Pharmacological Enhancement of Synaptic Efficacy, Spatial Learning, and Memory through Carbonic Anhydrase Activation in Rats

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Received December 8, 2000; accepted February 27, 2001

ABSTRACT
CA1 pyramidal cells were recorded in rat hippocampal slices. In the presence of carbonic anhydrase activators, comicrostimulation of cholinergic inputs from stratum oriens and γ-aminobutyric acid (GABAAergic inputs from stratum pyramidale at low intensities switched the hyperpolarizing GABA-mediated inhibitory postsynaptic potentials to depolarizing responses. In the absence of the activators, however, the same stimuli were insufficient to trigger the synaptic switch. This synaptic switch changed the function of the GABAAergic synapses from excitatory to inhibitory postsynaptic responses. Inhibition of carbonic anhydrase inhibitors, indicating a dependence on HCO₃⁻. Intralateral ventricular administration of these same carbonic anhydrase activators caused the rats to exhibit superior learning of the Morris water maze task, suggesting that the GABAAergic synaptic switch is critical for gating the synaptic plasticity that underlies spatial memory formation. Increased carbonic anhydrase activity might, therefore, also enhance perception, processing, and storing of temporally associated relevant signals and represents an important therapeutic target in learning and memory pharmacology.

Drugs that enhance acquisition and/or recall of associative memory represent important goals in the therapy of cognitive disorders. The effectiveness of such therapy should depend on whether the targeted mechanisms are actually involved in memory itself. Learning and memory are believed to require modifications of synaptic strength among relevant neurons in the network, through an interaction of multiple afferent pathways and signal molecules (Christie et al., 1994; Kornhauser and Greenberg, 1997; Ohno et al., 1997; Alkon et al., 1998; Paulsen and Moser, 1998; Xiang et al., 1998; Tang et al., 1999; Wu et al., 2000). A requirement for multiple synaptic interactions, versus a single glutamatergic pathway often studied experimentally, is in fact consistent with characterization of multiple deficits of neurotransmitters in memory impairments, including Alzheimer’s disease. Targeting the relevant synaptic/signal interactions within memory traces therefore might be an effective way to achieve a specific effect on learning and memory pharmacologically.

In mammals, the essential role of hippocampal CA1 pyramidal cells in spatial memory is well established. The CA1 pyramidal cells receive, in addition to glutamatergic input from the CA3 pyramidal neurons, abundant cholinergic and GABAAergic inputs. Activation of the medial septal afferents within the perforant pathway, a major cholinergic input to the hippocampus (Cooper and Sofroniew, 1996), is believed to be required for associative learning (Dickinson-Anson et al., 1998; Perry et al., 1999), since its disruption abolishes spatial memory (Winson, 1978; Winkler et al., 1995). GABAergic interneurons, on the other hand, control hippocampal network activity and synchronize the firing of pyramidal cells (Buhl et al., 1995; Cobb et al., 1995; Banks et al., 2000). One GABAergic interneuron is known to innervate some 1000 pyramidal cells, effectively shutting down the signal outflow when the interneurons are active (Sun et al., 2000). The functional interaction between these major inputs thus plays a significant role in hippocampus-dependent memory (Bartus et al., 1982; Winkler et al., 1995; Paulsen and Moser, 1998) and has attracted much attention in an effort to “dissect” the memory traces.

Consistent with the observations that the GABAAergic synaptic responses can be switched from inhibitory to excitatory (Alkon et al., 1992; Collin et al., 1995; Kaila et al., 1997; Taira et al., 1997; Sun et al., 2000, 2001b), evidence has been provided that such a synaptic switch depends on the increased HCO₃⁻ conductance through the GABAA receptor-channel complex and dramatically alters the operation of signal transfer through the hippocampal network (Sun et al., 1999, 2000). The synaptic switch appears to depend on carbonic anhydrase, a zinc-containing enzyme that catalyzes the reversible hydration of carbon dioxide. Carbonic anhydrase is

