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SHORT-TERM ENVIRONMENTAL ENRICHMENT ENHANCES SYNAPTIC PLASTICITY IN HIPPOCAMPAL SLICES FROM AGED RATS

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Abstract—Age-associated changes in cognition are mirrored by impairments in cellular models of memory and learning, such as long-term potentiation (LTP) and long-term depression (LTD). In young rodents, environmental enrichment (EE) can enhance memory, alter LTP and LTD, as well as reverse cognitive deficits induced by aging. Whether short-term EE can benefit cognition and synaptic plasticity in aged rodents is unclear. Here, we tested if short-term EE could overcome age-associated impairments in induction of LTP and LTD. LTP and LTD could not be induced in the CA1 region of hippocampal slices in control, aged rats using standard stimuli that are highly effective in young rats. However, exposure of aged littermates to EE for three weeks enabled successful induction of LTP and LTD. EE-facilitated LTP was dependent upon N-methyl-D-aspartate receptors (NMDARs). These alterations in synaptic plasticity occurred with elevated levels of phosphorylated cAMP response element-binding protein and vascular endothelial growth factor, but in the absence of changes in several other synaptic and cellular markers. Importantly, our study suggests that even a relatively short period of EE is sufficient to alter synaptic plasticity and molecular markers linked to cognitive function in aged animals.

Key words: environmental enrichment, long-term potentiation, long-term depression, N-methyl-D-aspartate receptor, aging.

INTRODUCTION

Memory deficits are widely recognized to occur in dementing diseases such as Alzheimer's disease. However, it is also known that memory deficits occur in the elderly in the absence of disease. Among individuals aged 71 and older, 22.2% have cognitive impairment without dementia (Plassman et al., 2008), while 13.9% have dementia (Plassman et al., 2007). Alarmingly, cognitive decline is already evident in humans by 45–49 years of age (Singh-Manoux et al., 2012), and progressively increases with age (Plassman et al., 2007, 2008; Borson, 2010; Daffner, 2010; Salthouse, 2010; Artegiani and Calegari, 2012; Singh-Manoux et al., 2012). Cognitive decline is also seen in aging primates (Aizawa et al., 2009) and rodents (Rapp and Gallagher, 1996; Chen et al., 2004; von Bohlen und Halbach et al., 2006; Villeda et al., 2011; Freret et al., 2012; Seib et al., 2013).

Memory deficit commonly experienced by the elderly in the absence of dementia is termed age-associated memory impairment (AAMI) or cognitive decline (AACD) (Levy, 1994). Even mild AAMI/AACD produces sufficient cognitive deficits to provide a substantial burden for those affected and their families (Langa and Levine, 2014). AAMI/AACD is an increasing burden on society as individuals over the age of 60 are the fastest growing age group (Daffner, 2010). Globally, the proportion of individuals over the age of 60 will increase from 10% in 2000 to 22% in 2050 and to 32% in 2100 (Lutz et al., 2008). This shift will increase the median age of the world's population from 26.6 years in 2000 to 37.3 years in 2050 and to 45.6 years in 2100. Given the prevalence of cognitive decline and its burden both to the individual and to society, it is critical that we seek to understand how to prevent or reverse age-related declines in cognition, and thus improve quality of life for the elderly.

The hippocampus is particularly vulnerable to aging (Mora et al., 2007). Indeed, age-related changes in synaptic plasticity have been reported for all hippocampal subregions (Foster, 2012). Two major forms of synaptic plasticity closely correlated with and critically involved in

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Abbreviations: ACSF, artificial cerebrospinal fluid; Creb, cAMP response element-binding protein; D,L-APV, 2-amino-5-phosphonovarelic acid; EE, environmental enrichment; fEPSP, field excitatory postsynaptic potential; H&E, hematoxylin and eosin; HFS, high-frequency stimulation; LFS, low-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; Map2, microtubule-associated protein 2; NMDAR, N-methyl-D-aspartate receptor; NPY, neuropeptide Y; PS, population spike; VDCC, voltage-dependent calcium channel; Vegf, vascular endothelial growth factor; VGlut1, vesicular glutamate transporter 1.

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learning and memory processes, are long-term potentiation (LTP) and long-term depression (LTD) (Sale et al., 2014). Aging impairs the induction, magnitude, and maintenance of LTP that can be evoked by weak stimulation (Landfield et al., 1978; Barnes, 1979; Burke and Barnes, 2006; Freret et al., 2012; Haxaire et al., 2012). In contrast to its effect on LTP, aging has increased susceptibility to induction of LTD (Norris et al., 1998; Foster and Kumar, 2007; Kumar and Foster, 2007).

