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Beta-hydroxy-beta-methylbutyrate (HMB) ameliorates age-related deficits in water maze performance, especially in male rats

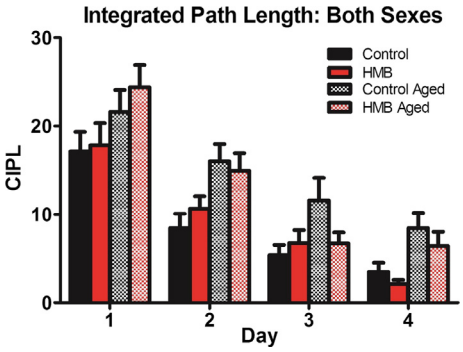
Daniel G. Kougias [b], Emily R. Hankosky [a], Joshua M. Gulley [a,b], Janice M. Juraska [a,b,*]

[a] Department of Psychology, University of Illinois, Urbana-Champaign, IL, USA
[b] Neuroscience Program, University of Illinois, Urbana-Champaign, IL, USA

HIGHLIGHTS

- Water maze performance was assessed in middle-aged and aged rats of both sexes.
- There were age-related deficits in water maze performance in both sexes.
- Dietary HMB countered age-related deficits in water maze performance in males.
- Dietary HMB reduced stress-related thigmotactic behavior in females.

GRAPHICAL ABSTRACT



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ABSTRACT

Beta-hydroxy-beta-methylbutyrate (HMB) is commonly supplemented to maintain muscle in elderly and clinical populations and has potential as a nootropic. Previously, we have shown that in both male and female rats, long-term HMB supplementation prevents age-related dendritic shrinkage within the medial prefrontal cortex (mPFC) and improves cognitive flexibility and working memory performance that are both age- and sex-specific. In this study, we further explore the cognitive effects by assessing visuospatial learning and memory with the Morris water maze. Female rats were ovariectomized at 11 months of age to model human menopause. At 12 months of age, male and female rats received relatively short- or long-term (1- or 7-month) dietary HMB (450 mg/kg/dose) supplementation twice a day prior to testing. Spatial reference learning and memory was assessed across four days in the water maze with four trials daily and a probe trial on the last day. Consistent with previous work, there were age-related deficits in water maze performance in both sexes. However, these deficits were ameliorated in HMB-treated males during training and in both sexes during probe trial performance. Thus, HMB supplementation prevented the age-related decrement in water maze performance, especially in male rats.

1. Introduction

In normal healthy aging, there is a decline in both physical and cognitive abilities. In both humans and animals, this age-related cognitive decline is marked by impairments in executive function, fluid intelligence, memory, processing speed, attention, and visuospatial abilities

* Corresponding author at: Department of Psychology, University of Illinois, 603 E Daniel St, Champaign, IL 61820, USA.
E-mail addresses: kougias2@illinois.edu (D.G. Kougias), hankosky@illinois.edu (E.R. Hankosky), jgulley@illinois.edu (J.M. Gulley), jjuraska@illinois.edu (J.M. Juraska).

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[1,2,3]. Recently, there has been increasing scientific and public interest in diet, and its role within the aging brain [4]. More specifically, particular dietary nutrients are being investigated and some can positively affect cognitive function in normal aging humans [5]. One such nutrient, a leucine metabolite known as beta-hydroxy-beta-methylbutyrate (HMB), may support not only muscle maintenance but also cognitive function in normal healthy aging.

Though HMB has been extensively studied in muscle tissue, less is known about its effects in the brain. HMB is known to have beneficial effects on muscle metabolism [6], and these underlying mechanisms are known to be dysregulated in the aging brain and contribute to age-related cognitive deficits [7,8,9]. More pertinently, we have previously shown that rats supplemented daily with dietary HMB have improved cognitive flexibility and working memory performance that are age- and sex-specific [10]. In particular, HMB supplementation enhanced working memory performances in old age male and female rats, ameliorated an age-related deficit in acquisition of a visual response strategy in control old age males, and improved acquisition of a visual response strategy in middle-aged females compared to their age-matched controls. Additionally, we have also shown that long-term HMB supplementation prevents age-related dendritic shrinkage within the medial prefrontal cortex (mPFC) in both male and female rats [11]. This dendritic shrinkage in aging is typically accompanied by a decline in performance on tasks involving working memory, reversals, and spatial learning [1,2], and thus presumably is in part the biological basis for these age-related cognitive deficits [12]. Given the deficit in Morris water maze performance of aged rats compared to young adults [13, 14], the current study seeks to assess the effects of dietary HMB supplementation on visuospatial learning and memory in a rat model of normal human aging.

