



# Age-related increase of $sI_{AHP}$ in prefrontal pyramidal cells of monkeys: relationship to cognition

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## Abstract

Reduced excitability, due to an increase in the slow afterhyperpolarization (and its underlying current  $sI_{AHP}$ ), occurs in CA1 pyramidal cells in aged cognitively-impaired, but not cognitively-unimpaired, rodents. We sought to determine whether similar age-related changes in the  $sI_{AHP}$  occur in pyramidal cells in the rhesus monkey dorsolateral prefrontal cortex (dlPFC). Whole-cell patch-clamp recordings were obtained from layer 3 and layer 5 pyramidal cells in dlPFC slices prepared from young ( $9.6 \pm 0.7$  years old) and aged ( $22.3 \pm 0.7$  years old) behaviorally characterized subjects. The amplitude of the  $sI_{AHP}$  was significantly greater in layer 3 (but not layer 5) cells from aged-impaired compared with both aged-unimpaired and young monkeys, which did not differ. Aged layer 3, but not layer 5, cells exhibited significantly increased action potential firing rates, but there was no relationship between  $sI_{AHP}$  and firing rate. Thus, in monkey dlPFC layer 3 cells, an increase in  $sI_{AHP}$  is associated with age-related cognitive decline; however, this increase is not associated with a reduction in excitability.

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**Keywords:** Slice; Patch-clamp; Voltage-clamp; Potassium channels; Excitability

## 1. Introduction

Working memory, which is largely mediated by the dorsolateral prefrontal cortex (dlPFC), declines significantly during normal aging in a large proportion of rhesus monkeys (Bartus et al., 1979; Lai et al., 1995; Moore et al., 2003, 2006; Rapp, 1990; Steere and Arnsten, 1997). It is increasingly evident that there is not a single causative factor; rather, a multitude of changes in neuronal structure and function occur with normal aging, which may together underlie cognitive decline (Dickstein et al., 2007). Relatively little is known about age effects on the functional electrophysiological properties of monkey neurons (Chang et al., 2005; Luebke et al., 2004; 2010); by contrast, the effects of age on rodent hippocampal pyramidal cells have been extensively studied with *in vitro* slice recordings (for review: Barnes, 2003; Burke and Barnes, 2010; Thibault et al., 2007). These studies have shown that there is an age-

related decrease in action potential firing rates of CA1 pyramidal cells, with a concomitant increase in the amplitude of the calcium-dependent slow afterhyperpolarization (sAHP) responsible for spike frequency adaptation (Disterhoft et al., 1996; Landfield and Pitler, 1984; Power et al., 2002; for review: Faber and Sah, 2003; Thibault et al., 1998). Other studies have demonstrated that the age-related increase in magnitude of the sAHP is inversely related to performance on tasks mediated largely by the hippocampus, with aged-impaired subjects having significantly higher sAHP amplitudes than both aged-unimpaired and young subjects, which do not differ (Matthews et al., 2009; Moyer et al., 2000; Tombaugh et al., 2005).

These *in vitro* slice studies have led to a widely held view that age-related decline in hippocampal function can be attributed to reduced excitability and reduced synaptic plasticity of CA1 pyramidal neurons, secondary to increased calcium influx and amplitude of calcium-dependent sAHPs (for review: Faber and Sah, 2003; Foster, 2007; Thibault et al., 2007). While some *in vivo* single unit studies have reported an age-related decrease in CA1 pyramidal cell

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firing rates (Sava and Markus, 2008; Shen et al., 1997), some others have reported no change in firing rates of CA1 pyramidal cells (Barnes et al., 1983; Oler and Markus, 2000; Tanila et al., 1997) or of CA3 pyramidal cells (Oler and Markus, 2000; Tanila et al., 1997). Further, Wilson et al. (2005) reported no change in firing rates of CA1 pyramidal cells, but an increase in firing rates of CA3 pyramidal cells in the aged rat. Age-related increases in firing rates have also been reported for layer 3 pyramidal cells in *in vitro* slices of aged monkey dIPFC (Chang et al., 2005), and for visual cortical pyramidal cells in the aged monkey *in vivo* (Leventhal et al., 2003; Schmolesky et al., 2000; Zhang et al., 2008). In each case where increased excitability with age has been seen, it has been associated with decline in sensory or cognitive function (Chang et al., 2005; Leventhal et al., 2003; Schmolesky et al., 2000; Wilson et al., 2005; Zhang et al., 2008). While certainly functionally relevant, the cellular basis for age-related increases in neuronal excitability is unknown, but one plausible mechanism is a reduction in the amplitude of the current underlying the sAHP (the  $sI_{AHP}$ ). This study was undertaken to determine whether there are age-related changes in the amplitude of the  $sI_{AHP}$  in monkey dIPFC pyramidal cells, and whether any such changes are related to alterations in excitability of these cells and/or to cognitive decline in the same monkeys.

## 2. Methods

### 2.1. Experimental subjects

Rhesus monkeys obtained from the Yerkes National Primate Research Center were used in this study, which was part of an ongoing program of studies of normal aging. Monkeys were maintained at both the Yerkes National Primate Research Center and at the Boston University Laboratory Animal Science Center (LASC) in strict accordance with animal care guidelines as outlined in the NIH Guide for the Care and Use of Laboratory Animals and the USA Public Health Service Policy on Humane Care and Use of Laboratory Animals. All procedures were approved by the Institutional Animal Care and Use Committees of both the Yerkes National Primate Research Center and the Boston University LASC. Both institutions are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

### 2.2. Assessment of cognitive function

Each monkey completed 6–9 months of testing on a variety of tasks designed to evaluate overall cognitive abilities. This testing consisted of the following: Delayed Non-Match to Sample (DNMS) basic (learning), DNMS performance at 2 and 10 minute delays, and the Delayed Recognition Span Task (DRST), with spatial and object modalities. Detailed descriptions of assessment and implementation of these tasks can be found in Herndon et al.

(1997). Significant impairment on individual behavioral tasks was defined as follows: greater than 210 errors for the DNMS basic task, less than 78% correct for the DNMS 2 and 10 minute delay tasks, and a span of less than 2.5 for the DRST spatial and object tasks (Herndon et al., 1997). The Cognitive Impairment Index (CII) is a composite score, calculated as an average of the standardized scores on the DNMS basic (10-second delay), DNMS 2-minute delay and the DRST spatial tasks using the guidance of a principal components analysis (Herndon et al., 1997). In the present study, monkeys were considered cognitively impaired if they received a CII *z*-score of more than 2.5 standard deviations greater than the mean for a large cohort of healthy, young monkeys.

