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Enhanced Neuronal Excitability in Rat CA1 Pyramidal Neurons Following Trace Eyeblick Conditioning Acquisition is Not Due to Alterations in I_M

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Abstract

Previous work done by our laboratory has demonstrated a reduction of the post-burst afterhyperpolarization (AHP) and accommodation following trace eyeblink conditioning in rabbit CA1 pyramidal neurons. Our laboratory has also demonstrated a reduction in the AHP in rat CA1 pyramidal neurons following spatial learning. In the current study we have extended our findings in rabbits by showing a reduction in both the AHP and accommodation in F344 X BN rat CA1 pyramidal neurons following acquisition of trace eyeblink conditioning. A component current of the AHP, I_M , was evaluated with a specific blocker of this current, and showed no apparent contribution to the learning-related increase in neuronal excitability. Rather, a reduction in an isoproterenol-sensitive component of the AHP, presumably sI_{AHP} , was observed to underlie the learning-specific change.

Introduction

Trains of action potentials in CA1 pyramidal neurons are followed by a prolonged post-burst afterhyperpolarization (AHP) that serves to limit further firing to a sustained depolarizing input. Prior work done by our laboratory has demonstrated that acquisition of the hippocampally-dependent trace eyeblink conditioning task results in a reduction of the AHP in CA1 neurons from rabbits (Moyer, Thompson & Disterhoft, 1996). In addition, we have shown that water maze acquisition results in a reduction of the AHP in rat CA1 pyramidal neurons (Oh, Kuo, Wu, Sametsky & Disterhoft, 2003). A reduction in the AHP has also been observed in rat piriform cortical neurons following acquisition of an odorant conditioning task (Saar, Grossman & Barkai, 1998). These findings suggest that a reduced AHP is a general cellular mechanism underlying learning, which is conserved across species, tasks and brain regions.

The AHP in hippocampal neurons can be separated into three components: a fast, medium and slow AHP based on pharmacological and kinetic criteria (Sah, 1996; Storm, 1990). These three components are mediated by four outward potassium currents: I_C , I_M , I_{AHP} , sI_{AHP} , and the mixed cationic current I_h (Maccaferri, Mangoni, Lazzari & DiFrancesco, 1993; Oh et al., 2003; Sah, 1996; Stocker, Krause & Pedarzani, 1999; Storm, 1990). The fast AHP occurs after single action potentials and is due to activation of I_C . The medium and slow AHP, seen after multiple action potentials, reflect the activation of I_M , I_{AHP} and I_h (medium AHP) and sI_{AHP}

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(slow AHP). Much evidence exists suggesting that these currents are important in learning for both invertebrates and vertebrates. A reduction in I_C has been seen in the invertebrate *Hermisenda* following associative training (Alkon, 1984) and in rat CA1 hippocampal pyramidal neurons after trace eyeblink conditioning (Matthews, Shah & Disterhoft, 2006). Work done by our laboratory demonstrating a reduction in both the medium and slow AHP following trace eyeblink conditioning acquisition in rabbits suggests alterations in I_M , I_{AHP} and/or sI_{AHP} (Moyer et al., 1996; Thompson, Moyer & Disterhoft, 1996). Additionally, voltage-clamp experiments have demonstrated a significant reduction in both the sI_{AHP} and I_{AHP} currents in rat CA1 pyramidal neurons following acquisition of the hippocampally-dependent spatial water maze task (Oh et al., 2003). However, voltage-clamp does not allow for a direct correlation of changes in the AHP currents with firing properties. Furthermore, neither of these studies examined the contribution of I_M to the learning-related changes in the AHP. I_M is modulated by acetylcholine and cholinergic agonists have been shown to facilitate trace eyeblink conditioning acquisition in rabbits, suggesting that this current may be altered as a consequence of learning (Kronforst-Collins, Moriearty, Schmidt & Disterhoft, 1997; Simon, Knuckley & Powell, 2004; Storm, 1990; Weiss, Preston, Oh, Schwarz, Welty & Disterhoft, 2000). Additionally, compounds known to block I_M , such as linopirdine, enhance cognitive function (Schnee & Brown, 1998). Taken together, these data suggest that alterations in I_M may underlie learning-related reductions in the AHP. Recently, a specific blocker of I_M , XE 991, has become available, making it possible to assess more accurately the contribution of this current to the AHP (Wang, Pan, Shi, Brown, Wymore, Cohen, Dixon & McKinnon, 1998).

