Intracellular Correlates of Spatial Memory Acquisition in Hippocampal Slices: Long-Term Disinhibition of CA1 Pyramidal Cells

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Gusev, Pavel A. and Daniel L. Alkon. Intracellular correlates of spatial memory acquisition in hippocampal slices: long-term disinhibition of CA1 pyramidal cells. J Neurophysiol 86: 881–899, 2001. Despite many advances in our understanding of synaptic models of memory such as long-term potentiation and depression, cellular mechanisms that correlate with and may underlie behavioral learning and memory have not yet been conclusively determined. We used multiple intracellular recordings to study learning-specific modifications of intrinsic membrane and synaptic responses of the CA1 pyramidal cells (PCs) in slices of the rat dorsal hippocampus prepared at different stages of the Morris water maze (WM) task acquisition. Schaffer collateral stimulation evoked complex postsynaptic potentials (PSP) consisting of the excitatory and inhibitory postsynaptic potentials (EPSP and IPSP, respectively). After rats had learned the WM task, our major learning-specific findings included reduction of the mean peak amplitude of the IPSPs, delays in the mean peak latencies of the EPSPs and IPSPs, and correlation of the depolarizing-shifted IPSP reversal potentials and reduced IPSP-evoked membrane conductance. In addition, detailed isochronal analyses revealed that amplitudes of both early and late IPSP phases were reduced in a subset of the CA1 PCs after WM training was completed. These reduced IPSPs were significantly correlated with decreased IPSP conductance and with depolarizing-shifted IPSP reversal potentials and reduced IPSP-evoked membrane conductance. Another subset of WM-trained CA1 PCs had enhanced amplitudes of action potentials but no learning-specific synaptic changes. There were no WM training-specific modifications of other intrinsic membrane properties. These data suggest that long-term disinhibition in a subset of CA1 PCs may facilitate cell discharges that represent and record the spatial location of a hidden platform in a Morris WM.

INTRODUCTION

A number of specific intracellular correlates of learning such as increased membrane excitability (Alkon et al. 1982; Coulter et al. 1989; Moyer et al. 1996; Schreurs et al. 1998, Thompson et al. 1996), facilitated synaptic transmission (McKernan and Shinnik-Gallagher 1997; Power et al. 1997), or both (LoTurco et al. 1988) have been found in brain slices prepared following behavioral training of animals. Furthermore experience-dependent plasticity has been associated with changes in synaptic dynamics found in neocortical slices (Fennerty et al. 1999; Rioult-Pedotti et al. 2000). The CA1 area of the dorsal hippocampus (HC) is critical for acquisition, consolidation, storage, and retrieval of spatial memory (Moser et al. 1995; Riedel et al. 1999; Whishaw et al. 1994) and contains a higher percentage of place cells with well-defined and focused place fields (Jung et al. 1994). Activity of these cells also has behavioral and reward correlates (Breeze et al. 1989; Kobayashi et al. 1997). Learning-related, long-term changes of the HC place cell responses have been found after spatial learning in new environments (Breeze et al. 1989; Hollup et al. 2001; Kobayashi et al. 1997; Moser et al. 1999; Nishijo et al. 1999; Wilson and McNaughton 1993). These modified place cell responses are thought to encode and subsequently store new information about spatial location of reinforcement by increased firing rates in old and newly developed place fields (Breeze et al. 1989; Hollup et al. 2001; Kobayashi et al. 1997; Moser et al. 1999; Nishijo et al. 1999). Long-term potentiation (LTP) of the excitatory synaptic inputs to the pyramidal cells (PCs) has been considered to be a likely, but as yet not entirely confirmed, mechanism for these place cell enhanced responses (Bliss and Collingridge 1993; Hoh et al. 1999; Kentros et al. 1998; Staubli et al. 1995, 1999; Tsien et al. 1996; Zamanillo et al. 1999). Interneurons, on the other hand, dynamically control the discharge rate (Miles et al. 1996) and collective activity (Cobb et al. 1995) of the CA1 PCs during rats’ exploratory behavior and rest intervals (Cicsvari et al. 1999; Paulsen and Moser 1998; Stewart 1993; Wilson and McNaughton 1993). To study possible membrane and/or synaptic modifications of CA1 PCs at different stages of spatial memory acquisition (Hoh et al. 1999; Morris 1984), we extended methods here that combine in vivo and in vitro preparations (Alkon et al. 1982; Coulter et al. 1989; Disterhoft et al. 1986; LoTurco et al. 1988; Moyer et al. 1996; Sanchez-Andres and Alkon 1991; Schreurs et al. 1998; Thompson et al. 1996) by recording from slices of the dorsal HC found to be critical for water maze (WM) spatial learning (Moser et al. 1995; Riedel et al. 1999; Whishaw et al. 1994) from animals exposed to training or control protocols. We found that CA1 PCs receive reduced synaptic inhibition following complete acqui-
sition of WM task. This mechanism may contribute to the previously observed relocations of the neuronal place fields and increases in the PCs’ place-related activity during spatial learning (Breese et al. 1989; Hollup et al. 2001; Kobayashi et al. 1997; Moser et al. 1999; Nishijo et al. 1999).

**Methods**

**Training procedure**

Male Wistar rats (ca 280 g) were trained on a Morris WM task in a swimming pool (1.5 m diameter and 0.6 m high, filled with milky water, 23–25°C range) that was located in a well-lit room with distinct extra maze cues. A 10-cm² transparent square platform was hidden in a constant location (quadrant center) within the pool with its top surface submerged 1.5 cm below the water level. On day 0, rats swam for 2.5 min in the pool in the absence of the platform. For the next 1 day (WMT1 group; clear water was used for the short-term WM training and swimming) or 3 days (WMT3 group), rats were trained to locate the hidden island in four trials per day. On the first day, an animal was guided to the platform if it did not find it in 2 min. Rats were then allowed to stay on the platform for 40 s before starting the next trial from another quadrant. Rats from the swim control groups swam for 2 min a day in the pool without the island for the next 1 (SW1 group) or 3 days (SW3 group), respectively. Spatial memory of rats used for in vitro recordings was assessed according to the time required to find the platform (escape latency). Transfer tests 24 h after 1 or 3 days of WM training were performed on parallel behavioral groups of the rats not used for recordings. During the transfer tests, rats were started in the quadrant opposite to the target and swam for 60 s in the pool without a platform. Spatial memory in these groups was assessed as dwell time and distance swum in four pool quadrants with video tracking system Poly-Track (San Diego Instruments). Naïve rats also housed individually spent approximately the same number of days (8–10) in their home cages at the National Institutes of Health (NIH) animal facility after delivery from the Charles River Laboratory and before intracellular recordings. The animals’ care was in accordance with the NIH guidelines.

**Slice preparation**

Twenty-four hours after the last behavioral session, rats were decapitated by a small animal guillotine in accordance with the NIH-approved protocol. The whole brain was removed, and both hippocampi were quickly dissected out in ice-cold sucrose-artifical cerebrospinal fluid ACSF (Moyer et al. 1996; Thompson et al. 1996) [containing (in mM) 248 sucrose, 5 KCl, 2 CaCl₂, 2 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 d-glucose, gassed with 95% O₂-5% CO₂, pH 7.4]. Transverse slices (400 µm) were cut from the dorsal parts of both HC with a McIlwain tissue chopper and placed in an interface chamber (31°C, Fine Science Tools) where they were incubated for 30 min under sucrose-ACSF, and for the next 1 h under normal ACSF before starting recordings. Slices were in contact with a solution containing (in mM) 119 NaCl, 2.5 KCl, 2.5 CaCl₂, 1.3 MgSO₄, 1 NaH₂PO₄, 26.2 NaHCO₃, and 10 d-glucose, gassed with 95% O₂-5% CO₂, pH 7.4, with a perfusion speed 5–6 ml per min.

**Electrophysiology**

These recording conditions were chosen to minimize alterations due to pharmacological intervention(s) and voltage-clamp manipulations that are required to record inhibitory postsynaptic currents (IPSCs). Intracellular recordings were made with microelectrodes fabricated from thick-walled glass (2 mm OD, 1 mm ID; FHC, Bowdoinham, ME) on an electrode puller (NE-2; Narishige, Tokyo) and filled with 3 M potassium acetate (DC resistance, 80–120 MΩ). An Axoclamp 2B amplifier was employed in the bridge mode (Axon Instruments, Foster City, CA). Electrodes were positioned with the aid of a binocular dissecting microscope (Wild, Switzerland, magnification was up to ×50). Recording microelectrodes were advanced by a Leitz micromanipulator (Wetzlar, Germany). Data were acquired and analyzed with the aid of pClamp 6 software (Axon Instrument) at A/D sampling rates 5–10 kHz using a DigiData-1200 series interface and PC Pentium (Dell, Austin, TX). Before starting recordings, a field response in the stratum pyramidale to a standard stimulation of Schaffer collaterals was measured. Experiments were continued if population spikes did not show any evidence of multiple afterpotentials. Recordings were performed from 519 neurons located in the st. pyramidale of the CA1p area (about the middle of the length of st. pyramidale between the fimbria and subiculum) to avoid differences in the cell properties due to the cells’ location (Masukawa et al. 1982). Recordings were identified as somatic and from PCs based on accepted electrophysiological criteria. CA1 neurons were included in the study as PCs if they had an action potential amplitude ≥70 mV from the spike threshold, an action potential duration 1.2 ms from rise threshold to return to baseline membrane potential (Vₘ), a postburst AHP, impulse frequency accommodation, and spontaneous activity well below 0.001 Hz (Moyer et al. 1996; Thompson et al. 1996). In addition, accepted cells had a stable input resistance ≥25 MΩ and resting Vₘ more negative than −60 mV. A constant hyperpolarizing current of ≤0.2 nA was applied to provide stability of Vₘ close to −65 mV, if necessary. Only one cell was recorded from a given slice. We positioned a microconcentric bipolar stimulating electrode (FHC) in the middle part of the st. radiatum to evoke complex postsynaptic potentials (PSPs) recorded from PCs by Schaffer collateral stimulation. We maintained the same distance (~120–150 µm) between the stimulating and recording electrodes throughout the experiments. Many of neuronal parameters were measured according to criteria of previous studies performed in this as well as other labs (Coulter et al. 1989; Disterhoft et al. 1986; LoTurco et al. 1988; Moyer et al. 1996; Thompson et al. 1996).

