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DIFFERENTIAL EFFECTS OF AGING ON DENDRITIC SPINES IN VISUAL CORTEX AND PREFRONTAL CORTEX OF THE RHESUS MONKEY

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Abstract—Aging decreases the density of spines and the proportion of thin spines in the non-human primate (NHP) dorsolateral prefrontal cortex (dlPFC). In this study, we used confocal imaging of dye-loaded neurons to expand upon previous results regarding the effects of aging on spine density and morphology in the NHP dlPFC and compared these results to the effects of aging on pyramidal neurons in the primary visual cortex (V1). We confirmed that spine density, and particularly the density of thin spines, decreased with age in the dlPFC of rhesus monkeys. Furthermore, the average head diameter of non-stubby spines in the dlPFC was a better predictor than chronological age of the number of trials required to reach criterion on both the delayed response test of visuospatial working memory and the delayed nonmatching-to-sample test of recognition memory. By contrast, total spine density was lower on neurons in V1 than in dlPFC, and neither total spine density, thin spine density, nor spine size in V1 was affected by aging. Our results highlight the importance and selective vulnerability of dlPFC thin spines for optimal prefrontal-mediated cognitive function. Understanding the nature of the selective vulnerability of dlPFC thin spines as compared to the resilience of thin spines in V1 may be a promising area of research in the quest to prevent or ameliorate age-related cognitive decline.

Key words: prefrontal cortex, visual cortex, dendritic spines, macaque, aging.

INTRODUCTION

Aging humans and non-human primates (NHPs) often develop cognitive impairment revealed by dorsolateral prefrontal cortex (dlPFC)-dependent cognitive tasks. Two tasks used to evaluate dlPFC function in NHPs are the delayed nonmatching-to-sample recognition memory task (DNMS) and the delayed response test of visuospatial working memory (DR) (Rapp and Amaral, 1989; Luebke et al., 2010). DR performance critically requires dlPFC integrity (Gross and Weiskrantz, 1962; Divac and Warren, 1971), and changes in dlPFC morphological and electrophysiological characteristics correlate with changes in DNMS performance (Peters et al., 1998; Chang et al., 2005; Shamy et al., 2011). Aging rhesus monkeys are generally impaired in both acquisition and performance across increasing memory delays on DR and DNMS (Rapp and Amaral, 1989; Roberts et al., 1997; Rapp et al., 2003; Nagahara et al., 2010), suggesting that dlPFC function may be degraded in aged animals. This can be contrasted with the relative preservation of function in aging humans and NHPs with respect to visual discrimination tasks not reliant on dlPFC (Bartus et al., 1979; Rapp, 1990; Rapp et al., 2003).

The delineation of the molecular and structural alterations that underlie these deficits is an important focus of research in cognitive aging. Normal aging is not associated with significant neuronal loss in the human (Pakkenberg and Gundersen, 1997) or macaque neocortex (Peters et al., 1994). Instead, age-related cognitive decline is thought to result from more subtle synaptic alterations (Morrison and Hof, 1997).

Most excitatory synapses between cortical neurons occur on dendritic protrusions called spines (Nimchinsky et al., 2002). Pyramidal neurons in the macaque dlPFC lose a significant proportion of their dendritic spines with age (Hao et al., 2007; Dumitriu et al., 2010), and morphologically distinct types of spines are differentially affected (Benavides-Piccione et al., 2013). Among spines with a discernable neck (non-stubby spines), the density of spines with large head diameters (mushroom spines) does not decrease with age in rhesus monkey dlPFC (Hao et al., 2007; Dumitriu et al., 2010). Instead, the age-related decrease in spine density is driven by the loss of long, thin spines (Hao et al., 2007; Dumitriu et al., 2010), thought to be highly plastic (Kasai et al., 2010) and critically important for working memory (Arnsten et al., 2012). We have hypothesized that the ''synaptic strategy'' underlying function of dlPFC requires the extensive ongoing synaptic plasticity and flexibility that thin

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Abbreviations: DAPI, 4,6-diamidino-2-phenylindole; dlPFC, dorsolateral prefrontal cortex; DNMS, nonmatching-to-sample recognition memory task; DR, delayed response test of visuospatial working memory; NHP, non-human primate.

