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Morphological and molecular changes in aging rat prelimbic prefrontal cortical synapses

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Abstract

Age-related impairments of executive functions appear to be related to reductions of the number and plasticity of dendritic spine synapses in the prefrontal cortex (PFC). Experimental evidence suggests that synaptic plasticity is mediated by the spine actin cytoskeleton, and a major pathway regulating actin-based plasticity is controlled by phosphorylated LIM kinase (pLIMK). We asked whether aging resulted in altered synaptic density, morphology, and pLIMK expression in the rat prelimbic region of the PFC. Using unbiased electron microscopy, we found an approximate 50% decrease in the density of small synapses with aging, while the density of large synapses remained unchanged. Postembedding immunogold revealed that pLIMK localized predominantly to the postsynaptic density where it was increased in aging synapses by approximately 50%. Furthermore, the age-related increase in pLIMK occurred selectively within the largest subset of prelimbic PFC synapses. Because pLIMK is known to inhibit actin filament plasticity, these data support the hypothesis that age-related increases in pLIMK may explain the stability of large synapses at the expense of their plasticity.

Keywords: Aging; Synapse; Prefrontal cortex; Actin; Electron microscopy; Postembedding immunogold; Perforated synapse; Postsynaptic density; Structural plasticity

1. Introduction

Neuronal networks in the prefrontal cortex (PFC) that mediate executive functions are known to be vulnerable to aging. While strong evidence suggests that age-related PFC dysfunction is not a result of neuronal death or outright degeneration (Peters et al., 1998), the neurobiological mechanisms that underlie vulnerability of PFC neurons to aging are only partly understood. Recent studies have demonstrated that aging may impair the functional integrity of PFC networks by altering the number, structure, and plasticity of axospinous synapses on pyramidal neurons. For example, aging results in a selective loss of small, thin-type dendritic spines on primate dorsolateral PFC pyramidal neurons, which correlates with impairments in learning (Dumitriu et al., 2010). Similar patterns of spine loss have been reported in aging rat prelimbic (PL) PFC, and it appears the remaining axospinous synapses on aged rat PL PFC pyramidal neurons have a reduced capacity for structural plasticity (Bloss et al., 2011). However, the mechanisms underlying synaptic vulnerability and resilience, as well as those mediating changes in the capacity for structural synaptic plasticity during aging remain unknown.

Experimental evidence suggests that dendritic spine plasticity is controlled by the actin spine cytoskeleton (Honkura et al., 2008; Matus, 2000; Okamoto et al., 2004). Actin dynamics are tightly regulated by upstream signaling pathways and actin-binding proteins (dos Remedios et al.,

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2003). A major pathway regulating actin-based plasticity within synapses is controlled by phosphorylated LIM kinase (pLIMK), which increases the stability of filamentous actin by inhibiting the filament severing actions of ADF/cofilin proteins via phosphorylation (Arber et al., 1998; Yang et al., 1998). In support of a prominent role in regulating structural and functional plasticity, LIMK1 knockout mice have altered synaptic morphology and plasticity (Meng et al., 2002). Furthermore, manipulation of the LIMK target cofilin modulates both synaptic potentiation (Fukazawa et al., 2003) and synaptic glutamate receptor plasticity (Gu et al., 2010; Yuen et al., 2010). Last, it should be noted that mutation of the LIMK gene in humans has been linked to Williams Syndrome (Tassabehji et al., 1996), a mental retardation consisting of deficits across several cognitive domains including those thought to be mediated by PFC circuitry (Meyer-Lindenberg et al., 2006).

In the present study, we took an anatomic approach using unbiased, quantitative postembedding immunoelectron microscopy to investigate potential age-related changes of pLIMK within the context of PL PFC synapse density and synapse size. We report here that the density of small synapses in the PL PFC was decreased by approximately 50% in aged rats while the density of large synapses remained unchanged between the ages. Immunogold labeling of pLIMK was abundant in PL PFC synapses and localized predominantly to the postsynaptic density (PSD), where it selectively increased in the largest subset of synapses during aging. These data provide the first evidence to connect age-related changes of PFC synapses to alterations of actin-related signaling pathways, and suggest that pathways controlling the phosphorylation state of pLIMK may explain the stability of aging synapses at the expense of their plasticity.