Rats were trained on the trace eyeblink conditioning task and current-clamp recordings were made from CA1 pyramidal neurons one day following task acquisition. Pharmacological blockade of I_M and of sI_{AHP} were used to assess the contributions of these currents on both the AHP and accommodation in neurons from control and trained animals. Thus, this study sought to determine if reductions in both the AHP and accommodation occur in rat CA1 pyramidal neurons following acquisition of the trace eyeblink conditioning task, as well as to determine the contributions of I_M and sI_{AHP} to the enhanced intrinsic excitability in CA1 pyramidal neurons observed after learning.

Methods

Male F1 hybrid Fischer-344 $\times$ Brown Norway rats (4 months old) were used as subjects. Rats were obtained from NIA or Harlan (Indianapolis, Indiana) and housed three per cage in a climate-controlled room on a 12/12 light/dark cycle with *ad libitum* access to food and water. All procedures were done following guidelines maintained by the National Institutes of Health and with approval of the Northwestern University Animal Care and Use Committee.

Surgery

Procedures of eyeblink conditioning as modified for the rat by Weiss et al. (1999) were employed in this study. Animals were anesthetized with a combination of Xylazine (13 mg/kg, i.p.) and Ketamine (87 mg/kg, i.p.) and placed in a stereotaxic device. An incision was made on the top of the skull allowing for retraction of the periosteum. Six bilateral holes were drilled into the skull for insertion of stainless steel screws. A strip connector with two Teflon-coated stainless steel wires was then placed on the skull. EMG activity was recorded from the *orbicularis oculi* muscle via these wires that were inserted underneath the skin until they penetrated the skin of the upper eyelid of the right eye. A tether holding a connector for relaying EMG activity and a tube for airpuff delivery was attached to the strip connector. Dental cement was then placed around the connector and over the screws until the connector was firmly in

place. Following surgery, animals were placed on a heating pad until fully mobile, returned to their home cages, and given 3 days minimum to recover before beginning behavioral training.

Eyeblink Conditioning

On the first day of training, rats were placed in the training environment in the morning for a habituation period lasting the duration of a training session. The training environment consisted of a Plexiglas cage placed in a sound attenuating chamber, allowing rats to move freely during training. Trained rats ($n=22$) received 5 sessions (2 sessions/day) of 30 paired presentations per session of a CS (a 250 ms, 8 kHz, 85 dB free field tone) and a US (a 100 ms, 4.5 psi corneal airpuff), with an intertrial interval (ITI) of 20–40 sec, with a 30 sec average. The tone and the airpuff were separated by a 250 ms blank trace interval. Pseudoconditioned animals ($n=15$) served as behavioral controls. Pseudoconditioning trials consisted of explicitly unpaired presentations of either tone (CS) alone or airpuff (US) alone trials, with a random ITI of 10–20 ms with an average of 15 sec. Only trained rats achieving a minimum criterion of 60% CRs by the final training session were used for subsequent biophysical studies. A Power Macintosh computer running customized LabView software controlled stimulus presentations and data collection, analysis and storage. The EMG output was amplified (5000x) and filtered to pass 100–5k Hz. This signal was digitized at 3 kHz and stored on the computer.

Slice preparation

Transverse hippocampal slices (300 μ m) were made using a vibrating slicer and procedures similar to those previously published (Oh et al., 2003). Briefly, ~18 hours after the last training session, the rats were anesthetized with isoflurane in a fume hood, and killed by decapitation. The brain was quickly exposed, hemisected in situ, removed, and immersed in an ice-cold ($< 1^{\circ}\text{C}$) oxygenated aCSF (in mM: 119 NaCl, 26 NaHCO_3 , 2.5 KCl, 2 CaCl_2 , 1.3 MgCl_2 , 1 NaH_2PO_4 , 11 glucose, gassed with 95% O_2 - 5% CO_2). The right hippocampus was dissected out, and a transverse chunk from the middle hippocampus was made. Slices were cut and transferred to a holding chamber filled with aCSF at room temperature ($\sim 22^{\circ}\text{C}$) for at least 45 minutes prior to any experiments. All of the biophysical recordings (at 31°C) and analyses were performed blind to the behavioral status of the animal.

