Watermaze Learning Enhances Excitability of CA1 Pyramidal Neurons

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Oh, M. Matthew, Amy G. Kuo, Wendy W. Wu, Evgeny A. Sametsky, and John F. Disterhoft. Watermaze learning enhances excitability of CA1 pyramidal neurons. J Neurophysiol 90: 2171–2179, 2003. First published June 18, 2003; 10.1152/jn.01177.2002. The dorsal hippocampus is crucial for learning the hidden-platform location in the hippocampus-dependent, spatial watermaze task. We have previously demonstrated that the postburst afterhyperpolarization (AHP) of hippocampal pyramidal neurons is reduced after acquisition of the hippocampus-dependent, temporal trace eyeblink conditioning task. We report here that the AHP and one or more of its associated currents (\(I_{AHP}\) and/or \(sI_{AHP}\)) are reduced in dorsal hippocampal CA1 pyramidal neurons from rats that learned the watermaze task as compared with neurons from control rats. This reduction was a learning-induced phenomenon as the AHP of CA1 neurons from rats that failed to learn the hidden-platform location was similar to that of neurons from control rats. We propose that reduction of the AHP in pyramidal neurons in regions crucial for learning is a cellular mechanism of learning that is conserved across species and tasks.

INTRODUCTION

The spatial watermaze task is one of the most widely used behavioral tests to assess the effects of various manipulations on spatial learning, ranging from genes to normal aging (Chen et al. 2000; D’Hooge and De Deyn 2001; Foster et al. 2001; Jeffery 1997; Poe et al. 2000; Silva et al. 1998; Zeng et al. 2001). The task depends on the proper function of the hippocampus (Morris et al. 1982), specifically, the dorsal hippocampus (Moser et al. 1993). In addition, there are a greater number of complex spike cells with finely tuned spatial receptive fields in the dorsal hippocampus (Jung et al. 1994), supporting the essential nature of the dorsal hippocampus in successful navigation.

To date, long-term potentiation (LTP) in the hippocampus has been the focus of attention as a possible cellular model of the synaptic changes that occur during spatial learning and memory. Many studies have correlated the necessity of LTP induction with spatial learning, but conflicting data also exist that suggest spatial learning can occur in the absence of LTP (D’Hooge and De Deyn 2001; Jeffery 1997; Silva et al. 1998). Thus a clear relationship between LTP and spatial learning currently does not exist and suggests that other mechanisms may be involved. Some of them may involve alterations of neuronal properties separate from those confined to the synapse (Johnston et al. 2000).

Studies from our laboratory have demonstrated enhanced neuronal excitability [i.e., reduced postburst afterhyperpolarization (AHP)] of hippocampal pyramidal neurons from both rabbits (Moyer et al. 1996, 2000; Thompson et al. 1996b) and rats (Oh et al. 1999a) that have acquired a temporal, hippocampus-dependent task, trace eyeblink conditioning. The AHP reduction was learning-specific as this reduction was not observed in neurons from animals that failed to acquire the task (Moyer et al. 1996, 2000; Thompson et al. 1996b). An AHP reduction was also observed in perirform cortical neurons after odor discrimination learning (Saar et al. 1998), demonstrating that this learning-induced biophysical alteration is not limited to hippocampal neurons after acquisition of a temporal, hippocampus-dependent task.

In the present study, we examined CA1 pyramidal neurons from dorsal and ventral subdivisions of the hippocampus of Fisher 344 × Brown Norway rats (F344 × BN) with current- and voltage-clamp recording techniques to determine if learning the spatial watermaze task would reduce the AHP and the current(s) underlying it. We found that the AHP and one or more of its associated currents (\(I_{AHP}\) and/or \(sI_{AHP}\)) were significantly reduced in dorsal hippocampal CA1 neurons from rats that learned the watermaze task as compared with those from neurons of control rats. These findings support the crucial nature of the dorsal hippocampus for learning this spatial, hippocampus-dependent task (Moser et al. 1993) and demonstrate that the learning-induced reductions of the AHP and one or more of its associated currents are localized to the neurons in the region of the hippocampus involved during learning. A portion of these findings has appeared in abstract form (Oh et al. 2001).

METHODS

Subjects were young (4–6 mo) male F344 × BN rats group housed (4/cage) in a climate-controlled room on a 12/12 light/dark cycle with ad libitum access to food and water. Animal care was provided and managed by the Northwestern University’s Animal Care personnel following the guidelines established by the university and the USDA.

