

Age-Related Enhancement of the Slow Outward Calcium-Activated Potassium Current in Hippocampal CA1 Pyramidal Neurons *In Vitro*

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Aging is associated with learning deficits and a decrease in neuronal excitability, reflected by an enhanced post-burst afterhyperpolarization (AHP), in CA1 hippocampal pyramidal neurons. To identify the current(s) underlying the AHP altered in aging neurons, whole-cell voltage-clamp recording experiments were performed in hippocampal slices from young and aging rabbits. Similar to previous reports, aging neurons were found to rest at more hyperpolarized potentials and have larger AHPs than young neurons. Given that compounds that reduce the slow outward calcium-activated potassium current (sI_{AHP}), a major constituent of the AHP, also facilitate learning in aging animals, the sI_{AHP} was pharmacologically isolated and characterized. Aging neurons were found to have an enhanced sI_{AHP} , the amplitude of which was significantly correlated to the amplitude of the AHP ($r = 0.63$; $p < 0.001$). Thus, an enhanced sI_{AHP} contributes to the enhanced AHP in aging. No differences

were found in the membrane resistance, capacitance, or kinetic and voltage-dependent properties of the sI_{AHP} . Because enhanced AHP in aging neurons has been hypothesized to be secondary to an enhanced Ca^{2+} influx via the voltage-gated L-type Ca^{2+} channels, we further examined the sI_{AHP} in the presence of an L-type Ca^{2+} channel blocker, nimodipine (10 μM). Nimodipine caused quantitatively greater reductions in the sI_{AHP} in aging neurons than in young neurons; however, the residual sI_{AHP} was still significantly larger in aging neurons than in young neurons. Our data, in conjunction with previous studies showing a correlation between the AHP and learning, suggest that the enhancement of the sI_{AHP} in aging is a mechanism that contributes to age-related learning deficits.

Key words: slow afterhyperpolarization; aging; L-type Ca^{2+} channels; whole-cell voltage clamp; current clamp; neuronal excitability; plasticity; nimodipine

Action potentials in hippocampal pyramidal neurons are followed by a post-burst afterhyperpolarization (AHP), which serves to limit further firing in response to sustained excitation in a process known as spike frequency adaptation (accommodation). Reductions in the AHP and accommodation have been observed in neurons from animals trained in various hippocampus-dependent (Moyer et al., 1996, 2000; Thompson et al., 1996b; Oh et al., 2001) and non-hippocampus-dependent tasks (Disterhoft et al., 1986; Coulter et al., 1989; Saar et al., 1998), suggesting that it is a general mechanism to increase neuronal excitability in learning.

The AHP and accommodation are reduced after the acquisition of the hippocampus-dependent trace eyeblink conditioning task in CA1 and CA3 hippocampal pyramidal neurons (Moyer et al., 1996, 2000; Thompson et al., 1996b). These biophysical changes are most likely learning induced, because they are not observed in neurons of pseudoconditioned controls (which receive the same but unpaired stimuli), naive controls, and animals that failed to acquire the task. Furthermore, reductions in the AHP and accommodation return to baseline in 7 d (Moyer et al., 1996; Thompson et al., 1996b), consistent with the hypothesis that

the hippocampus functions as an intermediate storage buffer during learning (Cohen and Eichenbaum, 1993; Kim et al., 1995; Disterhoft et al., 1996).

Acquisition of the trace eyeblink conditioned response is impaired in aging animals and aging humans (Thompson et al., 1996a; Weiss et al., 2000; Knuttinen et al., 2001a,b). Interestingly, the AHP and accommodation are enhanced in CA1 neurons from animals at ages that show learning deficits (Landfield and Pitler, 1984; Moyer et al., 1992, 2000). Although many aging animals failed to acquire the trace eyeblink conditioned response (Thompson et al., 1996a), those that did learn also showed a reduction in the AHP (Moyer et al., 2000). This inverse correlation between the AHP and learning has led us to hypothesize that AHP enhancement in aging is a mechanism involved in age-related learning deficits. Consistent with our hypothesis, drugs that reduce the AHP *in vitro* have also been shown to improve learning in aging animals (Deyo et al., 1989; Moyer et al., 1992; Kronforst-Collins et al., 1997; Oh et al., 1999; Weiss et al., 2000; Power et al., 2001).

The AHP is mediated by four outward K^+ currents (I_C , I_M , I_{AHP} , and sI_{AHP}), and its time course is modulated by the hyperpolarization-activated current, I_h (Storm, 1990; Maccaferri et al., 1993; Stocker et al., 1999; Oh et al., 2000). Given the kinetics of these currents, previous experiments showing prolonged AHP and enhanced accommodation in aging neurons strongly implicate alterations in the slower currents, particularly the sI_{AHP} , in aging (Landfield and Pitler, 1984; Moyer et al., 1992, 2000; Power et al., 2001). The following experiments were undertaken to determine whether the I_{AHP} - sI_{AHP} contributes to the enhanced AHP in aging neurons.

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MATERIALS AND METHODS

Subjects. Young (2–3 months old) and aging (>36 months old) female New Zealand albino rabbits (*Oryctolagus cuniculus*) were chosen as subjects. Animal use procedures were approved by Northwestern University's Animal Care and Use Committee, according to the standards of the United States Department of Agriculture.

Slice preparation. Slices were prepared following procedures similar to those described previously (Moyer et al., 1996). Briefly, two bathing solutions were used during the dissection: (1) normal artificial CSF (aCSF) composed of (in mM): 124 NaCl, 3 KCl, 1.3 MgSO₄, 1.24 NaH₂PO₄, 2.4 CaCl₂, 26 NaHCO₃, 10 D-glucose, and (2) sucrose-aCSF, containing an equimolar concentration of sucrose in place of NaCl. Both normal- and sucrose-aCSF solutions were oxygenated (bubbled with 95% O₂-5% CO₂, pH 7.4).

Rabbits were anesthetized with halothane and killed by decapitation. The brain was rapidly exposed *in situ*, hemisected, removed within 60 sec, and immediately immersed in oxygenated ice-cold (<1°C) sucrose-aCSF for ~4 min. Both hippocampi were quickly dissected out, cut into 3–4 mm chunks, and glued onto chilled chambers that were then filled with ice-cold sucrose-aCSF. Transverse slices (300 μm) along the dorsal-ventral gradient of the hippocampus were prepared using Vibratomes (TPI, O'Fallon, MO) and placed in holding chambers filled with normal-aCSF at room temperature (~23°C) for at least 45 min before any experiment.

Whole-cell electrodes and solutions. Patch electrodes were made from borosilicate glass using a Sutter Flaming-Brown horizontal puller (P-87; Sutter Instrument Company, Novato, CA) and heat-polished with a Narishige microforge (Model MF-930; Narishige International USA, Inc., East Meadow, NY) to a resistance of 1.5–6.0 MΩ. Three pipette solutions were used and consisted of the following (in mM): (1) 2 K-ATP, 10 HEPES, and 150 KMeSO₄; (2) 2 K-ATP, 10 HEPES, 140 KMeSO₄, and 10 KCl; and (3) 2 K-ATP, 10 HEPES, 130 KMeSO₄, and 10 KCl. The pH of these solutions was adjusted to 7.25 with KOH; the osmolarity of these solutions was 290 ± 10 mOsm. The results obtained with these solutions were indistinguishable. MeSO₄⁻ was used in place of Cl⁻ to reduce the rundown of the AHP current over time (Zhang et al., 1994). The use of KMeSO₄ in the electrode solution resulted in a ~10 mV junction potential with respect to the aCSF. This junction potential was measured for every batch of electrode solution according to procedures published by Neher (1992) and was corrected before data acquisition.

Data analysis and cell criteria. Hippocampal neurons were visualized using either a Zeiss Axioskop (Carl Zeiss, Inc., Oberkochen, Germany) or a Leica DM LFS microscope (Leica Microsystems AG, Wetzlar, Germany); both were equipped with a long working distance 40× water immersion objective and infrared differential interference contrast optics (Dodt and Zieglensberger, 1990; Stuart et al. 1993).

Two data acquisition systems were developed using either LabVIEW (National Instrument, Austin, TX) or C++ Builder (Borland Software Corporation, Scotts Valley, CA) with National Instrument NIDAQ driver. Data were acquired at 5 kHz and filtered at 2 kHz using a low-pass Bessel filter. Whole-cell recording procedures for young and aging neurons were variants of previously published procedures, using a single pipette to “clean” and patch onto the neuron (Blanton et al., 1989). Recordings were made from the soma of visually identified CA1 pyramidal neurons with seal resistances >1.5 GΩ before break through into the whole-cell mode. A few cells were labeled with Lucifer yellow to monitor the equilibration between the intracellular milieu and the pipette solution. All measurements were made at least 10 min after membrane rupture to allow for adequate solution equilibration. Only neurons with series resistance <15 MΩ, membrane resistance >60 MΩ, resting potential less than -60 mV, and for current-clamp recordings action potential amplitude >90 mV from resting potential were included in the data set.