Biophysical Recordings

Intracellular recordings were made from hippocampal neurons using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). Microelectrodes (30–50 $\text{M}\Omega$) filled with 3 M KCl were used. The membrane potential was held at -65 ± 3 mV for all measurements. Within a cell, initial measurements were made in aCSF, followed by serial bath application of XE 991 and isoproterenol. aCSF solution was the same as that used for slicing, with the addition of 10 μM bicuculline. Current-voltage (I-V) relations were studied using 400 msec current injections (-1.0 to $+2$ nA). Input resistance was calculated by measuring the average voltage deflection (last 75 msec of the 400 msec pulse) of a -0.2 nA hyperpolarizing current step. The depolarizing sag was calculated as the difference between the peak amplitude (during the first 150 msec) and the plateau deflection during a -1.0 nA hyperpolarizing step. The postburst AHP was triggered with a somatic depolarizing step sufficient to elicit 5 spikes within 100 ms. Total integral area of the AHP (calculated from the time that the voltage trace crosses the baseline from the depolarized state until it returns to baseline) as well as the duration of the AHP was analyzed. Each AHP measurement was an average of three trials. The duration of the AHP was measured as the time required for the AHP to return to baseline for at least 10 msec (maximum duration 5 sec), starting at the offset of the stimulus. The peak AHP amplitude was calculated as the maximum negative voltage deflection, relative to resting potential. Latency was calculated as the time at which the AHP achieved peak amplitude. Spike-frequency adaptation (accommodation) was studied using a 1 sec depolarizing step of the same stimulus intensity

used to study the AHP. Resting membrane potential was calculated as the difference in potential before and after withdrawing the microelectrode from the cell.

All chemicals were purchased from Sigma (St. Louis, MO), with the exception of XE 991 (Tocris). Isoproterenol and XE 991 were prepared as 10 mM stock solutions and diluted to final concentrations before use.

Data was digitized and analyzed using a Lab-NB board (National Instruments, Austin, TX) interfaced to a Pentium IV computer running customized LabView software (National Instruments). Data were acquired at 10 kHz for I-V relations, AHP and accommodation measurements, and 1 kHz for membrane potentials. Procedures developed with LabView were used for blind offline analysis. Statistical analysis was performed using paired and unpaired t-tests and ANOVA (StatView, Abacus Concepts, Berkley, CA). Significant main effects were computed using Fisher's post hoc tests. Consistent with previous studies, there were no differences in any of the current-clamp measures between naïve and pseudoconditioned animals, so these two groups were combined as the control group (Moyer et al., 1996; Thompson, Moyer & Disterhoft, 1996).

Results

Trace eyeblink conditioning acquisition

All animals used for the biophysical recordings achieved a minimum of 60% CRs by the final training session. Representative raw and integrated EMG recordings from a pseudoconditioned (Fig. 1A) and trained (Fig. 1B) animal on the final training session are shown. A repeated measures ANOVA indicated that trained animals ($n = 22$) acquired the task significantly better than pseudoconditioned animals ($n = 16$) ($F [1,35] = 125.1$, $p < 0.0001$). Pseudo animals never achieved greater than 20% CRs over the course of training. These data can be seen in Figure 1C.

Reduced AHP and accommodation in neurons from trained rats (Experiment 1)

The peak amplitude of the AHP was significantly reduced in CA1 pyramidal neurons from trained rats as compared to neurons from control animals (control: $n = 50$ neurons/18 animals (10 naïve, 8 pseudoconditioned), 6.8 ± 0.28 mV; trained: $n = 19$ neurons/6 animals, 5.5 ± 0.33 mV, unpaired t-test, $p = 0.01$) (Fig. 2 A,B). A reduction in the integrated area of the AHP was also observed in neurons from trained animals as compared to those from control animals (control: $n = 50$, 8184 ± 505 mVms; trained: $n = 19$, 5778 ± 583 mVms; unpaired t-test, $p = 0.01$) (Fig. 2 A,B). Additionally, a concomitant reduction in accommodation was seen in neurons from trained animals as compared to neurons from control animals. Neurons from trained animals fired an average of 3 more action potentials during the accommodation pulse than neurons from control animals (control: $n = 41$, 9 ± 0.48 action potentials; trained: $n = 17$, 12 ± 1.2 action potentials; unpaired t-test, $p = 0.02$) (Fig. 3 A,B). Instantaneous firing frequency for action potentials occurring during the accommodation pulse was also analyzed (1/ISI). In addition to an increase in total number of action potentials fired during the accommodation pulse, a repeated measures ANOVA performed on the instantaneous firing frequency revealed a significant difference for action potentials occurring in the later part of the accommodation train (after the initial 100 ms) for neurons from trained animals as compared to those from controls ($F [1,26] = 6.1$, $p = 0.02$) (Fig. 3C). There was no difference between neurons from trained and control animals in instantaneous firing frequency for action potentials occurring in the first 100 ms of the accommodation pulse ($F [1,57] = 1.87$, $p = 0.66$) (Fig. 3C). Also, no differences between groups for AHP duration (control animals: 3762.3 ± 858.8 ms; trained animals: 3293.2 ± 1009.9 ms) or latency (control animals: 126.1 ± 8.0 ms; trained animals: 110.9 ± 12.2 ms), sag (control animals: $5.09 \pm .3$ mV; trained animals: $4.7 \pm .5$ mV), input