### 2.3. Preparation of slices

Following completion of behavioral testing and structural magnetic resonance imaging (MRI) brain scans, monkeys were sacrificed. Monkeys were first tranquilized with ketamine, 10 mg/mL, then deeply anesthetized with sodium pentobarbital (to effect, 15 mg/kg I.V.). A thoracotomy was performed, and monkeys were killed by exsanguination while being perfused through the ascending aorta with ice-cold Krebs buffer (concentrations, in mM: 6.4  $\text{Na}_2\text{HPO}_4$ , 1.4  $\text{Na}_2\text{PO}_4$ , 137 NaCl, 2.7 KCl, 5 Glucose, 0.3  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ ; pH = 7.4, chemicals from Sigma, St. Louis, MO). Prior to perfusion, a craniotomy was performed, and, immediately following perfusion, the dura was opened and a 10 mm<sup>3</sup> block of the dIPFC (area 46) removed. The tissue was quickly mounted and cut into 400  $\mu\text{m}$  thick coronal slices with a vibrating microtome in ice-cold oxygenated Ringer's solution (concentrations, in mM: 26  $\text{NaHCO}_3$ , 124 NaCl, 2 KCl, 3  $\text{KH}_2\text{PO}_4$ , 10 Glucose, 1.3  $\text{MgCl}_2$ ; pH = 7.4, chemicals from Sigma). Immediately after cutting, slices were placed in oxygenated, room temperature Ringer's solution, where they were allowed to equilibrate for 1 hour. Following this equilibration period, a slice was placed in a submersion-type recording chamber (Harvard Apparatus, Holliston, MA), held down by a nylon mesh, and continuously superfused with oxygenated, room temperature Ringer's solution (at a rate of 2–2.5 ml/min). Chambers were located on the stages of Nikon E600 infrared-differential interference contrast (IR–DIC) microscopes (MicroVideo Instruments, Avon, MA).

### 2.4. Whole-cell patch-clamp recordings

Pyramidal cells from either layer 3 or layer 5 of area 46 were visually identified under IR–DIC optics, and standard, tight-seal, whole-cell patch-clamp recordings were performed. Pipettes were pulled from borosilicate glass on a horizontal Flaming and Brown micropipette puller (Model P–87, Sutter Instruments, Novato, CA). The internal solution used in recording pipettes was as follows (concentrations, in mM): 100 potassium aspartate, 15 KCl, 3  $\text{MgCl}_2$ , 5 EGTA, 10 Na-HEPES, 0.3 NaGTP, and 2 MgATP (pH = 7.4,

chemicals from Fluka, NY), and electrodes had resistances of 3–6 M $\Omega$  in the external, Ringer's solution. Experiments were performed with either List EPC-9 or EPC-10 patch-clamp amplifiers using either "Pulse" or "PatchMaster" acquisition software (HEKA Elektronik, Lambrecht, Germany). Access resistance was monitored throughout, and recordings were low-pass filtered at 10 kHz. To be included in the analyses, cells were required to exhibit repetitive action potential firing on depolarization, have a resting membrane potential negative to  $-55$  mV, an action potential overshoot and stable access resistance.

### 2.5. Determination of intrinsic membrane properties and action potential firing rates

To determine passive membrane properties, including resting membrane potential ( $V_r$ ) and input resistance ( $R_n$ ), a series of 200-ms current pulses (14 steps,  $-160$  to  $+100$  pA) were applied from a membrane potential of  $-70$  mV.  $V_r$  was measured as the potential present with 0 current input and  $R_n$  was determined as the slope of the best fit line through the plotted  $V$ - $I$  data. To examine repetitive action potential firing properties, 2000 ms depolarizing current pulses (30, 180 and 280 pA) were applied, also from a membrane potential of  $-70$  mV. Data were analyzed using "Pulse-Fit" or "FitMaster" analysis software from HEKA Elektronik.

### 2.6. Characterization of the slow afterhyperpolarization current $sI_{AHP}$

The  $sI_{AHP}$  was evoked with a series of 200 ms depolarizing prepulse steps in voltage clamp (eight steps,  $-50$  to  $+20$  mV) from a holding potential of  $-55$  mV (Fig. 1A). The amplitude of the  $sI_{AHP}$  was measured as the peak outward current  $\sim 20$  ms after cessation of the step, during the return to holding voltage (Fig. 1A). The calcium-dependence of the current was demonstrated by its increasing amplitude following increasing amplitude (Fig. 1A) and duration (Fig. 1B) depolarizing prepulse voltage steps used to evoke calcium influx through high voltage-activated calcium channels. The voltage-independence of the current was demonstrated by a lack of change in the amplitude of the tail currents at the offset of increasing holding potential steps (Fig. 1C, arrow). Finally, the current was identified pharmacologically as being primarily comprised of the  $sI_{AHP}$  as it was largely blocked by the noradrenergic  $\beta$ -receptor agonist isoproterenol (Fig. 1D), but not significantly reduced by apamin (not shown).

### 2.7. Statistical analyses

Both behavioral and electrophysiological data were analyzed for statistical significance using a two-tailed Student's  $t$ -test. To investigate relationships, linear (Pearson's Product-Moment correlations) and quadratic regression analyses were performed. For statistical analyses, significance was defined as  $p < 0.05$ . Monkeys within the aged

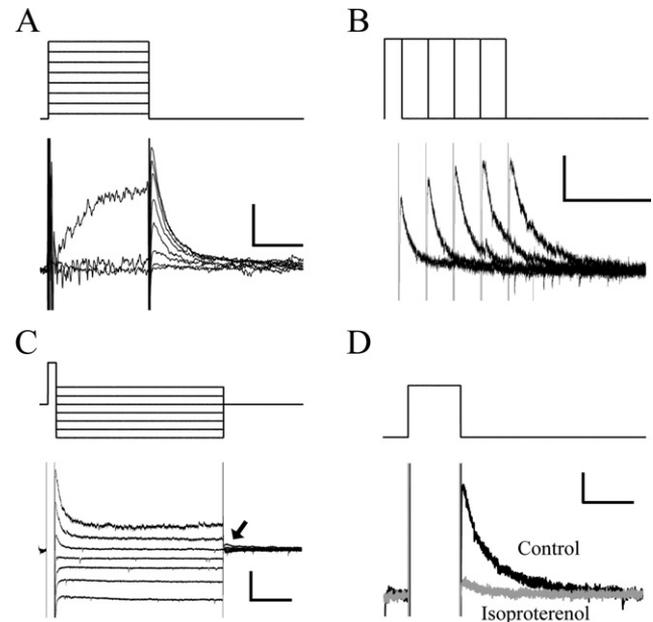


Fig. 1. Characterization of the  $sI_{AHP}$  in representative layer 3 pyramidal cells. (A) Response to increasing amplitude depolarizing 200-ms voltage prepulses. (B) Response of a representative layer 3 pyramidal cell to increasing duration depolarizing prepulses. (C) Voltage-independence of the  $sI_{AHP}$  tail current demonstrated by a consistent step to  $-5$  mV for 100 ms, followed by a voltage step to  $-95$  to  $-35$  mV. (D) Near complete block of the  $sI_{AHP}$  by bath application of isoproterenol,  $10 \mu\text{M}$ . Scale bars: A =  $35$  mV,  $100$  pA/100 ms; B =  $30$  mV,  $20$  pA/1 s; C =  $25$  mV,  $200$  pA/500 ms; D =  $30$  mV,  $100$  pA/200 ms.

cohort were grouped into aged-impaired (AI) and aged-unimpaired (AU) groups based on their CII  $z$ -score. In addition, for each behavioral task, aged monkeys were grouped into AI or AU groups based on their performance on the specific task (with the exception of the DNMS 10-minute delay on which all aged animals were impaired). All data are reported as  $\pm$  the standard error of the mean.