Current-clamp protocols. The AHP was first measured in current-clamp mode to provide a direct comparison for previous studies. Subsequently, the I_{AHP} - sI_{AHP} was pharmacologically isolated and characterized in voltage-clamp mode. Slices were individually transferred to small glass-bottom recording chambers and perfused continuously (~2 ml/min) with oxygenated aCSF heated to 31°C. Our laboratory has evidence for a dorsal-ventral gradient in excitability within the hippocampus (Sametsky et al., 2001). Thus, slices equally distributed across the septotemporal axis of the hippocampus in both age groups were used for recording. During current-clamp recordings, a membrane potential of neurons was maintained at -68 mV with either hyperpolarizing or depolarizing current injection, and the AHP was evoked using a 100 msec depolariz-

ing current step that reliably elicited a burst of four action potentials. The AHP measurements included the following: peak amplitude of the AHP (calculated as the maximum negative voltage deflection from the baseline potential during the first 250 msec after the current offset), duration of the AHP (measured as the time required for the membrane potential to return to the baseline potential for at least 10 msec after the 100 msec depolarizing current step offset), and integrated area of the AHP (calculated from the current offset for the entire duration of the AHP).

Voltage-clamp protocols. After current-clamp measurements, the I_{AHP} - sI_{AHP} were isolated by bath perfusion of modified aCSF containing the following: 500 nM tetrodotoxin (TTX) to block Na⁺ current; 2 mM CsCl to block inward K⁺ currents and I_h ; 2 mM 4-aminopyridine (4-AP) to block I_A and I_D ; 5 mM tetraethylammonium (TEA) to block I_C and I_M ; and 100–500 μM picrotoxin, and 2 mM kynurenic acid or 10 μM 6-cyano-7-nitroquinoxaline-2,3-(1H,4H)-dione (CNQX), and 25 μM D-2-amino-5-phosphonovaleric acid (D-AP5) to reduce synaptic current. 4-AP and TEA were substituted for equimolar NaCl. To block the L-type Ca²⁺ influx, nimodipine (dissolved in dimethyl sulfoxide) was added directly to the modified aCSF to achieve the desired concentration. Because nimodipine is light sensitive, all experiments involving nimodipine were performed in near-darkness. For all pharmacological experiments, slices were exposed to the modified medium for 10–20 min before measurements were taken.

Although both the I_{AHP} and sI_{AHP} coexist in CA1 hippocampus, the I_{AHP} accounts for only 20% of the AHP in rabbit CA1 pyramidal neurons (Oh et al., 2000). The apamin-sensitive I_{AHP} has a relatively fast onset (1–5 msec) 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_{AHP} is ~1.5 sec (Sah, 1996). Therefore, we differentiated the sI_{AHP} from the I_{AHP} by measuring the tail current 1 sec after pulse offset.

The AHP tail current was evoked by a 100 msec, 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. Although the membrane voltage was not precisely controlled during the depolarizing step because of the gain and space-clamp limitations, voltage control of the AHP tail current was well maintained (Lancaster and Adams, 1986; Constanti and Sim, 1987; Sah and McLachlan, 1991; Zhang et al., 1995). The AHP tail current measurements included the following: peak amplitude, latency to peak amplitude, amplitude of the tail current at 200 msec, 1 sec, and 2 sec after pulse offset, and decay rate. The decay kinetics of the AHP current were determined by measuring the time it took for the current to decay to half of the 200 msec (half decay 200) and 800 msec amplitudes (half decay 800, which revealed the decay of the sI_{AHP} alone). The decay time constant was also determined by fitting a single exponential to the tail current 400–3000 msec after pulse offset.

The voltage dependence and reversal potential of sI_{AHP} were examined by holding the neurons at -55 mV, activating the sI_{AHP} by a 100 msec, 50 mV voltage step, and then varying the holding potential of the tail current from -45 to -95 mV (see Fig. 4A). The voltage-dependence of sI_{AHP} activation was assessed by holding the neurons at -55 mV and varying the amplitude of a 100 msec step potential (see Fig. 3A). The time dependence of sI_{AHP} activation was assessed by holding the neurons at -55 mV and varying the duration of a 15–20 mV subthreshold activation pulse (see Fig. 3B).

Data analysis. All statistical analyses were performed using Statview (SAS Institute Inc., Cary, NC). Correlations were tested using Fisher's r to z . Age-related differences in amplitudes were tested using ANOVA, unpaired t test, or Mann-Whitney U test as appropriate. The F test was used to test for age-related differences in the variance. Data are reported as the mean ± SEM.

Drugs. KMeSO₄ was purchased from ICN (Aurora, OH); TTX was from Calbiochem (San Diego, CA); D-AP5 and CNQX were from Tocris (Ellisville, MO). All other drugs were purchased from Sigma (St. Louis, MO).

RESULTS

The age-related differences in the AHP and AHP currents are summarized in Table 1. No difference was observed in the membrane resistance or membrane capacitance between the two age groups. Similar to our previous report (Moyer et al., 1992), aging neurons rested at more hyperpolarized potentials (aging = -75.9 ± 1.2 mV; young = -70.2 ± 1.4 mV; $p < 0.01$) and exhibited greater AHP peak amplitudes (aging = -9.03 ± 0.64

Table 1. Age-related changes in the AHP and the underlying currents

	Young			Aging			<i>p</i> values		
	Mean	SEM	<i>n</i>	Mean	SEM	<i>n</i>	T	F	U
Post-burst AHP measures									
Amplitude (mV)	-7.21	0.45	15	-9.03	0.64	12	0.025	0.395	
Area (mV msec)	-11344	1588	15	-14106	1732	12	0.252		
Duration (msec)	5489	622	15	6009	636	12	0.568		
Latency (msec)	96.6	20.4	15	55.5	9.7	12		0.006	0.242
AHP tail current measures									
Peak amplitude (pA)	374.1	36.3	18	604.7	66.2	16		0.037	0.007
Latency to peak amplitude (msec)	154.9	39.2	18	92.4	26.1	16	0.205		
Area (pC)	468.9	57.8	17	830.8	111.2	16		0.019	0.012
200 msec amplitude (pA)	321.6	32.9	18	486.2	47.0	16	0.006	0.247	
1 sec amplitude (pA)	186.8	22.8	18	325.9	39.5	16	0.004	0.059	0.006
2 sec amplitude (pA)	101.3	16.4	18	206.2	30.9	16		0.027	0.006
Decay tau (msec)	-1814	141	16	-2122	183	16	0.193		
200 half decay (msec)	1256	127	18	1559	138	16	0.117		
800 half decay (msec)	1801	96	18	2141	92	16	0.016	0.684	
Reversal potential (mV)	-93.88	2.53	7	-93.66	1.94	11	0.944		
Membrane resistance (MΩ)	101.11	5.87	18	103.06	6.79	16	0.828		
Resting potential (mV)	-70.19	1.41	20	-75.87	1.23	16	0.006	0.307	

The mean, SEM, and number of neurons recorded (*n*) are given for young and aging neurons. Age-related comparisons were performed using unpaired *t* test (T), *F* test (F), and Mann-Whitney *U* test (U) as appropriate. Significant effects are highlighted in bold.

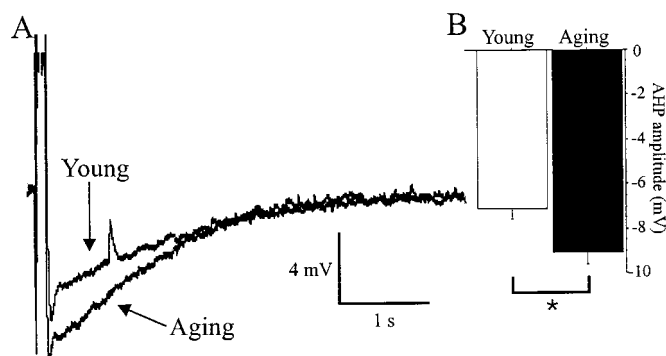


Figure 1. Current-clamp recordings showing an aging-related enhancement of the post-burst AHP. *A*, Voltage traces showing representative AHPs from young and aging neurons. *B*, Mean AHP amplitude was greater in aging neurons than in young neurons (mean \pm SEM; unpaired *t* test; **p* < 0.05).

mV; young = -7.21 ± 0.45 mV; *p* < 0.05) (Fig. 1) than young neurons. To control for resting membrane potential (RMP) differences, the neurons were maintained at -68 mV with either hyperpolarizing or depolarizing current injection, and the AHP was measured after a 100 msec current step that triggered four action potentials. The current used to hold the neurons at -68 mV was greater for aging neurons than for young neurons (aging = 64 ± 18 pA; young = 6 ± 15 pA; *p* < 0.05). Similarly, the current required to elicit four action potentials was greater for aging than for young neurons (aging = 966 ± 98 pA; young = 682 ± 52 pA; *p* < 0.01).