resistance (control animals: $63.6 \pm 2.3 \text{ M}\Omega$, trained animals: $59.0 \pm 4.2 \text{ M}\Omega$) or resting membrane potential (control animals: $63.7 \pm .9 \text{ mV}$; trained animals: $63.1 \pm 1.9 \text{ mV}$) were observed. A repeated measures ANOVA indicated that the width of the spikes used to generate the AHP was not altered by learning ($F[1,66]=2.08$, $p=.15$). There was also no significant difference between groups in the amount of current needed to elicit the 5 action potential burst used to generate the AHP (control: $.29 \pm .02 \text{ nA}$; trained: $.27 \pm .02 \text{ nA}$, $p=.5$, unpaired t-test).

Learning induced reductions in AHP are not sensitive to blockade of I_M but are sensitive to blockade of sI_{AHP} (Experiment 2)

The results from experiment 1 prompted a further investigation of the specific currents underlying the AHP that might be altered with learning, leading to the reduced AHP. The reductions in both the peak and area of the AHP seen in Experiment 1 indicated alterations of both the medium and slow AHP (Storm, 1990; Wu, Oh & Disterhoft, 2002). The medium AHP is mediated by several currents, I_{AHP} , I_M , I_h , and the slow AHP is mediated by the sI_{AHP} current. I_{AHP} was blocked in all recordings due to inclusion of bicuculline methiodide in the aCSF (Oh, Power, Thompson & Disterhoft, 2000; Stocker et al., 1999); all recordings were made at a potential at which I_h is not active or has very little contribution (-65 mV) (Storm, 1990; Williamson & Alger, 1990). Additionally, no difference was seen in the sag measure between neurons from trained and control animals, a measure used to assess I_h (Disterhoft, Coulter & Alkon, 1986; Maccaferri et al., 1993). Due to the slow kinetics of the sI_{AHP} , this current is thought to minimally impact the peak. Taken together, these data suggested that the likely current altered with learning resulting in the reduced peak AHP amplitude was I_M . A specific blocker of I_M , XE 991 (Wang et al., 1998) was bath applied to slices from both trained and control animals to assess the contribution of I_M to the AHP in both populations. In a subset of neurons, isoproterenol, a β -adrenergic agonist known to suppress the sI_{AHP} (Haas & Konnerth, 1983), was subsequently applied to inhibit this current and assess its contributions to the AHP in neurons from trained and control animals.

Pharmacological dissection of the AHP using serial application of XE 991 and isoproterenol yielded an interesting set of results. As observed in Experiment 1, both the peak AHP amplitude and total integrated area of the AHP were significantly reduced in neurons from trained animals (16/neurons $n = 34$) as compared to those from controls (30 neurons/18 animals (naïve=10, pseudoconditioned=8) (control: $7.3 \pm 0.34 \text{ mV}$; trained: $6.0 \pm 0.28 \text{ mV}$; peak amplitude: unpaired t-test, $p = 0.004$; control: $9430 \pm 641 \text{ mVms}$; trained: $6792 \pm 515 \text{ mVms}$; total area: unpaired t-test, $p = 0.003$) (Fig. 4D). Blockade of I_M with $10 \mu\text{M}$ XE 991 caused a significant reduction in the integrated area of the AHP occurring in the first 100 ms after pulse offset (Fig. 4A,C). This reduction was significant and comparable in neurons from both trained and control animals as compared to aCSF conditions (control neurons, $n = 27$: aCSF: $463.0 \pm 27.4 \text{ mVms}$; XE 991: $312.4 \pm 37.3 \text{ mVms}$, paired t-test, $p < 0.001$; trained neurons, $n = 22$: aCSF: $354.1 \pm 23.2 \text{ mVms}$; XE 991: $214.1 \pm 26.8 \text{ mVms}$, paired t-test, $p < 0.001$) (Fig. 4A,C). In neurons from both control and trained animals, application of XE-991 resulted in approximately a 40% reduction of the AHP area during the first 100 ms after pulse offset. As shown in Figure 4 A,D application of XE-991 to neurons from trained or control animals had no effect on the peak amplitude or integrated area of the AHP in neurons from animals in either group (peak amplitude: control neurons: aCSF: $7.3 \pm 0.47 \text{ mV}$; XE 991: $7.36 \pm 0.48 \text{ mV}$; trained neurons: aCSF: $6.0 \pm 0.28 \text{ mV}$; XE 991: $5.7 \pm 0.48 \text{ mV}$; total area: control neurons: aCSF: $9430 \pm 641 \text{ mVms}$; XE 991: $11262 \pm 1108 \text{ mVms}$; trained neurons: aCSF: $6656 \pm 514 \text{ mVms}$; XE 991: $8062 \pm 1139 \text{ mVms}$, NS paired t-test). After XE 991 application, a significant difference in the peak amplitude and total area of the AHP was still present between neurons from trained and control animals (peak amplitude: control, $7.3 \pm 0.47 \text{ mV}$; trained, $5.7 \pm 0.48 \text{ mV}$; unpaired t-test, $p = 0.02$; total area: control neurons, $11262 \pm 1108 \text{ mVms}$; trained, $8062 \pm 1139 \text{ mVms}$; unpaired t-test, $p < .05$). subsequent application of $10 \mu\text{M}$ isoproterenol in a subset of neurons