While most literature on the aging rat model focuses solely on males, it is pertinent to use both male and female rats to reflect the aging human population, which has proportionally more females. Normal human female aging is marked by reproductive senescence (menopause), where a dramatic decline in ovarian hormones coincides with the end of cyclicity [15]; whereas in rats, this does not include such a dramatic decline in ovarian hormones [16,17,18]. Rather, rat ovaries continue to secrete low to moderate levels of estrogen and progesterone at varying ratios after cyclicity ends. Moreover, there is evidence that circulating estrogens are neuroprotective in aged female rats [19,20, 21]. In particular, our laboratory has previously shown that performance on the water maze is influenced by the estrogen to progesterone ratio. Specifically, higher estrogen helps to preserve learning abilities in aged female rats whereas higher levels of progesterone appear to counter some of estrogen's positive effects [22,23]. Therefore in order to appropriately model human menopause and avoid the complications of rat estropause, in the present study, female rats were ovariectomized (OVX) in middle age to mimic the dramatic decline in ovarian hormones after human menopause. Furthermore, thigmotaxis, a behavior characterized by remaining near the perimeter of an apparatus in response to a novel environment, was assessed since sex differences often occur in this measure in the water maze [24].

2. Materials & methods

2.1. Subjects

Subjects were male (n = 45) and female (n = 38) Long-Evans hooded rats procured from Harlan Laboratories (Indianapolis, IN) at approximately 10 months of age. The rats were housed individually in standard clear Plexiglass laboratory cages, fed and hydrated ad libitum, weighed weekly, and through the duration of behavioral testing, handled daily. The colony was maintained on a 12-h light/dark cycle with lights on at 0800 h. At 11 months, all rats were anesthetized with isoflurane vapors and underwent surgery. Female rats were OVX via bilateral dorsal incisions while male rats underwent a sham surgery via bilateral dorsal incisions retaining their gonads to control for possible effects from anesthesia exposure. In accordance with animal care policy, rats were administered the analgesic carprofen (5 mg/kg delivered subcutaneously) immediately after anesthetization and again 6–12 h later. Animal care and experimental procedures were in accordance and approved by the Institutional Animal Care and Use Committee at the University of Illinois Urbana-Champaign and were within the guidelines of the National Institutes of Health.

2.2. Experimental schedule

Daily dosing with either vehicle or HMB solution began at 12 months of age for all groups and continued through the testing period and the subsequent two weeks until sacrificed. A relatively short-term treatment group was dosed for a month prior to the start of the behavioral testing procedure (refer to Section 2.4) at 13 months of age (middle-aged) and a relatively long-term treatment group for seven months, being tested at 19 months of age (aged). Both relatively short- and long-term HMB supplementation paradigms are employed to assess the potential effect of supplemental duration and HMB's putative action, whether it be cognitive-enhancing or ameliorative. Furthermore, the long-term (aged) vehicle-treated group served as a positive control for the expected age-related deficit in water maze performance in comparison to the short-term (middle-aged) vehicle-treated group. Within the short-term treatment (middle-aged) group, 11 males and 9 females were administered vehicle whereas 12 males and 10 females were administered HMB. Within the long-term treatment (aged) group, 11 males and 10 females were administered vehicle whereas 11 males and 9 females were administered HMB. Two weeks after the end of behavioral testing, rats were sacrificed with a lethal injection of sodium pentobarbital (100 mg/kg; Sigma, St. Louis, MO) and their brains were collected for Golgi Cox staining and processing [reported in 11].

2.3. Dosing procedure

There is no detectable sex difference in humans in the pharmacokinetics of HMB [25], though this has not been studied in rats. Furthermore, in humans circulating HMB levels peak by 1–2 h after ingestion and return to baseline by 9 h [26]. Thus, HMB was administered twice daily, except once on Sunday, with a target dose of 450 mg calcium-HMB (Ca-HMB)/kg body weight, which results in an HMB dose (~364 mg/kg) that others have shown to aid aging muscle [27]. Sipper tubes containing the vehicle (32 mg/mL calcium lactate + 20% sucrose) or HMB solution (50 mg/mL Ca-HMB + 20% sucrose) were placed into individual home cages twice daily Monday through Saturday (at approximately 0800 h and 2000 h) and once on Sunday (between 0800 and 1000 h). Given the range of weights, the volume of the HMB solution varied between 2.7 and 6.3 mL. Most rats started consuming the solution immediately and finished within 60 min of it being administered. However, tubes remained in cages until the next dose in order to maximize consumption. For each rat, the amount consumed was recorded and the actual dose ingested was estimated.

2.4. Behavioral testing: Morris water maze

2.4.1. Procedure

Since pretraining has previously been demonstrated to essentially eliminate sex differences in performance on the Morris water maze task [28], it was incorporated in the current study. At approximately 13 months of age (middle-aged) for the short-term treatment group and 19 months of age (aged) for the long-term treatment group, the rats had a day of pretraining followed by one day off, and then four consecutive days of testing. All pretraining and testing occurred during the early hours of the light cycle.