The current-clamp measurements of the AHP may be biased by differences in the depolarizing current pulse that may result in differences in Ca^{2+} influx or subtle changes in other conductances that control spike broadening. Therefore, we characterized the currents underlying the slow AHP ($I_{AHP} - sI_{AHP}$) with voltage-clamp protocols in the presence of Na^+ and K^+ channel

blockers (TTX, CsCl, 4-AP, and TEA). Recordings of the AHP current also revealed an aging-related increase in the peak current amplitude (mixed $I_{AHP} - sI_{AHP}$ amplitude; aging = 604.7 ± 66.2 pA; young = 374.1 ± 36.3 pA; *p* < 0.01), current amplitudes at 1 and 2 sec after pulse offset (sI_{AHP} amplitudes; 1 sec: aging = 325.9 ± 39.5 pA; young = 186.8 ± 22.8 pA; *p* < 0.01; 2 sec: aging = 206.2 ± 30.9 pA; young = 106.3 ± 16.4 pA; *p* < 0.01) (Fig. 2*A*), and integrated area of the tail current (aging = 830.8 ± 111.2 pC; young = 486.9 ± 57.8 pC; *p* < 0.01). The amplitude of the AHP showed correlations to all of the amplitude measurements, as well as the integrated area, of the AHP tail current (Table 2), indicating that the enhancements in the I_{AHP} and the sI_{AHP} underlie the enhancement in the AHP in aging.

Most of the amplitude measurements of the sI_{AHP} showed a greater variance in aging neurons. Although no differences were observed in the peak latency and the decay of the AHP tail current, the time required for the tail current to decay to half its amplitude at 800 msec after pulse offset was significantly longer in aging neurons than in young neurons (*p* < 0.05). Given that the apamin-insensitive, slow component of the AHP (the sI_{AHP}) accounts for $\sim 80\%$ of the total AHP in rabbit CA1 pyramidal neurons (Oh et al., 2000) and outlasts the I_{AHP} by seconds (Sah, 1996; Stocker et al., 1999), the correlations between our current-clamp and voltage-clamp measurements suggest that the enhanced sI_{AHP} predominantly underlies the enhanced AHP in aging neurons.

As step potentials were increased, a transient inward current appeared within a few milliseconds of pulse onset, followed by a slow outward current that continued as the AHP tail current. Increasing the amplitude of the step potential increased the amplitude of the sI_{AHP} in both age groups. Although no clear-cut threshold of sI_{AHP} activation could be determined, no significant tail current was observed unless the step potentials elicited an obvious Ca^{2+} transient (Fig. 3*A*). Increasing the duration of the step potential also increased the amplitude of sI_{AHP} in both age groups (Fig. 3*B*).

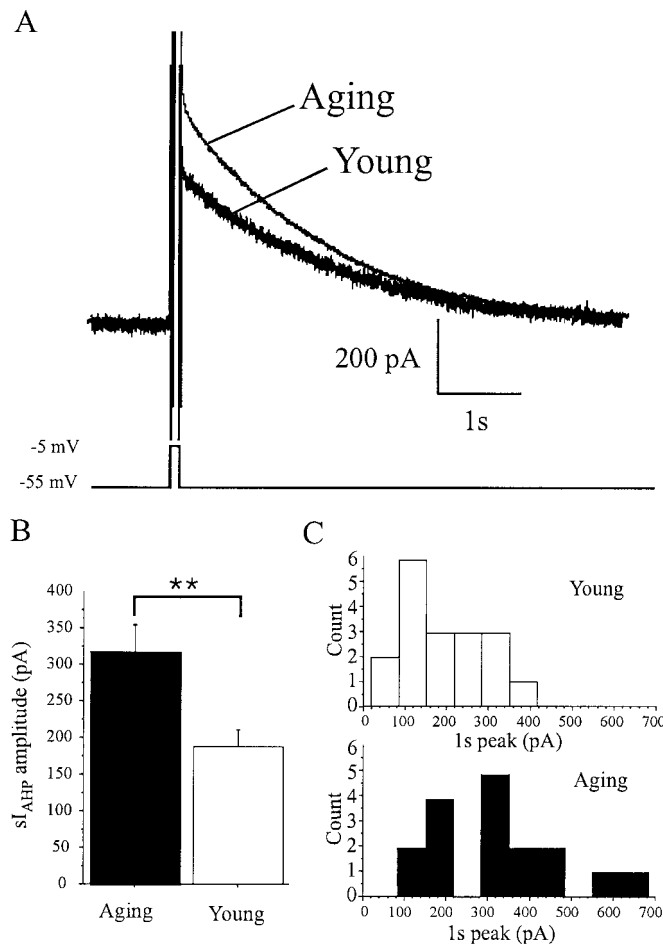


Figure 2. Voltage-clamp recordings showing an age-related enhancement of the sI_{AHP} . *A*, Representative sI_{AHP} tail currents from young and aging neurons, underlain with the voltage-clamp protocol for evoking the sI_{AHP} tail current. *B*, The sI_{AHP} amplitude is greater in aging neurons than in young neurons (mean \pm SEM; unpaired *t* test; ** $p < 0.01$). *C*, Frequency distribution of the sI_{AHP} (1 sec amplitude) in young and aging neurons.

The kinetics and voltage-dependence of the sI_{AHP} were not different in both age groups; these values were consistent with previous recordings from young hippocampal pyramidal neurons (Lancaster and Adams, 1986). The sI_{AHP} showed little voltage dependence from -45 to -85 mV for both age groups (Fig. 4*C*). Likewise, its decay rate was voltage independent (Fig. 4*B*). In both age groups, the sI_{AHP} reversed at approximately -94 mV, suggesting that the enhancement in the sI_{AHP} in aging was not caused by a change in the driving force for K^+ .

Our laboratory has reported previously that the AHP enhancement in aging is independent of the resting membrane potential difference (Moyer et al., 1992). Likewise in this study, when we compared the sI_{AHP} from cells with comparable resting membrane potentials (-82 mV $<$ RMP $<$ -77 mV), aging neurons still had significantly larger sI_{AHP} than young neurons (young = 153.63 ± 22.47 pA, $n = 14$; aging = 269.04 ± 34.64 pA, $n = 14$; $p < 0.01$). Thus, the difference in the resting membrane potentials did not contribute to the aging-related enhancement of the sI_{AHP} .

The sI_{AHP} is a Ca^{2+} -dependent K^+ current. Thus, its amplitude is affected by the amount of Ca^{2+} available to cause channel activation. Previous reports have demonstrated an aging-related enhancement in voltage-gated Ca^{2+} influx (Landfield and Pitler,

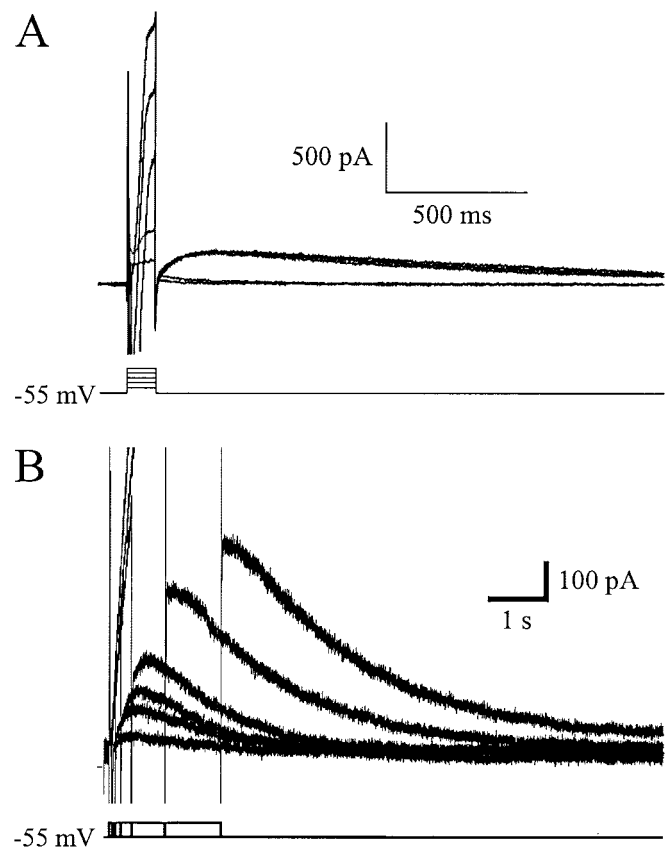


Figure 3. Ca^{2+} influx and sI_{AHP} . *A*, An example of the sI_{AHP} in response to depolarizing voltage steps from -50 to -10 mV, in increments of 10 mV. For leak subtraction, 2 mV step potentials were used. Note that although increasing the amplitude of the voltage pulse increased the amplitude of the sI_{AHP} , no significant sI_{AHP} was observed until an obvious Ca^{2+} transient was elicited by the step potentials. *B*, Increasing the pulse duration also increased the amplitude of the sI_{AHP} . The sI_{AHP} was activated with depolarizing steps from -50 to -30 mV with varying durations. Voltage steps with longer durations allowed for more Ca^{2+} influx, thereby increasing the sI_{AHP} .