## 3. Results

### 3.1. Most but not all aged monkeys were cognitively impaired

A total of 12 young (6.0–12.0 years old) and 16 aged (17.6–27.0 years old) rhesus monkeys were used in the present study. Table 1 provides information on the monkeys used for layer 3 cell recordings and Table 2 provides the same information for monkeys used for layer 5 cell recordings. In four of the young and five of the aged monkeys, both layer 3 and layer 5 cells were examined and thus there is overlap in the monkeys listed in Tables 1 and 2. Each monkey successfully completed testing on the DNMS-basic,  $-2$  and  $-10$  minute delay tasks and the DRST-spatial and -object tasks (Tables 1 and 2). As a group, the aged monkeys used for layer 3 recordings were significantly cog-

Table 1  
Layer 3 pyramidal cells – experimental subjects

Am#	Sex	Age	CII	Trials	Errors	DNMS 2' delay	DNMS 10' delay	DRST spatial	DRST object
AM204	M	6	-0.03	446	85	0.87	0.76	3.23	4.80
AM230	M	7.5	0.27	220	66	0.91	0.90	2.22	4.04
AM198	F	7.8	0.41	439	104	0.78	0.80	3.71	4.32
AM255	F	9.5	0.12	100	29	0.73	0.68	3.42	5.66
AM199	F	10.6	1.61	732	168	0.79	0.78	3.01	4.42
AM214	F	10.7	2.30	759	168	0.72	0.76	2.73	3.73
AM254	F	11	0.97	329	99	0.84	0.70	2.40	2.66
AM197	F	11.3	-0.02	361	46	0.91	0.82	2.31	2.91
Mean		9.3	0.70	423.3	95.6	0.82	0.78	2.88	4.07
SEM		0.74	0.32	86.6	19.4	0.03	0.03	0.21	0.37
AM257	F	17.6	1.66	420	98	0.74	0.58	2.36	2.66
AM253	F	18	0.55	329	99	0.83	0.74	3.03	2.93
AM256	F	20	2.20	883	202	0.75	0.64	3.09	3.01
AM200	F	21	4.95	1283	414	0.78	0.74	2.38	3.43
AM236	M	22.9	0.58	360	112	0.87	0.66	2.84	2.62
AM242	M	23	6.01	1600	431	0.58	0.55	2.97	3.19
AM234	F	23.5	3.48	1253	271	0.73	0.50	2.62	3.07
AM235	F	24	4.00	1140	375	0.87	0.72	2.26	2.59
AM243	M	24.4	11.9	3626	962	0.70	0.56	1.99	1.98
AM189	M	24.5	2.90	728	217	0.79	0.60	2.06	2.49
AM220	F	25.7	11.5	2880	948	0.74	0.68	2.03	2.46
AM181	F	27	9.63	2253	792	0.71	0.58	2.45	2.53
Mean		22.6	5.0	1396	410	0.76	0.63	2.51	2.75
SEM		0.88	1.22	314	96.3	0.02	0.02	0.12	0.12
<i>p</i> <		0.0001	0.005	0.009	0.007	0.05	0.0003	0.05	0.0002

CII: *z*-score; DNMS basic: total number of trials or errors; DNMS delay: % correct; DRST: average total span.

nitively impaired, as demonstrated by the mean CII *z*-score of  $5.0 \pm 1.2$  compared with  $0.7 \pm 0.3$  in the young group ( $p < 0.005$ ). In the young layer 3 cohort, the CII *z*-score ranged from  $-0.03$  to  $2.3$ , with none considered impaired overall, while in the aged group the range was  $0.55$  to  $11.9$ , with 8 of the 12 monkeys classified as

significantly impaired and four as unimpaired (Table 1). Performance on every task was significantly worse in the aged group of monkeys compared with young (Table 1). Monkeys used for layer 5 recordings demonstrated a similar pattern of age-related cognitive impairment (Table 2).

Table 2  
Layer 5 pyramidal cells – experimental subjects

Am#	Sex	Age	CII	Trials	Errors	DNMS 2' delay	DNMS 10' delay	DRST spatial	DRST object
AM204	M	6	-0.03	446	85	0.87	0.76	3.23	4.80
AM205	M	6.2	0.08	231	54	0.80	0.88	3.24	6.32
AM198	F	7.8	0.41	439	104	0.78	0.80	3.71	4.32
AM255	F	9.5	0.12	100	29	0.73	0.68	3.42	5.66
AM202	F	10.3	0.45	360	99	0.86	0.64	2.90	4.15
AM214	F	10.7	2.30	759	168	0.72	0.76	2.73	3.73
AM194	F	11.9	2.26	764	156	0.74	0.66	2.43	2.99
AM195	F	12	-0.03	255	56	0.83	0.68	3.07	5.11
Mean		9.3	0.70	419.3	93.9	0.79	0.73	3.09	4.64
SEM		0.9	0.38	90.7	18.5	0.02	0.03	0.15	0.40
AM257	F	17.6	1.66	420	98	0.74	0.58	2.36	2.66
AM190	F	18	1.79	737	149	0.81	0.72	2.33	2.81
AM253	F	18	0.55	329	99	0.83	0.74	3.03	2.93
AM177	F	20.7	6.73	2060	518	0.69	0.42	2.37	2.79
AM208	M	22	1.09	300	74	0.75	0.60	2.66	3.66
AM234	F	23.5	3.48	1253	271	0.73	0.50	2.62	3.07
AM179	F	23.8	6.99	1559	505	0.69	0.67	1.84	1.82
AM189	M	24.5	2.90	728	217	0.79	0.60	2.06	2.49
AM220	F	25.7	11.5	2880	948	0.74	0.68	2.03	2.46
Mean		21.5	4.1	1141	319.9	0.75	0.61	2.37	2.74
SEM		1.1	1.3	313	102.2	0.02	0.04	0.13	0.18
<i>p</i> <		0.0001	0.03	0.05	0.05	0.16	0.02	0.002	0.001

CII: *z*-score; DNMS basic: total number of trials or errors; DNMS delay: % correct; DRST: average total span.