Watermaze training

Rats were trained to find the hidden platform using procedures adapted from those previously described (Gallagher et al. 1993). Briefly, the rats were randomly assigned to one of three behavioral groups: trained (\(n = 16\)), swim control (\(n = 14\)), and naïve (\(n = 9\)). On the first day of training, the trained and swim control rats were

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given eight cued trials on the watermaze. For these trials, a black platform (raised 2 cm above the water level) was randomly placed in the center of a quadrant for each trial (60 s max: 60 s ITI). After the rat climbed onto the platform or was hand-guided to it, the animal was allowed to rest on the platform for an additional 15 s before being removed from the training tank to a drying cage.

Twenty-four hours after the cued trials, rats in the trained group were given two training sessions per day with four trials per session (60 s max: 60 s ITI) for 2 days. The rats were placed in a start position in the maze at one of four locations around the tank that varied in a pseudorandom manner for each trial. The last trial of each day was a probe trial, during which the hidden platform was retracted to the bottom of the tank for 60 s and raised back to its location at the end of the trial making it available for escape. Although latency to the platform during training trials is commonly used to assess learning, a more stringent criterion is time spent in the target quadrant during the probe trials (Gallagher et al. 1993; Morris et al. 1982). Thus, to assess if a trained rat did learn the hidden platform location and be classified as a learner, we performed $\chi^2$ tests (StatView V5.01, SAS Institute, Cary, NC) comparing the amount of time spent in the target and opposite quadrants during the probe trial at the end of the second training day.

Each swim control rat was time-yoked to a trained rat and swam in the pool without an escape platform for an equal number of sessions and trials and for the same period of time on each trial. Naïve rats were handled daily for 3 days prior to slice preparation.

The data collection and analysis was performed using a VP200 tracker and HVS Water for Windows (HVS Image: Hamptonn, UK). As the path length data were highly correlated with the latency to the tracker and HVS Water for Windows (HVS Image; Hampton, UK).

Cary, NC) comparing the amount of time spent in the target and

Hippocampal slice preparation

Briefly, ~18 h after the last training session, the rats were anesthetized with halothane in a fume hood and killed by decapitation. The brain was quickly exposed, hemisected in situ, removed, and immediately immersed in an ice-cold (~1°C) oxygenated modified artificial cerebrospinal fluid (ACSF; which contained (in mM) 124 NaCl, 26 NaHCO₃, 3 KCl, 1 CaCl₂, 2.4 MgSO₄, 1.25 NaH₂PO₄, and 10 n-glucose, gassed with 95% O₂-5% CO₂). Transverse hippocampal slices (300 μm) were made using vibratomes in the modified ACSF and placed in a holding chamber filled with normal ACSF (same as modified except: 2.4 mM CaCl₂, and 2 mM MgSO₄; ~22°C) for ≥45 min prior to any experiments. Only slices from the dorsal and ventral subdivisions of the hippocampus were used for the biophysical recordings (Fig. 3A). Typically, about four to six slices per subdivision from each hemisphere were obtained for the biophysical recordings. All of the biophysical recordings (at 31°C), and analyses were performed blind to the behavioral status of the animal.

Biophysical recordings

Two distinct intracellular recording techniques were used to assess the potential postsynaptic alteration(s) that occur with learning. However, not all of the rats were used for both of the assessments. In the initial phase of the study, sharp-electrode, current-clamp measurements were made from 157 CA1 pyramidal neurons from learners (n = 9), nonlearners (n = 3), swim control (n = 11), and naïve (n = 4) rats using an Axoclamp 2A amplifier in bridge mode and previously published protocols (Moyer et al. 1996, 2000; Thompson et al. 1996b). Microelectrodes were made from thin-walled capillary glass, and filled with 3 M KCl (30–50 MΩ). Once the neuron had stabilized for 5 min after the initial impalement and met the cell health criteria, the membrane properties were measured with the neuron held near −68 mV to ensure that any differences observed were not due to voltage-dependent membrane properties. The current-voltage (I-V) relations were studied by using 400-ms current steps (range: −1.0 to +0.2 nA). The depolarizing sag was calculated as the difference between the peak amplitude (during the 1st 150 ms) and the plateau voltage deflection during a −1.0-nA hyperpolarizing current injection. The input resistance was calculated by measuring the plateau voltage deflection during the last 75 ms of a 400-ms, −0.2-nA hyperpolarizing step. The AHP was studied using a 100-ms depolarizing current step that reliably elicited a burst of four action potentials.