1984; Pitler and Landfield, 1990; Moyer and Disterhoft, 1994; Campbell et al., 1996), partially attributable to an increase in the functional L-type Ca^{2+} channel density (Thibault and Landfield, 1996; Chen et al., 2000), in neurons from aging animals. Furthermore, the enhancements in the AHP and the plateau phase of the Ca^{2+} action potential in aging neurons are both reduced by nimodipine, an L-type Ca^{2+} channel blocker (Moyer and Disterhoft, 1994), raising a possibility that the enhanced sI_{AHP} in aging neurons is secondary to an enhanced L-type Ca^{2+} influx. To investigate whether age-related enhancement of the sI_{AHP} is secondary to an enhanced L-type Ca^{2+} influx, we examined the sI_{AHP} before and after bath applications of nimodipine ($10 \mu M$). Aging-related enhancement of the sI_{AHP} ($p < 0.0005$) was replicated and reported in Table 3. Bath application of nimodipine significantly reduced the integrated area of the I_{AHP} - sI_{AHP} , as well as the amplitude of the sI_{AHP} , at 1 sec after pulse offset ($p < 0.0001$) (Fig. 5). Nimodipine reduced both the area of the I_{AHP} - sI_{AHP} and the amplitude of the sI_{AHP} in young and aging neurons by <25 and $<30\%$, respectively (Fig. 6*A*). Consistent with our previous study (Moyer and Disterhoft, 1994), nimodipine caused quantitatively greater reductions in the integrated area of the I_{AHP} - sI_{AHP} and the amplitude of the sI_{AHP} in aging

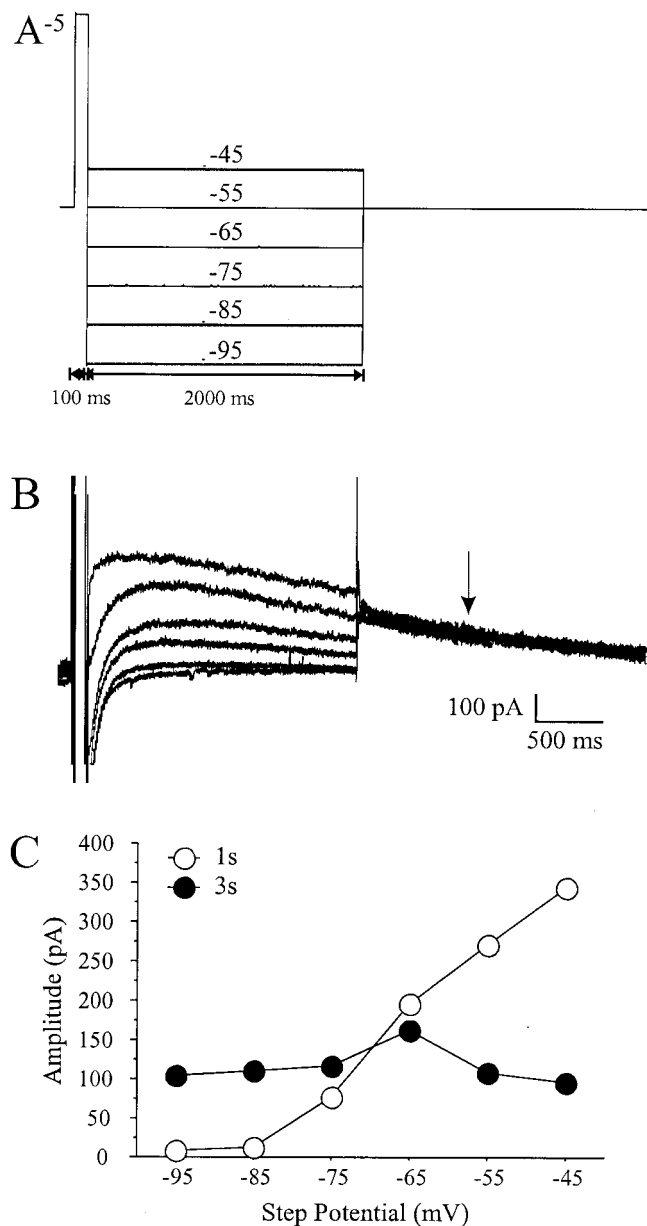


Figure 4. Voltage independence and reversal potential of the AHP tail current. *A*, The protocol for measuring the voltage dependence of the sI_{AHP} tail current. The sI_{AHP} tail current was evoked by a 100 msec, 50 mV pulse from a holding potential of -55 mV. After the voltage pulse, membrane potential was stepped to various test potentials for 2 sec before returning to the -55 mV holding potential. For leak subtraction, the neurons were stepped to the test potentials for 2 sec without the 100 msec, 50 mV prepulse to elicit the sI_{AHP} tail current. *B*, Representative currents evoked from the voltage-dependence protocol. *C*, The reversal potential was obtained by linear extrapolation of the voltage versus sI_{AHP} amplitude at 1 sec to the voltage axis. The reversal potential of the sI_{AHP} in this example was -90 mV. Similar to previous reports, the sI_{AHP} conductance did not show any voltage dependence at either age (young: $n = 7$; aging: $n = 11$). The test potentials did not alter the decay rate of the current, and amplitude of the sI_{AHP} measured at 1 sec after the various test potentials (labeled as 3s and denoted with an arrow) remained the same.

neurons than in young neurons ($p < 0.05$) (Fig. 6*B*). The residual area of the $I_{AHP} - sI_{AHP}$ and the amplitude of the sI_{AHP} were still significantly larger in aging neurons than in young neurons (amplitude for young = 117.3 ± 17.65 pA; for old, 190.0 ± 21.47 pA;

$p = 0.01$) (Fig. 6*C*). Hence, our data are consistent with observations of an enhanced L-type Ca^{2+} influx in aging neurons. However, this enhanced L-type Ca^{2+} influx cannot fully account for the aging-related enhancement in the sI_{AHP} .

DISCUSSION

In hippocampal pyramidal neurons, excitability is controlled by a pronounced AHP. Based on kinetics and pharmacological criteria, the AHP can be separated into fast, medium, and slow components (for review, see Storm, 1990; Sah, 1996). In neurons from trace eyeblink conditioned animals, both the peak amplitude of the AHP and its duration are decreased (Moyer et al., 1996, 2000; Thompson et al., 1996b). In contrast, both parameters are increased in neurons from naive aging animals (Landfield and Pitler, 1984; Moyer et al., 1992). After pharmacologically isolating the slow components of the AHP ($I_{AHP} - sI_{AHP}$), we found age-related enhancements in the integrated area (mixed $I_{AHP} - sI_{AHP}$), the peak amplitude (mixed $I_{AHP} - sI_{AHP}$), and the amplitudes at 1 and 2 sec after pulse offset (sI_{AHP} alone) for the AHP tail current. We did not pharmacologically separate the I_{AHP} and the sI_{AHP} . However, in CA1 pyramidal neurons from rabbits and rats, the apamin-sensitive I_{AHP} accounts for only a small percentage ($\sim 20\%$) of the total AHP (Stocker et al., 1999; Oh et al., 2000). Furthermore, blocking I_{AHP} with saturating concentrations of apamin had only small effects on accommodation (Oh et al., 2000). Thus by inference, the reduced neuronal excitability observed in aging neurons can mostly be attributed to an enhanced sI_{AHP} . Consistent with this hypothesis, the amplitude measurements of the sI_{AHP} correlated with the integrated area, as well as amplitude measurements, of the AHP, further supporting the possibility that an enhanced sI_{AHP} underlies an enhanced AHP in aging.

Many factors can lead to an enhanced sI_{AHP} in aging. Increased leak conductance is not likely to be one of them, because neurons of both age groups shared similar membrane resistances. Although aging neurons rested at more hyperpolarizing potentials, the reversal potentials for the sI_{AHP} were similar in both young and aging neurons. Furthermore, when the cells were grouped by resting membrane potentials, aging neurons still had significantly larger sI_{AHP} than young neurons, suggesting that changes in the driving force for K^+ ions do not contribute to an enhanced sI_{AHP} in aging. The slow AHP is also affected by conductances that control spike broadening, and hence the amount of Ca^{2+} influx, such as the A-type voltage-dependent K^+ current (Giese et al., 1998). The enhanced sI_{AHP} that we observed in aging neurons is not caused by alterations in these conductances, because all voltage-clamp recordings were performed in the presence of 4-AP and TEA.