Table 3  
Electrophysiological properties of dIPFC pyramidal cells

	Young		Aged		<i>p</i> <
	Mean	SEM	Mean	SEM	
Layer 3					
V <sub>r</sub>	-67.9	0.58	-67.1	0.49	<i>ns</i>
R <sub>n</sub> (MΩ)	114.5	7.29	118.0	4.36	<i>ns</i>
Firing rate (Hz)					
30-pA step	0.99	0.43	1.42	0.32	<i>ns</i>
180-pA step	10.3	0.95	13.6	0.62	0.004
280-pA step	13.5	0.87	15.9	0.69	0.03
sI <sub>AHP</sub> Amp (pA)					
-10-mV step	45.1	5.07	57.3	5.27	<i>ns</i>
0-mV step	78.3	7.64	95.7	7.84	<i>ns</i>
10-mV step	84.7	9.09	112.4	8.90	0.05
20-mV step	108.2	11.1	139.1	9.97	0.05
Layer 5					
V <sub>r</sub>	-66.3	0.83	-67.1	0.76	<i>ns</i>
R <sub>n</sub> (MΩ)	150.0	7.21	148.8	7.49	<i>ns</i>
Firing rate (Hz)					
30-pA step	1.7	0.53	3.0	0.64	<i>ns</i>
180-pA step	13.4	0.78	16.0	1.14	<i>ns</i>
280-pA step	16.9	0.66	18.9	1.14	<i>ns</i>
sI <sub>AHP</sub> Amp (pA)					
-10-mV step	63.2	8.68	68.1	11.1	<i>ns</i>
0-mV step	89.4	11.9	98.5	15.4	<i>ns</i>
10-mV step	116.4	14.8	126.7	18.7	<i>ns</i>
20-mV step	139.6	16.3	152.7	21.5	<i>ns</i>

### 3.2. Population of cells examined

For layer 3 pyramidal cell analyses, recordings were obtained from a total of 48 cells in slices prepared from 8 young monkeys and 82 cells from 12 aged monkeys, and for layer 5 analyses recordings were obtained from 38 cells from 8 young monkeys and 33 cells from 9 aged monkeys (Tables 1 and 2). Passive membrane properties (resting membrane potential and input resistance), action potential firing rates evoked by depolarizing current steps and slow afterhyperpolarization current (sI<sub>AHP</sub>) properties were assessed for all layer 3 and layer 5 pyramidal cells (Table 3).

### 3.3. Passive membrane properties and action potential firing rates

Neither the resting membrane potential (V<sub>r</sub>) nor the input resistance (R<sub>n</sub>) were changed with age in layer 3 or layer 5 pyramidal cells (Table 3). Pyramidal cells responded to depolarizing current steps with trains of regular spiking action potentials that exhibited little spike frequency adaptation. Layer 3 pyramidal cells from aged monkeys fired action potentials at significantly higher rates than did those from young subjects at the +180 and +280 pA current steps (*p* < 0.004 and 0.03, respectively; Table 3). Cells in layer 5, however, did not display an age-related change in action potential firing rate (Table 3). These data are consistent with those previously published by our group (Chang et al., 2005; Luebke and Chang, 2007).

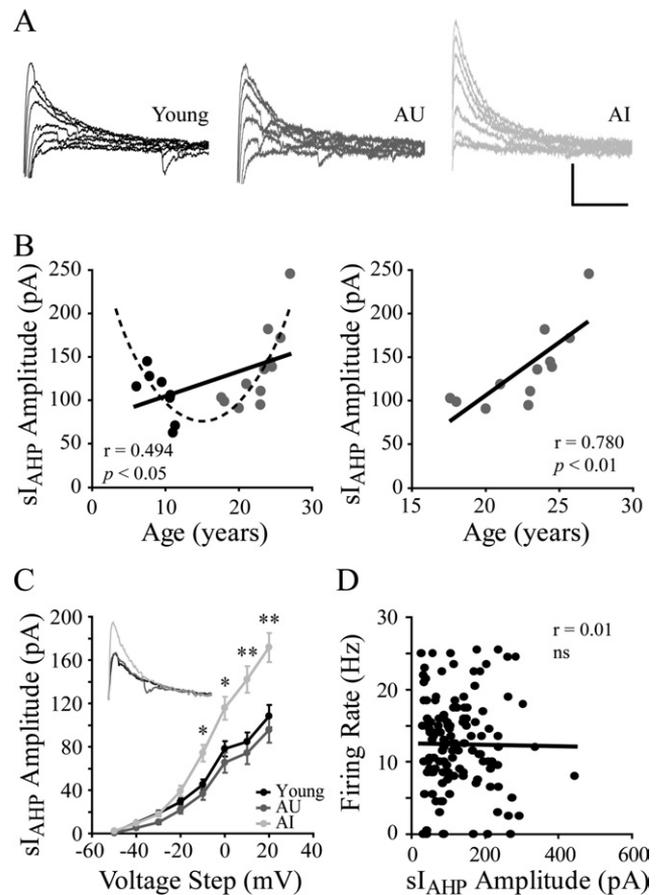


Fig. 2. The amplitude of the sI<sub>AHP</sub> increases significantly with age in layer 3 pyramidal cells. (A) sI<sub>AHP</sub>s evoked by increasing depolarizing voltage steps in representative cells from young (left), aged-unimpaired (middle) and aged-impaired monkeys (right). (B) Significant positive correlation between sI<sub>AHP</sub> amplitude and age in all monkeys (left) and those within the aged cohort only (right). Additionally, a U-shaped quadratic equation was fit to the data obtained from all monkeys, and a significant relationship observed (dashed line). (C) Line graph plotting mean sI<sub>AHP</sub> amplitude as a function of voltage step for cells from young, aged-unimpaired and aged-impaired monkeys. Inset: superimposed traces of sI<sub>AHP</sub>s evoked by +20 mV prepulses in cells from young, aged-unimpaired and aged-impaired monkeys. (D) Mean sI<sub>AHP</sub> amplitude vs firing rate evoked by a +180 pA 2-second current step. Linear regression line demonstrates no significant relationship between sI<sub>AHP</sub> amplitude and firing rate. Scale bars: 50 pA/100 ms. \**p* < 0.01; \*\**p* < 0.001.

### 3.4. sI<sub>AHP</sub> amplitude is significantly increased with age in layer 3 but not layer 5 cells

Increasing depolarizing voltage steps led to increased sI<sub>AHP</sub> amplitude in all cells (Figs. 2A, C, 3A, C). At the voltage steps which activated the highest amplitude current (+10 and +20 mV) a significantly greater amplitude sI<sub>AHP</sub> was seen in layer 3 cells from aged compared with young monkeys (*p* < 0.05; Table 3). This contrasts with layer 5 cells, which demonstrated no significant age-related change in sI<sub>AHP</sub> amplitude (Table 3). When the mean maximal sI<sub>AHP</sub> amplitudes of layer 3 cells from each monkey were plotted vs. age and a linear regression performed, a sig-

