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EFFECTS OF AGING ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF LAYER 5 PYRAMIDAL CELLS IN THE MONKEY PREFRONTAL CORTEX

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Abstract—A significant decline in executive system function mediated by the prefrontal cortex (PFC) often occurs with normal aging. *In vitro* slice studies have shown that layer 2/3 pyramidal cells in the monkey PFC exhibit increased action potential (AP) firing rates which may, in part, contribute to this decline. Given that layer 5 cells also play a role in executive system function, it is important to determine if similar age-related changes occur in these cells. Whole-cell patch-clamp recordings in *in vitro* slices prepared from the PFC of young and aged behaviorally characterized rhesus monkeys were employed to answer this question. Basic membrane and repetitive AP firing properties were unaltered with age. Aged cells exhibited significantly decreased single AP amplitude, duration and fall time and increased slow afterhyperpolarization (sAHP) amplitude, but these changes were not associated with cognitive performance. This study demonstrates that layer 5 pyramidal cells, unlike layer 2/3 pyramidal cells, undergo only modest electrophysiological changes with aging, and that these changes are unlikely to contribute to age-related cognitive decline.

Key words: *in vitro* slice, whole cell patch clamp, prefrontal cortex, normal aging, cognitive performance.

The prefrontal cortex (PFC) mediates executive function domains such as working memory, cognitive flexibility, and set shifting (review: Fuster, 1997; Constantinidis and Procyk, 2004). Layer 2/3 cortico-cortical connections and a closed loop layer 5 cortico-striatal–thalamic–cortical circuit are both involved in the mediation of these functions, which are essential for normal daily living (for review: Alexander et al., 1986; Owen, 1997; Cavada et al., 2000). The process of normal, non-pathological aging is often accompanied by a significant decline in executive function in humans (review: Albert and Moss, 1999) and monkeys (review: Moss et al., 1999). Since changes in the action potential (AP) firing rates of pyramidal cells in the PFC are directly associated with transitions between different epochs of PFC-dependent cognitive tasks (for review: Goldman-Rakic, 1995; Fuster, 1997; Owen, 1997; Cavada et al., 2000), it is reasonable to postulate that perturbations in the electrophysiological properties of these neurons, such as AP firing rates, may contribute to cognitive deficits in senescence. Indeed, age-related alterations in firing rates have been reported. For example, *in vivo* single unit recordings of supragranular cells in the visual cortex (Schmolesky et al., 2000; Leventhal et al., 2003) and *in vitro* whole cell patch clamp recordings of layer 2/3 pyramidal cells in the PFC (Chang et al., 2005) demonstrate that neurons from aged monkeys exhibit significantly increased AP firing rates compared with those from young monkeys. In the aged monkey visual cortex *in vivo*, increased AP firing rates are associated with decreased stimulus selectivity (Schmolesky et al., 2000), and the increased AP firing rates of layer 2/3 pyramidal cells in *in vitro* PFC slices are associated with degree of cognitive impairment in aged monkeys (Chang et al., 2005). Wilson and coworkers (2005) have also demonstrated that the firing rates of CA3 pyramidal cells recorded in freely behaving rats are significantly increased with age, resulting in a failure to rapidly encode new spatial information. Taken together, these studies indicate that principal cells in a number of brain areas exhibit significantly altered AP firing properties with age, which may, at least in part, underlie age-associated cognitive decline.

Very little is known about the effects of age on the basic electrophysiological properties of layer 5 pyramidal cells. Given the importance of AP firing rates in the normal modulation of cognition, and the importance of the cortico-striatal–thalamic–cortical circuit in the modulation of executive functions, the current study was undertaken to determine whether there are significant alterations in the basic electrophysiological properties of neurons in layer 5 from the PFC of aged, cognitively characterized rhesus monkeys.

EXPERIMENTAL PROCEDURES

Experimental subjects

Seven young (6–12 years old) and eight aged (20–29 years old) rhesus monkeys (*Macaca mulatta*) were obtained from the Yerkes National Primate Research Center at Emory University (Atlanta, GA, USA) and then housed 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 U.S. *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (Table 1). Both the Boston University LASC and the Yerkes Center are fully

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Abbreviations: AHP, afterhyperpolarization; AP, action potential; DNMS, delayed non-match to sample; DRST, delayed recognition span task; FA, fast adapting; fAHP, fast afterhyperpolarization; IR-DIC, infrared-differential interference contrast; ISI, interspike interval; KAsp, potassium aspartate; LASC, Laboratory Animal Science Center; mAHP, medium afterhyperpolarization; PFC, prefrontal cortex; RS, regular spiking; sAHP, slow afterhyperpolarization.

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Table 1. Experimental subjects

Monkey	Age (y)	Sex	DNMS basic	DNMS delay	DRST spatial
Young					
AM188	6	F	64	0.83	2.76
AM204	6	M	85	0.87	3.23
AM198	7	F	104	0.78	3.71
AM205	7.3	M	54	0.80	3.24
AM199	10	F	168	0.79	3.01
AM194	11.9	F	156	0.74	2.43
AM195	12	F	56	0.83	3.07
Mean	8.6		98.1	0.81	3.06
SD	2.7		47.1	0.04	0.40
Aged					
AM190	20	F	149	0.81	2.33
AM177	20.7	F	518	0.69	2.37
AM162	22.3	F	163	0.79	2.03
AM165	22.7	M	227	0.78	2.9
AM179	23.8	F	505	0.69	1.84
AM189	24.5	M	217	0.79	2.06
AM181	27	F	792	0.71	2.45
AM180	29	F	242	0.75	1.98
Mean	23.8		351.6*	0.75^{+}	2.25**
SD	3.1		229.1	0.05	0.34

DNMS basic, total number of errors; DNMS delay, % correct; DRST, average total span.