**Experimental protocol to study intrinsic membrane properties**

The experimental protocol to study membrane properties of PCs was as follows: 1) 700-ms 0.5-nA hyperpolarizing current pulses were applied to evaluate an input resistance (Rᵢₙ) and to control the balanced bridge throughout the recording. The averages of 10 plateau voltage deflections were used for the Rᵢₙ evaluation. 2) One-hundred-millisecond depolarizing current pulses were applied to establish the current strength to evoke robust one or four spikes and also to study action potential parameters. Ten to 15 samples were collected per cell. 3) The average maximum voltage deflection following current offsets obtained during five injections of 100-ms current pulses was used to evaluate the slow afterhyperpolarization (sAHP) following one and four spikes. The second maximum was measured in the biphasic AHPs. We studied both single- and postburst slow AHPs to avoid possible masking effects of saturation on learning-induced changes. 4) The average numbers of action potentials obtained during each of five 800-ms intracellular current pulses of the same intensity that was used for the postburst sAHP study served to evaluate spike frequency accommodation of CA1 PCs. 5) Current-voltage relations (I-V) were studied using 700-ms current pulses with intensities ranging from −0.5 up to +0.7 nA. Three samples of I-V traces were collected for each cell. Average peak and plateau voltage deflections were used to evaluate slope membrane Rᵢₙ in response to hyper- and depolarizing current pulses (I < 0, I > 0). Rᵢₙ, Rᵢₙ-Iₙ, Rᵢₙ-Iₙ ratios were used to evaluate membrane rectification properties.

**Experimental protocol to study synaptic inputs**

Stimulation of the Schaffer collateral input was employed to assess synaptic properties of the CA1 PCs after spatial learning. The follow-
ing measurements were made: 1) input-output relations (I-O) of the excitatory component of the complex PSPs were studied by applying 0.4-ms current pulses at 0.1 Hz to the st. radiatum. To standardize the stimulus intensity, current intensity was used to elicit 2-mV excitatory PSP (EPSP) increments until action potential generation. Twenty measurements of PSPs were taken for all current intensities. Average EPSP amplitudes were used to construct I-O plots and to calculate the slopes of linear regression of the current intensity with the EPSP amplitude. 2) The initial EPSP rising slopes were measured as EPSP amplitudes elicited in the first milliseconds above the baseline $V_m$ level. Maximum subthreshold EPSPs were used in this analysis. 3) Analysis of the averaged PSPs containing maximum subthreshold EPSPs (maximum PSPs) was performed at peaks of the EPSP and inhibitory PSP (IPSP) phases and at 40-, 200-, and 350-ms time points relative to the stimulus artifact. 4) Reversal potentials ($E_{IPSP}$) for the early and late IPSP phases were evaluated at 40- and 150-ms latencies, respectively. Averages of 5 PSPs elicited by Schaffer collateral stimulation (approximately at half of spike threshold) at different holding $V_m$ were measured and plotted against $V_m$. Data points for the early IPSP were fit with linear functions, and $E_{IPSP}$ was determined from a single linear fitting equation by interpolating to zero PSP amplitude (Jensen et al. 1993). Data points for the late IPSP were fit by one or two linear functions, and $E_{IPSP}$ was determined from the second linear fitting equation usually applied with data obtained at the $V_m$ held below −70 mV. And 5) changes in membrane conductance introduced during early and late IPSPs ($g_{IPSP}$) were estimated according to the relation: $g_{IPSP} = 1/R_{IPSP} = 1/R_{m}$, where $R_{IPSP}$ is the total input resistance during the IPSP. $R_{m}$ is the resting input membrane resistance (Hablitz and Thalmann 1987). I-V relationships were plotted for the resting state, for 40- and 150-ms IPSP time points. Analyses of I-V relationships were performed on the portion of the I-V curves that was linear. The slopes of the resulting I-V regression lines were used to estimate $R_m$ and $R_{IPSP}$, respectively.

**Data analysis**

The rats were trained, slices were prepared, and recordings were performed by the same person. To avoid bias, data were processed only after the final sizes of the WMT1 and SW1, WMT3 and SW3 samples of cells were reached. Thus the records were taken without benefit of knowledge of effects that emerged after the extensive data analyses were performed. All data sets were first tested for the normality of their distributions with the Kolmogorov-Smirnov test. Acquisition of spatial memory in the Morris WM task and the effects of behavioral training on the frequency distribution of the events (and therefore on the relative frequency of the events) was used to test the null hypothesis that the rats acquired a spatial learning strategy in the WM task. The results were analyzed with chi-squared ($\chi^2$) test (Microsoft Excel, Microsoft). The bins were not chosen arbitrarily. Rather the bin size for the raw data selected to be large enough to ensure adequate sample size per bin for statistical testing (Hays 1963). Once selected, the same bin size was applied, in accordance with accepted practice, to all experimental and control groups. Physiological changes were considered learning-specific when there was a statistically significant difference between the WM trained rats and both control groups (comparably treated swim control and naive animals). Comparing swim control animals to naive control allowed us to extract nonspecific changes related to the learning context (e.g., arousal, stress, and physical activity).

**R E S U L T S**

**Water-maze learning**

Escape latency for the first trials of WM training was already significantly improved in the second day of training as compared with the first day [$F(1,18) = 6.33$, $P < 0.05$, 1-way ANOVA; Fig. 1A, trials 1 and 5]. However, the escape latency is not a sufficient indicator of spatial learning since it does not necessarily reflect spatial memory formation. Past work suggests that the rats have mainly learned a strategy for the WM task including climbing a hidden platform and searching for it (Hoh et al. 1999). Indeed transfer tests performed 24 h after 1 day of training indicated that rats did not show clear spatial preference for the trained quadrant as was measured by dwell time [$F(3,36) = 1.082$, $P > 0.3$; Fig. 1B] and by distance swim in the target and control quadrants [$F(3,36) = 0.601$, $P > 0.6$, Fig. 1C]. SW1 rats spent most of the time swimming along the walls and trying to climb them.

Twenty-four hours after 3 days of WM training, during transfer tests, however, rats showed strong spatial bias in their swim pathways as was indicated by increased mean dwell time and length of swim tracks in the trained quadrant as compared with control quadrants [$F(3,36) = 5.25$, $P < 0.01$; $P < 0.01$, $P < 0.02$, $P < 0.001$, Fisher’s post hoc test; $F(3,36) = 6.26$, $P < 0.01$; $P < 0.01$; $P < 0.001$, $P < 0.01$ Fig. 1, B and C, respectively]. There was no spatial bias in the dwell time and swim track lengths in the SW1 [$F(3,36) = 1.719$, $P > 0.2$; $F(3,36) = 2.747$, $P > 0.05$, respectively; Fig. 1, B and C] and SW3 control groups [$F(3,36) = 1.26; P > 0.3$; $F(3,36) = 1.439$, $P > 0.2$, respectively; Fig. 1, B and C]. After 3 days, the swim controls did not change their pattern of swimming. Swim tracks of control rats were mainly along the walls of the pool. These behavioral results suggest that acquisition of the WM task was complete (Morris 1984) and a spatial representation had been formed in the HC after 3 days of WM training but not after 1 day of WM training.

**Time course of EPSP and IPSP phases and isochronal analyses**

The complex PSPs induced by Schaffer collateral stimulation include activation of several conductances underlying the EPSP and IPSP phases initiated by glutamate release from the CA3 PC presynaptic terminals (Andreasen and Lambert 1998; Andreasen et al. 1989; Collingridge et al. 1988) and GABA release from a number of different interneurons which target different structural domains of the CA1 PCs (Buhl et al. 1994; Nurse and Lacaille 1997; Paulsen and Moser 1998). Somatic (early) and dendritic (late) IPSPs in the HC have been implicated in the control of the output and input of the PCs. Powerful inhibitory control by interneurons is mediated both through PC hyperpolarization and decreases in PC membrane resistance. Fast IPSPs arise mostly in the somata, while IPSPs with slow rise to peak are of dendritic origin (Pearce 1993). Early IPSPs with fast and slow onsets are mediated by $\gamma$-aminobutyric acid A (GABA$_A$) and $\gamma$-aminobutyric acid B (GABA$_B$) receptors that control both chloride and bicarbonate conductances (Perkins and Wong 1996; Staley et al. 1995). Monosynaptic and isolated early (GABA$_A$) IPSPs evoked by strong stimulation of the st. radiatum mostly return to the baseline in 300–400 ms (Billard et al. 1995; Davies and Collingridge 1993; Nathan and Lambert 1991; Ropertoff and Lambert 1994; Xie et al. 1995). Late (GABA$_B$) IPSPs last for ~600–1,000 ms, are maximal after ~150 ms, and are mediated by diverse subclasses of GABA$_B$ postsynaptic receptors regulating several potassium conductances (Billard et al. 1995; Hablitz and Thalmann 1987; Lopantsev and Schwartzkroin...
Because of the complex and diverse nature of the PSPs in the HC, we focused our study on those IPSPs which peaked in #70 ms. The vast majority of the maximum IPSPs that showed this property had similar time courses, showing large early IPSP phases and smaller amplitude late IPSP phases (Fig. 2, B and C). [The minor fraction of the total PSP population with IPSP time-to-peak >70 ms had very distinct responses either with 2 almost equal size peaks or small IPSP components (Fig. 2, B and D).]