ABBREVIATIONS: GABA, γ-aminobutyric acid; PSP, postsynaptic response; IPSP, inhibitory postsynaptic potential; NMDA, N-methyl-D-aspartate; EPSP, excitatory postsynaptic potential; Sch, Schaffer collateral pathways.
present within the intracellular compartments of the pyramidal cells (Pasternack et al., 1993). The fact that a membrane-impermeant carbonic anhydrase inhibitor, benzolamide, was effective in blocking the synaptic switch when introduced into the recorded pyramidal cells, but not when applied extracellularly (Sun et al., 1999), indicates that the underlying enzyme is intracellular. Blocking the rapid HCO₃⁻ formation that depends on carbonic anhydrase activity thus prevents the synaptic switch in vitro and impairs rat spatial learning in vivo (Sun et al., 2001b). To test whether the GABAergic synaptic switch is crucial for gating synaptic plasticity and memory, we investigate here the effects of carbonic anhydrase activators on this GABAergic synaptic switch. We found that in the presence of the activators, the synaptic switch was induced with associated activation of heterosynaptic inputs at intensities that were insufficient to trigger the synaptic switch by the stimulation alone. Furthermore, intraventricular administration of the activators significantly enhanced the rats’ ability to learn a water maze task and to recall that maze from memory.

**Materials and Methods**

**Brain Slices.** Male Sprague-Dawley rats (150–180 g) were anesthetized with pentobarbital and decapitated. The hippocampal formation was removed and sliced (400 μm) with a McIlwain tissue chopper (Sun et al., 1999). Slices were maintained in an interface chamber (Medical Systems Corp., Greenvale, NY) at 31°C with continuous perfusion of artificial cerebrospinal fluid. Artificial cerebrospinal fluid consisted of 125 mM NaCl, 3 mM KCl, 1.3 mM MgSO₄, 2.4 mM CaCl₂, 26 mM NaCHO₃, 1.25 mM NaH₂PO₄, and 10 mM C₆H₁₂O₆.

**Electrophysiology.** Intracellular recordings were obtained from CA1 pyramidal neurons using glass micropipette electrodes filled with 2 M potassium acetate (pH 7.25), with measured tip resistance in the range 70 to 120 MΩ. Cells that show obvious accommodation, an identifying characteristic of pyramidal cells, were used in the study. Labeling the recorded cells exhibiting this characteristic with dye has previously revealed that the recorded cells are indeed pyramidal cells (Sun et al., 1999). Signals were amplified, digitized, and dye has previously revealed that the recorded cells are indeed pyramidal cells (Sun et al., 1999). Signals were amplified, digitized, and recorded using a videocamera. The escape latency and the route of rats’ swimming across the pool to the platform were recorded. The quadrant test (1 min) was performed after removing the platform, 24 h after the last training trial.

Statistical analyses were performed using the Student’s t test for paired or unpaired data or ANOVA whenever appropriate. The values are expressed as means ± S.E.M., with n indicating the 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**

Microstimulation of stratum pyramidale with a single pulse elicited a hyperpolarizing inhibitory postsynaptic potential (IPSP; Fig. 1a). The IPSP was, mainly if not exclusively, from activation of the GABAergic inputs from the Basket interneurons, whose cell bodies and axons are restricted to stratum pyramidale. As described in our previous publications (Sun et al., 1999, 2000), the IPSPs exhibited a reversal potential of about −78 mV. No detectable minor PSP components that exhibit a different reversal potential were observed. Bath application of kynurenic acid (500 μM, 20 min), a broad-spectrum competitive antagonist for both N-methyl-d-aspartate (NMDA) and non-NMDA receptors (Collingridge and Lester, 1989), effectively abolished EPSPs of CA1 pyramidal cells evoked by stimulation of the Schaffer collateral pathways (Sch; by 96.3 ± 4.1%, n = 6 from six different rats, p < 0.05). At this concentration, kynurenic acid did not increase the IPSP amplitudes (−8.2 ± 0.6 mV prekynurenic acid versus −8.3 ± 0.7 mV during the application; n = 7 from seven different rats, p > 0.05; Fig. 1a), suggesting that the single-pulse stratum pyramidale microstimulation did not evoke a significant glutamatergic EPSP component. The EPSPs, however, were blocked by bicuculline, the selective GABA_A receptor antagonist (by 97.9 ± 4.4% on average, n = 6 from six different rats, p < 0.05; 1 μM, 30-min perfusion; Fig. 1b), indicating that the EPSPs were predominantly mediated by activation of the GABA_A receptors and vivo, agents (2 μl/site/day) were bilaterally injected during training days about 30 min before the training, at a speed of 1 μl/min. The control rats received the same volume of saline.