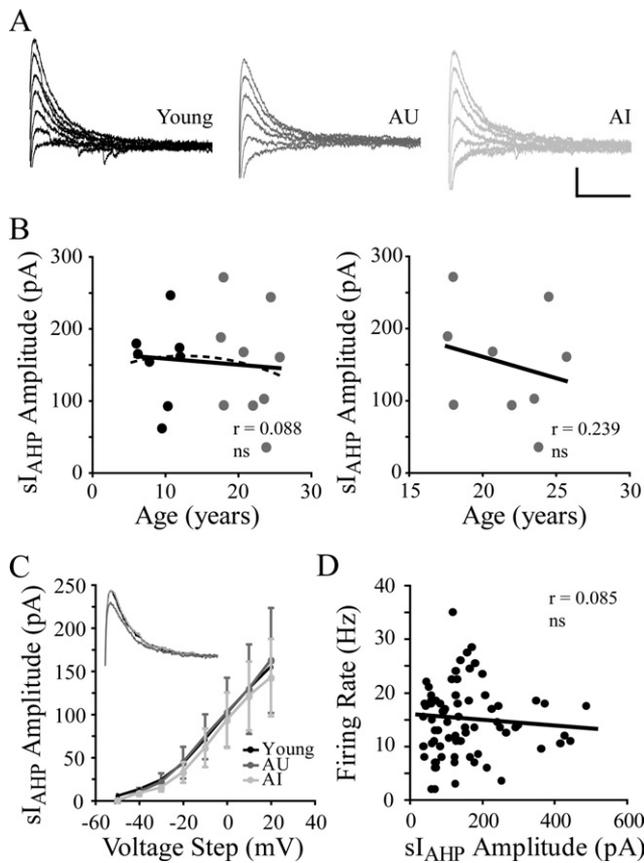


Fig. 3. The amplitude of the sI<sub>AHP</sub> is unaltered with age in layer 5 pyramidal cells. (A) sI<sub>AHP</sub>s evoked by increasing depolarizing voltage steps in representative cells from young (left), aged-unimpaired (middle) and aged-impaired monkeys (right). (B) No significant correlation between sI<sub>AHP</sub> amplitude and age is seen in all monkeys (left) or in the aged cohort only (right). (C) Line graph plotting mean sI<sub>AHP</sub> amplitude as a function of voltage step for cells from young, aged-unimpaired and aged-impaired monkeys. Inset: superimposed traces of sI<sub>AHP</sub>s evoked by +20 mV prepulses in cells from young, aged-unimpaired and aged-impaired monkeys. (D) Mean sI<sub>AHP</sub> amplitude vs. firing rate evoked by a +180 pA 2-second current step. Linear regression line demonstrates no significant relationship between sI<sub>AHP</sub> amplitude and firing rate. Scale bars: 50 pA/100 ms.

nificant linear relationship was seen—with increasing amplitude sI<sub>AHP</sub> associated with increased age ( $p < 0.05$ ,  $r = 0.494$ , d.f. = 18; Fig. 2B, left). Interestingly, these data were also well-fit by a quadratic function ( $p < 0.01$ ,  $r = 0.850$ , d.f. = 18; Fig. 2B, left, dashed line). The mean sI<sub>AHP</sub> amplitude of layer 3 cells from monkeys within only the aged cohort also correlated with age ( $p < 0.01$ ,  $r = 0.780$ , d.f. = 10; Fig. 2B, right), demonstrating that the mean maximal sI<sub>AHP</sub> amplitude increases progressively with advancing age. These relationships were not observed for sI<sub>AHP</sub> amplitude in layer 5 cells vs. age (Fig. 3B).

The maximal amplitude of the sI<sub>AHP</sub> was significantly greater in layer 3 cells from AI compared with AU and young monkeys (AI v. AU:  $p < 0.001$ ; AI v. young:  $p <$

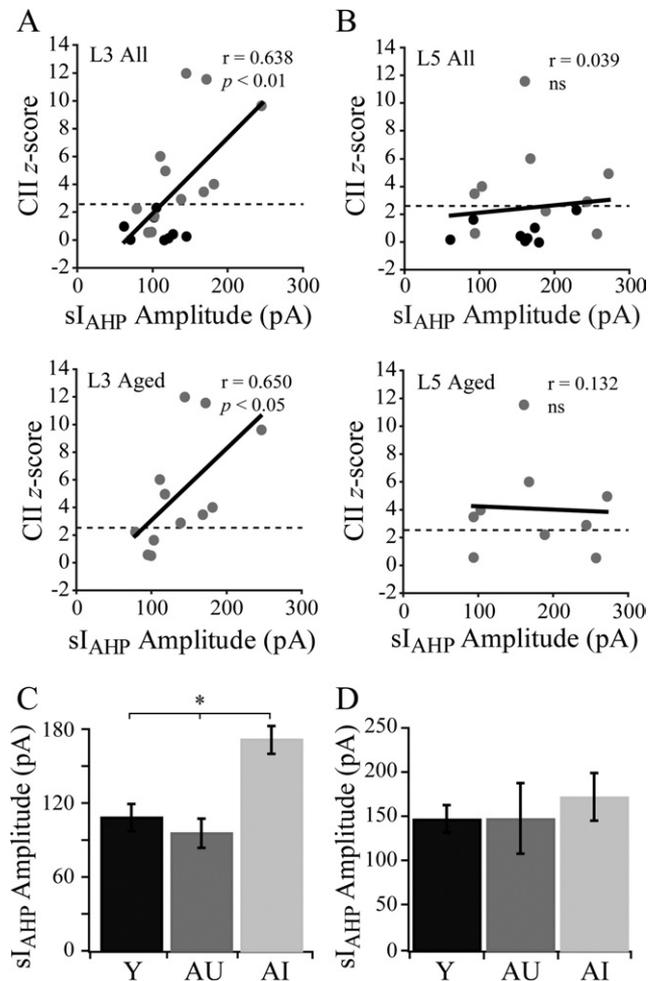


Fig. 4. Relationship of sI<sub>AHP</sub> amplitude to overall CII z-score. (A) Top, all subjects: mean sI<sub>AHP</sub> amplitude in layer 3 pyramidal cells from a given monkey vs the CII z-score for that monkey. Linear regression line demonstrates a significant positive correlation. Bottom, aged subjects only: mean sI<sub>AHP</sub> amplitude for a given monkey vs. the CII z-score for that monkey. Linear regression line demonstrates a significant positive correlation. (B) Top, all subjects: mean sI<sub>AHP</sub> amplitude in layer 5 pyramidal cells for a given monkey vs the CII z-score for that monkey. Linear regression line demonstrates no correlation. Bottom, aged subjects only: mean sI<sub>AHP</sub> amplitude for a given monkey vs. the CII z-score for that monkey in the aged cohort of monkeys only. Linear regression line demonstrates no correlation. (C) Bar graph giving mean sI<sub>AHP</sub> amplitudes in layer 3 cells from young ( $n = 8$ ), aged-unimpaired ( $n = 4$ ) and aged-impaired monkeys ( $n = 8$ ). (D) Bar graph giving mean sI<sub>AHP</sub> amplitudes in layer 5 cells from young ( $n = 8$ ), aged-unimpaired ( $n = 4$ ) and aged-impaired monkeys ( $n = 5$ ). Dashed lines in A and B correspond to a z-score of 2.5, subjects with z-scores above this line are considered significantly impaired. \* $p < 0.001$ .

0.001; AU v. young: not significant; Figs. 2C, 4C). This was not the case for layer 5 pyramidal cells, where there was no significant difference in maximal sI<sub>AHP</sub> amplitude between cells from AI, AU and young monkeys (Figs. 3C, 4D). Interestingly, when sI<sub>AHP</sub> amplitude was plotted versus firing rate evoked by a strong depolarizing current step in the same cells, there was no significant relationship between the two variables

in either layer 3 ( $r = 0.01$ , d.f. = 125; Fig. 2D) or layer 5 cells ( $r = 0.085$ , d.f. = 72; Fig. 3D).