The duration of the AHP was measured as the time required from the 100-ms depolarizing current step offset to the membrane potential returning to the baseline potential for ≥10 ms. The peak AHP amplitude was calculated as the maximum negative voltage deflection from the baseline potential during the first 250 ms after the current offset. The integrated area of the AHP was calculated from the current offset for the entire duration of the AHP. A total of five AHP measurements were made from each neuron at 30-s intervals. Accommodation was studied using an 800-ms depolarizing current step of the same stimulus intensity used to evoke the AHP. The number of action potentials elicited was noted for three trials at 30-s intervals. A cell was classified as a CA1 neuron and included in the study if it had a stable resting membrane potential < −60 mV, an action potential duration >1.2 ms from rise threshold to repolarizing the resting potential, an input resistance ≥20 MΩ, and an action potential amplitude >80 mV from rest. The initial findings from the sharp-electrode, current-clamp recordings supported a learning-induced alteration of the current(s) underlying the AHP. Thus whole cell patch-clamp recording techniques were used to isolate and examine two of the component currents underlying the AHP: $I_{AHP}$ and $I_{sAHP}$.

Whole cell patch, voltage-clamp recordings were made from the soma of 141 visually identified CA1 pyramidal neurons (8 learners, 9 swim controls, and 8 naïve rats) with seal resistances >1.5 GΩ prior to break through into the whole cell mode using a Zeiss Axioskop or a Leica DM LFS microscope equipped with a 40× water-immersion lens and IR-DIC optics, and an Axopatch 1C or 200B amplifier. Patch electrodes (3–6 MΩ) were filled with (in mM) 130 KMeSO₄, 10 KCl, 2 K-ATP, and 10 HEPES; pH adjusted to 7.25 with KOH; 290 ± 10 mOsm. The use of KMeSO₄ (ICN, Aurora, OH) in the electrode solution resulted in a ~10–20% junction potential with respect to the ACSF and was corrected for prior to data collection. All measurements were made ≥5 min after membrane rupture to allow for adequate solution equilibration. Series resistance was measured but not compensated. Only neurons with seal resistance <15 MΩ, membrane resistance >60 MΩ, and resting potential < −55 mV were included in the data set.

The $I_{AHP}$-$sAHP$ were isolated by adding the following to the perfusate: 500 nM tetrodotoxin (TTX, Calbiochem, San Diego, CA) to block Na⁺ current; 2 mM CsCl to block inward K⁺ and $I_{Ca}$; 2 mM 4-aminopyridine (4-AP) to block $I_{Na}$ and $I_{Ca}$; 5 mM tetraethylammonium (TEA) to block $I_{TEA}$ and $I_{Ca}$; and 500 μM picrotoxin, 10 μM 6-cyano-7-nitroquinoxaline-2,3-(1H,4H)-dione (CNQX, Tocris, Ellisville, MO), and 25 μM 2-aminophosphonopentanoic acid (z-AP5, Tocris) to reduce synaptic currents. TEA and 4-AP were substituted for equimolar NaCl. Unless specified, all drugs were purchased from Sigma (St. Louis, MO).

The AHP tail current was evoked by a 100-ms, 50-mV voltage step from a holding potential of −55 mV. This protocol elicited a single robust, unclamped Ca²⁺ current followed by the AHP tail current. The membrane voltage was not precisely controlled during the depolarizing step because of the gain and space-clamp limitations (although these were minimized with the blockade of distal dendritic currents, especially $I_{Na}$ and $I_{Ca}$). However, the voltage control of the AHP tail current was well maintained (Constanti and Sim 1987; Lancaster and Adams 1986; Sah and McLachlan 1991; Zhang et al. 1995). Both the $I_{AHP}$ and $sAHP$ coexist in CA1 hippocampus, with the $I_{AHP}$ account-
ing for ~20% of the AHP in CA1 pyramidal neurons (Oh et al. 2000; Stocker et al. 1999). The apamin-sensitive $I_{\text{AHP}}$ has a relatively fast onset (1–5 ms) and a slow offset lasting between 50 and several hundred milliseconds (Sah 1996; Stocker et al. 1999). In contrast, the decay time constant of the $sI_{\text{AHP}}$ is ~1.5 s (Sah 1996). Therefore the $sI_{\text{AHP}}$ amplitude was measured as the tail current at 1 s after the pulse offset, and the $f_{\text{AHP}}$ was measured as the peak amplitude of the tail current.