Given that the average half-width of isolated EPSPs is \sim 18 – 21 ms (Andreasen and Lambert 1998; Collingridge et al. 1988), the IPSP phase at 40 ms will not be significantly contaminated by the EPSP, and IPSPs at 200 and 350 ms should not be affected by EPSPs at all. In current-clamp recordings performed in normal ASCF, brief EPSPs are mostly mediated by AMPA receptors. Expression of the N-methyl-D-aspartate (NMDA) component of the EPSP is prevented by concurrently activated IPSPs, which rapidly hyperpolarize neurons into a range of \( V_m \) at which \( Mg^{2+} \) blocks NMDA channels. NMDA currents have much longer half-width (\sim 60 ms) if measured at holding \( V_m \) about \sim -30 mV and under the presence of the \( GABA_A \) antagonist picrotoxin (Collingridge et al. 1988). Furthermore even with effective blockade of inhibition, the \( I-V \) relation for the NMDA component does not demonstrate a conductance increase in the holding \( V_m \) range from \sim -80 up to \sim -60 mV (Andreasen et al. 1989).

To analyze learning-induced between-group differences, we chose a sample of IPSPs with as similar time courses as possible—i.e., with peaks \sim 70 ms. The sampling rate (10 kHz) for the recorded PSPs was associated with minimal error for the PSP amplitude measurement. Figure 2A illustrates the reproducibility of the maximum IPSP amplitude and waveform. Furthermore each reported value for the isochronal analyses was the average of 20 responses. Note the transition of the early IPSP phase into the late IPSP phase. The early IPSPs were measured at 40-ms latency relative to the time of the st. radiatum stimulation. The late IPSP phases were measured at 350-ms latency when they were not significantly contaminated by early responses as indicated by the difference in the \( g_{IPSP} \) and \( E_{IPSP} \) that was below \( E_{CI} \) (about \sim -85 mV) (Kaila et al. 1999; Pham and Lacaille 1996; Xie et al. 1995). Finally, IPSP diversity appears as differences in the IPSP time courses, membrane conductance increases, and levels of the reversal potentials.
1993; see also Swearengen and Chavkin 1989) (Figs. 5 and 6, this study). Bicuculline did not significantly affect the late IPSP conductance measured at the late IPSP peaks (165–340 ms after strong Schaffer collateral stimulation) (Swearengen and Chavkin 1989). The transitional phases between both the early and late IPSP components were measured at a 200-ms time point.

Reduced IPSP amplitude after water-maze learning

Slices were prepared from the dorsal HC 24 h after completing one of the four behavioral procedures and from the HC of naive control animals (n = 20, number of animals). These procedures included 1 or 3 days of WM training (WMT1 and WMT3 groups; n = 21 and 20, respectively) and 1 or 3 days of swimming without a platform in the pool (SW1 and SW3 groups; n = 20 and 20, respectively).

First, analyses of the IPSPs with time to peak ≤70 ms showed that the WMT3 group had a learning-specific reduction in the mean peak IPSP amplitude. This IPSP amplitude was smaller as compared with naive and SW3 controls, and WMT1 group [F(4, 219) = 3.435, P < 0.01; P < 0.01, P < 0.05, respectively, Fisher’s post hoc test; Table 1]. There were no learning-specific changes in the mean peak IPSP amplitude in the WMT1 cells as compared with the SW1 and naive cells (P > 0.1, P > 0.7, respectively; Table 1).

In addition to this major learning-specific synaptic correlate of spatial memory acquisition in the WMT3 rats, we studied specifically the role of the early and late IPSPs in the observed disinhibition. The findings described in the following text are based on the results of isochronal analyses and, in general, demonstrate that animals trained for 3 days had IPSPs with reduced early and late phases. These early and late IPSP amplitude reductions were consistently manifest in significant between-group differences in IPSP distributions. These significant IPSP distribution differences occurred in the absence of learning-specific differences of passive membrane properties. Between-group differences in isochronal mean IPSP measurements, however, were not significantly different in all cases.

Analyses of the recorded PSPs from whole populations of neurons were also conducted (see following). Our main conclusions about the learning-specific synaptic changes in the dorsal HC were similar and independent of this classification of the data on the basis of IPSP dynamics.

Increased IPSP and EPSP peak latencies after water-maze learning

There was a learning-specific increase in the mean times to IPSP peaks in the WMT3 rats as compared with the naive (P < 0.05) and SW3 rats [F(4, 219) = 2.765, P < 0.05; P < 0.05; Table 1]. At the early stage of training in the WMT1 cells, this increase was not learning-specific as compared with SW1 (P > 0.9, Table 1). Representative PSP traces from the WMT3 and control (SW3, naive) animals illustrated longer times to the IPSP peak (Fig. 4). A weaker inhibition may account for an
Isochronal analysis of the complex postsynaptic potentials (PSPs) revealed disinhibition of the CA1 pyramidal cells (PCs) that was correlated with spatial memory formation. The learning-specific disinhibition was accompanied by the reduced peak inhibitory PSP (IPSP) amplitudes and longer times to the IPSP and excitatory PSP (EPSP) peaks (underlined). In general, the SW3 cells recorded after 3 days of swimming without platform had a mean hyperpolarized as compared to the naïve, SW1, WMT1 groups. The WMT3 cells had a mean

observed learning-specific increase in the mean latency of the EPSP peak in the WMT3 group as compared with the naïve, SW3, WMT1, and naïve slices. Representative traces from nine PCs recorded in the dorsal hippocampal slices obtained from the WMT3 and control (SW3, naïve) animals are illustrated in Fig. 4. There were no learning-specific changes in the WMT1 mean IPSP amplitude and distribution as compared with the SW1 and naïve groups (P > 0.1, P > 0.4; P > 0.07, P > 0.5, respectively).

Early IPSP phase

Because of the complexity of the membrane conductances underlying the PSPs, an isochronal measure (e.g., 40-ms time point) provided an objective means of comparing IPSP amplitudes between groups.

Analyses of IPSPs at a 40-ms latency also showed reduction of the mean IPSP amplitude in the WMT3 group as compared with naïve, WMT1, and SW1 rats [F(4,219) = 4.249, P < 0.01; P < 0.05, P < 0.001, P < 0.05, respectively], but not as compared with SW3 rats. However, as was shown in previous studies, learning-specific intracellular changes may appear only in a subset of and not in the entire population of the recorded cells. In such cases mean values may not sufficiently reflect learning-related modifications (Sanchez-Andres and Alkon 1991; Schreurs et al. 1998). In this study, therefore we analyzed between-group differences of IPSP distributions.

Based on the total number of recordings and the range of IPSP amplitudes for each isochronal measure, the minimum binwidth that provided a testable bin size proved to be 20% of the overall amplitude range (Fig. 3). The distribution analyses with \( \chi^2 \) test did reveal a learning-specific increase in the fraction of cells with smaller early IPSPs in the WMT3 group as compared with the SW3 group (P < 0.05; Fig. 3A). There were more IPSPs with amplitudes ≤7 mV in the WMT3 slices as compared with the SW3, SW1, WMT1, and naïve slices. Representative traces from nine PCs recorded in the dorsal hippocampal slices obtained from the WMT3 and control (SW3, naïve) animals are illustrated in Fig. 4. There were no learning-specific changes in the WMT1 mean IPSP amplitude and distribution as compared with the SW1 and naïve groups (P > 0.1, P > 0.4; P > 0.07, P > 0.5, respectively).

Late IPSP phase

Isochronal measures (e.g., 200- and 350-ms time points) provided an objective means of comparing late IPSP amplitudes between groups. Late phases had reduced mean amplitudes in the WMT3 group of rats as compared with naïve, WMT1 and SW1 rats at 200 ms [F(4,219) = 14.947, P < 0.0001; P < 0.01; P < 0.0001, P < 0.0001, respectively; Table 1, Fig. 3B], and at 350 ms [F(4,219) = 15.952, P < 0.0001; P < 0.05, P < 0.0001, P < 0.0001, respectively; Table 1, Fig. 3C] but not as compared with the SW3 rats. The observed IPSP reduction was a learning-specific effect, however, as indicated by the larger fraction of the WMT3 neurons with reduced late IPSP amplitudes as compared with naïve, SW1 and WMT1 groups and distribution as compared with the SW1 and naïve groups (P < 0.05, respectively).