The sI_{AHP} is a Ca^{2+} -dependent K^+ current that is modulated by many neurotransmitters. Therefore, its amplitude also depends on (1) the amount of Ca^{2+} available to cause channel activation, (2) the degree of modulation, and (3) channel conductance and functional density of the sI_{AHP} channels. Many studies have shown altered Ca^{2+} homeostasis in aging neurons. Changes in voltage-gated Ca^{2+} influx (Landfield and Pitler, 1984; Moyer et al., 1992; Thibault and Landfield, 1996) and intracellular Ca^{2+} release (Martini et al., 1994), as well as an elevation in the free cytosolic Ca^{2+} concentration, most likely resulting from a combination of disrupted Ca^{2+} handling, buffering, sequestration, and efflux mechanisms (Khachaturian, 1989; Disterhoft et al., 1993, 1994; Thibault et al., 1998; Verkhatsky and Toescu, 1998), have all been implicated in aging. Exactly how these changes

Table 2. Correlation between current-clamp and voltage-clamp measurements

AHP tail current	Post-burst AHP (mV)		Post-burst AHP area (mV msec)	
	<i>r</i>	<i>p</i> values	<i>r</i>	<i>p</i> values
Peak amplitude (pA)	-0.683	0.0002	-0.463	0.0248
Latency to peak amplitude (msec)	0.327	0.1292	0.200	0.3635
200 msec amplitude (pA)	-0.654	0.0005	-0.564	0.0043
1 sec amplitude (pA)	-0.631	0.0009	-0.582	0.0029
2 sec amplitude (pA)	-0.592	0.0023	-0.514	0.0110
Area (pC)	-0.618	0.0012	-0.542	0.0066
Half decay 200 (msec)	-0.141	0.5241	-0.177	0.4245
Half decay 800 (msec)	-0.291	0.1802	-0.167	0.4500
Decay tau (msec)	0.252	0.2494	0.219	0.3193

Amplitude and area of the AHP were correlated to amplitudes and area of the slow outward tail current. Correlations, *r*, were performed using Fisher's *r* to *z*. Significant effects are highlighted in bold.

Table 3. Effect of nimodipine on young and aging neurons

AHP tail measures	Young			Aging			<i>p</i> values		
	Mean	SEM	<i>n</i>	Mean	SEM	<i>n</i>	T	F	U
<i>sI</i> _{AHP} -aCSF									
Peak amplitude (pA)	214.8	21.0	30	353.6	27.5	35	0.0002	0.0567	0.0005
Latency to peak amplitude (msec)	403.4	33.4	30	445.8	33.9	35	0.3796		
200 msec amplitude (pA)	189.5	23.6	30	317.3	43.2	35	0.0008	0.0704	
1 sec amplitude (pA)	143.2	15.8	30	262.4	23.5	35		0.0096	0.0002
2 sec amplitude (pA)	74.0	11.1	30	128.5	18.1	35		0.0021	0.0095
Area (pC)	253.7	36.9	30	646.8	67.4	35		0.0012	0.0005
With nimodipine									
Peak amplitude (pA)	164.5	22.9	25	266.0	26.7	31	0.007	0.1859	
Latency to peak amplitude (msec)	475.8	37.1	25	460.9	38.8	31	0.785		
200 msec amplitude (pA)	127.6	24.0	25	226.4	26.3	31	0.009	0.3072	
1 sec amplitude (pA)	117.3	17.7	25	190.0	21.5	31	0.014	0.1231	
2 sec amplitude (pA)	59.4	9.4	25	107.8	15.5	31		0.0027	0.040
Area (pC)	286.5	46.0	25	465.9	57.4	31	0.022	0.0953	

Age-related changes in the I_{AHP} - sI_{AHP} were replicated and reported in this table. The mean, SEM, and number of neurons recorded (*n*) are given for young and aging neurons. Statistical analyses were performed using unpaired *t* test (T), *F* test (F), and Mann-Whitney *U* test (U) as appropriate. Significant *p* values are highlighted in bold.

affect the sI_{AHP} is unclear. For example, it has been hypothesized that the age-related enhancement of the AHP is secondary to an enhanced Ca^{2+} influx (Pitler and Landfield, 1990), particularly that through the L-type Ca^{2+} channels (Campbell et al., 1996; Thibault and Landfield, 1996; Chen et al., 2000). In this study, we addressed this possibility by examining the effect of nimodipine on the sI_{AHP} in the context of aging. Saturating concentrations of nimodipine caused quantitatively greater reductions in the sI_{AHP} in aging neurons than in young neurons, suggesting that the contribution of the L-type Ca^{2+} influx to activate the sI_{AHP} is enhanced in aging. However, after blocking the L-type Ca^{2+} influx, the residual sI_{AHP} from aging neurons remained significantly larger than that of the young neurons, indicating that the enhanced L-type Ca^{2+} influx alone cannot account for the aging-related enhancement of the sI_{AHP} .

Although nimodipine is a highly lipophilic compound, and its diffusion through slices can be problematic, it is not likely that the enhanced sI_{AHP} in aging neurons observed in the presence of nimodipine is caused by differences in the amount of unblocked L-type Ca^{2+} currents. In this study, saturating concentrations of nimodipine were applied for at least 10 min before recording. In addition, recordings were typically made from CA1 pyramidal neurons at no more than 100 μ m below the surface of the slice.

Previously our laboratory has shown that the L-type Ca^{2+} current in aging neurons can be blocked with lower concentrations of nimodipine than that of the young neuron (Moyer et al., 1992). In the unlikely event of a diffusion problem, these data suggest a greater likelihood for residual L-type Ca^{2+} current to exist in young neurons than in aging neurons. In such a case, the aging-related enhancement of the sI_{AHP} that is independent of the L-type Ca^{2+} influx reported here would be an underestimation.

Ca^{2+} that activates the sI_{AHP} also comes from intracellular Ca^{2+} store release through a process known as Ca^{2+} -induced Ca^{2+} release (CICR) (Sah and McLachlan, 1991; Torres et al., 1995, 1996; Tanabe et al., 1998; Shah and Haylett, 2000). Whether altered CICR contributes to an enhanced sI_{AHP} in aging remains speculative.

The amplitude of the sI_{AHP} also depends on the degree of neuromodulation it receives. The sI_{AHP} is reduced by metabotropic glutamate agonists (Liu et al., 1993), acetylcholine (Madison et al., 1987), serotonin (Colino and Halliwell, 1987), histamine (Haas and Greene, 1986), dopamine (Malenka and Nicoll, 1986), noradrenaline (Haas and Konnerth, 1983; Madison and Nicoll, 1986), corticotropin releasing factor (Fox and Gruol, 1993), vasoactive intestinal peptide (Haas and Gahwiler, 1992), and calcitonin gene-related peptide (Nohmi et al., 1986). Many of

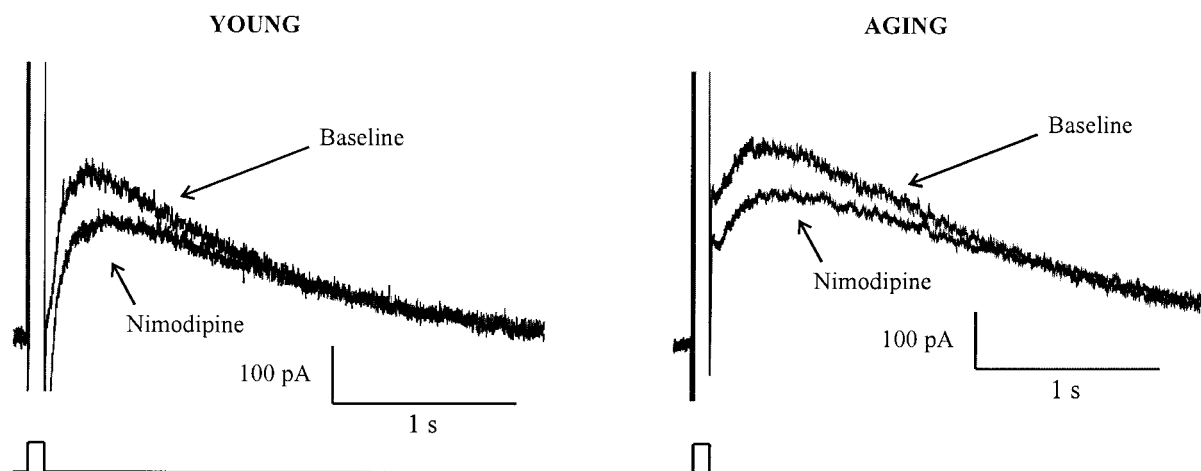


Figure 5. The effect of L-type Ca^{2+} influx on the sI_{AHP} . Shown are representative current traces from young and aging neurons before and after bath applications of nimodipine.