**Spatial Maze Tasks.** Effects of increasing HCO₃⁻ formation in vivo on spatial memory were evaluated in rats with the Morris water maze task. Male adult Wistar rats (200–250 g) were housed in a temperature-controlled (20–24°C) room for a week, allowed free access to food and water, and kept on a 12-h light/dark cycle. Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p) and placed in a stereotactic apparatus (Kopf Instruments, Tujunga, CA). The core temperature of rats was monitored and kept constant (38.0 ± 0.5°C) with warming light and pad. Two stainless steel guide cannulas were placed with the tips positioned at the coordinates (anterior-posterior, 0.5 mm; lateral, 1.5 mm; horizontal, 3.2 mm), under aseptic conditions. At the end of surgery and under appropriate anesthesia, rats received (s.c.) banamine (1 mg/kg) and ketoprofen (5 mg/kg) in lactate/Ringer’s solution. A 7-day recovery period was allowed before any further experimentation.

On the first day of experiments, all rats were randomly assigned to different groups (10 each) and swam for 2 min in a 1.5- (diameter) × 0.6-m (depth) pool (22 ± 1°C). On the following day, rats were trained in a two-trial per day task for four consecutive days. Each training trial lasted for up to 2 min, during which rats learned to escape from water by finding a hidden platform that was placed at a fixed location and submerged about 1 cm below the water surface. The navigation of the rats was tracked by a videocamera. The escape latency and the route of rats’ swimming across the pool to the platform were recorded. The quintuple test (1 min) was performed after removing the platform, 24 h after the last training trial.

Statistical analyses were performed using the Student’s t test for paired or unpaired data or ANOVA whenever appropriate. The values are expressed as means ± S.E.M., with n indicating the 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.
Two carbonic anhydrase activators, imidazole (100 μM, starting at the vertical arrow in d) reduces the IPSP slightly when applied alone (c) but induces a lasting synaptic reversal of the GABAergic responses when associated with costimulation (at the arrowhead in d). The application of phenylalanine (100 μM, 20 min, also starting at the vertical arrow in d). Arrowheads indicate the time when single-pulse stimulation of stratum pyramidale is delivered. In d, the data points are illustrated as means ± standard errors of the means and for clarity, only every other minute is illustrated.

were therefore referred to as Basket interneuron-CA1 responses.