Environmental enrichment (EE) is an experimental setting in which animals are put in surroundings designed to enhance social interactions, sensory and motor stimulation, and learning and memory. Cages are typically large and contain tunnels, platforms, toys, and running wheels (Mora et al., 2007). Impressively, EE prevents or ameliorates deficits in both hippocampal-dependent and -independent forms of learning and memory in multiple species of aged mammals (Escorihuela et al., 1995; Soffie et al., 1999; Duffy et al., 2001; Leggio et al., 2005; Bennett et al., 2006; Sale et al., 2014). Moreover, in young rodents, LTP in the hippocampal CA1 region are strengthened after EE (Duffy et al., 2001; Artola et al., 2006; Huang et al., 2006). While one notable study found that 10–12 weeks of EE reversed age-related changes in LTD and LTP (Kumar et al., 2012), the effect of EE on synaptic plasticity in the context of aging requires further examination.

Despite these encouraging findings, critical details regarding the implementation of EE and its downstream molecular mechanisms remain uncertain. In attempts to understand how EE improves cognitive function, existing studies have identified a plethora of cellular and molecular changes associated with the beneficial effects of EE. For example, rodents housed in enriched conditions show increased brain weight and size, dendritic branching, synapse formation, number of astrocytes, and neurogenesis in several areas of the brain such as the hippocampus, cerebral cortex, and basal ganglia (Comery et al., 1996; Soffie et al., 1999; van Praag et al., 2000; Kolb et al., 2003; Leggio et al., 2005; Mora et al., 2007; Diniz et al., 2010). However, we do not know the age of exposure to EE or duration of EE required to evoke these changes since existing studies have largely used chronic protocols with widely differing ages of EE onset and durations of EE exposure. Given these knowledge gaps, we have investigated the effects of short-term EE instated in old age on synaptic plasticity and a panel of hippocampal molecular markers. We hypothesized that short-term EE instated in old age would ameliorate aging-related decays in LTP and LTD in hippocampal slices.

EXPERIMENTAL PROCEDURES

Animals

All animal procedures were approved by the Washington University Animal Studies Committee, Division of Comparative Medicine, Washington University School of Medicine, St. Louis, MO, and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). All efforts were made to minimize the number of animals used and their suffering. Four to six rats per group were used in each experiment.

Environmental enrichment (EE)

Male Sprague–Dawley rats were obtained from Harlan (Indianapolis, IN) at 21 months of age. Upon arrival, rats were single-housed for 7–10 days before being randomly divided into two groups: standard housing (control) or environmental enrichment (EE) training. All rats remained single-housed; however, rats in the EE group were transferred in groups of three to a large (L 15″ × W 30″ × D 30″), two-story cage, for three hours a day (from 5:00 pm to 8:00 pm), seven days a week, for three weeks. The cage contained toys and various objects whose locations were rearranged every day. Both groups had food available *ad libitum*.

Hippocampal slice preparation

Rats were moved to the dissection room the night prior to sacrifice. Dissections were performed between 10:30 am and 12:30 pm to account for possible effects of transportation and the light/dark cycle, respectively. Rats were deeply anesthetized with halothane or isoflurane in a chemical fume hood and decapitated via guillotine. The brain was quickly removed and hippocampi were rapidly dissected and placed in gassed (95% O_2–5% CO_2), 30 °C standard artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 22 $NaHCO_3$, and 10 D-glucose. Transverse slices (500 μm thick) of the dorsal hippocampus were cut with a custom-made rotary slicer (Tokuda et al., 2010). Slices were then maintained in an incubation chamber for 1 h at 30 °C in ACSF. Individual slices were transferred to a submersion recording chamber where they were constantly perfused with standard solution (2 ml/min) at 30 °C.

Electrophysiology

Extracellular recordings in gassed ACSF were obtained from the dendritic layer of the CA1 region with the use of 5- to 10 MΩ glass electrodes filled with 2 M NaCl. A bipolar electrode was placed in stratum radiatum to stimulate the Schaffer collateral/commissural pathway. The stimulus intensity was set to evoke 40–50% of the maximal amplitude of field excitatory postsynaptic potentials (fEPSPs). Different types of afferent stimulation were performed at the same relative intensity in individual slices. LTP was induced by delivering 100-Hz × 1-sec high-frequency stimulation (HFS) to the Schaffer collateral pathway. LTD was induced by delivering low-frequency stimulation (LFS) consisting of 900 pulses at 1 Hz to the Schaffer collateral pathway. These stimulus parameters were chosen because they produce robust and reliable LTP and LTD, respectively, in hippocampal slices from young (30 day old) rats (Izumi and Zorumski, 1995).

fEPSPs were monitored and analyzed with an IBM computer-based data acquisition system. The