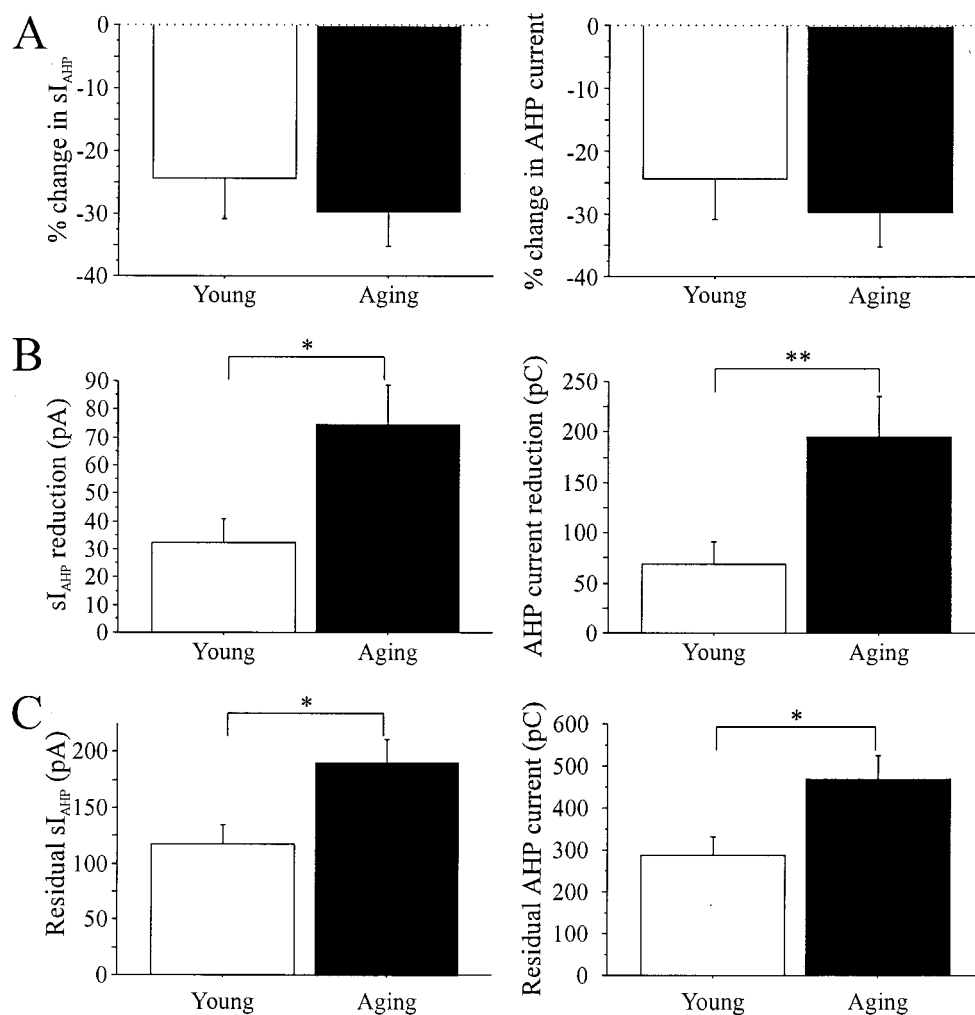


Figure 6. *A*, Bath application of nimodipine caused a comparable percentage (25–30%) of reduction in the sI_{AHP} of young and aging neurons. *B*, The amount of reductions in the sI_{AHP} (1 sec amplitude) and the AHP current (integrated area) was significantly larger in aging than in young neurons (unpaired t test; $*p < 0.05$ and $**p < 0.001$, respectively; $n = 31$ for aging; $n = 25$ for young). *C*, After eliminating the contribution of the L-type Ca^{2+} influx on the sI_{AHP} with bath-applied nimodipine, the residual sI_{AHP} and AHP current were still significantly larger in the aging neurons than in the young neurons (unpaired t test; $*p < 0.05$).

these molecules were shown to suppress the sI_{AHP} through protein kinase activities (Gerber et al., 1992; Müller et al., 1992; Pedarzani and Storm 1993, 1995, 1996; Torres et al., 1995; Abdul-Ghani et al., 1996; Haug and Storm, 2000). Changes in many of these neurotransmitter systems (Luine et al., 1990; Fischer et al., 1992; Smith and Booze, 1995; Richter-Levin and Segal, 1996;

Miguez et al., 1999; Stemmelin et al., 2000; Segovia et al., 2001), as well as their effector kinases (Colombo et al., 1997; Pascale et al., 1998), have been implicated in aging. Conceivably, altered neurotransmission in aging, coupled with altered kinase and phosphatase activities that normally maintain the sI_{AHP} (Pedarzani et al.,

1998) and alter this current. The enhanced sI_{AHP} in aging neurons that we report in this study is a postsynaptic phenomenon and does not reflect changes in basal neurotransmission, because our slices were perfused in aCSF containing TTX as well as glutamatergic and GABAergic antagonists.

Age-related changes in the functional sI_{AHP} channel density as well as the channel properties can also affect the sI_{AHP} . Whether these mechanisms contribute to the enhanced sI_{AHP} in aging remains to be addressed.

Molecules that affect the sI_{AHP} have also been implicated in other forms of plasticity. For example, kinases known to modulate the sI_{AHP} (PKC, PKA, and calcium-calmodulin kinase II) are also important for the induction of long-term potentiation (LTP), a model for cellular mechanisms of learning and memory (for review, see Soderling and Derkach, 2000). Currently, the sI_{AHP} channels are thought to be located on apical or basal dendrites close to the soma (Sah and Bekkers, 1996; Bekkers, 2000), although a recent study by Lancaster et al. (2001) questions the basal dendritic location, suggesting a role for the sI_{AHP} in controlling dendritic excitability and synaptic integration (LoTurco et al., 1988; Andreassen and Lambert, 1995). Consistent with this hypothesis, activation of the sI_{AHP} dampens temporal summation of the EPSPs as well as speeds up their decay rate (Lancaster et al., 2001). Pharmacological manipulations that facilitated LTP have also been shown to reduce the AHP (Cohen et al., 1999), suggesting that the AHP and its underlying currents can serve as an adjustable gain control, variably hyperpolarizing and shunting synaptic potentials arising in the distal dendrites and controlling the induction of further plasticity (Sah and Bekkers, 1996). Accordingly, the enhanced sI_{AHP} in aging can hamper the formation of further plastic alterations important for learning and memory (Dunwiddie et al., 1992; Sah and Bekkers, 1996; Giese et al., 2001).

The correlation between the AHP (and thus, accommodation) and learning have led us to hypothesize that the enhancement in the AHP is involved in aging-related memory deficits. Here we report that this AHP enhancement in aging is attributable to an enhanced sI_{AHP} . In addition, the variance of the sI_{AHP} amplitude increases with age, strikingly similar to the age-related heterogeneity of learning observed in rabbits of the same age (Thompson et al., 1996a). The data presented in this study have allowed us to refine our initial hypothesis: enhancement of the sI_{AHP} is involved in age-related learning deficits. In support of our hypothesis, we have shown that nimodipine facilitates acquisition of the trace eyeblink conditioned response and reduces the AHP in animals (Deyo et al., 1989; Moyer et al., 1992; Kowalska and Disterhoft, 1994). In this study, we have further shown that nimodipine reduces the sI_{AHP} in young and aging animals. Similarly, chronic treatment with metrifonate, a cholinesterase inhibitor, or CI1017, an M1 muscarinic agonist, both reduce the sI_{AHP} and the slow AHP and facilitate trace eyeblink conditioning in aging rabbits (Kronforst-Collins et al., 1997; Oh et al., 1999; Weiss et al., 2000; Power et al., 2001). The cholinergic reduction of the sI_{AHP} is independent of Ca^{2+} entry (Muller and Connor, 1991), suggesting that the cognitive benefits of procholinergic treatments are caused by a reduction of the sI_{AHP} itself. We are unaware of experiments designed to disrupt learning by enhancing the sI_{AHP} . However, treatment with the central cholinergic blocker scopolamine, which should eliminate cholinergic reduction of the sI_{AHP} , disrupts trace eyeblink conditioning in young rabbits (Kaneko and Thompson, 1997). The results of the present study provide additional support for an involvement of the en-

hanced sI_{AHP} in age-related learning deficits, and further suggest that key modulators of this current are good candidates for future therapeutic interventions in age-related cognitive impairments.