resulted in near complete abolition of the AHP. Suppression of sI_{AHP} resulted in a significant reduction of the peak amplitude of the AHP in neurons from both control and trained animals as compared to that recorded in aCSF plus XE-991 conditions (control neurons ($n=19$), XE 991: 7.3 ± 0.47 mV, ISO: 2.8 ± 0.34 mV, paired t-test $p < .0001$; trained neurons ($n=13$), XE 991: 5.7 ± 0.47 mV, ISO: 2.3 ± 0.18 mV, paired t-test, $p < .0001$) (Fig. 4B,D). Isoproterenol application also caused a significant reduction in the integrated area of the AHP in neurons from both groups of animals as compared to XE991 (control animals, XE 991: 11262 ± 1108 mVms, ISO: 2409 ± 620 mVms, paired t-test $p < 0.0001$; trained animals, XE 991: 8062 ± 1139 mVms, ISO: 2095 ± 430 mVms, paired t-test, $p = 0.003$; Fig. 4B,D). subsequent to application of isoproterenol, the significant difference in either the amplitude or total area of the AHP no longer existed between neurons from control and trained animals (amplitude, control neurons: $2.8 \pm .34$; trained neurons: $2.3 \pm .18$, unpaired t-test, NS. area, control neurons: 2409 ± 620 ; trained neurons: 2095 ± 430 , unpaired t-test, NS)

Learning-related alterations in accommodation were not sensitive to blockade of I_M but were sensitive to blockade of sI_{AHP}

The effect of I_M blockade on accommodation was also examined in Experiment 2. As shown in Figure 5 A,B, application of $10 \mu\text{M}$ XE-991 significantly reduced accommodation in neurons from both trained and control animals. The increase in number of action potentials and firing frequency after blockade of I_M was restricted to an early portion of the accommodation pulse (first 100 ms) (Fig. 5 A,B,C). ANOVA analysis performed on all cells for which accommodation measures were taken in both aCSF and XE 991 revealed that for the first four spikes of the accommodation pulse, a significant effect in instantaneous firing frequency was seen for neurons from both trained and control animals (control neurons: $F [1,38] = 17.9$, $p = 0.0001$; trained neurons: $F [1,36] = 33.4$, $p < 0.0001$). XE-991 application had no effect on the firing frequency for spikes occurring in the later part of the accommodation pulse ($>$ initial 100 ms) in CA1 pyramidal neurons from either trained or control animals (control neurons: $F [1,19] = 4.4$, $p > .05$; trained neurons: $F [1,30] = 3.4$, $p > 0.05$). Subsequent application of $10 \mu\text{M}$ isoproterenol did not cause a further enhancement of firing during the early phase for neurons from either trained or control animals (control neurons: $F [1,29] = .025$, $p > .05$; trained neurons: $F [1,30] = .007$, $p > .05$), but did cause a significant enhancement in firing frequency for spikes occurring during the later phase for neurons from both groups (control neurons: $F [1,17] = 17.8$, $p = 0.0006$; trained neurons: $F [1, 21] = 25.3$, $p < 0.0001$) (Fig. 4C).

