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Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice

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Abstract

The present study tested whether environmental enrichment can reduce age-related spatial reference memory deficits and alter synaptic protein levels in aged female mice. Female C57BL/6 mice, (4 or 27–28 months), were tested in spatial and cued Morris water maze tasks. Prior to (14 days) and during testing, a subset of aged females was exposed to rodent toys and running wheels for 3 h per day. The remaining aged females were group housed but were not exposed to enriching objects. At the conclusion of testing, levels of the presynaptic protein synaptophysin were measured in hippocampus and frontoparietal cortex. Enrichment improved spatial memory acquisition; relative to young controls, aged enriched females performed similarly, whereas aged control females were impaired. Enrichment also accelerated the development of a spatial bias in spatial probe trials. In contrast, the cued task was not significantly affected by enrichment. Hippocampal and cortical synaptophysin levels were increased in aged enriched females relative to young and aged controls. These data suggest that environmental enrichment can be a potent cognitive enhancer for aged females and suggests a potential neurobiological mechanism of this effect.

Keywords: Environmental enrichment; Spatial reference memory; Synaptophysin; Hippocampus; Frontoparietal cortex; ELISA

1. Introduction

Memory decline is a common feature of aging in humans [19,30,67]. It is thought that this decline is the result of deterioration in brain regions critical for memory, such as the neocortex, hippocampus, and basal forebrain. The discovery that non-human animals, from rodents to primates, experience similar memory and neural dysfunction to that of humans has spurred decades of animal research designed to develop treatments to alleviate age-related memory loss (see [2,3] for reviews). Most often, these treatments involve administration of drugs that alter the function of various neurotransmitter or hormone systems. In contrast, few investigations have utilized behavioral manipulations to reduce or prevent age-related memory loss. Emerging evidence suggests that one such intervention, environmental enrichment, may be a particularly effective cognitive enhancer. For example, intellectual abilities in humans are maintained longer in people who continue to engage in intellectually stimulating activities throughout their lives [64,69]. Although animal models afford the ability to understand the neurobiological basis of this benefit, relatively few studies have examined the effects of this treatment in aging animals.

The impact of environmental stimulation on cognitive and neural development has been known for several decades. Seminal work in the 1960s and 1970s revealed that the cerebral cortices of rats raised in enriched environments (group housed, exposed to stimulus objects) were profoundly altered compared to the brains of rats raised in impoverished conditions (singly housed, no stimulation). Alterations in environmentally enriched rats included increased enzyme levels, cortical thickness, dendritic spines and branching, synaptic contacts and transmission, and neuron size (e.g. see [14,16,28,31,34–36,49,62] for review). Further, enriched rats exhibited enhanced learning and memory abilities relative to impoverished littermates [9,37,73,80].

Subsequent work indicates that this effect is not limited to early development. Environmental enrichment appears to benefit cortical plasticity and learning abilities at any point in the lifespan [32,62,71]. For example, enrichment can completely protect against learning and memory deficits in adult mice with a conditional knockout of the NMDAR1 receptor subunit in the hippocampal CA1 region [61]. Moreover, enrichment can enhance the proliferation of new neurons [47] and dendritic spines [61] in the hippocampus of adult mice. Particularly relevant to aging are studies

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examining enrichment-induced alterations in middle-aged and aged rodents. In rodents examined at middle-age, enrichment initiated during middle-age increases cortical dendritic branching [32], forebrain weight [13], neurotrophin levels [38,60], and hippocampal neurogenesis [48], as well as enhances learning of a Hebb-Williams maze [13] and the spatial reference memory version of the Morris water maze [23,48,60]. In rodents examined at old age, enrichment initiated either at weaning or during old age, results in a variety of alterations in the brain including increased Purkinje cell dendritic branching [33], increased cortical thickness, RNA content, and presynaptic vesicle number [15,57,76], reduced aging-induced hippocampal gliosis [71], and increased cortical and hippocampal levels of the presynaptic protein synaptophysin [63]. Furthermore, enrichment in aged male rodents improves several types of learning, reverses short-term memory deficits, increases spontaneous alternation, and increases food-seeking behaviors [71,75,76,79]. However, the benefit to behavior is not universal; enrichment in aged male rodents does not increase reactivity to spatial novelty or general motor activity, nor does it improve performance in the Lashley III maze or a brightness discrimination [75,76]. Nevertheless, the data in aging rodents suggest that environmental enrichment can profoundly alter neural structure and improve several types of behavior.