### Table 1. PSP parameters of CA1 pyramidal cells as functions of training procedures

<table>
<thead>
<tr>
<th></th>
<th>WMT1</th>
<th>SW1</th>
<th>Naïve</th>
<th>WMT3</th>
<th>SW3</th>
</tr>
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<tbody>
<tr>
<td>Maximum peak IPSP, mV</td>
<td>−10.3 ± 0.4</td>
<td>−9.2 ± 0.3</td>
<td>−10 ± 0.5</td>
<td>−8.3 ± 0.5***</td>
<td>−9.5 ± 0.5</td>
</tr>
<tr>
<td>n</td>
<td>43</td>
<td>39</td>
<td>44</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>Time to IPSP peak, ms</td>
<td>34 ± 1.2</td>
<td>34 ± 1.1</td>
<td>30.8 ± 1.2</td>
<td>35.7 ± 1.7**</td>
<td>30.8 ± 1.3</td>
</tr>
</tbody>
</table>
| Maximum        | −9.7 ± 0.4 | −8.7 ± 0.2 | −9.3 ± 0.5 | −7.5 ± 0.5** | −8.5 ± 0.4 |**
| IPSP at 40 ms, mV | −5.6 ± 0.3* | −5.2 ± 0.3 | −4.6 ± 0.3 | −2.8 ± 0.3*** | −3.4 ± 0.3*** |
| IPSP at 200 ms, mV | −2.9 ± 0.2** | −2.7 ± 0.2* | −2.2 ± 0.2 | −1.3 ± 0.2*** | −1.5 ± 0.1*** |
| IPSP at 350 ms, mV | 6.5 ± 0.3* | 6.3 ± 0.3** | 7.6 ± 0.4 | 8.5 ± 0.3§ | 7.8 ± 0.3§ |
| Initial rising  | 3.9 ± 0.2 | 3.7 ± 0.2 | 4.3 ± 0.2 | 3.9 ± 0.2 | 3.9 ± 0.2 |
| EPSP slope, mV/msec | 5.1 ± 0.2 | 5 ± 0.1 | 5 ± 0.1 | 5.8 ± 0.2** | 5.2 ± 0.2 |
| EIPSP at 40 ms  | −76.7 ± 1.3 | −81 ± 0.7 | −79.3 ± 0.8 | −77.7 ± 1.1 | −80.2 ± 1 |
| n              | 13   | 17  | 42    | 45   | 44  |
| EIPSP at 150 ms | −94 ± 3.3 | −100.8 ± 1.9 | −100.1 ± 1.5 | −97.8 ± 1.6 | −102.1 ± 1.5 |
| n              | 13   | 17  | 41    | 42   | 43  |
| ISPSP at 40 ms | 25 ± 3.7 | 23 ± 2.9 | 25.9 ± 2.2 | 22 ± 2.1 | 25.8 ± 2.7 |
| n              | 12   | 17  | 43    | 45   | 44  |
| ISPSP at 150 ms | 5.3 ± 0.5 | 5.6 ± 0.8 | 4.2 ± 0.4 | 3.1 ± 0.4§ | 3.5 ± 0.4§ |
| n              | 12   | 17  | 42    | 42   | 43  |
| Vm, mV         | 63.7 ± 0.3 | 63.2 ± 0.3* | 64.3 ± 0.4 | 64.8 ± 0.3§ | 64.7 ± 0.3** |
| n              | 43   | 39  | 44    | 50   | 48  |
respectively; also refer to the legend of Fig. 3 for detailed discussion of the IPSP distributions in the SW1 and SW3 groups).

### Whole population of the recorded IPSPs

The vast majority of the maximum IPSPs peaked in ≤70 ms and had a large early phase followed by a late smaller phase (see Fig. 2 for methods). While we focused our study on this major class of IPSPs, we did check the whole population of the recorded IPSPs regardless of their classification based on IPSP kinetics and peak latency. While there were no learning-specific differences of mean IPSP amplitudes (using the reference parameters discussed in the preceding text), there was a larger fraction of cells with small IPSP in the WMT3 group as compared with the SW3 group \((P < 0.01)\) was not accompanied by significant changes in the late SW1 IPSPs as compared with the WMT1 group. Also we cannot rule out the effect of the slightly depolarized \(V_m\) on the nonspecific increase of mean IPSP amplitude in the WMT1 and WMT3 cells as compared with the naive cells \((P < 0.05, P < 0.01, \text{respectively}; \text{Table 1})\). A slightly higher frequency of cells with smaller late IPSPs was also present in the SW3 as compared with naive rats \((P < 0.01; P < 0.05)\). Nevertheless we cannot exclude a small effect of slightly hyperpolarized \(V_m\) on the IPSP amplitude in the SW3 cells (Table 1) and, most important, there was no difference in the IPSP conductance between SW3 and naive cells (see Fig. 6). Symbols (*) and (**) indicate statistically significant difference between whole WMT3 and SW3 distributions at \(P < 0.05\) and \(P < 0.01\), respectively, \(\chi^2\) test.

\(V_m\)

The data analysis revealed a small but statistically significant difference in the mean values of the baseline \(V_m\) among the five groups of cells during PSP study \([F(4,219) = 8.043, P < 0.0001]\). However, there were no learning-specific changes in \(V_m\) (Table 1). Conductance and reversal potential studies were not dependent on the \(V_m\) level.

### Membrane conductance evoked during IPSP phase \(g_{IPSP}\)

Analysis of the membrane conductance changes evoked during the IPSP phases provided compelling evidence of the reduced inhibition after WM learning. IPSPs have a powerful control over neuron excitability not only by determining the level of the \(V_m\) but mainly due to their shunting effect on membrane resistance. This shunting significantly reduces the EPSPs' ability to bring \(V_m\) close to an action potential threshold. Given that learning-induced changes in the isochronal IPSP amplitudes were revealed by the distribution analyses, we concentrated on relationships between the isochronal IPSP amplitude and conductance to assess the effect(s) of the IPSP decrease on neuronal properties.

Although there were no learning-specific changes in mean \(g_{IPSP}\) measured both at 40- and 150-ms time points \([F_{(4,156)} = 0.551, P > 0.6; F_{(4,151)} = 3.965, P < 0.01, \text{respectively}; \text{Table 1})\], analyses performed on scatter plots of the \(g_{IPSP}\) versus maximum IPSP amplitudes showed a significant linear correlation between these parameters. IPSPs with smaller amplitudes were associated with smaller increases in membrane conductance (Fig. 6).
FIG. 4. Reduced early and late IPSP phases 24 h after water-maze training. A: superimposed representative traces of the complex postsynaptic responses from 3 dorsal CA1 pyramidal cells evoked by a subthreshold Schaffer collateral stimulation after 3 days of training (WMT3 group). Numbers at the left and ⋯⋯ V_n. Each trace is an average of 15–20 responses. Artifacts are truncated at 5-mV amplitudes. B and C: representative synaptic responses from 3 neurons recorded after 3 days of swimming without platform (SW3 group, B) and from naïve rats (C). A–C, bottom: Same synaptic responses with a faster time scale. Note the smaller amplitudes of the early and late IPSP phases in the WMT3 group as compared with the SW3 and naïve controls. At the same time, the amplitudes of the EPSP phases in the WMT3 group were not different from both controls. However, the duration of the EPSP amplitudes of the early and late IPSP phases in the WMT3 group as compared with the SW3 and naïve controls was larger in neurons from the WMT3 group compared with the naïve and SW3 controls (P < 0.05; Fig. 5A). At the same time, both SW1 and SW3 controls had late IPSP amplitudes reduced early and late IPSPs as well as depolarized early and late IPSPs. This subset was larger in neurons from the WMT3 rats as compared with the naïve and SW3 controls (P < 0.05; Fig. 5B). At the same time, g_ipsp was not different among SW3, naïve, WMT1, and SW1 animals at both latencies (Fig. 5, A and B). Therefore long-term learning-specific disinhibition in a subset of the CA1 PCs was accompanied by reduced g_ipsp evoked during the early and late IPSP phases, and, consequently by reduced shunting effect on neuron R_in.

IPSP reversal potential (E_ipsp)

To examine the ionic basis of the disinhibition of PCs as a result of spatial learning, we measured IPSP E_ipsp in all groups. The effects of the WM training could not be seen in the mean E_ipsp measured at 40-ms latency [F(4,156) = 2.03, P > 0.05; Table 1] and at 150-ms latency [F(4,151) = 2.11, P > 0.05; Table 1]. There was, however, a significant linear correlation between IPSP amplitude and the level of the early E_ipsp—i.e., smaller IPSPs had more depolarized E_ipsp (Fig. 6A). To identify learning-specific changes within the isochronal IPSP distributions, we focused our analysis on those cells with early IPSP amplitudes that were ≤7.5 mV (mean IPSP amplitude at 40 ms in the WMT3 group) and E_ipsp ≥−78 mV (mean E_ipsp at 40 ms in the WMT3 group) in all five groups. This subset was larger in neurons from the WMT3 rats as compared with the naïve and SW3 controls (P < 0.05; P < 0.05; Fig. 6A). At the same time, both SW1 and SW3 controls were not different from naïve subjects (P > 0.6; P > 0.6; Fig. 6A) and WMT1 group was not different from SW1 control (P > 0.06).