Effects of I_M and sI_{AHP} blockade on intrinsic properties of neurons

Application of XE 991 did not significantly alter the input resistance of neurons from either group of animals (control animals, aCSF: 73.5 ± 3.5 M Ω , XE 991: 76.7 ± 5.3 M Ω ; trained animals, aCSF: 76.4 ± 3.0 M Ω , XE 991: 76.5 ± 2.9 M Ω .) Application of XE 991 did have a significant effect on the resting membrane potential of neurons from both groups of animals (control animals, aCSF: -62.6 ± 1.3 mV, XE 991: -57.8 ± 1.7 mV, paired t-test $p < .001$; trained animals, aCSF: -63.9 ± 1.5 mV, XE 991: -58.6 ± 1.3 mV, paired t-test $p < .002$). Application of ISO did not have a significant effect on the RMP of neurons from either group of animals (control animals, XE 991: -57.8 ± 1.7 mV, ISO: -58 ± 2.6 mV; trained animals, XE 991: -58.6 ± 1.3 mV, ISO: $-59.7 \pm .8$ mV) Application of ISO did not significantly effect the input resistance of neurons from either the control or trained group (control group, XE 991 76.7 ± 5.3 M Ω , ISO: 73.3 ± 2.6 M Ω ; trained animals, XE 991: 76.5 ± 2.9 M Ω , ISO: 69.6 ± 5.0 M Ω).

Discussion

The results of this study demonstrate that a reduction in the AHP and accommodation occur in rat CA1 pyramidal neurons following acquisition of trace eyeblink conditioning, as previously seen in rabbits (Moyer et al., 1996; Thompson et al., 1996). Specifically a reduction

in both the peak amplitude and area of the AHP was observed, as well as an increase in instantaneous firing frequency for action potentials occurring later in the accommodation train. Additionally, our data indicate that alteration of I_M does not underlie these reductions, but that a reduction in an isoproterenol-sensitive component, presumably sI_{AHP} , occurs.

These findings are not only consistent with previous findings in rabbits, but also with those observed in rat CA1 pyramidal neurons following watermaze learning (Oh et al., 2003). Together, the previous findings as well as those from the current study provide support for a reduction in the AHP as a cellular mechanism occurring with learning that is conserved across species and learning tasks. These data are also consistent with a reduction in accommodation as a consequence of learning as a phenomenon conserved across species.

Interestingly, the learning-related reduction of both the peak and total area of the AHP were observed in the presence of blockade of I_{AHP} (I_{AHP} was blocked in all recordings due to inclusion of bicuculline methiodide in recording aCSF). I_{AHP} is thought to be primarily involved in the medium AHP. Voltage-clamp analysis of I_{AHP} revealed a learning-related reduction in this current in rat CA1 pyramidal neurons after water maze acquisition (Oh et al., 2003). Although the present findings do not rule out the possibility that reductions in I_{AHP} occur and contribute to the learning-related reduction in the total AHP after eyeblink conditioning, they do suggest that another current(s) is changing as well. At a recording potential of -65 mV (all recordings in this study were made at -65 ± 3 mV), the AHP is composed of I_{AHP} , I_M and sI_{AHP} . Due to kinetics, the likely candidate current contributing to the peak of the AHP at -65 mV is I_M (Storm, 1990). We found that I_M does not play a significant role in the peak of the AHP. Blockade of I_M with XE 991 had no effect on either the peak amplitude or area of the AHP in neurons from either control or trained animals, demonstrating that it is not this current that is altered with learning as reflected in the reduced AHP. Blockade of I_M , however, affected the early component of the AHP (occurring in the first 100 ms after pulse offset) in neurons from both control and trained animals. Application of XE 991 resulted in a comparable reduction of this component of the AHP in neurons from both groups, about 40%. This time window for an XE 991 sensitive component is consistent with a recent finding by Peters and colleagues (Peters, Hu, Pongs, Storm & Isbrandt, 2005). Additionally, blockade of I_M by XE 991 had a significant effect on early, but not late spike frequency adaptation in neurons from both control and trained animals. This is consistent with prior reports detailing the role of I_M in this phase of spike frequency adaptation (Madison & Nicoll, 1984; Storm, 1990). As there was a difference between neurons from trained and control animals for the late, but not early phase of accommodation, our data suggest that alterations in I_M are not responsible for the learning-related reduction in accommodation in CA1 pyramidal neurons seen after training.