REFERENCES

- Abdul-Ghani MA, Valiante TA, Carlen PL, Pennefather PS (1996) Tyrosine kinase inhibitors enhance a $Ca(2+)$ -activated K^+ current (IAHP) and reduce IAHP suppression by a metabotropic glutamate receptor agonist in rat dentate granule neurones. *J Physiol (Lond)* 496:139–144.
- Andreassen M, Lambert JD (1995) The excitability of CA1 pyramidal cell dendrites is modulated by a local $Ca(2+)$ -dependent $K(+)$ -conductance. *Brain Res* 698:193–203.
- Bekkers JM (2000) Distribution of slow AHP channels on hippocampal CA1 pyramidal neurons. *J Neurophysiol* 83:1756–1759.
- Blanton MG, LoTurco JJ, Kriegstein AR (1989) Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J Neurosci Methods* 30:203–210.
- Campbell LW, Hao SY, Thibault O, Blalock EM, Landfield PW (1996) Aging changes in voltage-gated calcium currents in hippocampal CA1 neurons. *J Neurosci* 16:6286–6295.
- Chen KC, Blalock EM, Thibault O, Kaminker P, Landfield PW (2000) Expression of alpha 1D subunit mRNA is correlated with L-type Ca^{2+} channel activity in single neurons of hippocampal “zipper” slices. *Proc Natl Acad Sci USA* 97:4357–4362.
- Cohen AS, Coussens CM, Raymond CR, Abraham WC (1999) Long-lasting increase in cellular excitability associated with the priming of LTP induction in rat hippocampus. *J Neurophysiol* 82:3139–3148.
- Cohen NJ, Eichenbaum H (1993) Memory, amnesia, and the hippocampal system. Cambridge, MA: MIT.
- Colino A, Halliwell JV (1987) Differential modulation of three separate K -conductances in hippocampal CA1 neurons by serotonin. *Nature* 328:73–77.
- Colombo PJ, Wetsel WC, Gallagher M (1997) Spatial memory is related to hippocampal subcellular concentrations of calcium-dependent protein kinase C isoforms in young and aged rats. *Proc Natl Acad Sci USA* 94:14195–14199.
- Constanti A, Sim JA (1987) Calcium-dependent potassium conductance in guinea-pig olfactory cortex neurones in vitro. *J Physiol (Lond)* 387:173–194.
- Coulter DA, LoTurco J, Kubota M, Disterhoft JF, Moore JW, Alkon DL (1989) Classical conditioning reduces the amplitude and duration of the calcium-dependent afterhyperpolarization in rabbit hippocampal pyramidal cells. *J Neurophysiol* 61:971–981.
- Deyo RA, Straube KT, Disterhoft JF (1989) Nimodipine facilitates associative learning in aging rabbits. *Science* 243:809–811.
- Disterhoft JF, Coulter DA, Alkon DL (1986) Conditioning-specific membrane changes of rabbit hippocampal neurons measured in vitro. *Proc Natl Acad Sci USA* 83:2733–2737.
- Disterhoft JF, Moyer Jr JR, Thompson LT (1993) The calcium rationale in Alzheimer's disease. New York: Raven.
- Disterhoft JF, Gispen WH, Traber J, Khatchaturian AS (1994) Calcium hypothesis of aging and dementia. *Ann NY Acad Sci* 747.
- Disterhoft JF, Thompson LT, Moyer Jr JR, Mogul DJ (1996) Calcium-dependent afterhyperpolarization and learning in young and aging hippocampus. *Life Sci* 59:413–420.
- Doty HU, Zieglerberger W (1990) Visualizing unstained neurons in living brain slices by infrared DIC-videomicroscopy. *Brain Res* 537:333–336.
- Dunwiddie TV, Taylor M, Heginbotham LR, Proctor WR (1992) Long-term increases in excitability in the CA1 region of rat hippocampus induced by β -adrenergic stimulation: possible mediation by cAMP. *J Neurosci* 12:506–517.
- Fischer W, Chen KS, Gage FH, Bjorklund A (1992) Progressive decline in spatial learning and integrity of forebrain cholinergic neurons in rats during aging. *Neurobiol Aging* 13:9–23.
- Fox EA, Gruol DL (1993) Corticotropin-releasing factor suppresses the afterhyperpolarization in cerebellar Purkinje neurons. *Neurosci Lett* 149:103–107.
- Gerber U, Sim JA, Gähwiler BH (1992) Reduction of potassium conductances mediated by metabotropic glutamate receptors in rat CA3 pyramidal cells does not require protein kinase C or protein kinase A. *Eur J Neurosci* 4:792–797.
- Giese KP, Storm JF, Reuter D, Fedorov NB, Shao LR, Leicher T, Pongs O, Silva AJ (1998) Reduced K^+ channel inactivation, spike broadening, and after-hyperpolarization in $Kv\beta 1.1$ -deficient mice with impaired learning. *Learn Mem* 5:257–273.
- Giese KP, Peters M, Vernon J (2001) Modulation of excitability as a learning and memory mechanism: a molecular genetic perspective. *Physiol Behav* 73:803–810.
- Haas H, Greene RW (1984) Adenosine enhances afterhyperpolarization and accommodation in hippocampal pyramidal cells. *Pflügers Arch* 402:244–247.