All biophysical data were digitized and analyzed on-line using PCI-MIO-16E-4 boards (National Instruments, Austin, TX) interfaced to personal computers using custom software routines written in LabVIEW (National Instruments) or C++ (Borland Software, Scotts Valley, CA). Complete analyses were performed off-line using procedures developed with LabVIEW or C++. Statistical analyses were performed using StatView. Significant main effects were evaluated using Fisher’s PLSD post hoc tests. All data are reported as the means ± SE.

Both current- and voltage-clamp biophysical data from swim control and naïve rats were nearly identical, consistent with our previous reports (Moyer et al. 1996, 2000; Thompson et al. 1996b). Thus the biophysical data from neurons of these animals were combined to form the “control” group. Additionally, the mean group biophysical data averaged by animal or by individual neurons were nearly identical. Thus to perform meaningful statistical analyses, the biophysical data averaged by neurons were presented for this report.

RESULTS

Performance of the trained animals on the watermaze

The F344×BN rats trained to find the hidden-platform in the spatial watermaze task were able to quickly do so: $F(3,45) = 9.98, P < 0.0001$. As illustrated in Fig. 1A, the latency on the fourth training session was significantly reduced as compared with the previous sessions (Fisher’s PLSD, $P < 0.02$) and was similar to those previously reported (Bannerman et al. 1999; Moser and Moser 1998).

Analysis of the probe data revealed two distinct groups, learners and nonlearners, although all the trained rats had similar latencies, swim speeds, and path lengths during the training trials. Learners ($n = 13$) spent a significantly greater percentage of time in the target quadrant as compared with the swim control animals ($n = 14$) on the second training day (Fig. 1B). Nonlearners ($n = 3$) did not spend significantly more time in the target quadrant and had similar probe data as the swim control animals (Fig. 1B). In addition, the proximity measure (Gallagher et al. 1993) analysis illustrated that the learners searched closer to the hidden platform location during the probe trial as compared with the swim controls (Fig. 1C). Again, the proximity data for nonlearners were very similar to those of the swim controls. There were no differences among groups on the probe trial on training session 1 as would be expected from the learning curves (Fig. 1A).

No difference in performance on the cued version of the watermaze task was found between the learners, nonlearners, and control rats: $F(2,27) = 0.380, P > 0.68$. All the rats were able to quickly swim to the visible platform during the cued trials: $F(7,189) = 13.505, P < 0.0001$. Thus the differences in time spent in the target quadrant (Fig. 1B) and the proximity measure (Fig. 1C) reflect learning the hidden platform location and not a difference in sensorimotor behavior or lack of motivation (Gallagher et al. 1993; Morris et al. 1982).

AHP reduction in dorsal, but not ventral, CA1 neurons from learners

The peak amplitude of the postburst AHP was significantly reduced [$F(2,74) = 4.219, P < 0.02$] in CA1 neurons from dorsal hippocampus of learners as compared with those from controls (Fisher’s PLSD, $P < 0.02$) and nonlearners ($P < 0.03$; Table 1). The integrated area of the AHP was also significantly reduced [$F(2,74) = 4.439, P < 0.02$] in dorsal CA1 neurons from learners as compared with those from controls ($P < 0.02$) and nonlearners ($P < 0.02$) (Table 1). No significant differences in either peak amplitude or integrated area of the AHP were observed between the dorsal CA1 neurons from controls and nonlearners ($P > 0.5$). The AHP duration and latency, accommodation, input resistance, sag, and membrane potential of the dorsal CA1 neurons were also not statistically different between the groups (Table 1, Fig. 2).

No significant effect of training was observed for any of the measures from CA1 neurons from the ventral hippocampus (Table 1). Although there was a lack of a statistically signifi-
significant reduction in the AHP from ventral CA1 neurons of learners, the mean AHP of ventral CA1 neurons from learners was smaller than that from controls and nonlearners (Table 1). These findings illustrate that the AHP reduction of CA1 pyramidal neurons was most prominent in the hippocampal subdivision crucial for spatial learning, the dorsal hippocampus, but was apparent to some degree in the ventral hippocampus as well.

Reduced AHP of CA1 neurons from learners at multiple time points

The postburst AHP is composed of four outward potassium currents ($I_h$, $I_C$, $I_{AHP}$, and $s_{AHP}$), and the AHP time course is modulated by $I_h$ (for reviews see, Sah 1996; Storm 1990; Wu et al. 2002). To explore which current(s) was altered, we examined the amplitudes of the AHP at 200, 800, and 1,000 ms after the pulse offset.