### 3.5. The amplitude of the $sI_{AHP}$ in layer 3 (but not layer 5) cells is significantly related to cognitive performance

The relationship between the amplitude of the  $sI_{AHP}$  and overall cognitive impairment was assessed by plotting the mean maximal amplitude of the current for all layer 3 or all layer 5 cells from a given monkey vs. that monkey's CII z-score (Fig. 4A, B). A linear regression was performed, and a significant relationship was found between mean  $sI_{AHP}$  amplitude in layer 3 cells and CII z-score ( $p < 0.01$ ,  $r = 0.638$ , d.f. = 18; Fig. 4A, top), but not between  $sI_{AHP}$  amplitude in layer 5 cells and CII z-score (Fig. 4B, top). The mean  $sI_{AHP}$  amplitude of layer 3 (but not layer 5) cells from monkeys within the aged cohort only also correlated with increased CII z-score ( $p < 0.05$ ,  $r = 0.650$ , d.f. = 10; Fig. 4A, B, bottom), demonstrating that mean maximal  $sI_{AHP}$  amplitude increases with increasing overall cognitive impairment within the aged group. The mean amplitude of the  $sI_{AHP}$  was significantly greater in layer 3 cells from AI than from AU and young monkeys with mean maximal amplitudes of  $172 \pm 13$  pA vs  $96 \pm 12$  pA and  $108 \pm 11$  pA, respectively ( $p < 0.001$ ; Fig. 4C), but there was no difference in the mean amplitude of the current in layer 5 cells from the three groups (Fig. 4D).

The relationship between the maximal amplitude of the  $sI_{AHP}$  in layer 3 cells and impairment on each behavioral task was assessed by plotting the mean amplitude of the current for a given monkey vs that monkey's performance on the task. No relationship between layer 5  $sI_{AHP}$  amplitude and any behavioral task was found (CII: Fig. 4B; other tasks not shown). Performance on the DNMS basic task was significantly negatively correlated with the mean amplitude of the  $sI_{AHP}$  in layer 3 cells, both in all monkeys ( $p < 0.01$ ,  $r = 0.661$ , d.f. = 18; Fig. 5A, top) and within the aged cohort only ( $p < 0.02$ ,  $r = 0.669$ , d.f. = 10; Fig. 5A, bottom). By contrast, there was no significant linear relationship between the mean amplitude of the  $sI_{AHP}$  in layer 3 cells and performance on the DNMS 2 or 10 minute delay tasks (Fig. 5B, C). The mean amplitude of the  $sI_{AHP}$  in layer 3 cells from aged monkeys that were impaired on each individual task was compared with the amplitude in cells from those monkeys that were unimpaired on the task. The mean amplitude of the  $sI_{AHP}$  was significantly greater in layer 3 cells from aged animals that were impaired on the DNMS basic task compared with those that were from AU or from young monkeys ( $p < 0.001$ ; Fig. 5D). The mean amplitude of the  $sI_{AHP}$  was also significantly greater in layer 3 cells from aged animals that were impaired on the DNMS 2-minute delay task compared with young monkeys ( $p < 0.01$ ; Fig. 5E); however, these cells did not differ from those from aged monkeys that were unimpaired on this task. All aged animals were impaired on the DNMS 10-minute delay task (thus, no

AU data are presented in Fig. 5F) and a significant difference in the amplitude of the  $sI_{AHP}$  between young and aged monkeys was seen ( $p < 0.05$ ).

There was a trend toward a significant relationship between increased mean amplitude of the  $sI_{AHP}$  in layer 3 cells and poorer performance on the DRST spatial task within the aged group ( $p < 0.10$ ,  $r = 0.528$ , d.f. = 10; Fig. 6A, bottom), but not within the overall cohort (Fig. 6A, top). Finally, there was no significant relationship between the mean amplitude of the  $sI_{AHP}$  in layer 3 cells and performance on the DRST object task (Fig. 6B). The mean amplitude of the  $sI_{AHP}$  was significantly greater in layer 3 cells from animals that were impaired on the DRST spatial tasks compared with AU or to young monkeys ( $p < 0.01$ ; Fig. 6C), but only between AI and young on the DRST object task ( $p < 0.02$ ; Fig. 6D).

Given that both CII and  $sI_{AHP}$  significantly increase with age, a partial correlation analysis was performed to determine if cognitive impairment per se is related to the increase in  $sI_{AHP}$ , or if the increase is simply an unrelated consequence of the aging process. Partial correlation analyses allow for the examining of one variable while controlling for variance in another, in this case controlling for age while examining the relationship between  $sI_{AHP}$  and CII. The  $p$ -value approached but did not quite meet statistical significance ( $p = 0.059$ ). It seems likely that this is due to the variance in this relatively small sample. Hence it is suggestive of an overall nonzero association (i.e. a relationship of  $sI_{AHP}$  to CII, when age is controlled for) in the general population of monkeys.

## 4. Discussion

### 4.1. Age-related increase in the amplitude of the $sI_{AHP}$ in layer 3 pyramidal cells

One of the most consistent findings in the aging literature is of a significant increase in the amplitude of the sAHP and/or its underlying current – the  $sI_{AHP}$  – in aged rodent hippocampal CA1 pyramidal cells (for review: Faber and Sah, 2003; Foster, 2007; Thibault et al., 2007). Here, we demonstrate that layer 3 pyramidal cells in *in vitro* slices of the primate dlPFC exhibit a similar age-related increase in  $sI_{AHP}$  amplitude. Thus, in contrast to neuronal excitability, which has been reported to decrease, increase or remain the same with age depending on brain area and species, the  $sI_{AHP}$  amplitude has consistently been demonstrated to increase with age across brain areas and species. However, it should be noted that this phenomenon is not ubiquitous, given that the amplitude of the  $sI_{AHP}$  does not change with age in layer 5 pyramidal cells from the same monkeys. The observation of an age-related increase in  $sI_{AHP}$  amplitude in layer 3 but not layer 5 cells is of interest given the different roles of cells in the two cortical laminae; corticocortical layer 3 cells are thought to play a key role in cognitive