Similar analysis of the relationships between the late IPSP amplitude and late E_ipsp revealed that only the group showing complete acquisition of the spatial memory task also showed an increased fraction of cells with both reduced late IPSPs and late depolarized E_ipsp. The larger fraction of CA1 PCs that had late IPSP amplitudes ≤2.8 mV (mean IPSP amplitude at 200 ms in the WMT3 group) and E_ipsp ≥−98 mV (mean E_ipsp at 150 ms in the WMT3 group) was also larger in neurons from the WMT3 rats as compared with the naïve and SW3 controls (P < 0.05; P < 0.05; Fig. 5B). At the same time, g_ipsp was not different among SW3, naïve, WMT1, and SW1 animals at both latencies (Fig. 5, A and B). Therefore long-term learning-specific disinhibition in a subset of the CA1 PCs was accompanied by reduced g_ipsp evoked during the early and late IPSP phases, and, consequently by reduced shunting effect on neuron R_in.

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Learning-specific relation between $E_{\text{IPSP}}$ and $g_{\text{IPSP}}$

To further characterize the possible mechanisms of the learning-induced depolarizing shifts in the $E_{\text{IPSP}}$, we analyzed for a correlation between the $E_{\text{IPSP}}$ and $g_{\text{IPSP}}$ for the above-described early and late-phase IPSP components. For both early and late IPSPs, there was a statistically significant linear correlation between the $E_{\text{IPSP}}$ and $g_{\text{IPSP}}$ in the WMT3 group ($r = 0.55$, slope of the linear regression $= 1.09$; $r = 0.43$, slope $= 0.1$). This is another major finding that emerged without any segregation into subpopulations for exactly the same early IPSP and late IPSP components that showed learning-specific reduction of amplitudes. This correlation was not characteristic of the SW3 ($r = 0.07$, slope $= 0.18$, NS; $r = 0.11$, slope $= 0.03$, nonsignificant (NS)) and naïve group ($r = 0.33$, slope $= 0.86$; $r = 0.19$, slope $= 0.05$, NS; plots not shown).

A more detailed analysis of the relationship between $E_{\text{IPSP}}$ and $g_{\text{IPSP}}$ further showed that the fraction of cells with early $g_{\text{IPSP}}$ ≤ 22 nS (mean $g_{\text{IPSP}}$ in the WMT3 cells) and $E_{\text{IPSP}}$ ≥ −78 mV (mean $E_{\text{IPSP}}$ in the WMT3 cells) was larger in the WMT3 group as compared with SW3 and naïve cells ($P < 0.05$, $P < 0.01$). The fraction of cells with late $g_{\text{IPSP}}$ ≤ 3.1 nS (mean $g_{\text{IPSP}}$ in the WMT3 cells) and $E_{\text{IPSP}}$ ≥ −98 mV (mean $E_{\text{IPSP}}$ in the WMT3 group) was larger in the WMT3 group as compared with SW3 ($P < 0.05$), although, it was not statistically significant larger as compared with naïve cells ($P = 0.11$).

Representative cells in Fig. 7 demonstrate depolarized $E_{\text{IPSP}}$ and smaller estimated changes in the membrane conductance $g_{\text{IPSP}}$ during the early and late IPSP phases after acquisition of the water maze task as compared with the SW3 and naïve control groups.

Thus correlation analyses suggest that a learning-induced reduction in the total $g_{\text{IPSP}}$ is also associated with an increased role of the conductance(s) that have reversal potentials more depolarized than $E_{\text{Cl}^-}$ and $E_{\text{K}^+}$. At this stage, we cannot distinguish between such candidates as HCO$_3^-$ or Na$^+$ and Ca$^{2+}$ conductances to explain the learning-induced depolarizing shifts in the early $E_{\text{IPSP}}$ of cells displaying reduced $g_{\text{IPSP}}$. Application of pharmacological agents to isolate monosynaptic EPSP and IPSP should further clarify the remaining uncertainty.
Relations between \(E_{\text{IPSP}}\) and EPSP rising slope

We observed an \(-28\)-mV range of the early \(E_{\text{IPSP}}\) (from \(-88\) mV up to \(-60\) mV; Fig. 6A) and a 45-mV range for the late \(E_{\text{IPSP}}\) (from \(-120\) mV up to \(-75\) mV; Fig. 6B) in all experimental and control groups. One of the possible explanations for such variability could be difference in summation of the EPSP- and IPSP-related \(\text{Na}^+, \text{Ca}^{2+}, \text{Cl}^-, \text{and K}^+\) conductances (Andreasen and Lambert 1998; Collingridge et al. 1988; Hablitz and Thalmann 1987; Jensen et al. 1993; Otis et al. 1993). As in previous studies (Lopantsev and Schwartzkroin 1999; Otis et al. 1993), we found here that \(E_{\text{IPSP}}\) for some late IPSPs were more negative than the theoretical equilibrium potential for \(K^+\) current (\(-98\) mV at 2.5 mM \(K^+\) in ACSF) (Otis et al. 1993). It has been suggested that a clear late \(E_{\text{IPSP}}\) is not demonstrable under physiological levels of \(K^+\) because of the rectification in membrane properties at hyperpolarized \(V_m\) and activation of a parallel shunting conductance (Hablitz and Thalmann 1987). Bilinear late IPSP versus \(V_m\) plots in the present study (not shown) as well in previous studies may reflect this possible effect of rectification (Lopantsev and Schwartzkroin 1999; Otis et al. 1993).

Potentiation of both non-NMDA and NMDA EPSP components is expressed as an increase of the EPSP rising slopes (Blitzer et al. 1995; Buonomano 1999; Huang and Hsu 1999; Manabe et al. 2000). There was, however, no correlation between the IPSP peak amplitude and the EPSP initial slope (\(r = -0.16, P > 0.2\)) nor was there a correlation between the \(E_{\text{IPSP}}\) at 40 ms and the EPSP initial rising slope (\(r = -0.24, P > 0.1\)) in the WMT3 group. These data do not support the hypothesis that learning-specific depolarizing shifts in the \(E_{\text{IPSP}}\) and IPSP amplitude reduction were caused by EPSP facilitation. We cannot entirely rule out, however, the possibility that potentiated EPSPs may be masked by a potentiated IPSP conductance i.e., one offsetting the other.

**EPSP input-output relationships and initial rising slope**

The following findings from the five groups of neurons provide additional evidence that the Schaffer collateral-induced excitatory inputs of the CA1 PCs were not facilitated as a result of the spatial learning. EPSP amplitudes were measured from the baseline \(V_m\).

1) Slopes from linear regression equations for stimulating current-EPSP amplitude (I-O) relations were determined individually for each cell in all groups and comparisons were performed separately for the same ranges of stimulating currents. There were no learning-specific differences in the intensity of stimulating current applied to Schaffer collateral input. The intensities of both average and maximum stimulating currents to elicit EPSPs in the WMT3 and SW3 groups were not different from naïve control and from each other (\(P > 0.9, P > 0.9, P > 0.9, P > 0.9\), respectively; Table 2). However, ANOVA and post hoc analyses showed higher intensities of the stimulating current to elicit EPSPs in slices in the WMT1 group as compared with the SW3, SW1, and naïve group. The WMT3 distribution was not different from the WMT1 distribution. \(\dagger\) \(P < 0.05; \chi^2\) test.

![Fig. 6](http://jn.physiology.org/ by 10.220.32.246 on May 19, 2017)
ranges, however, one-way ANOVA did not reveal any specific effect of WM training on the linear regression slopes of stimulating current versus EPSP amplitude after 1 day of training [$F(2,70) = 0.1946, P > 0.8$; $F(1,58) = 0.0622, P > 0.8$; Table 2, WMT1, SW1, and naïve groups] or after 3 days of WM training [$F(2,29) = 0.6133, P > 0.5$; $F(2,56) = 0.5666, P > 0.5$; $F(2,26) = 0.3393, P > 0.7$; Table 2, WMT3, SW3, and naïve groups]. Synchronous responses and stimulus-response relationships for the EPSP phase from the WMT3 and SW3 representative cells are illustrated in Fig. 8.

2) The initial rising slopes of the maximum subthreshold EPSPs frequently used as an indicator of EPSP potentiation (Blitzer et al. 1995; Buonomano 1999; Huang and Hsu 1999; Manabe et al. 2000) were also not affected by WM training [$F(4,219) = 1.179, P > 0.3$ for the subgroup of cells; Table 1, $F(4,256) = 0.5666, P > 0.5$ for the whole population; not shown]. Representative traces from all five groups of cells and EPSP rising slope frequency distributions demonstrate no effect of the behavioral procedures (Fig. 9, A and B).

3) While EPSPs had larger mean amplitudes in slices from the WMT3 and SW3 rats as compared with EPSPs recorded in slices from the WMT1 and SW1 rats [$F(4,255) = 6.227, P < 0.01$], they did not reveal any specific effects of WM training on the linear regression slopes of stimulating current versus EPSP amplitude after 1 day of training [$F(2,70) = 5.0.1946, P > 0.8$; $F(1,58) = 0.0622, P > 0.8$; Table 2, WMT1, SW1, and naïve groups] or after 3 days of WM training [$F(2,29) = 0.6133, P > 0.5$; $F(2,56) = 0.5666, P > 0.5$; $F(2,26) = 0.3393, P > 0.7$; Table 2, WMT3, SW3, and naïve groups]. Synaptic responses and stimulus-response relationships for the EPSP phase from the WMT3 and SW3 representative cells are illustrated in Fig. 8.

