ARCHIVAL REPORT

Age-Specific Effects of Voluntary Exercise on Memory and the Older Brain

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Background: Physical exercise in early adulthood and mid-life improves cognitive function and enhances brain plasticity, but the effects of commencing exercise in late adulthood are not well-understood.

Method: We investigated the effects of voluntary exercise in the restoration of place recognition memory in aged rats and examined hippocampal changes of synaptic density and neurogenesis.

Results: We found a highly selective age-related deficit in place recognition memory that is stable across retest sessions and correlates strongly with loss of hippocampal synapses. Additionally, 12 weeks of voluntary running at 20 months of age removed the deficit in the hippocampally dependent place recognition memory. Voluntary running restored presynaptic density in the dentate gyrus and CA3 hippocampal subregions in aged rats to levels beyond those observed in younger animals, in which exercise had no functional or synaptic effects. By contrast, hippocampal neurogenesis, a possible memory-related mechanism, increased in both young and aged rats after physical exercise but was not linked with performance in the place recognition task. We used graph-based network analysis based on synaptic covariance patterns to characterize efficient intrahippocampal connectivity. This analysis revealed that voluntary running completely reverses the profound degradation of hippocampal network efficiency that accompanies sedentary aging. Furthermore, at an individual animal level, both overall hippocampal presynaptic density and subregional connectivity independently contribute to prediction of successful place recognition memory performance.

Conclusions: Our findings emphasize the unique synaptic effects of exercise on the aged brain and their specific relevance to a hippocampally based memory system for place recognition.

Key Words: Aging, exercise, memory, network, neurogenesis, presynaptic density

Western societies are rapidly aging, with attendant increases in the prevalence of dementia (1). Interest has therefore focused on physical exercise as an effective, inexpensive, and low-risk strategy for maximizing brain health in later life (2). For example, prospective studies have shown that physical activity acts as a protective factor in the incidence of dementia (3), and randomized clinical trials have found that regular aerobic exercise can reduce rates of cognitive decline in older adults (4). Whether such exercise can arrest cognitive decline associated with aging when begun later in life, however, is mostly unknown.

The neural substrates underlying the protective effects of exercise on cognitive performance in elderly people are not well-understood (5). In rodents, voluntary running at 2–3 months of age leads to several changes in the hippocampus, including increased neurogenesis (6), neurotrophin gene expression (7), dendritic spine density (8), synaptogenesis (9), synaptic plasticity (10), and enhanced survival of neural progenitor cells (11). Consistent with these changes, exercise leads to improvements in various hippocampal-dependent tasks such as spatial reference memory (6,10,12) and context fear conditioning (13). Initiating exercise in older rodents might also lead to changes in the hippocampus and improve performance on hippocampal-dependent tasks. For instance, van Praag *et al.* (14) provided aged mice (18-month-old) with access to running wheels for 45 days and found increased neurogenesis in the dentate gyrus, as well as improved acquisition and retention of spatial reference memory in the Morris water maze (MWM).

We used a combination of place and object recognition memory paradigms as well as a spatial learning task to determine whether exercise begun when rats are old can arrest or even reverse age-related cognitive decline and whether these effects are independent of age-related changes in noncognitive factors (e.g., motor ability). Short-term place recognition memory is dependent on hippocampal integrity (15), whereas object recognition involves the perirhinal cortex and is largely independent of the hippocampus (16,17). In our study, aging was associated with deficits in place but not object recognition performance, and this deficit was correlated with loss of presynaptic density. Twelve weeks of voluntary running restored place recognition performance in aged rats to that of younger adults but did not produce any detectable differences in the place recognition performances of the younger adults. Running also led to increased neurogenesis in both younger and older rats, but these effects did not predict place recognition performance. Running also led to a marked increase in presynaptic density in older rats, surpassing levels seen in younger animals, and was linked to changes in intrahippocampal synaptic connectivity and predicted place recognition performance. Our findings show that exercise begun in old age reverses some forms of cognitive decline and increases presynaptic density as well as connectivity, raising the possibility that the former is mediated by changes in the latter.

