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Environmental enrichment modulates 5-hydroxymethylcytosine dynamics in hippocampus

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ABSTRACT

Gene–environment interactions mediated at the epigenetic level may provide an initial step in delivering an appropriate response to environmental changes. 5-Hydroxymethylcytosine (5hmC), a DNA base derived from 5-methylcytosine (5mC), accounts for ~40% of modified cytosine in the brain and has been implicated in DNA methylation-related plasticity. To identify the role of 5hmC in gene–environment interactions, we exposed both young (6-week-old) and aged (18-month-old) mice to both an enriched environment and a standard environment. Exposure to EE significantly improves learning and memory in aged mice and reduces 5hmC abundance in mouse hippocampus. Furthermore, we mapped the genome-wide distribution of 5hmC and found that the alteration of 5hmC modification occurred mainly at gene bodies. In particular, genes involved in axon guidance are enriched among the genes with altered 5hmC modification. These results together suggest that environmental enrichment could modulate the dynamics of 5hmC in hippocampus, which could potentially contribute to improved learning and memory in aged animals.

1. Introduction

Environmental factors are known to have physiological and behavioral effects on aging and related disease states in mammals (reviewed in [1]). Prolonged exposure to environmental enrichment (EE), which includes but is not limited to stimuli such as physical exercise, exposure to novel objects, and increased social interactions, is found to improve learning and memory, increase neurogenesis and angiogenesis in the hippocampus of aged mice [2–5], and potentially slow the progress of brain aging in rodents [6–8]. In addition to improving health and cognitive function in humans, voluntary physical exercise can also delay the cognitive deficits associated with aging and related neurodegenerative disorders, such as Alzheimer's disease (AD) [9,10], mitigate the disease phenotype of fatal neurodegenerative diseases, such as spinocerebellar ataxia type 1 (SCA) [11], and induce dynamic changes in promoter methylation in human skeletal muscle [12].

There is ample evidence that environmental factors, such as physical exercise, nutrient deficiency, pharmacological agents, and pollutants, change DNA methylation states in a gene/promoter-specific manner, while changing the expression of DNA methyl transferases (DNMTs) [12–16]. These findings suggest that the epigenetic landscape of genomic DNAs is responsive to changes in environmental signals during the lifetime of organisms. Gene–environment interactions mediated at the epigenetic level may be an intermediary step to providing an appropriate response of the gene/tissue/organism to the changes in the environment.

5mC has generally been viewed as a stable and long-lasting covalent modification to DNA; however, the fact that ten-eleven translocation (TET) proteins, including TET1, TET2, and TET3, can convert 5mC to 5-hydroxymethylcytosine (5hmC), a hydroxymethylated form of 5mC, gives a new perspective on the previously observed plasticity in 5mC-dependent regulatory processes [17–19]. Furthermore, TET enzymes are also known to further oxidize 5hmC into 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), which can be readily repaired by DNA repair enzymes (see also the review by Hajkova and colleagues, this issue). 5hmC has been detected in heart and lung tissue, though much higher levels have been found in the central nervous system [17,20,21] (see also review by Li and colleagues, this issue). Using a specific chemical-labeling method for 5hmC detection, we recently generated the first genome-wide maps of 5hmC in mouse cerebellum and hippocampus during development and aging [22]. Our group and others showed that genomic 5hmC levels are age-specific, involved in active DNA demethylation, and may be important for on-demand gene regulation [22–26]. Nevertheless, we do not know whether 5hmC levels or the genomic distribution of 5hmC is affected by external signals in the environment, including diet, exercise, and social interactions, which are all components of an enriched environment (EE).

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As such, a genome-wide analysis of 5hmC distribution in young and aged animals exposed to EE is needed to determine the role of 5hmC in gene–environment interactions. Here we exposed both young (6-week-old) and aged (18-month-old) mice to both an enriched environment and a standard environment, and mapped the dynamics of 5hmC in hippocampus induced by EE. We found that exposure to the EE significantly improves learning and memory in aged mice and reduces 5hmC abundance in mouse hippocampus. Genome-wide profiling of 5hmC suggests that the alteration of 5hmC modification occurs mainly at gene bodies, in particular the genes involved in axon guidance. Together these results suggest that environmental enrichment could modulate the dynamics of 5hmC in hippocampus, which may potentially contribute to the improved learning and memory in aged animals.

2. Results

2.1. Environmental enrichment improves cognitive function in aged mice

To understand the effects of environmental signals on cognitive function during the aging process, we exposed young and aged mice to either an enriched environment (EE, as described in Materials and methods) for 4 weeks, or kept them in their standard cages (Ctrl) commonly used for housing (Fig. S1). After EE exposure, mice were tested in Morris water maze assay (MWM, as described in Materials and methods) for their cognitive function, specifically learning and memory improvements. MWM analysis revealed that, over time, aged mice exposed to EE (hereafter AE) showed a reduced latency to locate a hidden platform in the water maze tank (i.e. learning) compared to aged mice kept in Ctrl (hereafter AC) (Fig. 1A). Furthermore, once the AE mice learned the location of the platform in a quadrant of the MWM during the initial training period, they spent significantly more time in the same quadrant even after the removal of the platform compared to AC mice (Fig. 1B), which points to improved memory retention in AE mice. It was interesting to observe that EE exposure led to aged mice performing as well as the young mice in the MWM (Fig. S2), suggesting that EE treatment made aged mice appear, at least cognitively, to behave more like young mice. As expected, the swim distance of the AE mice also improved, although their swim speed was not changed (Fig. S3) compared to AC mice, suggesting that the cognitive improvements are due to EE exposure alone. There were no statistically significant differences in learning and memory between young mice exposed to EE (YE) and young mice kept in Ctrl (YC) (Figs. 1C and D).