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spines provide (Morrison and Baxter, 2012). We hypothesize further that while synaptic plasticity also occurs in sensory areas such as the primary visual cortex (Trachtenberg et al., 2002; Gilbert and Li, 2012), the balance between stability and plasticity likely differs from dlPFC, which should be reflected in regional differences in both spine populations and vulnerability to age. Thus, we chose V1 for a regional comparison of age-related effects relevant to synaptic stability vs. plasticity. Other groups have also drawn regional comparisons between V1 and dlPFC in the context of synaptic aging (Peters et al., 1998, 2001; Amatrudo et al., 2012). To address this issue, we imaged pyramidal neurons in layer III of Brodmann areas 46 (dlPFC) and 17 (V1) and examined the density and morphology of dendritic spines along segments of apical and basal dendrites. We found that the density of thin spines is reduced with age in dlPFC, but not in V1, and that the density of other spine types does not change with age in either area.

EXPERIMENTAL PROCEDURES

All neurons analyzed were newly loaded for this experiment using tissue that had been stored at 4 °C in a solution of 0.1% sodium azide in phosphate-buffered saline for up to 9 years prior to loading. Tissue slices were stored free-floating in well plates sealed with Parafilm and checked periodically to ensure adequate solution was present. Storage solution was changed whenever there was visible evidence of evaporation in one or more plates. Our replication and extension of previous findings from Dumitriu et al. (2010) using these methods from a subset of the same animals using an entirely different set of neurons is key given the current discussions regarding difficulty in replication of preclinical findings (Landis et al., 2012). In addition, demonstrating the validity of the use of stored tissue opens up opportunities for multiple additional studies of other cortical regions.

Animals

Seven young adult (9–13 years old; mean, 10.9 ± 0.6 years old; one male, six females) and 10 aged (22–33 years old; mean, 27.2 ± 1.2 years old, one male, nine females) rhesus monkeys (*Macaca mulatta*) were used for the analysis of area 46. Data from different sets of neurons from three of the young and two of the aged animals were reported previously in Dumitriu et al. (2010). For the analysis of V1, four young adult (9–11 years old; mean, 10.6 ± 0.5 years old; all female) and four aged (24–27 years old; mean, 26.1 ± 0.5 years old; all female) rhesus monkeys were used, including seven of the same animals used in the analysis of area 46. Behavioral results from several of these animals have been reported previously (Dumitriu et al., 2010). Animals were singly housed in colonies of approximately 40 individuals under the same conditions used in previous studies (Rapp et al., 2003; Hao et al., 2006, 2007), and water and monkey chow were provided in excess of nutritional needs. All experiments were conducted in compliance with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals approved by the Institutional Animal Care and Use Committee at the University of California, Davis and Icahn School of Medicine at Mount Sinai.

Behavioral testing

DR test. Cognitive assessment included a DR test of spatiotemporal working memory, as previously described (Rapp et al., 2003; Shamy et al., 2011). Monkeys sat in a manual Wisconsin General Testing Apparatus and watched through a clear Plexiglass panel while the experimenter baited one of two wells and covered both with identical opaque covers. The left/right position of the rewarded location was varied pseudorandomly and balanced across trials. The Plexiglass panel was raised and the monkey was allowed to uncover one well to find the food reward. The task was first acquired with no delay for 30 trials per day until a criterion of 90% correct choices in 90 consecutive trials was reached. A one-second delay was then imposed, during which an opaque screen was lowered, and monkeys were again required to reach a criterion of 90% correct choices in 90 consecutive trials. Monkeys were then tested on increasing delay intervals of 5, 10, 15, 30, and 60 s for 90 trials each.

DNMS test. Monkeys were tested on a DNMS test of visual recognition memory as previously described (Rapp and Amaral, 1991; Rapp et al., 2003; Hara et al., 2012). Monkeys were placed in the same manual Wisconsin General Testing Apparatus as above and presented with a sample object covering the central well of the test tray, which contained a food reward. After the reward was retrieved, an opaque screen was lowered for the duration of the delay period. When the screen was again raised, the monkey was presented with two objects: the pre-delay sample item and a novel object. The novel object covered the food reward. The intertrial interval was 30 s. The task was acquired using a delay period of 10 s, tested for 20 trials per day until a criterion of 90% correct was met across 100 consecutive trials. After reaching criterion, monkeys were tested on successively longer delay periods. Retention intervals of 15, 30, 60, and 120 s were tested for 100 trials each, 20 trials per day. The final delay period of 10 min was tested for 50 trials, five trials per day.

Quantitative analysis of dendritic spine density and morphology

Perfusion and tissue processing. Monkeys were perfused after behavioral testing was completed. Animals were first deeply anesthetized with ketamine hydrochloride (25 mg/kg) and sodium pentobarbital (20–35 mg/mg), intubated, and mechanically ventilated. Sodium nitrate (0.5%, 1.5 ml) was injected into the left ventricle of the heart, and the descending aorta was clamped. Transcardial perfusion was performed with cold 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at a rate of 250 ml/min for 2 min, followed by