FIG. 7. Representative cells demonstrate depolarized IPSP reversal potentials and smaller estimated changes in membrane conductance during the early and late IPSP phases after acquisition of the water-maze task in the WMT3 group as compared with the SW3 and naïve control groups. A: after 3 days of training in the WMT3 group, PSP with reduced IPSP phase was evoked in CA1 pyramidal cell by stratum radiatum stimulation (↑) at different holding membrane potentials as indicated on the left of each trace. Each trace is an average of 5 sweeps evoked during current pulses of equal intensity (ranging usually from −0.9 to 0.15 nA). At each membrane potential, the voltage was measured at rest prior to stimulation of st. radiatum ($V_m$ rest), at 40 ms, and at 150 ms following stimulus artifact. Time points for the measurements are indicated (---). Artifacts are truncated. B and C: PSPs with larger IPSP phase were recorded from the dorsal hippocampus slices from rat swum for 2 min/day for 3 days without the platform in the pool (SW3 group, B) and from naïve rat (C). D–F: current-voltage ($I$-$V$) relationships for the IPSPs in the cells shown in A–C, respectively. Membrane potentials at rest ( ■) and during the IPSP phases at 40 ms ( ●) and at 150 ms ( △) were plotted as a function of injected current. —, the linear regressions of each set of data. The decrease in slope of regression lines for the IPSPs relative to the resting state indicated an increased membrane conductance during IPSPs. The estimated early and late conductance changes (gIPSP, calculated from the slope of the regression lines, see METHODS) for the WMT3 neuron (18.7 and 2.7 nS, respectively) were less than that for SW3 (31.3 and 4 nS, respectively) and naïve (33.9 and 3.4 nS, respectively) neurons. Note also a larger slope of the $I$-$V$ function for IPSPs for the WMT3 cell as compared with the SW3 and naïve cells. — — — intersections of IPSP regression lines with the $I$-$V$ function for the resting state at the $E_{IPSP}$ level at 40 and 150 ms. Note the more depolarized $E_{IPSP}$ levels both for the early and late IPSPs in the WMT3 cell (−74 and −91 mV, respectively) as compared with the $E_{IPSP}$ level for the IPSPs in the SW3 (−87 and −104 mV, respectively) and naïve (−85 and −107 mV, respectively) neurons.
The slope mean values of linear regressions of stimulating current versus EPSP amplitude obtained for the same stimulation intensity. Parentheses enclose number of neurons. There were no learning-specific changes in the I-O functions: the slopes were also not different among the WMT1, SW1, and naïve cells, and the slopes were also not different among the WMT3, SW3, and naïve cells. A minor group of cells with 0.05-ms stimulating impulses are not included in this analysis. Average stimulating current calculated only for neurons with 3 points in the I-O functions. Maximum stimulating current calculated for all neurons. I, μA mean current intensity applied for the str. radiatum stimulation. * P < 0.05, ** P < 0.01 significantly different from naïve control; † P < 0.05, ‡ P < 0.01 significantly different from the SW1 and WMT1 groups, Fisher’s and Scheffe’s (for stimulating current only) post hoc tests.

0.0001; P < 0.0001, P < 0.01, P < 0.0001, P < 0.01 for total PSP population, not shown; F(4,219) = 7.11, P < 0.0001; P < 0.01, P < 0.001; P < 0.05 for the PSP with IPSP peaks at ≥70 ms; Table 1], they were not different from naïve neurons (P > 0.1; P > 0.3 for the whole population, not shown; P > 0.07; P > 0.8 for PSP with early IPSP peaks, Table 1). Mean EPSP amplitude and EPSP distribution in the WMT3 group were also not different from the SW3 EPSPs (P > 0.1, P > 0.7; P > 0.4, P > 0.1, respectively for the whole population (not shown) and subset of cells, Table 1). There may have also been a nonspecific enhancement of EPSP amplitude due to the slightly hypopolarized V_m in the WMT3 and SW3 groups as compared with the WMT1 and SW1 rats (Table 1).

Based on the preceding studies of the complex PSPs, we conclude, therefore that learning-specific disinhibition in a subset of the dorsal CA1 PCs is due to reduced synaptic inhibition with smaller membrane conductances evoked during IPSPs and depolarizing-shifted reversal potentials. At the same time, it is unlikely that IPSP reduction can mask EPSP facilitation.

**FIG. 8.** EPSP input-output (I-O) functions do not show EPSP phase facilitation after WM training. A: increasing intensities of stimulating current were applied to the str. radiatum to obtain ~2-mV increments in the EPSP peak amplitude until the action potential was triggered. Each synaptic response includes a short-latency EPSP component followed by a biphasic IPSP with both early and late phases. IPSP amplitudes that followed subthreshold EPSPs are not shown) indicate the absence of a learning-specific effect on EPSP I-O relations. Each slope (cf. Table 2 for means) is taken from the linear regression for all points determined within the I-O range. Equations for the WMT3 cell (Y = 0.6 + 2.12X) and for the SW3 cell (Y = −4.31 + 2.03X) indicate similar slopes of linear regressions of stimulating current vs. the EPSP amplitude for representative neurons.

**TABLE 2.** EPSP input-output functions during stratum radiatum stimulation obtained for the same ranges of stimulating current

<table>
<thead>
<tr>
<th></th>
<th>WMT1</th>
<th>SW1</th>
<th>Naïve</th>
<th>WMT3</th>
<th>SW3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average stimulating I, μA</td>
<td>17.2 ± 3.7* (30)</td>
<td>19.2 ± 4.2** (30)</td>
<td>6.2 ± 1.9 (48)</td>
<td>7.5 ± 1.3‡ (55)</td>
<td>6 ± 0.5‡ (56)</td>
</tr>
<tr>
<td>Maximum subthreshold I, μA</td>
<td>26.1 ± 4.1** (39)</td>
<td>27.4 ± 5** (38)</td>
<td>9.4 ± 3 (51)</td>
<td>12.7 ± 2.5‡ (57)</td>
<td>9.3 ± 0.9‡ (58)</td>
</tr>
<tr>
<td>I, μA</td>
<td>17.2 ± 3.7 (30)</td>
<td>19.2 ± 4.2 (30)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Slope</td>
<td>1.2 ± 0.3 (30)</td>
<td>1.38 ± 0.5 (30)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>I, μA</td>
<td>—</td>
<td>—</td>
<td>2.2 ± 0.1 (15)</td>
<td>2.4 ± 0.2 (10)</td>
<td>2.4 ± 0.3 (7)</td>
</tr>
<tr>
<td>Slope</td>
<td>—</td>
<td>—</td>
<td>4.4 ± 0.6 (15)</td>
<td>3.7 ± 0.7 (10)</td>
<td>4.7 ± 0.6 (7)</td>
</tr>
<tr>
<td>I, μA</td>
<td>—</td>
<td>—</td>
<td>3.5 ± 0.2 (15)</td>
<td>4.1 ± 0.1** (21)</td>
<td>4.1 ± 0.1** (23)</td>
</tr>
<tr>
<td>Slope</td>
<td>—</td>
<td>—</td>
<td>2 ± 0.2 (15)</td>
<td>2.3 ± 0.2 (21)</td>
<td>2.3 ± 0.2 (23)</td>
</tr>
<tr>
<td>I, μA</td>
<td>—</td>
<td>—</td>
<td>5.7 ± 0.3 (7)</td>
<td>6 ± 0.2 (9)</td>
<td>5.9 ± 0.2 (13)</td>
</tr>
<tr>
<td>Slope</td>
<td>—</td>
<td>—</td>
<td>0.9 ± 0.1 (7)</td>
<td>1.4 ± 0.2 (9)</td>
<td>1.5 ± 0.2 (13)</td>
</tr>
<tr>
<td>I, μA</td>
<td>5.3 ± 0.7* (14)</td>
<td>5.8 ± 0.4** (17)</td>
<td>3.9 ± 0.3 (42)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Slope</td>
<td>2.2 ± 0.5</td>
<td>2.2 ± 0.8</td>
<td>2.6 ± 0.3</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Membrane properties after water-maze training

In addition to the receptor-regulated (e.g., GABA) postsynaptic excitation, intrinsic neuronal excitability parameters were also examined.

Action potential

There was a learning-specific increase in the variance of spike amplitude in the WMT3 group as compared with the SW3 (43.4 vs. 25.8, ratio = 1.68; \( P < 0.05 \), \( F \) test) and naive (43.4 vs. 21.7, ratio = 2; \( P < 0.01 \)) controls due to an apparent increase in the fraction of neurons with enhanced spike amplitudes after 3 days of WM training (Table 3; Fig. 10). There were no learning-specific differences in the spike width (Table 3). There was no observed learning-specific change of the input resistance that could account for the observed increase of action potential amplitude (see Table 4). The observed learning-specific increase of spike amplitude might be explainable by a decreased input conductance following long-term disinhibition. However, such an explanation can be excluded by the absence of a significant correlation between action potential amplitude and the amplitude of early (\( r = -0.07, P > 0.6 \)) and late IPSPs (\( r = 0.06, P > 0.6 \)) in the WMT3 neurons.

These results suggest that one subset of CA1 PCs showed a learning-related change in intrinsic neuronal properties following WM training.