Significant effects of training ($F(2,74) = 6.302, P < 0.004$), of repeated AHP measures ($F(2,148) = 264.627, P < 0.0001$), and interaction of training and AHP amplitudes ($F(4,148) = 2.631, P < 0.04$) were observed in the dorsal CA1 neurons.

The AHP amplitudes measured at all time points from the dorsal CA1 neurons of learners were significantly smaller as compared with those from nonlearners and controls (Fig. 3,B and C).

No statistically significant differences in the AHP amplitudes were observed between the ventral CA1 neurons from the three groups at any of the time points (Fig. 3D). These results again illustrate that the alteration of the AHP in CA1 neurons occurred in the subdivision that is crucial for learning this task.

No differences in spike width after training

Previous reports have demonstrated that $I_A$ and $I_C$ are reduced after associative learning (Alkon 1984) and are important contributors in determining action potential duration (spike width; Giese et al. 1998b; Shao et al. 1999). We measured the spike width of the action potentials elicited during the AHP measurements at 1/3 of the action potential amplitudes and compared the width of the fourth action potential to that of the 1st action potential as an indication that $I_A$ and/or $I_C$ was altered after watermaze learning (Giese et al. 1998b; Shao et al. 1999). No difference in spike width was observed between the groups in either subdivision: dorsal [$F(2,74) = 1.705, P > 0.18$] (Fig. 4), ventral [$F(2,77) = 0.413, P > 0.66$]. The lack of differences in spike width with training suggested that these currents ($I_A$ and $I_C$) may not be altered in CA1 neurons after learning this task.

Currents associated with AHP are reduced in CA1 neurons from learners

The reductions of the peak and 200-ms AHP amplitudes in the dorsal hippocampal CA1 neurons of learners implicated a reduction of the medium AHP ($I_{AHP}$) after learning. Furthermore, reductions of the AHP amplitudes at 800 and 1,000 ms strongly suggested an alteration of the $s_{AHP}$ in dorsal CA1 neurons after learning this hippocampus-dependent spatial task. Thus we isolated the $I_{AHP}$ and $s_{AHP}$ pharmacologically and characterized these currents using whole cell patch voltage-clamp protocols. For these voltage-clamp recordings, all of the trained rats met our learning criteria; hence, there was no nonlearner group for these biophysical comparisons.

Consistent with the current-clamp data, the reductions in $I_{AHP}$ and $s_{AHP}$ were only observed in dorsal CA1 neurons of learners ($s_{AHP}$, 77.5 ± 11.1 pA, $n = 19$; $I_{AHP}$–$s_{AHP}$, 197.0 ± 24.4 pA; area, 197.1 ± 29.2 pC) as compared with those from neurons of controls ($s_{AHP}$, 142.1 ± 13.3 pA, $n = 49$; $I_{AHP}$–$s_{AHP}$, 289.7 ± 24.9 pA; area, 320.8 ± 28.8 pC; unpaired t-test, $P < 0.05$; Fig. 5). No significant differences were observed for

### Table 1. Biophysical properties of CA1 hippocampal pyramidal neurons after watermaze training

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<th>Control</th>
<th>Nonlearners</th>
<th>Learners</th>
<th>Control</th>
<th>Nonlearners</th>
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<td>Postburst AHP</td>
<td>-4.37 ± 0.24</td>
<td>-4.55 ± 0.56</td>
<td>-3.35 ± 0.23</td>
<td>-5.16 ± 0.24</td>
<td>-5.02 ± 0.40</td>
<td>-4.63 ± 0.35</td>
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<td>Peak, mV</td>
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<td>Area, mV</td>
<td>-4.16 ± 0.38</td>
<td>-4.64 ± 0.83</td>
<td>-2.65 ± 0.29</td>
<td>-4.34 ± 0.43</td>
<td>-3.96 ± 0.65</td>
<td>-3.40 ± 0.46</td>
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<tr>
<td>Duration, s</td>
<td>2.73 ± 0.14</td>
<td>2.69 ± 0.22</td>
<td>2.30 ± 0.20</td>
<td>2.19 ± 0.18</td>
<td>1.94 ± 0.28</td>
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<tr>
<td>Latency, ms</td>
<td>108 ± 7</td>
<td>109 ± 10</td>
<td>95 ± 10</td>
<td>97 ± 9</td>
<td>99 ± 14</td>
<td>88 ± 10</td>
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<td><strong>Ventral</strong></td>
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<td>Postburst AHP</td>
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<td>Peak, mV</td>
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Values are means ± SE. Numbers of cells are 40, 13, and 24 and 40, 14, and 26 for dorsal and ventral control, nonlearners, and learners, respectively. Numbers in bold indicate a significant difference as compared to both the control and nonlearners groups using the Fisher’s protected least-significant difference test. Spike width is the ratio of the width of the fourth action potential divided by the width of the first action potential measured during the four-spike after hyperpolarization measurements. Accom, accommodation; RMP, resting membrane potential; IR, input resistance.
any of the measures from the ventral subdivision of the hippocampus (Fig. 5B). These voltage-clamp results not only support the findings of the current-clamp recordings that the mAHP (IAHP) and sAHP (sIAHP) are reduced in dorsal CA1 neurons from rats that learned this hippocampus-dependent, spatial task, but also suggest that reduction in either IAHP, sIAHP, or both IAHP and sIAHP may underlie the reductions in the postburst AHP.