Isoproterenol was applied following application of XE 991 to determine if the residual AHP was sensitive to β -adrenergic modulation. As both I_M and I_{AHP} were blocked prior to isoproterenol application, and this drug's inhibition of sI_{AHP} is well documented (Lancaster & Batchelor, 2000; Madison & Nicoll, 1986; Martin, Araque & Buno, 2001), isoproterenol presumably suppressed sI_{AHP} in these recordings. In neurons from both trained and control animals, application of isoproterenol significantly reduced, and nearly abolished, the residual AHP. There was no significant difference in either the peak or area of the AHP between neurons from control and trained animals following isoproterenol application. That is, isoproterenol application eliminated the learning-related difference in AHP size between neurons from control and trained animals. Since sI_{AHP} activates slowly over several hundred milliseconds and peaks between 400 and 700 ms (Sah, 1996; Storm, 1990), it was our expectation that sI_{AHP} would contribute minimally to the peak of the AHP. However, our data clearly demonstrate that sI_{AHP} has a significant contribution to the peak of the AHP when I_{AHP} was

blocked. In fact, these data strongly suggest that the AHP in rat CA1 pyramidal neurons is dominated by an isoproterenol-sensitive component, most likely sI_{AHP} .

The effects of isoproterenol application on accommodation in neurons from both control and trained animals are also consistent with well known effects of sI_{AHP} suppression on accommodation (Madison & Nicoll, 1984; Storm, 1990). Application of isoproterenol did not result in further enhancement of firing frequency for action potentials occurring during the first 100 ms of the accommodation pulse over that seen with XE 991 application. However, isoproterenol application did significantly enhance firing frequency for spikes occurring later in the train in neurons from all groups, a phase unaffected by XE 991 application, but significantly enhanced after learning. Although application of isoproterenol did decrease the AHP and increase firing in neurons from both groups, this does not negate the necessity of alterations in the sI_{AHP} for learning related reductions of the AHP and accommodation. Learning does not result in a complete abolition of the AHP or accommodation; there is a residual AHP and accommodation following learning that can still be further modulated. Thus, it is not surprising that application of isoproterenol causes a further reduction of the AHP and accommodation in neurons from both trained and control animals. However, it is important to note that application of isoproterenol eliminates the significant difference in the peak and area of the AHP between neurons from control and trained animals, as well as impacts only the phase of accommodation that is reduced after learning. Taken together, the effects of isoproterenol application on both the AHP and accommodation suggest that the isoproterenol-sensitive component, presumably the sI_{AHP} , is altered with learning, leading to a reduction in the AHP and accommodation.

Supporting evidence exists for the sI_{AHP} as one current underlying the AHP that is altered with learning. In addition to the reductions in this current seen after water maze learning in rat CA1 pyramidal neurons (Oh et al., 2003), classical eyeblink conditioning in rabbit CA1 pyramidal neurons (Sanchez-Andres & Alkon, 1991) and in rat piriform cortex neurons after olfactory discrimination learning (Brosh, Rosenblum & Barkai, 2006), the sI_{AHP} has been shown to be reduced by many of the same compounds that reduce the AHP. Additionally, these compounds affect learning. Our laboratory has shown that the cholinesterase inhibitor and cognitive enhancer metrifonate reduces the AHP as well as the sI_{AHP} (Kronforst-Collins et al., 1997; Oh, Power, Thompson, Moriearty & Disterhoft, 1999; Power, Oh & Disterhoft, 2001). Interestingly, metrifonate had no effect on either I_M or the apamin sensitive I_{AHP} thus it appears that metrifonate reduces the AHP solely via its actions on the sI_{AHP} (Oh et al., 1999; Power et al., 2001). Additionally, the PKC, PKA and CAMKII pathways have all been shown to be involved in learning, and *in vitro* application of activators of these pathways reduced the AHP via reductions in the sI_{AHP} (Malenka, Madison, Andrade & Nicoll, 1986; Micheau & Riedel, 1999; Pedarzani & Storm, 1993; Pedarzani & Storm, 1996; Selcher, Weeber, Varga, Sweatt & Swank, 2002; Van der Zee, Kronforst-Collins, Maizels, Hunzicker-Dunn & Disterhoft, 1997; Vazquez, Vazquez & Pena de Ortiz, 2000; Wu et al., 2002). Additional evidence for the importance of sI_{AHP} in learning-related alterations of the AHP comes from studies of aging animals. The AHP and sI_{AHP} are enhanced in CA1 pyramidal neurons from aging rabbits (36 months) as compared to neurons from young animals (Power et al., 2001). The size of the sI_{AHP} and AHP were highly correlated, suggesting that enhancements in the sI_{AHP} have a significant impact on AHP size in aging animals. Interestingly, this is an age at which rabbits are significantly impaired in their ability to learn the trace eyeblink conditioning task (Thompson et al., 1996). As a whole, these data suggest that the sI_{AHP} is exquisitely sensitive to modulation by both neurotransmitters and compounds that reduce the AHP and are involved in learning. They suggest that the sI_{AHP} is the component current of the AHP whose alterations lead to learning-related reductions in the AHP.