Single-pulse stimulation of stratum orients (1 Hz, 10 s) coincident with trains of stimulation of stratum pyramidale produced a small but lasting decrease in the IPSP amplitudes (Fig. 1, d and f). For instance, at 40 min after the costimulation, the peak IPSPs were −4.9 ± 0.7 mV, significantly smaller than −7.4 ± 0.9 mV before the associated stimulation (n = 8 from seven different rats, p < 0.05; paired t test). Two carbonic anhydrase activators, imidazole (100 μM, 20 min; Parkes and Coleman, 1989) or phenylalanine (100 μM, 20 min; Clare and Supuran, 1994), were applied. In the presence of phenylalanine, the peak IPSPs in response to single-pulse stimulation of stratum pyramidale were slightly but significantly reduced (Fig. 1c; to −4.5 ± 0.8 mV in the presence of phenylalanine from prephenylalanine, peak IPSPs of −7.6 ± 1.2 mV; n = 7 from seven different rats, p < 0.05). In the presence of the carbonic anhydrase activator, the same intensities of costimulation of stratum pyramidale and stratum oriens produced a lasting reversal of the IPSPs to EPSPs, observed when the membrane potentials were maintained at their control levels (Fig. 1, d and e). Thus, 40 min after the costimulation (under Materials and Methods) and in the presence of phenylalanine, the peak PSPs were 6.4 ± 1.1 mV, significantly different (n = 8 from eight different rats, p < 0.05) from their prephenylalanine values (−7.2 ± 1.2 mV) or from those in the presence of phenylalanine but before the costimulation (Fig. 1d). In the presence of imidazole, similar effects on the IPSPs (−5.3 ± 0.7 mV in the presence of imidazole versus preimidazole of −7.8 ± 0.6 mV; n = 7 from seven different rats, p < 0.05) and effects of the costimulation (peak PSPs: 4.2 ± 0.6 mV, in the presence of imidazole and 40 min after the costimulation versus preimidazole values of −7.5 ± 0.7 mV; n = 6 from six different rats, p < 0.05) were observed, although in general, less potent. Thus, the results with imidazole were not illustrated in detail.

Both the reducing effect of carbonic anhydrase activators on the IPSPs and the synaptic switching effect with costimulation of the cholinergic and GABAergic inputs depend on activity of the carbonic anhydrase. For instance, in the presence of acetazolamide (10 μM, 20 min), a blocker of carbonic anhydrase and thus the synthesis of HCO₃⁻ (Staley et al., 1995), phenylalanine did not significantly reduce the peak IPSPs (−7.7 ± 0.9 mV in the presence of phenylalanine versus prephenylalanine peak IPSPs of −7.9 ± 1.1 mV, n = 6 from six different rats, p < 0.05). Nor did imidazole, in the presence of acetazolamide, significantly change the size of the IPSPs (−7.5 ± 1.0 mV in the presence of imidazole versus preimidazole peak IPSPs of −7.4 ± 0.8 mV, n = 5 from five different rats, p > 0.05). The same intensities of costimulation did not induce the synaptic switch (Fig. 1d and g) in the presence of acetazolamide and phenylalanine or imidazole. Thus, in the presence of acetazolamide and phenylalanine, these IPSPs were not significantly altered by the co-stratum oriens-stratum pyramidale stimulation (−7.8 ± 1.3 mV, 40 min after compared with −7.6 ± 0.9 mV control value, n = 8 from eight different rats, p > 0.05). Furthermore, the co-stimulation did not significantly alter the IPSPs in the presence of acetazolamide and imidazole (−7.7 ± 1.1 mV, 40 min after compared with −7.5 ± 0.8 mV control value, n = 6 from six different rats, p > 0.05).

The influence of the GABAergic synaptic switch on the
signal passage through the CA1 cells was evaluated when the glutamatergic Sch inputs were costimulated. In eight cells, single-pulse stratum pyramidale stimulation evoked an IPSP (Fig. 2a). Excitatory Sch input was stimulated at intensities 30% above the action potential threshold (100% of 20 trials) of the recorded cells (Fig. 2b). Costimulation of the GABAergic inputs and Sch blocked (100% of 20 trials; n = 10 from eight different rats, p < 0.05) the effects of excitatory Sch input, stimulated at above-action-potential-threshold intensities (Fig. 2c) in all eight cells tested. The effective signal-filtering period in each single-pulse-evoked inhibitory response was ≥100 ms, during which no action potential (0% of 20 trials) was evoked by Sch stimulation at the above-threshold intensity. After the synaptic switch (Fig. 2d) induced by costimulation of the GABAergic and cholinergic inputs in the presence of phenylalanine, below-threshold Sch stimulation, which by itself did not evoke action potentials (0% of 20 trials; Fig. 2e), became sufficient to evoke action potentials (100% of 20 trials; n = 8 from eight different rats, p < 0.05) when delivered during the period of ≥100 ms of single-pulse stimulation of the GABAergic input (Fig. 2f; n = 8 from eight different rats). Multiple spikes were evoked when the Basket interneurons-CA1 PSP was costimulated with above-threshold Sch stimulation after inducing the synaptic switch (data not shown). Thus, after the synaptic switch, activity of the GABAergic interneurons amplified excitatory Sch inputs. Therefore, weak signals are amplified after synaptic switch to trigger action potentials, while strong excitatory signals cannot successfully pass through the network under associated inhibition.