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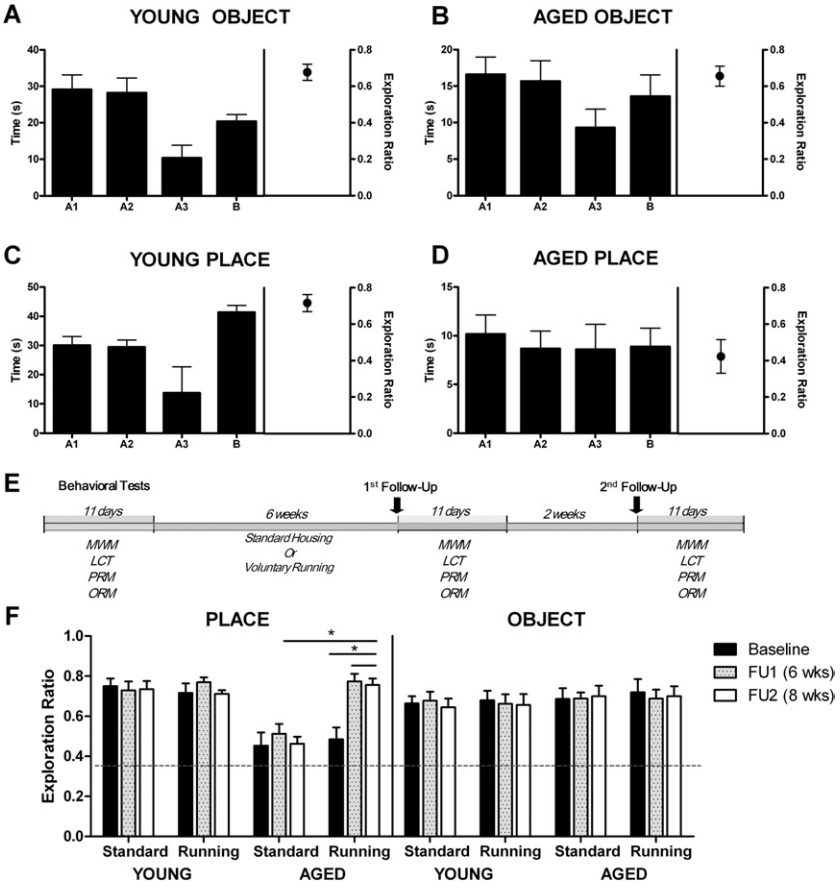

Figure 1. Age-related impairment in hippocampal-dependent place recognition memory (PRM) in older rats is rescued by exercise. Young ($n = 10$) and aged ($n = 10$) animals were assessed on the object recognition memory (ORM) **(A, B)** and PRM **(C, D)** behavioral paradigms. These involved exposing the rat to two identical objects (A1, A2) and then either replacing one of them with a new object (B in **A** and **B**) or to moving one of the original objects to a new location (B in **C** and **D**). Exploration Ratio (chance performance indicative of recognition memory failure = .5) was computed as the amount of time spent exploring the novel object or place over the entire time spent exploring. Compared with young rats, aged rats were impaired in PRM but not ORM. **(E)** Behavioral timeline of exercise experiment. Animals were initially tested on a battery of behavioral tests before being allocated to running or standard housing conditions. Animals remained in these conditions for the remaining duration of the study. First follow-up (FU) testing occurred at 6 weeks post running/standard housing, and second FU testing started 2 weeks later. **(F)** Voluntary running increased PRM performance in aged rats from impaired levels to that of young animals. There was no effect of running on ORM in older rats or on either object or PRM in young rats. Error bars in all bar graphs depict SEM. LCT, localized cue task; MWM, Morris water maze.

Methods and Materials

Subjects

Aged subjects were 28 experimentally naïve 20-month-old female ex-breeder Fischer 344 rats, whereas younger subjects were 30 experimentally naïve 7-week-old female virgin Fischer 344 rats, both obtained from a commercial supplier (Australian Research Centre, Perth, Australia). Experimental procedures were consistent with the ethical guidelines established by the American Psychology Association and were approved by the Animal Care and Ethics Committee of the University of New South Wales. Further details are provided in Supplement 1.

Apparatus

The water maze consisted of a circular pool constructed from fiberglass. A circular escape platform was submerged 1.5 cm below water level. Black curtains enclosed the pool, with three external cues within the curtains to reduce the amount of visuospatial information available to the animals. Object and place recognition memory tests were conducted in an open field arena constructed from black polyvinyl chloride plastic. Running wheels were made of plastic with a solid back running surface (Wodent Wheels, Salem, Oregon). There were two wheels/cage. Body weight and number of wheel revolutions/cage were measured weekly. Further details and behavioral procedures are described in Supplement 1.

Immunohistochemistry

At the completion of the behavioral tests, rats were anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneal) and perfused transcardially. Brains were postfixed for 1 hour and placed in 20% sucrose solution overnight. The entire hippocampus was cut in 40-μm coronal sections in alignment with the atlas of Paxinos and Watson (18) with a cryostat (Microm HM560; Microm International, Walldorf, Germany). Six serially adjacent sets of sections were obtained from each brain and stored in .1% sodium azide in .1 mol/L phosphate-buffered saline, pH 7.2. Further immunohistochemical details can be found in Supplement 1.

Statistical Analysis

Data were analyzed by Student *t* test or a one-way analysis of variance for multiple comparisons in the recognition paradigms and neural measures. A repeated measures analysis of variance was used for testing outcomes from multiple learning trials in the MWM, and analysis of covariance was used when controlling for covariates. Differences were considered significant when $p < .05$. A matrix to matrix test was computed on the basis of all possible pairwise cross-correlational presynaptic density differences between groups (19). Computational details for graph network analyses are available in Supplement 1.

Results

Aged Rats Are Selectively Impaired in Hippocampally Dependent Short-Term Place Recognition Memory

We used object and place recognition tasks to evaluate whether there is a loss of short-term memory associated with advanced age (20). Place recognition was significantly impaired in aged rats compared with young rats [$F(1,18) = 16.497$, $p < .001$] (Figure 1C, D). In contrast, there were no significant age differences on the hippocampally independent object recognition memory task [$F(1,18) = .025$, $p > .87$] (Figure 1A, B). Among the aged rats only, place recognition performance was significantly worse than object rec-