The findings described in this paper build upon previous studies demonstrating learning-related reductions in the AHP in CA1 pyramidal neurons. In addition to demonstrating that a learning-related reduction of the AHP and accommodation occurs in rat CA1 pyramidal neurons after trace eyeblink conditioning acquisition, the data presented here clearly show that these alterations do not reflect a reduction in I_M after training, but more likely the sI_{AHP} . It should be stressed that the fact that I_M blockade does not affect the learning-specific excitability change observed in neurons measured at a time point after learning does not preclude the possible contribution of I_M to the cellular mechanisms that establish the increased excitability during the learning process. Future studies will explore this possibility.

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Figure 1.

Acquisition of trace eyeblink conditioning. Examples of eyeblink responses exhibited by a pseudoconditioned rat receiving unpaired presentations of the CS and US (A) and a trained rat receiving paired presentations of the CS and US (B). Examples are taken from the final training session. Both raw EMG (bottom) and rectified/integrated EMG responses (top) are shown for each trace. Shaded bars indicate the timing of the tone CS (dark gray) and airpuff US (light gray). (C) Acquisition rates of trace eyeblink conditioning for trained and pseudoconditioned animals. Trained animals (filled circles) acquired the task significantly better than pseudoconditioned animals (open circles) ($p < .0001$). Pseudo animals never achieved greater than 20% CRs over the course of training.

Figure 2. Learning-related alterations in the AHP. (A) Representative traces of the AHP in CA1 pyramidal neurons from trained and control animals. (B) Both the peak amplitude and total area of the AHP (right side) are significantly reduced in CA1 pyramidal neurons from trained animals (gray bars) as compared to controls (open bars) following acquisition of trace eyeblink conditioning. * $p = 0.01$, unpaired t-test.

Figure 3. Learning-related alterations in accommodation. (A) Representative accommodation traces recorded in neurons from control and trained animals. (B) A significant reduction in accommodation was seen in neurons from trained animals (gray bars) as compared to neurons from control animals (open bars). Neurons from trained animals fired an average of 3 more spikes during the accommodation pulse as compared to neurons from control animals. (C) Instantaneous firing frequency for spikes occurring during the accommodation pulse. Intervals 1–4 occurred during the first 100 ms of the pulse. A significant increase in firing frequency was seen for intervals 5–9 in neurons from trained animals (closed circles) as compared to controls (open circles). * $p = 0.02$, unpaired t-test.

Figure 4.

Effects of XE 991 and isoproterenol on the AHP. (A) and (B) Representative AHP traces recorded from control (left) and trained (right) neurons. AHP measurements were made first in aCSF followed by XE 991 (A) followed by isoproterenol (B). *Inset*: Enlarged view of the first 300 ms after pulse offset. (C) XE 991 application significantly reduced the area of the AHP occurring in the first 100 ms after pulse offset in neurons from both control (open bars) and trained animals (gray bars). (C) XE 991 application had no significant effect on AHP amplitude (left bar graph) or total area of the AHP (right bar graph) for neurons from either trained (gray bars) or control animals (open bars). isoproterenol application significantly reduced both the amplitude (left bar graph) and total area of the AHP (right bar graph) in

neurons from both trained (gray bars) and control animals (open bars). * $p < 0.001$, paired t-test, as compared to aCSF values. † $p < 0.05$, unpaired t-test, as compared to control values. ** $p < .01$ as compared to XE 991 values.

Figure 5. Effects of XE 991 and isoproterenol on accommodation. (A) Representative accommodation traces from control (top) and trained (bottom) neurons in aCSF, XE 991 and isoproterenol conditions. (B) Instantaneous firing frequency for spikes occurring during the accommodation pulse. Intervals 1–4 for the first 100 ms of the accommodation pulse (left) and the first 5 intervals after the initial 100 ms (right) are plotted. In neurons from both control (open squares) and trained animals (closed squares), a significant increase in firing frequency was seen for intervals 1–4 following application of XE 991 (initial 100ms), but there was no effect on intervals 1–5 (post 100ms). Isoproterenol application did not cause a further increase in firing frequency for intervals 1–4 (initial 100ms) for neurons from either trained (closed triangles) or control animals (open triangles), but did significantly enhance firing frequency for intervals 1–5 (post 100ms) for neurons from both groups.