**DISCUSSION**

The main finding of this report is that learning the location of the hidden platform in the watermaze task is associated with a reduced postburst AHP in dorsal CA1 hippocampal pyramidal neurons from rats that learned the hidden platform location. A: diagram illustrating the dorsal and ventral subdivisions of the hippocampus from which the biophysical recordings were made. B: typical examples of the postburst AHP from dorsal CA1 neurons of learner, nonlearner, and controls are illustrated. These neurons were current-clamped to near −68 mV (horizontal dashed line). The vertical dashed lines illustrate the time points of AHP measurements at 200, 800, and 1,000 ms after the pulse offset. C: a repeated-measures ANOVA for the dorsal CA1 neurons revealed a significant group by time interaction: F(4,148) = 2.631, P < 0.04. Further analyses revealed that there were significant effects of training at all time points, with the amplitudes from learners significantly reduced as compared with those from nonlearners and control rats. Fisher’s protected least-significant difference (PLSD): *P < 0.02; **P < 0.002. D: no significant learning-induced reduction of the AHP was observed for any of the time points measured in the ventral subdivision of the hippocampus.
nal neurons. This reduction was learning specific as no significant differences were observed for these measurements between the neurons from control rats and rats that were trained but failed to acquire the task. Additionally, the reduction was observed in CA1 neurons from the dorsal, but not ventral, subdivision of the hippocampus—a finding that coincides with the essential nature of the dorsal hippocampus in spatial tasks (Bannerman et al. 1999; Moser et al. 1993). Similar to the current-clamp recordings, whole cell voltage-clamp results indicated a learning-related reduction in one or more of the component currents underlying the AHP (I_{AHP} and/or sI_{AHP}) only in dorsal CA1 neurons. These data support our hypothesis that learning a hippocampus-dependent task results in enhanced neuronal excitability of CA1 neurons in subdivision(s) crucial for learning the task via a reduction of the AHP and one or more of its associated currents.

Space-clamp errors are an issue when performing whole cell voltage-clamp experiments. We have minimized the space-clamp error by blocking synaptic transmission and other currents besides I_{AHP} and sI_{AHP} (such as I_C, I_M, I_A, I_K, I_h) with our pharmacological compounds (see METHODS). In addition, the channels thought to underlie the sAHP have been reported to be in the proximal dendrites or on the soma of CA1 neurons (Bekkers 2000; Sah and Bekkers 1996). Therefore our whole cell voltage-clamp measures should have been minimally impacted by space-clamp issues.

In addition to the I_{AHP} and sI_{AHP}, other currents may have been altered (enhanced or reduced) after learning the watermaze task, such as I_C, I_A, and/or I_h. Spike width is commonly used to assess I_C (Shao et al. 1999) and I_A (Giese et al. 1999b), and sag measurement is commonly used to assess I_h (Disterhoft et al. 1986; Maccarferri et al. 1993). We did not find a difference in either measurement after training. However, as our biophysical recording protocols did not specifically isolate and measure these currents, we cannot be certain that these currents were not altered in CA1 neurons with our training protocol.

Surprisingly, we did not find a robust difference in either the duration of the postburst AHP or the amount of spike-frequency adaptation in CA1 neurons after spatial learning as we have previously reported after the acquisition of a temporal, trace eyeblink conditioning task in rabbit hippocampal pyramidal neurons (Moyer et al. 1996, 2000; Thompson et al. 1996b). These negative findings could be due to a species and/or task difference(s) and warrant future exploration.