We tested the effects of carbonic anhydrase activators on spatial learning in rats, using the hidden-platform water maze. As shown in Fig. 3a, the latency to escape to the platform in all three groups of rats decreased following the training sessions. Statistical analysis revealed significant effects of groups (F2,27 = 9.192, p < 0.001), trials (F4,218 = 7.83, p < 0.001), and groups × session of trials (F14,218 = 3.70, p < 0.001), indicating that spatial learning in rats injected with phenylalanine (phenylalanine rats) was faster than in rats injected with saline (control rats). Moreover, a post hoc analysis reveals a significant difference from the second to sixth trials (p < 0.05), confirming better learning in phenylalanine rats. In fact, the escape latency of the phenylalanine rats reached a plateau on the fifth trial. Three additional trials were needed for the control rats to show the same escape latency as the phenylalanine rats (Fig. 3a). Quadrant tests 24 h after the last training trial revealed that the control rats (F3,36 = 159.9, p < 0.0001; ANOVA and Newman-Keuls post hoc test), and the phenylalanine rats (F3,36 = 201.2, p < 0.0001) spent more time searching in the target quadrant (quadrant 4) where the platform was previously placed and had been removed. However, in comparison with control rats, phenylalanine rats exhibited a clearly greater preference for the target quadrant (by 24.8 ± 1.8%, p < 0.05; unpaired t test) (Fig. 3, d and e). The target quadrant ratios, target/average of the nontarget quadrants, between the phenylalanine and the control rats were significantly different (p < 0.001; Fig. 3b). Similarly, rats injected with imidazole (imidazole rats) also showed a faster learning and a significant shorter escape latency from the third to sixth trials (p < 0.05) than the control animals. Quadrant tests revealed that imidazole rats had a greater preference for the target quadrant (by 15.1 ± 1.6%, p < 0.05) than the control rats. Thus, the rats injected with the carbonic anhydrase activators performed better than their controls in this spatial memory retention task. The average swim speeds for all eight trials, however, did not differ between all the groups (Fig. 3c; p > 0.05), including the imidazole and acetazolamide/imidazole groups (data not shown), indicating that the carbonic anhydrase activators and inhibitor did not grossly affect their sensory or locomotor activities. During the experimental periods, no rats showed any apparent sign of discomfort or abnormal behaviors such as hypo- or hyperactivity.

The effects of carbonic anhydrase activators on spatial learning are depicted in Fig. 2. Single-pulse stimulation of stratum pyramidale evokes an IPSP (a). Single pulse stimulation of Sch at above-threshold intensity evokes an action potential (b). Cosingle-pulse stimulation of stratum pyramidale and Sch (the same as a and b) eliminates the EPSP and no action potential is evoked (c). After the associated costimulation of stratum pyramidale and stratum oriens (under Materials and Methods) in the presence of phenylalanine, the IPSP is reversed to EPSP, observed at the same resting membrane potential (d). Single-pulse stimulation of Sch at below-threshold intensities evokes an EPSP (e). Cosingle-pulse stimulation of stratum pyramidale and Sch (the same as d and e) evokes an action potential (f). Arrowheads indicate the time when single-pulse stimulation of stratum pyramidale or costimulation is delivered. The calibration bar units are the same for the traces and insets (as in a) except b and f.
learning were sensitive to carbonic anhydrase inhibitors. Bilateral intraventricular injections of acetazolamide not only eliminated the effects of the carbonic anhydrase activators on the learning but also produced memory impairment (Fig. 3a). The acetazolamide/phenylalanine group showed a strikingly smaller reduction ($F_{1,18} = 40.38$, $p < 0.0001$) in escape latency during training trials than the control group did. Quadrant tests revealed that the acetazolamide/phenylalanine rats showed no significantly different preference for a particular quadrant ($F_{3,36} = 1.43$, $p > 0.05$; Fig. 3f) and a significantly different ($p < 0.001$) target quadrant ratio from those of the phenylalanine and the control rats (Fig. 3b). Identical results were also observed in rats injected with acetazolamide and imidazole (data not shown).