$^{+}$ $P<0.04$.

* $P<0.01$.

** $P<0.001$.

accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and all procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of both institutions. Every effort was made to minimize the number of animals used and their suffering.

Assessment of cognitive function

All animals completed a battery of cognitive tasks that assessed learning and working memory functions. Monkeys were tested on the following tasks: delayed non-match to sample (DNMS) basic (acquisition), DNMS with a delay period of 2 min, and the spatial modality of the delayed recognition span task (DRST). For a detailed description of implementation and assessment of performance on these tasks, see Herndon et al. (1997). Significant impairment on a task was defined as: >200 errors for DNMS basic, <78% correct for DNMS 2 min delay, and a span of <2.5 for DRST (Herndon et al., 1997).

Preparation of slices

Coronal brain slices were obtained from monkeys perfused as part of other on going studies. Monkeys were tranquilized with ketamine (10 mg/ml) and then deeply anesthetized with sodium pentobarbital (to effect 15 mg/kg, i.v.). While under deep anesthesia, monkeys were thoracotomized and craniotomies performed. Ten millimeter thick blocks of the PFC (area 46) containing both the upper and lower banks of the sulcus principalis were obtained by biopsy. Following the biopsy monkeys were killed by exsanguination with a 4% paraformaldehyde solution perfused through the ascending aorta. Coronal 400 μm thick slices were cut on a vibrating microtome, placed in 26 °C oxygenated (95% O_2/5% CO_2) Ringer's solution (concentrations in mM: 26 $NaHCO_3$, 124 NaCl, 2 KCl, 3 KH_2PO_4, 10 glucose, 2.5 $CaCl_2$, 1.3 $MgCl_2$; pH=7.4, chemicals obtained from Sigma, St. Louis, MO, USA). Slices were then allowed to equilibrate for at least 1 h prior to use, and slices remained viable for up to 12 h. The time from the beginning of the perfusion of the monkey to obtaining slices of PFC was approximately 10–15 min. At the time of recording, a single slice was positioned under a nylon mesh in a submersion type slice-recording chamber (Harvard Apparatus, Holliston, MA, USA) and constantly superfused with 26 °C, oxygenated Ringer's solution at a rate of 2–2.5 ml per minute.

Whole cell patch clamp recordings

Layer 5 pyramidal cells in the lower bank of the sulcus principalis were visually identified with a Nikon E600 infrared-differential interference contrast (IR-DIC) microscope (Micro Video Instruments, Avon, MA, USA). Standard, tight-seal whole-cell patch clamp recordings (Luebke and Rosene, 2003; Luebke et al., 2004; Chang et al., 2005) were made with electrodes fabricated on a Flaming and Brown horizontal micropipette puller (Model P-87, Sutter Instruments, Novato, CA, USA) from nonheparinized microhematocrit capillary tubes (World Precision Instruments, Sarasota, FL, USA). Recording pipettes were filled with a potassium aspartate (KAsp) internal solution containing (in mM): 100 KAsp, 15 KCl, 3 $MgCl_2$, 5 EGTA, 10 Na–Hepes, 0.3 NaGTP and 2 MgATP (pH=7.4, chemicals obtained from Fluka, NY, USA). The electrodes had a final resistance of 3–6 Mohm in Ringer's solution. Experiments were performed with List EPC-7 or EPC-9 patch clamp amplifiers and "Pulse" acquisition software from HEKA Electronik (Lambrecht, Germany). Recordings were low-pass filtered at 10 kHz and access resistance was monitored throughout each experiment.

Characterization of intrinsic membrane and AP firing properties

Cells were recorded in the current clamp mode throughout the course of all experiments. Resting membrane potential was determined by measuring the membrane voltage in the absence of current input. PFC layer 5 pyramidal cell types were classified based on membrane responses to a series of 2 s steps (ranging from +30 to +330 pA) applied from a potential of −70 mV. Passive membrane characteristics were assessed as described in detail elsewhere (Chang et al., 2005). Briefly, a series of 200 ms current steps (11 steps, ranging from −120 to +80 pA) was applied to the cell from a potential of −70 mV and input resistance determined by the slope of the best-fit line on the resulting voltage–current (V–I) graph. The membrane time constant (tau) was determined by fitting the membrane potential response to a small hyperpolarizing pulse to a single exponential function. Single AP characteristics, including amplitude, threshold, and kinetics (rise time, duration at half AP amplitude, and fall time) were analyzed. The first AP produced by the 200-ms current-clamp series was used for single AP measurements. The threshold for firing was measured by expanding the time scale of the digitized trace in the PulseFit oscilloscope window to 1 ms per gradation and, using a linear measurement software function, measuring the voltage at the point in the trace when upward deflection begins. Maximal AP amplitude was measured from threshold to the peak of the spike on the voltage axis. Duration at half-maximal amplitude was measured at half-amplitude from threshold to peak, while rise time and decay time were measured as the duration from threshold to peak amplitude, and peak to the beginning of the fast afterhyperpolarization (AHP), respectively. AHP amplitudes were measured from baseline (membrane potential during prepulse) to maximal amplitude. Medium afterhyperpolarization (mAHP) was measured following the first AP spike generated at rheobase from a 200 ms current step. Frequency–current (f–I) plots were generated by