Our present findings are contrary to those recently reported by Gusev and Alkon (2001), who showed no alterations of the AHP after watermaze training. There are several differences in their methodology and ours that may account for the different observations. The major difference is the learning criterion used to identify the rats that had learned the hidden-platform location after the watermaze training for the biophysical studies. Gusev and Alkon used only the escape latency as their learning criterion. They did not gather probe trial data from the trained animals that they used for their biophysical comparisons. They inferred that these rats learned the watermaze task by using probe data from a different group of trained rats (not used for biophysical recordings). However, escape latency alone is not an accurate indicator of learning (Gallagher et al. 1993), which the authors acknowledge. We found that rats can have a short escape latency but perform poorly on the probe trials (a more sensitive measure of learning in the watermaze) as demonstrated by our nonlearners. The CA1 neurons from these nonlearners have significantly different AHPs than those from rats that clearly learned the location of the hidden platform as demonstrated by the probe trials.

The most-often proposed cellular mechanism for spatial learning and for spatial learning impairment with aging is the modulation of synaptic transmission, specifically by LTP (D’Hooge and De Deyn 2001). It has been demonstrated that LTP is impaired in vitro from hippocampal slices of aging animals that were impaired in learning a hippocampus-dependent spatial task (Bach et al. 1999; Rosenzweig et al. 1997; Tombaugh et al. 2002a). Notably, pharmacological interventions that promote LTP in hippocampal slices of aging animals in vitro reversed the learning deficits in the aging animals (Bach et al. 1999; Tombaugh et al. 2002a). Additionally, it has been shown that spatial learning is impaired in transgenic animals that fail to demonstrate LTP in CA1 region of the hippocampus in vitro (Giese et al. 1998a; Tsien et al. 1996). However, conflicting reports about the necessity of LTP for spatial learning (D’Hooge and De Deyn 2001; Jeffery 1997; Silva et al. 1998) have cast doubt on its validity as the definitive cellular model of learning and memory.

An alternative or additional cellular mechanism for learning to LTP is the enhanced excitability of pyramidal neurons via the reduction of the postburst AHP. Numerous studies have demonstrated that the postburst AHP is reduced in hippocampal pyramidal neurons from rabbits and rats in vitro after learning the temporal, trace (Moyer et al. 1996, 2000; Oh et al. 1999a; Thompson et al. 1996b) or delay (Coulter et al. 1989; Disterhoft et al. 1986; Sanchez-Andres and Alkon 1991) eyeblink conditioning tasks. The data from the present report demonstrate a reduction of the AHP in the dorsal hippocampal pyramidal neurons after spatial learning. The AHP has also been shown to be reduced in piriform cortical neurons after odor discrimination learning (Saar et al. 1998). The fact that the AHP reduction is a reproducible phenomenon that occurs across species, across tasks, and in other cortical neurons besides hippocampal pyramidal neurons makes it an appealing potential cellular mechanism for learning.

The postburst AHP has been shown to be enhanced in hippocampal pyramidal neurons from aging animals as compared with that in neurons from young animals, i.e., neurons from aging animals are less excitable (Landfield and Pitler 1984; Moyer et al. 1992, 2000; Oh et al. 1999b; Wu et al. 2002). In addition to having an enhanced AHP, aging animals are impaired in learning various hippocampus-dependent tasks. For example, >60% of aging rabbits (>36 mo old) fail to meet the learning criteria in the trace eyeblink conditioning task (Thompson et al. 1996a). Using spatial, hippocampus-dependent tasks, numerous studies have demonstrated the severity of learning-deficits observed in aged animals as compared with the performance in the young (Bach et al. 1999; D’Hooge and De Deyn 2001; Gallagher et al. 1993; Poe et al. 2000; Tombaugh et al. 2002a). However, not all aging animals are impaired in acquiring hippocampus-dependent tasks. Examination of hippocampal pyramidal neurons from aging animals that learned the trace eyeblink conditioning task revealed that the postburst AHP was reduced, and the values observed were similar to those from neurons of young animals that learned the task (Moyer et al. 2000). Pharmacological treatments that
ameliorate the age-related learning deficits (such as cholinesterase inhibitors, muscarinic agonists, L-type calcium channel blockers) have also been shown to reduce the AHP of hippocampal pyramidal neurons in vitro (Kronforst-Collins et al. 1997; Moyer et al. 1992; Oh et al. 1999b; Weiss et al. 2000; Wu et al. 2002). CA1 neurons from aging rats that performed similarly to young rats on the water maze task had near identical slow AHP values as that observed from the young animals when measured well after training; whereas, neurons from aging behaviorally impaired rats had significantly enhanced slow AHPs as compared with those from aging unimpaired or young animals (Tombaugh et al. 2002b). Thus successful learning of hippocampus-dependent tasks may result in an enhanced excitability of hippocampal pyramidal neurons via reduction of the AHP, or reduced AHP in hippocampal pyramidal neurons may be a prerequisite for successful learning of hippocampus-dependent tasks. Either possibility leads us to postulate that the reduction of the postburst AHP is a cellular substrate of learning and memory.