**Discussion**

The importance of GABAergic synaptic switch in controlling signal processing in the hippocampal network, as demonstrated in previous studies (Sun et al., 1999, 2000), suggested that enhancement of the efficacy of that switch would lead to improved learning and memory. The present study is the first to directly show that such an enhancement can be achieved through the use of carbonic anhydrase activators and that these carbonic anhydrase activators increase efficacy of temporally associated activity of the cholinergic and GABAergic inputs in switching the hyperpolarizing GABAergic IPSPs to excitatory PSPs. The synaptic switch can be induced by associative postsynaptic stimulation (Collin et al., 1995), activation of the calexcitin signal cascade, or costimulation of the cholinergic and GABAergic inputs at greater intensities and more prolonged periods of stimulation (Sun et al., 2001a). The results shown above indicate that the presence of the enzyme activators facilitates induction of the synaptic switch so that weaker and fewer trains of costimulation were required. These results provide further evidence in support of the notion that neural information in recognition memory is more likely coded by the temporal association of heterosynaptic inputs rather than by a single neurotransmitter type (Steckler et al., 1998).

Two enzyme activators from different classes of compounds, which have different spectra of biological actions, were used in the study, yielding similar results. They were administered directly into the brain to avoid the limitation of accumulation in the brain by the blood-brain barrier. Competitive transport and rapid peripheral hydroxylation are known to limit the phenylalanine concentration in the brain of systemically administered phenylalanine-containing substances (such as aspartame, whose metabolites include 5-benzyl-3,6-dioxo-2-piperazineacetic acid, phenylalanyl-aspartic acid, asparaginyl-phenylalanine, phenylalanine methyl ester, phenylalanine, aspartic acid, methanol, and formate). These effects will limit phenylalanine’s access to the brain and possibly its behavioral impact. In addition to activation of carbonic anhydrase, high concentrations of phenylalanine in the brain might facilitate the synthesis of catecholamines and catecholaminergic transmission. Imidazole-like structures, on the other hand, may react with many biologically active molecules, including monoamine oxidase,
histamine H3 receptors, angiotensin II type 1 receptors, ethanol binding sites in GABA receptor channel complex, GABA<sub>A</sub> receptors, the nicotinic-cholinergic receptor channel complex, the prosthetic heme group of the nitric-oxide synthase, some K<sub>a</sub>TP channels, and imidazole binding sites. The biological consequences and specificity of an increased brain imidazole concentration, therefore, still remain to be clarified. Thus, our results do not rule out a possible contribution of synaptic/signal interaction in other brain regions or an action of the substances and their metabolites at the α-adrenoceptors, dopaminergic receptors, and/or histaminergic receptors to the enhancement of spatial learning and memory. The common denominator of the two carbonic anhydrase activators, the action on carbonic anhydrase, however, is the likely underlying mechanism for the observed effects. The critical role of carbonic anhydrase activation in the observed effects of carbonic anhydrase activators was further directly demonstrated by the effectiveness of acetazolamide, a carbonic anhydrase inhibitor, in blocking the synaptic switch. Acetazolamide has been shown to be able reduce or eliminate flux of HCO<sub>3</sub><sup>−</sup> in hippocampal pyramidal neurons underlying a depolarizing PSP (Staley et al., 1995). Activity of carbonic anhydrase in the CA1 pyramidal cells is essential since intracellular application of benzolamide, a membrane-impermeant carbonic anhydrase inhibitor, was previously found to effectively block the GABAergic synaptic switch (Sun et al., 1999). Our present (and previous) results are consistent with an induction of a depolarizing transmembrane HCO<sub>3</sub><sup>−</sup> flux that underlies the synaptic switch. The effects of acetazolamide on rat water maze spatial learning are also in line with a report that a single dose of acetazolamide is sufficient to significantly reduce the EEG θ power during rat rapid eye movement sleep (Sone et al., 1998). Our behavioral data, however, do not provide a direct link to a depolarizing GABA dependence. Therefore, possible contribution from non-GABAergic mechanisms (such as depolarization, proton effects on other channels) remains to be evaluated.