Table 3. Intrinsic membrane characteristics of pyramidal neurons without learning-specific changes after water-maze training

<table>
<thead>
<tr>
<th></th>
<th>WMT1</th>
<th>SW1</th>
<th>Naive</th>
<th>WMT3</th>
<th>SW3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-spike sAHP amplitude, mV</td>
<td>-1.25 ± 0.11</td>
<td>-1.16 ± 0.11</td>
<td>-1.07 ± 0.11</td>
<td>-1.37 ± 0.08</td>
<td>-1.24 ± 0.08</td>
</tr>
<tr>
<td>Latency of 1-spike sAHP peak, ms</td>
<td>273 ± 20.3</td>
<td>283 ± 25.5</td>
<td>250 ± 23.8</td>
<td>261 ± 14.9</td>
<td>254 ± 16</td>
</tr>
<tr>
<td>( I_i ), nA to evoke 4 spikes</td>
<td>0.32 ± 0.02*</td>
<td>0.31 ± 0.02**</td>
<td>0.38 ± 0.02</td>
<td>0.45 ± 0.03</td>
<td>0.45 ± 0.03‡</td>
</tr>
<tr>
<td>‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postburst sAHP amplitude, mV</td>
<td>-6 ± 0.3</td>
<td>-6.3 ± 0.3</td>
<td>-6.8 ± 0.4</td>
<td>-7.7 ± 0.4‡</td>
<td>-7.4 ± 0.3†</td>
</tr>
<tr>
<td>Time to peak 4-spike sAHP, ms</td>
<td>284 ± 8.9</td>
<td>287 ± 8.7</td>
<td>285 ± 9.8</td>
<td>278 ± 8.7</td>
<td>279 ± 8.3</td>
</tr>
<tr>
<td>Decay time to 1/3 of sAHP peak, ms</td>
<td>1,514 ± 51.7</td>
<td>1,561 ± 53.3</td>
<td>1,516 ± 63.1</td>
<td>1,427 ± 55.7</td>
<td>1,480 ± 63.5</td>
</tr>
<tr>
<td>Total time of interspike intervals in 4-spike burst, ms</td>
<td>68.7 ± 1.9</td>
<td>65 ± 1.6</td>
<td>67.3 ± 1.1</td>
<td>66.3 ± 1.2</td>
<td>67.7 ± 1.3</td>
</tr>
<tr>
<td>Spike height, mV</td>
<td>78.1 ± 0.7</td>
<td>77.6 ± 0.6</td>
<td>77.3 ± 0.6</td>
<td>81.6 ± 0.8§ †††</td>
<td>80.1 ± 0.6**‡ †††</td>
</tr>
<tr>
<td>Spike width, ms</td>
<td>1.42 ± 0.2</td>
<td>1.36 ± 0.1</td>
<td>1.43 ± 0.02</td>
<td>1.43 ± 0.02</td>
<td>1.48 ± 0.02</td>
</tr>
<tr>
<td>Number of spikes per 800-ms</td>
<td>5.5 ± 0.2</td>
<td>5.5 ± 0.2</td>
<td>5.7 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>( n )</td>
<td>60</td>
<td>63</td>
<td>62</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>( V_m ) below 1-st spike threshold in 4-spike burst, mV</td>
<td>8.4 ± 0.3</td>
<td>8.2 ± 0.3</td>
<td>8.7 ± 0.3</td>
<td>8.8 ± 0.3</td>
<td>8.4 ± 0.3</td>
</tr>
<tr>
<td>Membrane potential, mV</td>
<td>-64.4 ± 0.3</td>
<td>-64.2 ± 0.3</td>
<td>-64.4 ± 0.3</td>
<td>-64.8 ± 0.26</td>
<td>-64.1 ± 0.28</td>
</tr>
<tr>
<td>Resting ( V_m ) before electrode was withdrawn, mV</td>
<td>-61.7 ± 0.6</td>
<td>-61.4 ± 0.6</td>
<td>-61.3 ± 0.6</td>
<td>-63.5 ± 0.7</td>
<td>-62.7 ± 0.6</td>
</tr>
</tbody>
</table>

There was no learning-specific modification of intrinsic membrane excitability following water-maze training in CA1 pyramidal cells, sAHP, slow after hyperpolarization, \( * P < 0.05 \), \( ** P < 0.01 \), \( *** P < 0.0001 \) significantly different from naive control; \( † P < 0.05 \), \( ‡ P < 0.01 \), \( †† P < 0.001 \) significantly different from the SW1 and WMT1 cells, Fisher’s post hoc test; \( § P < 0.001 \) significantly different from the naive cells, Scheffe’s post hoc test.
learning-specific reduction of IPSPs (see preceding text), but no changes of intrinsic membrane properties. Another subset of the PCs in the WMT3 slices showed increased spike amplitude but no synaptic changes.

**Intrinsic membrane excitability**

There were no learning-specific changes in spike threshold, resting $V_r$ level, or input resistance values (Table 3). Nor were there learning-specific changes in the sAHP following a single spike or a four-spike train (Fig. 11A, Table 3; see Fig. 12A for representative traces). There were also no learning-specific changes in the spike frequency accommodation during the 800-ms depolarizing current pulses (Fig. 11B, Table 3; see Fig. 12B for representative traces). Enhancement of the sAHP and spike frequency accommodation was not learning-specific. The mean values of the peak latencies for the one- and four-spike sAHPs, mean values of the decay times of the postburst sAHP, interspike time intervals between first, second, third, and fourth action potentials (not shown), and mean values of the total duration of four-spike bursts were also not affected by behavioral treatment (Table 3).

Reduced sAHPs and spike frequency accommodation were observed, however, in the CA1 and CA3 PCs with a different learning paradigm, namely acquisition of the classical delayed and trace eyeblink conditionings in rabbits (Coulter et al. 1989; Disterhoft et al. 1986; Moyer et al. 1996; Sanchez-Andres and Alkon 1991; Thompson et al. 1996).

Although membrane properties of neurons can be affected by synaptic activity, it has been shown previously (Alger and Nicoll 1980; Gusev and Alkon 1998), that sAHP is not contaminated by the evoked IPSP. Averaging large numbers of samples for each individual neuron’s measurement should minimize effects of spontaneous synaptic activity on measurements.

**Membrane slope input resistance and rectification**

To exclude the possibility that synaptic modifications could simply reflect a difference in passive membrane properties, we performed a detailed analysis of current-voltage relations for the pyramidal cells. Slope membrane input resistance mea-

---

**TABLE 4. Changes in passive membrane properties of CA1 pyramidal neurons after 3 days of water-maze training were not learning-specific**

<table>
<thead>
<tr>
<th></th>
<th>WMT1</th>
<th>SW1</th>
<th>Naive</th>
<th>WMT3</th>
<th>SW3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak slope $R_m$ (I &gt; 0), $\Omega$</td>
<td>43.6 ± 1.34</td>
<td>43.5 ± 1.14</td>
<td>45.4 ± 1.1</td>
<td>41.1 ± 1.1**</td>
<td>39.8 ± 1.1**</td>
</tr>
<tr>
<td>$n$</td>
<td>53</td>
<td>53</td>
<td>54</td>
<td>54</td>
<td>60</td>
</tr>
<tr>
<td>Peak rectification $R_m$ (I &gt; 0)/$R_m$ (I &lt; 0)</td>
<td>NA</td>
<td>NA</td>
<td>1.94 ± 0.1</td>
<td>1.94 ± 0.1</td>
<td>1.87 ± 0.1</td>
</tr>
<tr>
<td>$n$</td>
<td>42</td>
<td>59</td>
<td>56</td>
<td>59</td>
<td>56</td>
</tr>
<tr>
<td>Peak latency at −0.5 nA, ms</td>
<td>61.7 ± 1.8</td>
<td>60 ± 1.7</td>
<td>61.6 ± 1.5</td>
<td>60.2 ± 1.4</td>
<td>59.9 ± 1.4</td>
</tr>
<tr>
<td>$n$</td>
<td>53</td>
<td>53</td>
<td>54</td>
<td>54</td>
<td>60</td>
</tr>
<tr>
<td>Plateau slope $R_m$ (I &lt; 0), $\Omega$</td>
<td>35.6 ± 1.07</td>
<td>35.4 ± 0.95</td>
<td>36.2 ± 0.9</td>
<td>33.6 ± 0.9**</td>
<td>32.6 ± 0.8***</td>
</tr>
<tr>
<td>$n$</td>
<td>44</td>
<td>59</td>
<td>57</td>
<td>59</td>
<td>57</td>
</tr>
<tr>
<td>Plateau rectification $R_m$ (I &gt; 0)/$R_m$ (I &lt; 0)</td>
<td>NA</td>
<td>NA</td>
<td>1.68 ± 0.09</td>
<td>1.66 ± 0.06</td>
<td>1.62 ± 0.07</td>
</tr>
<tr>
<td>$n$</td>
<td>44</td>
<td>59</td>
<td>57</td>
<td>59</td>
<td>57</td>
</tr>
<tr>
<td>Time to −0.5-nA voltage response peak, ms</td>
<td>61.7 ± 1.8</td>
<td>60.1 ± 1.7</td>
<td>61.5 ± 1.5</td>
<td>60.2 ± 1.4</td>
<td>59.9 ± 1.4</td>
</tr>
<tr>
<td>$n$</td>
<td>53</td>
<td>53</td>
<td>54</td>
<td>54</td>
<td>60</td>
</tr>
</tbody>
</table>

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significantly different from naive group, Fisher’s post hoc test.
In addition, the sAHP distribution in the WMT3 group was not significantly different from the SW3 group. However, there were differences in the SW1 group: a larger fraction of the neurons with sAHPs more than 5 mV was also accompanied by somewhat increased fraction of cells with sAHP amplitude more than 5 mV range as compared with naıve group. *R* = number of neurons. 

