induced by cholinergic agonists in vitro seems not directly comparable to the $\theta$ under anesthesia (Muir and Bilkey 1998; Ylinen et al. 1995).

Not only does the discharge phase relationship between pyramidal cells and interneurons differ between the anesthetized and behaving animals, but the phase relationship is also dynamic. In behaving animals, the phase forward shift of the discharges of place cells on each $\theta$ cycle occurs during traversal of the place field of the cell (O’Keefe and Recce 1993; Shen et al. 1997). Place cells thus fire in phase with progressive stronger GABAergic inputs from interneurons and at earlier phases of the $\theta$ cycles as the rat moves toward the center of their place field (Fig. 7B) (Cscsvari et al. 1999; O’Keefe and Recce 1993; Shen et al. 1997). Mechanism(s) responsible for the $\theta$ initiation or entraining of the pyramidal cell activity should be able to code for timing (Trussell 1999) or entrain pyramidal cells at different $\theta$ phases, including those with minimal delay (Cscsvari et al. 1999). A rebound excitation following hyperpolarization is unlikely to have such multi-phase capability as the interneurons become more active during $\theta$-related activity. A brief switch toward or to GABAergic depolarization would be more effective and reliable in processing dynamic information. Strong GABAergic inputs after the
synaptic switch can entrap the activity of pyramidal cells so that the delay would be relatively short and evoke an “in phase” activity.

The present results suggest that the GABAergic entraining could result through in three ways. In a small percentage of cells, CCH was able to elevate the reversal potential to levels that were above the threshold for spike activity. GABAergic inputs thus could directly drive, even if briefly, activity of the pyramidal cells with sufficient transformation of hyperpolarizing to depolarizing responses. The reversed response, although often not strong enough to reach threshold by itself, can entrain the pyramidal cells when stimulated at a θ frequency (Fig. 7). Furthermore, the reversed response can effectively enhance weak excitatory inputs to reach threshold (Fig. 7C) (Sun et al. 1999). When the inputs are not very strong and require summation of multiple synaptic activation or other associative inputs, such as glutamatergic inputs (Sun et al. 1999) to reach the threshold, the entrained action potentials are likely to be delayed. It should also be noted that for this summation effect to occur, there must be sufficient spatial proximity on the pyramidal cells for the glutamatergic excitatory postsynaptic potentials to spread from the dendrites to the somata where they would interact with the transformed GABAergic IPSPs. Thus place cells would be capable of firing at all phases of the θ rhythm in relation to the activity of GABAergic interneurons.

We have shown reversed, HCO₃⁻-dependent GABAergic postsynaptic responses and their effectiveness in entraining activity of pyramidal cells. The most reasonable explanation for our results is an essential requirement of carbonic anhydrase activity in the molecular signaling pathways for learning and memory. This explanation is consistent with the occurrence of mental rigidity or posttraumatic stress disorder (e.g., in survival from formed memory. Such compounds may have clinical value when temporarily suppressed memory is beneficial (e.g., in synaptic functions (Sone et al. 1998). ACET-regulated HCO₃⁻

bicarbonate-dependent GABAergic depolarization, as defined in vitro, also directly contributes to spatial memory. The critical role of pyramidal cells when stimulated at a θ frequency (Fig. 7). Furthermore, the reversed response can effectively enhance weak excitatory inputs to reach threshold (Fig. 7C) (Sun et al. 1999). When the inputs are not very strong and require summation of multiple synaptic activation or other associative inputs, such as glutamatergic inputs (Sun et al. 1999) to reach the threshold, the entrained action potentials are likely to be delayed. It should also be noted that for this summation effect to occur, there must be sufficient spatial proximity on the pyramidal cells for the glutamatergic excitatory postsynaptic potentials to spread from the dendrites to the somata where they would interact with the transformed GABAergic IPSPs. Thus place cells would be capable of firing at all phases of the θ rhythm in relation to the activity of GABAergic interneurons.

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