An interesting feature of the research to date examining enrichment in aging rodents is that the vast majority of these studies utilized males as their subjects. This discrepancy may be important, as numerous studies have reported sex differences in both the brain and behavior of young rodents in response to environmental enrichment. For example, sex differences in rats have been reported in cortical and hippocampal dendritic branching [42,43], myelinated axons in corpus callosum [45], and exploratory behavior [41], despite the fact that male and female rats interact with the enriched environment similarly [46]. Spatial memory, a type of memory particularly vulnerable to decline in aged humans (e.g. [19,64,67]), is enhanced by enrichment in both young males and females [44,66,73]. However, no study has thus far examined whether enrichment can improve spatial memory or alter synaptic plasticity in aged females. Spatial reference memory, as tested in the Morris water maze, is enhanced by enrichment in middle-aged females [23,48], as is cortical dendritic branching [32] and hippocampal neurogenesis [48], suggesting that the female brain retains plasticity well into aging. Thus, we hypothesized that enrichment in aged females would reduce spatial memory decline, perhaps by augmenting synaptic plasticity.

The present study was designed to test this hypothesis. Aged group-housed female mice were exposed to an enriched environment for 3 h per day both prior to and during testing in the spatial and cued (non-spatial) versions of the Morris water maze. Their performance was compared to that of young and aged controls who were also group-housed, but not exposed to the enriched environment. Aged female mice, like aged male mice and aged rats, are typically impaired in the spatial version of the Morris water maze, but not the cued version ([20,22,25,26,29,50,52], but see [10,39]). Thus, enrichment was predicted to affect the spatial, but not the cued, water maze task. Synaptic alterations were examined by measuring levels of the protein synaptophysin in the hippocampus and neocortex, brain regions critical for spatial memory. Synaptophysin is a 38-kDa calcium-binding glycoprotein found in the membranes of neurotransmitter-containing presynaptic vesicles [40,78]. In non-demented humans, synaptophysin decreases with age in the hippocampus and various cortical regions [17,51,55]. In patients with Alzheimer's disease, synaptophysin immunoreactivity is significantly reduced in frontal cortex and hippocampus, and is correlated with impaired cognitive abilities [51,72,74,82]. In rodents, some studies have found similar age-related reductions in cortical or hippocampal synaptophysin [11,63], whereas other studies report no age-related changes in this protein [10,59] or reductions restricted to specific hippocampal subregions [70]. Despite these discrepancies, several studies report significant correlations between synaptophysin levels and spatial memory in the water maze, such that more synaptophysin is associated with better spatial memory [10,11,70]. In addition, findings in aged male rats indicate that environmental enrichment can significantly increase presynaptic vesicle number in the frontal cortex [57] and synaptophysin expression in the hippocampus and several cortical regions [63]. Thus, modulation of synaptophysin may play a role in enrichment-induced alterations in memory. The present study will be the first to examine this potential relationship. Our findings suggest that environmental enrichment, even if initiated during old age, can significantly accelerate spatial memory acquisition and increase cortical and hippocampal synaptophysin levels.

2. Methods

2.1. Subjects

Subjects were 8 young and 14 aged female C57BL/6 mice obtained at the ages of 3 months and 26–27 months from Hilltop Lab Animals, Inc. (Scottdale, PA). Upon arrival at Yale, the mice were quarantined at our medical school animal facility for 3 weeks. They were then moved to an animal facility adjacent to the laboratory in the Department of Psychology, and handled for five days prior to testing to habituate them to being picked up by the experimenter. Thus, at the beginning of behavioral testing, the mice were approximately 4 months or 27–28 months of age. Mice were housed up to 4 per shoebox cage in a room with a 12:12 light/dark cycle (lights on at 06:00 h) and behavioral testing was performed during the light phase of the cycle. Food (Purina LabDiet 5P00 ProLab RMH 3000) and water were provided ad libitum. All procedures conformed to the standards set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the