**DISCUSSION**

Our principal learning-specific findings are reduction of the mean peak IPSP amplitude as well as increased mean latencies to the IPSP and EPSP peaks and correlation of the depolarizing-shifted IPSP reversal potentials and reduced IPSP-evoked membrane conductance. Furthermore this long-term disinhibition in a subset of the dorsal CA1 PCs was a clear and significant intracellular correlate of spatial memory acquisition. 

Disinhibition became apparent as the rats progressed from strategy learning (Hoh et al. 1999) to the complete representation of the spatial water maze (Morris 1984). At this advanced stage of the spatial training (WMT3 group), learning-specific synaptic correlates of the WM task acquisition included reduced early and late IPSP phases in the complex PSPs and delayed IPSP and EPSP peaks. Reduced early and late IPSPs were significantly correlated with decreased IPSP-evoked membrane conductance and depolarized IPSP reversal potential. At the early stage of WM learning (WMT1 group), we did not observe any learning-specific changes, although there was a statistically nonsignificant tendency to an increased proportion of the cells with a depolarized reversal potential for the early IPSP.

We defined the maximum IPSP as that elicited with a stimulus intensity that also elicits a preceding EPSP just subthreshold for an action potential. The changes in the maximum IPSP amplitude were not influenced by the level of pyramidal cell excitability since we found no learning-specific differences in
intrinsic membrane excitability. Namely, there were no learning-specific differences in the current intensity required to evoke a single action potential or four-spike burst, single-spike, or postburst sAHP amplitudes, spike frequency accommodation, membrane resistance, capacity or rectification, resting \( V_{\text{m}} \), and spike threshold.

These reductions in the WMT3 IPSPs were not correlated with EPSP facilitation. Analyses of the EPSP facilitation indices such as I-O relations, initial rising slopes and amplitudes of the EPSP did not indicate learning-specific changes. Therefore we suggest that disinhibition occurred primarily due to the reduction in the IPSPs’ amplitudes and conductances and shifts in the balance of ionic conductances responsible for the GABA-mediated responses.

The learning-specific synaptic changes observed here during concurrent stimulation of the excitatory and inhibitory synapses suggest that a subset of the CA1 PCs will be less inhibited and thus show more effective temporal summation of excitatory inputs. The smaller membrane conductance during the late IPSPs would be expected to increase the weight of the excitatory inputs on the CA1 PC dendrites mediated by Schaffer collaterals. Thus conditions may be provided for opening of the NMDA channels and induction of the NMDA-dependent changes in excitatory synapses (Andreasen and Lambert 1998; Andreasen et al. 1989; Bliss and Collingridge 1993; Collingridge et al. 1988). The smaller membrane conductance during early somatic IPSPs would be expected to facilitate spike generation (Buhl et al. 1994; Miles et al. 1996; Nurse and Lacaille 1997; Paulsen and Moser 1998). Backpropagating action potentials would, in turn, enhance \( \text{Ca}^{2+} \) entry into dendrites and possibly would trigger further modifications such as LTP of excitatory synapses (Markram et al. 1997; Tsukokawa and Ross 1996). Our previous intracellular study of rabbit eyeblink conditioning demonstrated learning-specific changes of EPSP summation but not of individual EPSPs (LoTurco et al. 1988). The lack of a learning-specific change in EPSPs or intrinsic neuronal excitability is most likely not due to the pyramidal cell selection, since only 7 of 326 neurons were rejected based on predetermined criteria (see METHODS). Although our data do not indicate a net facilitation of the EPSPs recorded from PCs’ soma, to make a conclusion about
the real distribution of learning-related EPSP modifications, multiple dendritic intracellular recordings would be necessary. Nevertheless long-term disinhibition could contribute to the previously observed place-related increases in the activity of place cells after learning the position of a platform to escape from the water (Hollup et al. 2001; Moser et al. 1999). This is consistent with in vivo observations of suppressed interneuronal activity during exploration of an unfamiliar environment (Paulsen and Moser 1998) and learning of a novel spatial representation (Wilson and McNaughton 1993).

Approximately 20% of place cells recorded in vivo during spatial learning gradually increased their firing rates in already established or newly acquired place fields (Kobayashi et al. 1997; Nishijo et al. 1999). Although we cannot claim that a subset of PCs with reduced IPSPs are themselves the place cells or that disinhibition itself creates the place cells, we suggest that long-term disinhibition may promote the place cells’ ability to transiently increase their firing rates and to discharge at early phases of theta cycles when rats cross these place fields (Hollup et al. 2001; Kamondi et al. 1998; Kentros et al. 1998; Kobayashi et al. 1997; Magee 1999; Moser et al. 1999; O’Keefe and Recce 1993; Wilson and McNaughton 1993) and anticipate a reward (Nishijo et al. 1999). Finally, long-term disinhibition could also facilitate involvement of the particular PCs in neuronal ensemble reactivation and depolarization of specific downstream CA1 targets during the animal’s rest cycle and period of sharp waves in the HC electroencephalogram (Csicsvari et al. 1999; Kudrimoti et al. 1999; Nadasy et al. 1999).

An increase in bicarbonate flow through the GABAA receptor-mediated channels or increase in proportional conductance of GABAergic (depolarizing) channels (Perkins and Wong 1996) may account for the observed depolarizing shifts of $E_{IPSP}$ and reduced amplitudes of the early IPSPs. Consistent with these possibilities, positive shifts of $E_{IPSP}$ were correlated with reduction of the early IPSPs. Long-term postsynaptic transformation of the fast IPSPs into the EPSPs may also contribute to the observed learning-related long-term disinhibition (Collin et al. 1995). It has been shown that pairings of GABA application or basket cell stimulation with the CA1 PC depolarization caused IPSP transformation associated with depolarizing shifts in $E_{IPSP}$. A sole reduction in chloride conductance did not cause a depolarizing shift in the early $E_{IPSP}$ in the CA3 area (Thompson and Gahwiler 1989b) due, for instance, to an increase in the EPSP conductance. Pharmacological isolation of the IPSPs in future studies may reveal or exclude more subtle interactions of the EPSP- and IPSP-related conductances.

Decreases in the IPSP conductance could result from either presynaptic processes through the decrease in the evoked GABA release, or postsynaptic processes, such as desensitization (Thompson and Gahwiler 1989a,b). Disinhibition due to decreased GABA release from the terminals targeting spiking CA1 PCs or due to both decreased GABA release and driving force for Cl$^-$ ions in CA3 PCs (following repetitive mossy fiber stimulation) lasted only for minutes (Alger et al. 1996; Thompson and Gahwiler 1989a). It is unlikely therefore that these pre-and postsynaptic changes may underlie the persistent disinhibition observed in slices after WM learning. GABAA receptor phosphorylation is a possible postsynaptic mechanism for reduced IPSP conductance (Moss et al. 1992).

On the other hand, modified relations between hyperpolarizing potassium currents and depolarizing current(s) all regulated by GABAB receptors (Pham and Lacaille 1996) may cause the observed positive shifts of the late $E_{IPSP}$ and reduced amplitudes of the late IPSPs. Because the fast EPSPs are not present at the 150-ms time point and the NMDA component is suppressed with recording conditions used here (Andreasen and Lambert 1998; Andreasen et al. 1989; Collingridge et al. 1988), we suggest that EPSP-evoked conductances do not contribute to the observed depolarizing shifts in the late $E_{IPSP}$. Therefore our present data indicate a learning-induced depolarizing shift in the balance of postsynaptic ionic conductances underlying the late GABA-mediated synaptic inhibition in a subset of cells with reduced IPSPs. The relative importance of the post-versus presynaptic mechanisms of learning-specific disinhibition will be a subject of future study.

After complete learning of the water maze following 3 days of training, we also found learning-specific increased action potential amplitudes in a different subset of neurons. Reduction in the $I_h$-like potassium current similar to that observed after classical conditioning in rabbit Purkinje cell dendrites (Schurr et al. 1998) and the mollusk *Hermisenda* (Alkon et al. 1982) may account for the increased action potential amplitude that we observed in the CA1 PCs.

The nonspecific reduction of PCs’ membrane excitability and somewhat higher current intensities applied to elicit EPSPs may be related to stress-induced increase in the slow Ca$^{2+}$-dependent K$^+$ conductance ($I_{AHP}$) and depression of amino acid-mediated transmission (Joels and de Kloet 1992, 1993; Teschemacher et al. 1996).

In conclusion, our data provide the first intracellular synaptic correlate of spatial memory acquisition in the dorsal CA1 area of the HC. These results suggest that neuronal ensembles may be formed during associative learning by prolonged reduction of somatic and dendritic inhibition in a subset of the PCs. This prolonged disinhibition represents, therefore a novel long-term synaptic change that can contribute importantly to hippocampal function during learning and memory.

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