The exact subcellular mechanism of learning-induced AHP reduction is unclear as the AHP and the currents underlying it are modulated by all known neurotransmitters that act via second messenger cascades (for reviews see, Sah 1996; Storm 1990; Wu et al. 2002). However, one transmitter system that may be intricately involved with learning is the cholinergic system (for review see, Disterhoft et al. 1999; Wu et al. 2002). Studies from our laboratory have demonstrated that cholinergic compounds that directly or indirectly reduce the AHP in vitro reverse the age-related learning deficits in vivo (Kronforst-Collins et al. 1997; Oh et al. 1999b; Weiss et al. 2000). Using microdialysis technique, it has also been demonstrated that there is an enhanced release of acetylcholine in the hippocampus during learning various tasks: eyeblink conditioning (Meyer et al. 1996), contextual fear conditioning (Nail-Boucherie et al. 2000), and radial-arm maze (Fadda et al. 2000). A product of muscarinic acetylcholine receptor activation is the activation of protein kinase C (PKC), and PKC activation and translocation have been shown to be learning-dependent (Bank et al. 1988; Van der Zee et al. 1997; Young et al. 2002). Additionally, a recent report by Barkai and colleagues suggests that the learning-induced reduction of the AHP measured in brain slices in vitro occurs via a PKC-dependent mechanism (Seroussi et al. 2002). Thus modulation of cholinergic transmission may be a pathway that impacts learning and also leads to the reduction of the AHP.

The characteristics of place fields, like the AHP, tend to be altered with spatial learning and with normal aging. The place fields of young adult rats undergo experience-dependent alteration with spatial exploration (Shen et al. 1997; Wilson et al. 2003), demonstrating the plasticity of the hippocampal pyramidal neurons. For example, sharpening and increase of place fields representing the hidden platform location have been demonstrated in the dorsal hippocampus of young adult rats after annular watermaze training (Hollup et al. 2001). On the other hand, aged rats with spatial learning-impairment have place fields that do not change with experience (Shen et al. 1997) or, in other words, are “rigid” (Wilson et al. 2003). The plasticity observed in young animals and the “rigidity” observed in aging animals may be due to the differences in the neuronal properties of the hippocampal pyramidal neurons. The postburst AHP and spike-frequency adaptation are smaller in hippocampal pyramidal neurons from young animals as compared with those from aging animals (Landfield and Pitler 1984; Moyer et al. 1992, 2000; Oh et al. 1999b). Thus hippocampal pyramidal neurons in young animals are more excitable, and perhaps more plastic, than those in aging animals. Interestingly, the place field rigidity can be mimicked in young rats by selectively removing the cholinergic input to the hippocampus by immunotoxic lesion of the medial septum and vertical limb of the diagonal band (Ikonen et al. 2002). In addition, young rats given a muscarinic antagonist, scopolamine, have reduced firing of place cells and spatial coherence of the place fields (Brazhnik et al. 2003) and are impaired in the acquisition of the watermaze task (Riekkinen and Riekkinen 1997). As discussed in the preceding text, modulation of hippocampal pyramidal neuronal excitability with cholinergic transmission may play an important role in hippocampal-dependent learning and ameliorating the learning-deficits observed with normal aging. Therefore the reduced AHP in the dorsal CA1 neurons that accompanies learning the location of the hidden platform in the watermaze may help mediate the sharpening of spatial fields observed in this neuronal population after spatial learning.

The data from this report along with those demonstrating a reduction of the postburst AHP after trace (Moyer et al. 1996, 2000; Thompson et al. 1996b) and delay eyeblink conditioning (Coulter et al. 1989; Disterhoft et al. 1986; Sanchez-Andres and Alkon 1991), and odor discrimination learning (Saar et al. 1998) present a strong case for the reduction of the calcium-activated potassium current(s) that underlie the afterhyperpolarization as one cellular substrate or marker of learning in different learning tasks, cortical regions, and species.

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DISCLOSURES

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