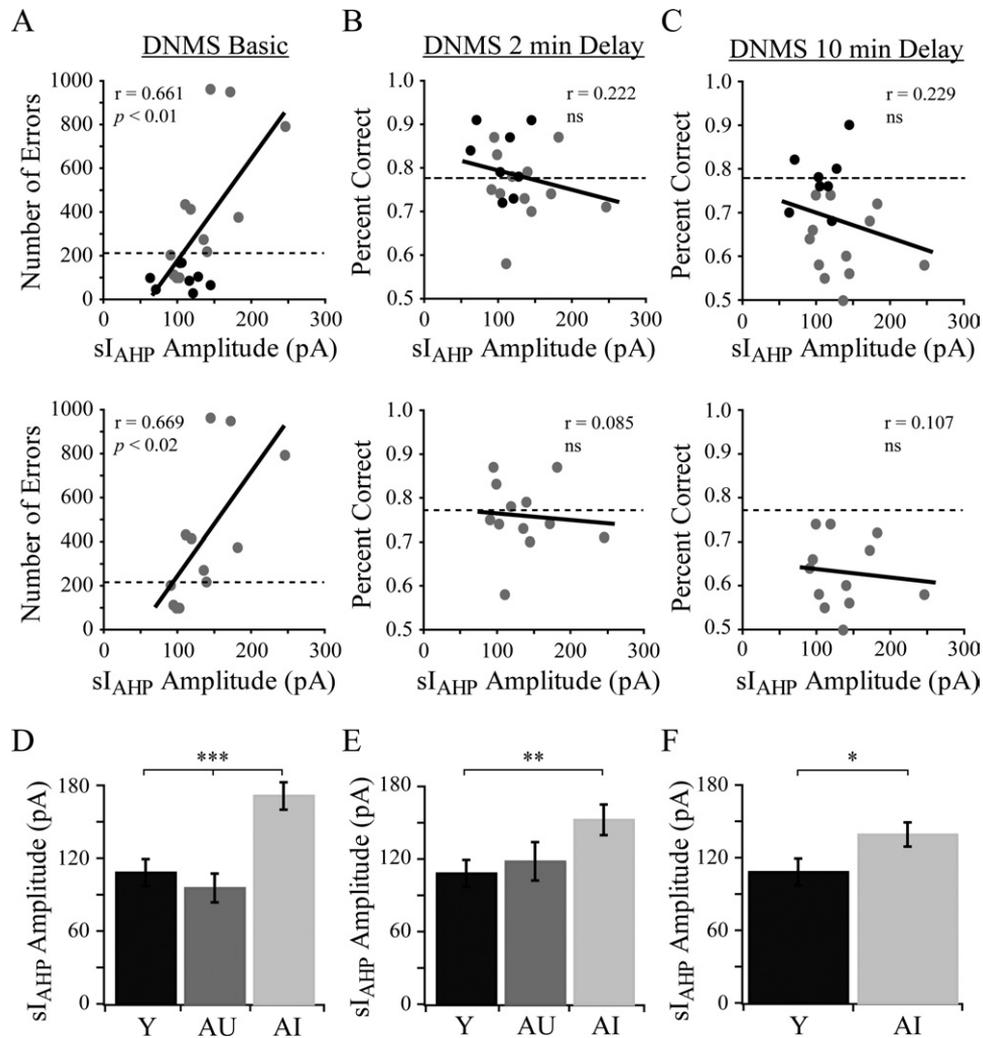


Fig. 5. Relationship of  $sI_{AHP}$  amplitude to performance on Delayed Non-Match to Sample behavioral tasks. Mean  $sI_{AHP}$  amplitude in layer 3 pyramidal cells from a given monkey vs the monkey's performance on (A) DNMS basic, (B) DNMS 2-minute delay, and (C) DNMS 10-minute delay. Top: data from all monkeys, bottom: data from aged monkeys only. Bar graphs depicting mean  $sI_{AHP}$  amplitude in layer 3 pyramidal cells from young monkeys and aged monkeys that were either unimpaired or impaired on (D) DNMS basic, (E) DNMS 2-minute delay, or (F) DNMS 10-minute delay. Note that because all aged monkeys were impaired on the DNMS 10-minute delay task there is no aged-unimpaired group. Dashed lines in A, B and C correspond to the level above which (DNMS errors) or below which (DNMS 2 and 10 minute delay) a subject is considered significantly impaired. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

function and to be especially vulnerable during aging, while primarily subcortically projecting layer 5 cells play a lesser role in cognitive function and may be relatively spared during aging (Morrison and Hof, 2002; Morrison and Hof, 2007).

The present study did not directly address the mechanism of the age-related increase in  $sI_{AHP}$ . Perhaps the most straightforward explanation would be an age-related increase in the somatic membrane surface area, and thus in the total number of channels underlying the  $sI_{AHP}$ . However, layer 3 pyramidal cell soma size does not change with age (Peters, personal communication), and input resistance was the same in young and aged cells in the present study, arguing against this idea. Studies in the rodent hippocampus

have clearly demonstrated that age-related changes in calcium homeostasis can directly impact the amplitude of this calcium-dependent current (Kumar and Foster, 2002; Norris et al., 1998; for review: Faber and Sah, 2003; Foster, 2007; Thibault et al., 2007). There is increased calcium influx in CA1 pyramidal cells with aging (Moyer et al., 1992; Power et al., 2002) due to an increase in density of high voltage-activated L-type calcium channels (Thibault and Landfield, 1996). There is also evidence for changes in ryanodine sensitive calcium-dependent calcium release with age, although consensus on this is lacking (Clodfelter et al., 2002; Gant et al., 2006; Kumar and Foster, 2004; for review: Foster, 2007; Thibault et al., 2007). In addition to being influenced by intracellular calcium lev-

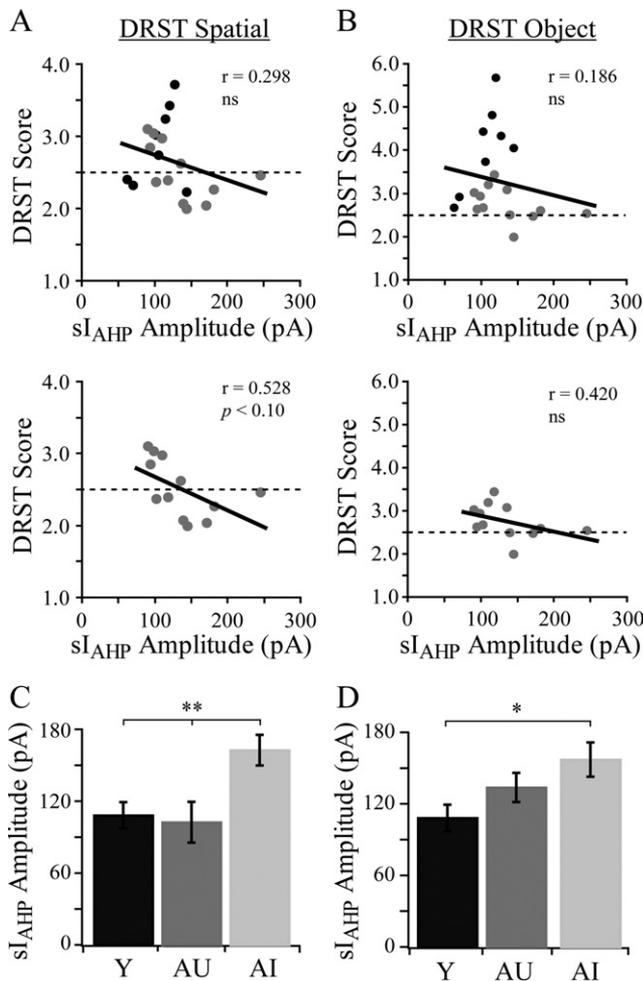


Fig. 6. Relationship of sI<sub>AHP</sub> amplitude to performance on Delayed Recognition Span Tasks. Mean sI<sub>AHP</sub> amplitude in layer 3 pyramidal cells from a given monkey vs. the monkey's performance on (A) DRST spatial and (B) DRST object behavioral tasks. Top: data from all monkeys, bottom: data from aged monkeys only. Bar graphs depicting mean sI<sub>AHP</sub> amplitude in layer 3 pyramidal cells from young monkeys and aged monkeys that were either unimpaired or impaired on (C) DRST spatial or (D) DRST object tasks. Dashed lines in A and B correspond to the level below which a subject is considered significantly impaired. \* $p < 0.02$ ; \*\* $p < 0.01$ .