2.2. Environmental enrichment reduces global 5hmC level in the hippocampus

Genome-wide distributions of 5hmC in mouse brain tissue (cerebellum and hippocampus) have been mapped previously [24], revealing age- and tissue-specific 5-hmC dynamics [25]. To determine the effect of a prolonged environmental signal on 5hmC, we measured its overall abundance in genomic DNA isolated from the hippocampus, cortex, and cerebellum of all mice, using antibodies specific to 5hmC by dot-blot analysis. When compared to AC mice, we saw a significant reduction in the total genomic 5hmC signal intensity in AE mice (a 2.5-fold decrease, $n = 10$, $P < 0.05$, Student's t-test; means ± S.E.M.) (Fig. 2A). A similar reduction was also observed in young mice (Fig. 2B).

The TET protein family consists of TET1, TET2, and TET3, all of which can convert 5mC to 5hmC. To determine whether EE could alter the expression of Tet1, Tet2 or Tet3, we performed quantitative RT-PCR and did not observe any change of their expressions (data not shown).

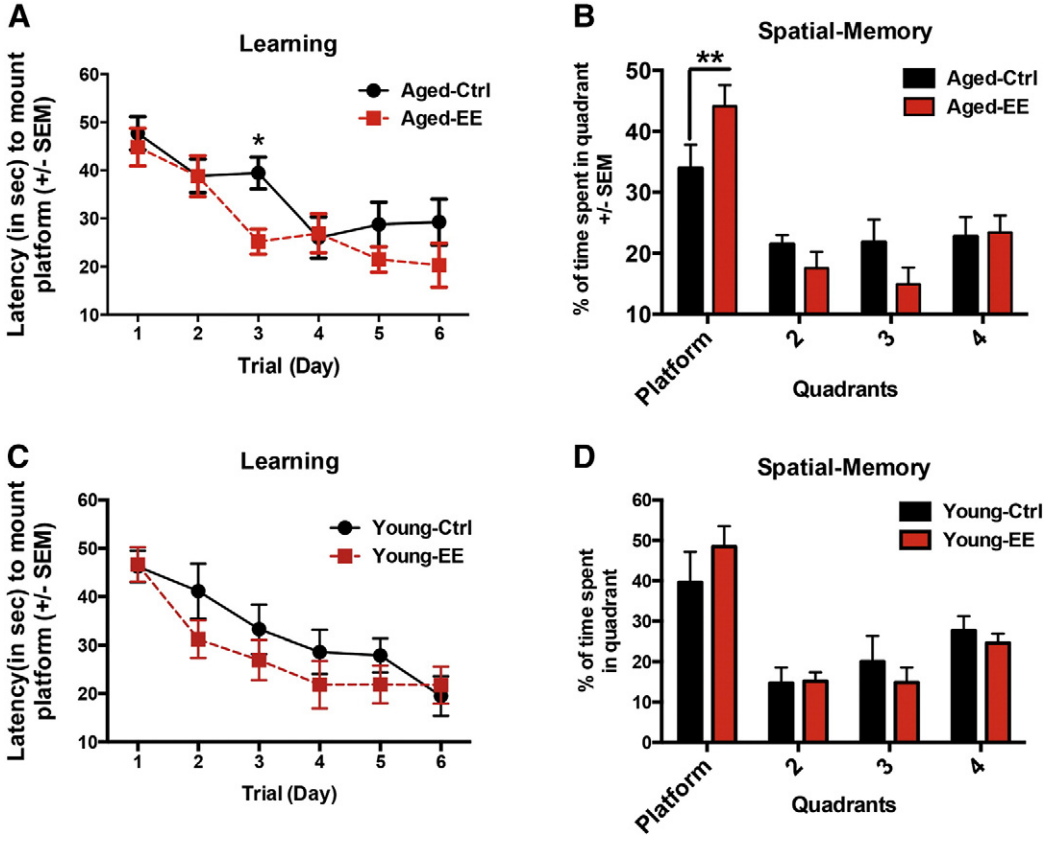

Fig. 1. Behavioral outcomes of mice subjected to Morris water maze (MWM). (A and C) Latency to mount platform (in seconds) is used to measure learning (i.e. learning the location of hidden platform); (B and D) the fraction of time spent in the quadrant after the removal of the hidden platform, which is used to measure the retention of spatial memory (i.e. recalling the location of the platform after it is removed from the MWM). Learning and spatial memory of mice exposed to either EE or Ctrl were scored. A four-trial-per-day procedure was conducted for six consecutive days. Each point represents the mean ± SEM, $n = 10$–12 mice per group. Unpaired t-test two-tailed for each time point (day) was used.