2. Methods

2.1. Animals and tissue preparation

Male Sprague–Dawley rats were purchased form Harlan (Harlan, Indianapolis, IN, USA) at 3 and 20 months of age (i.e., young and aged; $n = 5$ per age), and were housed under standard laboratory conditions for 6 weeks as part of previously reported experiments (Bloss et al., 2010, 2011). At approximately 21 months of age in this rat strain there is an approximate 90% survival rate of male rats, and survival rates decline precipitously several months later (Altun et al., 2007). All experiments were conducted in compliance with the National Institutes of Health guidelines for the Care and Use of Experimental Animals and approved by the Institutional Animal Care and Use Committees at Mount Sinai School of Medicine and Rockefeller University.

Animals were perfused at 4.5 and 21.5 months of age as described (Bloss et al., 2010). Coronal sections (250 μm) encompassing the PL cortex of the medial PFC (between 3.7 and 2.7 mm from bregma; Fig. 1A) according to Paxinos and Watson (2005) were prepared for immunoelectron microscopy as reported previously (Janssen et al., 2005). Be-

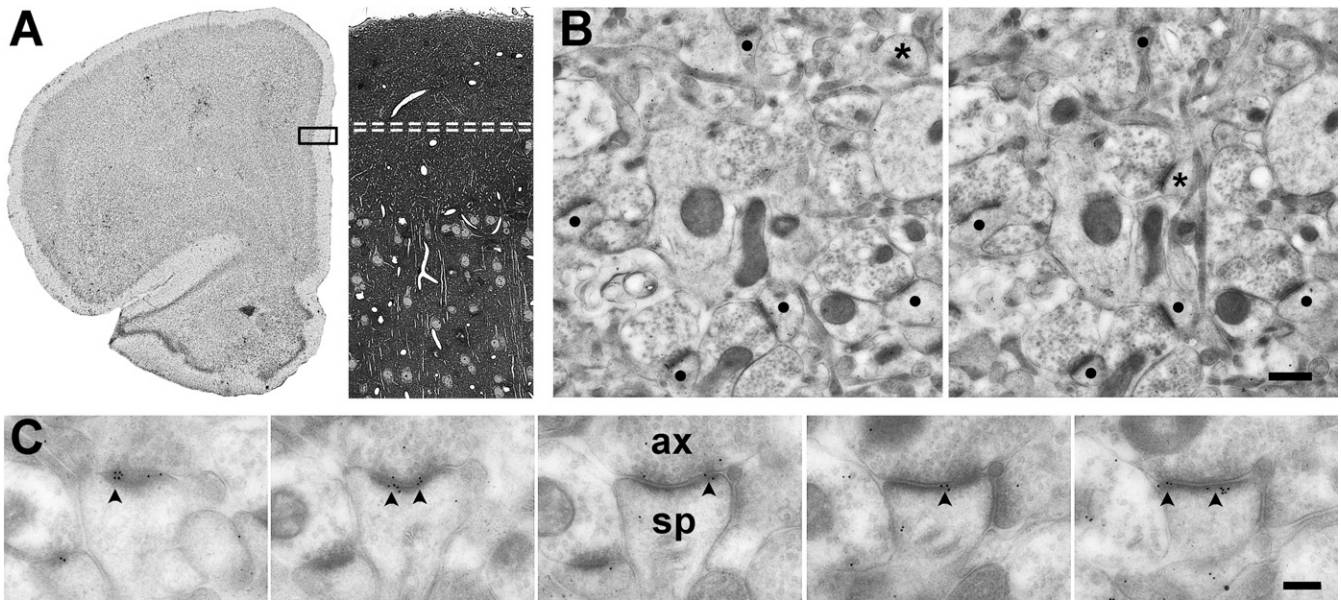

Fig. 1. Electron microscopy (EM) sampling, disector analysis, and phosphorylated LIM kinase (pLIMK) immunogold. (A) EM blocks encompassed rat prelimbic (PL) prefrontal cortex (PFC) (black box), and serial sections were collected from layer I (white dashed lines, approximately 100 μm from layer II). (B) Example of axospinous synapses identified by the disector analysis (note: black dots denote synapses contained in both planes; asterisks denote synapses contained only in 1 plane). (C) Representative serial images of a PL layer I synapse with pLIMK immunogold labeling. Note the prominent localization of pLIMK to the postsynaptic density (PSD) (arrowheads). "ax" denotes an axonal bouton, "sp" denotes a dendritic spine. Scale bars: (B) 500 nm; (C) 200 nm.