Carbonic anhydrase is a highly efficient enzyme. If its activity is crucial for coding and storing learned information, one would expect the existence of cellular mechanisms to control activity of the enzyme. There are indications that intracellular Ca<sup>2+</sup> release increases HCO<sub>3</sub><sup>−</sup> conduction through the GABA<sub>A</sub> receptor-mediated IPSPs and that the effect is sensitive to carbonic anhydrase inhibition (Sun et al., 2000). Membrane association is another efficient mechanism to activate carbonic anhydrase (Parkes and Coleman, 1989). It remains to be determined whether translocation and membrane association of the cytosol carbonic anhydrase participate in memory acquisition and/or consolidation. Nevertheless, the involvement of carbonic anhydrase in cognitive functions is consistent with the evidence (Meier-Ruge et al., 1984) of a significantly diminished activity of the enzyme in Alzheimer’s disease than in age-matched controls and with increasing age.

It is also important to point out that the observed synaptic switch does not appear to involve a masked excitatory component, such as glutamatergic EPSPs. First, microstimulation was delivered to the area remote to major excitatory terminal inputs. Second, the stimulation at the tested intensity did not directly activate the pyramidal cells. Third, the evoked IPSPs showed little change in magnitude with an effective blockade of the glutamatergic receptors. Finally, the evoked IPSPs were abolished by blocking the GABA<sub>A</sub> receptors. On the other hand, the relatively brief time course of the switched PSPs, compared with that of IPSPs, might suggest that the HCO<sub>3</sub><sup>−</sup> flux may be limited by its availability. Alternatively, the kinetics of the transformed IPSP may reflect a flux direction-dependent property.

Postynaptic GABAergic depolarizing responses have been reported by several groups under a variety of conditions (Wong and Watkins, 1982; Alkon et al., 1992; Staley et al., 1995; Burg et al., 1998; Leinekugel et al., 1999). The present results demonstrate that the switched synaptic responses provide a postsynaptic mechanism to direct or gate signal flow through the hippocampal network. The GABAergic interneurons, especially the Basket interneurons, whose cell bodies and axons are restricted in the cell layer, are known to innervate the perisomatic region of the pyramidal cells. Thus, bursting activity from the interneurons in the absence of synaptic switch inhibits the pyramidal cells, powerfully blocking excitatory signal transfer through the hippocampal circuit. An associated activation of the cholinergic and GABAergic inputs can trigger the synaptic switch, especially when the carbonic anhydrase is activated. After the synaptic switch, however, the same type of GABAergic activity amplifies excitatory signal. The mechanism thus differentiates responses according to the nature and temporal association of relevant signals and the neural activity states, a phenomenon that may underlie synaptic plasticity in learning and memory (Liu and Cull-Candy, 2000; Shulz et al., 2000). The synaptic switch mechanism enables the network to perform signal processing and gate information flow and direction accordingly.

Altering neural activity states that learning depends on, rather than the afferent signals themselves, may represent an effective therapeutic strategy to achieve memory therapy. Agents that activate carbonic anhydrase may have clinical value for enhanced memory and for the treatment of spatial memory decline. Phenylalanine may be used in the majority of individuals who do not have genetic lack of phenylalanine hydroxylase, whereas the development of more potent and selective nonphenylalanine activators (such as imidazole- and histamine-derivatives) might apply to those individuals with hydroxylase dysfunction.

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