els, the sI<sub>AHP</sub> is modulated by norepinephrine and acetylcholine, which reduce the sI<sub>AHP</sub> open channel probability and thus its amplitude (Sah and Isaacson, 1995). Moore et al. (2005) reported significant reductions in noradrenergic markers, and Vannucchi and Goldman-Rakic (1991) reported a decreased affinity of M1 receptors for the muscarinic agonist carbachol in the dlPFC of aged monkeys. An age-related decline in these sI<sub>AHP</sub> inhibitors could result in a disinhibition of the current and an increase in its amplitude. Further studies are required to determine which, if any, of these mechanisms underlie the significant increase in amplitude of the sI<sub>AHP</sub> in the aged primate dlPFC.

#### 4.2. Functional electrophysiological implications: firing rates of layer 3 pyramidal cells are not associated with sI<sub>AHP</sub> amplitude

Changes in neuronal excitability and synaptic plasticity are frequently proposed to underlie cognitive decline during normal aging (for review: Barnes, 2003; Burke and Barnes, 2006, 2010; Faber and Sah, 2003; Foster, 2007; Thibault et al., 2007). Both excitability and synaptic plasticity can be strongly modulated by the sI<sub>AHP</sub>, which acts by increasing spike frequency adaptation in the first case, and by shunting dendritic synaptic currents in the second (for review: Faber and Sah, 2003). Many previous *in vitro* slice studies have suggested that reduced excitability of CA1 pyramidal cells plays an important role in age-related impairment in hippocampal function in rodents. It is worth noting, however, that while some *in vivo* recording studies report a reduction in the firing rates of CA1 pyramidal cells with age (Sava and Markus, 2008; Shen et al., 1997), several others report that the firing rates of these cells are unaltered with age (Barnes et al., 1983; Oler and Markus, 2000; Tanila et al., 1997; Wilson et al., 2005). Furthermore, several studies have shown increased excitability of aged neurons – with recordings of pyramidal cells in *in vitro* slices of the monkey dlPFC (Chang et al., 2005), and with *in vivo* single unit recordings of CA3 pyramidal cells in rats (Wilson et al., 2005) and visual cortical pyramidal cells in the rhesus monkey (Leventhal et al., 2003; Schmolesky et al., 2000; Zhang et al., 2008). Importantly, in these studies, increased firing rates were associated with age-related cognitive impairment in monkeys and rats (Chang et al., 2005; Wilson et al., 2005; respectively), and with degradation of visual stimulus selectivity in monkeys (Leventhal et al., 2003; Schmolesky et al., 2000; Zhang et al., 2008).

Age-related reduction in rodent CA1 pyramidal cell excitability is due to increased action potential firing frequency adaptation likely related to an age-related increase in sAHP amplitude (Disterhoft et al., 1996; Landfield and Pitler, 1984; Power et al., 2002; for review: Faber and Sah, 2003; Thibault et al., 1998). A key question addressed by the present study was whether the age-related increase in excitability of layer 3 dlPFC pyramidal cells in the monkey could be due to a decrease in the amplitude of the sI<sub>AHP</sub>. The lack of a relationship between the amplitude of this current and action potential firing rates of layer 3 and layer 5 pyramidal cells argues strongly against this idea and suggests a dissociation between sI<sub>AHP</sub> amplitude and action potential firing rates in these cells, which exhibit little spike frequency adaptation compared with hippocampal pyramidal cells. Our findings indicate that the age-related sI<sub>AHP</sub> amplitude increase is not related to excitability changes in layer 3 pyramidal cells of the monkey dlPFC. Thus, the mechanism(s) underlying increased excitability of neurons with normal aging remains an open question.

#### 4.3. Relationship of $sI_{AHP}$ amplitude to performance on cognitive tasks

It is well known that significant cognitive impairment occurs with normal aging in many species, and that within any cohort of aged animals or humans there are successful-agers that are not impaired and unsuccessful-agers that are significantly impaired (Bartus et al., 1979; Herndon et al., 1997; Lai et al., 1995; Moore et al., 2003, 2005, 2006; Rapp, 1990; Steere and Arnsten, 1997). Thus, the demonstration that most, but not all, aged monkeys were significantly cognitively impaired in the present study is consistent with many previous studies. The findings in the aged rhesus monkey are consistent with findings in aged rodent CA1 pyramidal cells in that  $sI_{AHP}$  amplitude in layer 3 cells of aged monkey dlPFC correlated positively with increased CII z-score. Further, cells from monkeys that were impaired on the DNMS basic, DNMS 2-minute delay and DRST (both spatial and object) tasks exhibited significantly increased mean  $sI_{AHP}$  amplitude compared with those from young monkeys. The amplitude of the  $sI_{AHP}$  in cells from aged monkeys that were unimpaired on these tasks, by contrast, did not differ from young. The key question is—what discriminates between successful and unsuccessful aging individuals at a single neuron or network level? The inverse relationship between the amplitude of the sAHP in hippocampal pyramidal neurons recorded in *in vitro* slices and performance on both hippocampal-dependent and -independent conditioning tasks by subjects from which slices were prepared is well-established (Disterhoft et al., 1986, 1996; Matthews et al., 2009; Thompson et al., 1996; Tombaugh et al., 2005). This consistent finding has led to the hypothesis that reduction in sAHP amplitude may be a general mechanism by which neuronal excitability is increased during learning, and that an increase in sAHP amplitude leads to reduction in excitability and cognitive impairment in aging (e.g. Faber and Sah, 2003). The question arises as to whether similar mechanisms hold true in other brain areas, including the dlPFC of the aged monkey. Given the dissociation between  $sI_{AHP}$  amplitude and firing rate in dlPFC pyramidal cells shown in this study, a change in excitability due to a change in  $sI_{AHP}$  is not a plausible mechanism. The  $sI_{AHP}$  could also impact cognition by shunting incoming depolarizing synaptic potentials, thus raising the threshold for induction of synaptic plasticity (Sah and Bekkers, 1996; Thibault et al., 2001). This mechanism is consistent with the report of reduced synaptic excitation of these cells in aged monkey dlPFC slices (Luebke et al., 2004). Further work is needed to address the important question of precisely how the increase in  $sI_{AHP}$  amplitude in layer 3 pyramidal cells relates to the decline in functions mediated by the dlPFC with normal aging in the primate.

#### Disclosure statement

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