- Haas H, Greene RW (1986) Effects of histamine on hippocampal pyramidal cells of rats in vitro. *Exp Brain Res* 62:123–130.
- Haas HL, Gahwiler BH (1992) Vasoactive intestinal polypeptide modulates neuronal excitability in hippocampal slices of the rat. *Neuroscience* 47:273–277.
- Haas HL, Konnerth A (1983) Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells. *Nature* 302:432–434.
- Haug T, Storm JF (2000) Protein kinase A mediates the modulation of the slow $\text{Ca}(2+)$ -dependent $\text{K}(+)$ current, $\text{I}(\text{sAHP})$, by the neuropeptides CRF, VIP, and CGRP in hippocampal pyramidal neurons. *J Neurophysiol* 83:2071–2079.
- Kaneko T, Thompson RF (1997) Disruption of trace conditioning of the nictitating membrane response in rabbits by central cholinergic blockade. *Psychopharmacology* 131:161–166.
- Khachaturian ZS (1989) The role of calcium regulation in brain aging: reexamination of a hypothesis. *Aging* 1:17–34.
- Kim J, Clark R, Thompson RF (1995) Hippampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behav Neurosci* 109:195–203.
- Knuttinen MG, Gamelli AE, Weiss C, Power JM, Disterhoft JF (2001a) Age-related effects on eyeblink conditioning in the F344 x BN F1 hybrid rat. *Neurobiol Aging* 22:1–8.
- Knuttinen MG, Power JM, Preston PR, Disterhoft JF (2001b) Awareness in classical differential eyeblink conditioning in young and aging humans. *Behav Neurosci* 115:747–757.
- Kowalska M, Disterhoft JF (1994) Relation of nimodipine dose and serum concentration to learning enhancement in aging rabbits. *Exp Neurol* 127:159–166.
- Kronforst-Collins MA, Moriearty PL, Schmidt B, Disterhoft JF (1997) Metrifonate improves associative learning and retention in aging rabbits. *Behav Neurosci* 111:1031–1040.
- Lancaster B, Adams P (1986) Ca^{2+} -dependent current generating the AHP of hippocampal neurons. *J Neurophysiol* 55:1268–1282.
- Lancaster B, Hu H, Ramakers GM, Storm JF (2001) Interaction between synaptic excitation and slow afterhyperpolarization current in hippocampal pyramidal cells. *J Physiol (Lond)* 536:809–823.
- Landfield PW, Pitler TA (1984) Prolonged Ca^{2+} -dependent afterhyperpolarizations in hippocampal neurons of aged rats. *Science* 226:1089–1092.
- Liu YB, Disterhoft JF, Slater NT (1993) Activation of metabotropic glutamate receptors induces long-term depression of GABAergic inhibition in hippocampus. *J Neurophysiol* 69:1000–1004.
- LoTurco JL, Coulter DA, Alkon DL (1988) Enhancement of synaptic potentials in rabbit CA1 pyramidal neurons following classical conditioning. *Proc Natl Acad Sci USA* 85:1672–1676.
- Luine V, Bowling D, Hearn M (1990) Spatial memory deficits in aged rats: contributions of monoaminergic systems. *Brain Res* 537:271–278.
- Maccaferri G, Mangoni M, Lazzari A, DiFrancesco D (1993) Properties of the hyperpolarization-activated current in rat hippocampal CA1 pyramidal cells. *J Neurophysiol* 69:2129–2136.
- Madison D, Nicoll RA (1986) Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 pyramidal neurons, in vitro. *J Physiol (Lond)* 372:221–244.
- Madison DV, Lancaster B, Nicoll RA (1987) Voltage-clamp analysis of cholinergic action in the hippocampus. *J Neurosci* 7:733–741.
- Malenka RC, Nicoll RA (1986) Dopamine decreases the calcium-activated afterhyperpolarization in hippocampal CA1 pyramidal cells. *Brain Res* 379:210–215.
- Martini A, Battaini F, Govoni S, Volpe P (1994) Inositol 1, 4, 5-trisphosphate receptor and ryanodine receptor in the aging brain of Wistar rats. *Neurobiol Aging* 15:203–206.
- Miguez JM, Aldegunde M, Paz-Valinas L, Recio J, Sanchez-Barcelo E (1999) Selective changes in the contents of noradrenaline, dopamine and serotonin in rat brain areas during aging. *J Neural Trans* 106:1089–1098.
- Moyer Jr JR, Disterhoft JF (1994) Nimodipine decreases calcium action potentials in rabbit hippocampal CA1 neurons in an age- and concentration-dependent manner. *Hippocampus* 4:1–18.
- Moyer Jr JR, Thompson LT, Black JP, Disterhoft JF (1992) Nimodipine increases excitability of rabbit CA1 pyramidal neurons in an age- and concentration-dependent manner. *J Neurophysiol* 68:2100–2109.
- Moyer Jr JR, Thompson LT, Disterhoft JF (1996) Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. *J Neurosci* 16:5536–5546.
- Moyer Jr JR, Power JM, Thompson LT, Disterhoft JF (2000) Increased excitability of aged rabbit CA1 neurons after trace eyeblink conditioning. *J Neurosci* 20:5476–5482.
- Müller W, Connor JA (1991) Cholinergic input uncouples Ca^{2+} changes from K^{+} conductance activation and amplifies intradendritic Ca^{2+} changes in hippocampal neurons. *Neuron* 6:901–905.
- Müller W, Petrozzino JJ, Griffith LC, Danho W, Connor JA (1992) Specific involvement of $\text{Ca}(2+)$ -calmodulin kinase II in cholinergic modulation of neuronal responsiveness. *J Neurophysiol* 68:2264–2269.
- Neher E (1992) Correction for liquid junction potentials in patch clamp experiments. *Methods Enzymol* 207:123–131.
- Nohmi M, Shinnick-Gallagher P, Gean PW, Gallagher JP, Cooper CW (1986) Calcitonin and calcitonin gene-related peptide enhance calcium-dependent potentials. *Brain Res* 367:346–350.
- Oh MM, Power JM, Thompson LT, Moriarty P, Disterhoft JF (1999) Metrifonate increases neuronal excitability in CA1 pyramidal neurons from both young and aging rabbit hippocampus. *J Neurosci* 19:1814–1823.
- Oh MM, Power JM, Thompson LT, Disterhoft JF (2000) Apamin increases the excitability of CA1 hippocampal pyramidal neurons. *Neurosci Res Commun* 27:135–142.
- Oh MM, Gamelli AE, Wu WW, Sametsky E, Disterhoft JF (2001) Morris watermaze learning enhances neuronal excitability of CA1 hippocampal pyramidal neurons in rats. *Soc Neurosci Abstr* 27:921.1.
- Pascale A, Nogue X, Marighetto A, Micheau J, Battaini F, Govoni S, Jaffard R (1998) Cytosolic hippocampal PKC and aging: correlation with discrimination performance. *NeuroReport* 9:725–729.
- Pedarzani P, Storm JF (1993) PKA mediates the effects of monoamine transmitters on the K^{+} current underlying the slow spike frequency adaptation in hippocampal neurons. *Neuron* 11:1023–1035.
- Pedarzani P, Storm JF (1995) Dopamine modulates the slow $\text{Ca}(2+)$ -activated K^{+} current IAHP via cyclic AMP-dependent protein kinase in hippocampal neurons. *J Neurophysiol* 74:2749–2753.
- Pedarzani P, Storm JF (1996) Evidence that $\text{Ca}/\text{calmodulin}$ -dependent protein kinase mediates the modulation of the Ca^{2+} -dependent K^{+} current, IAHP, by acetylcholine, but not by glutamate, in hippocampal neurons. *Pflügers Arch* 431:723–728.
- Pedarzani P, Krause M, Haug T, Storm JF, Stuhmer W (1998) Modulation of the Ca^{2+} -activated K^{+} current sIAHP by a phosphatase-kinase balance under basal conditions in rat CA1 pyramidal neurons. *J Neurophysiol* 79:3252–3256.
- Pitler TA, Landfield PW (1990) Aging-related prolongation of Ca^{2+} spike duration in rat hippocampal slice. *Brain Res* 508:1–6.
- Power JM, Oh MM, Disterhoft JF (2001) Metrifonate decreases sIAHP in CA1 pyramidal neurons in vitro. *J Neurophysiol* 85:319–322.
- Richter-Levin G, Segal M (1996) Serotonin, aging and cognitive functions of the hippocampus. *Rev Neurosci* 7:103–113.
- Saar D, Grossman Y, Barkai E (1998) Reduced afterhyperpolarization in rat piriform cortex pyramidal neurons is associated with increased learning capability during conditioning. *Eur J Neurosci* 10:1518–1523.
- Sah P (1996) $\text{Ca}(2+)$ -activated K^{+} currents in neurones: types, physiological roles and modulation. *Trends Neurosci* 19:150–154.
- Sah P, Bekkers JM (1996) Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: implications for the integration of long-term potentiation. *J Neurosci* 16:4537–454.
- Sah P, McLachlan EM (1991) $\text{Ca}(2+)$ -activated K^{+} currents underlying the afterhyperpolarization in guinea pig vagal neurons: a role for $\text{Ca}(2+)$ -activated Ca^{2+} release. *Neuron* 7:257–264.
- Sametsky EA, Wu WW, Oh MM, Moskal JR, Disterhoft JF (2001) Dorsal-ventral gradient of excitability in hippocampal pyramidal neurons. *Soc Neurosci Abstr* 27:382.7.
- Segovia G, Porras A, Del Arco A, Mora F (2001) Glutamatergic neurotransmission in aging: a critical perspective. *Mech Ageing Dev* 122:1–29.
- Shah M, Haylett DG (2000) $\text{Ca}(2+)$ channels involved in the generation of the slow afterhyperpolarization in cultured rat hippocampal pyramidal neurons. *J Neurophysiol* 83:2554–2561.
- Soderling TR, Derkach VA (2000) Postsynaptic protein phosphorylation and LTP. *Trends Neurosci* 23:75–80.
- Smith ML, Booze RM (1995) Cholinergic and GABAergic neurons in the nucleus basalis region of young and aged rats. *Neuroscience* 67:679–688.
- Stemmelin J, Lazarus C, Cassel S, Kelche C, Cassel JC (2000) Immunohistochemical and neurochemical correlates of learning deficits in aged rats. *Neuroscience* 96:275–289.
- Stocker M, Krause M, Pedarzani P (1999) An apamin-sensitive Ca^{2+} -activated K^{+} current in hippocampal pyramidal neurons. *Proc Natl Acad Sci USA* 96:4662–4667.
- Storm JF (1990) Potassium currents in hippocampal pyramidal cells. *Prog Brain Res* 83:161–187.
- Stuart GJ, Dodt HU, Sakmann B (1993) Patch-clamp recordings from the soma and dendrites of neurons in brain slices using infrared video microscopy. *Pflügers Arch* 423:511–518.
- Tanabe M, Gahwiler BH, Gerber U (1998) L-Type Ca^{2+} channels mediate the slow Ca^{2+} -dependent afterhyperpolarization current in rat CA3 pyramidal cells in vitro. *J Neurophysiol* 80:2268–2273.
- Thibault O, Landfield PW (1996) Increase in single L-type calcium channels in hippocampal neurons during aging. *Science* 272:1017–1020.
- Thibault O, Porter NM, Chen KC, Blalock EM, Kaminker PG, Clodfelter GV, Brewer LD, Landfield PW (1998) Calcium dysregulation in neuronal aging and Alzheimer's disease: history and new directions. *Cell Calcium* 24:417–433.
- Thompson LT, Moyer Jr JR, Disterhoft JF (1996a) Trace eyeblink conditioning in rabbits demonstrates heterogeneity of learning ability both between and within age groups. *Neurobiol Aging* 17:619–629.

- Thompson LT, Moyer Jr JR, Disterhoft JF (1996b) Transient changes in excitability of rabbit CA3 neurons with a time course appropriate to support memory consolidation. *J Neurophysiol* 76:1836–1849.
- Torres GE, Chaput Y, Andrade R (1995) Cyclic AMP and protein kinase A mediate 5-hydroxytryptamine type 4 receptor regulation of calcium-activated potassium current in adult hippocampal neurons. *Mol Pharmacol* 47:191–197.
- Torres GE, Arfken CL, Andrade R (1996) 5-Hydroxytryptamine₄ receptors reduce afterhyperpolarization in hippocampus by inhibiting calcium-induced calcium release. *Mol Pharmacol* 50:1316–1322.
- Verkhatsky A, Toescu EC (1998) Calcium and neuronal aging. *Trends Neurosci* 21:2–7.
- Weiss C, Preston AR, Oh MM, Schwarz RD, Welty D, Disterhoft JF (2000) The M1 muscarinic agonist CI-1017 facilitates trace eyeblink conditioning in aging rabbits and increases the excitability of CA1 pyramidal neurons. *J Neurosci* 20:783–790.
- Zhang L, Weiner JL, Valiante TA, Velumian AA, Watson PL, Jahromi SS, Schertzer S, Pennefather P, Carlen PL (1994) Whole-cell recording of the Ca(2+)-dependent slow afterhyperpolarization in hippocampal neurones: effects of internally applied anions. *Pflügers Arch* 426:247–253.
- Zhang L, Pennefather P, Velumian A, Tymianski M, Charlton M, Carlen PL (1995) Potentiation of a slow Ca(2+)-dependent K⁺ current by intracellular Ca²⁺ chelators in hippocampal CA1 neurons of rat brain slices. *J Neurophysiol* 74:2225–2241.