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A Reduction in Behavioral Pattern Separation Is Attenuated by Dietary Supplementation with a Magnesium-Rich Marine Mineral Blend in Middle-Aged Rats

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ABSTRACT Middle age is increasingly accepted as a key period during which individuals are susceptible to the effect of environmental and lifestyle factors. Emerging research indicates that dietary factors play a crucial role in brain health and cognitive function, and studies in both animals and humans have demonstrated that dietary interventions can mitigate cognitive impairment. Specifically, magnesium has been shown to enhance learning and memory, and magnesium deficiency is associated with impaired hippocampal-dependent memory formation in animal studies. The aim of this study was to examine if supplementation with a magnesium-rich marine mineral blend (MMB) could alter middle-age-related cognitive impairment. Young and middle-aged rats were given access to a control diet or an experimental diet formulated with an MMB for 4 weeks before undergoing a series of behavioral assessments. Supplementation of MMB to middle-aged rats rescued a deficit in cognitive impairment, specifically a pattern separation paradigm that is sensitive to alterations in a type of brain plasticity called neurogenesis. It had no effect on general activity in the open field or performance on other hippocampal-associated tasks. Changes in cognitive function occur as a predictable consequence of aging. Research into whether modification of dietary factors, such as this MMB, may play a role in the prevention of age-related cognitive impairment warrants further investigation.

KEYWORDS: • ***hippocampus*** • ***magnesium*** • ***marine extract*** • ***memory*** • ***neurogenesis*** • ***pattern separation*** • ***middle-age***

MIDDLE AGE is a critical period during which individuals are sensitive to the impact of environmental and lifestyle factors such as exercise and diet. Memory performance and processing speed have been shown to be vulnerable to various lifestyle factors during middle age.[1] Deterioration in hippocampal-associated memory occurs as a consequence of aging[2] and cognitive impairments are associated with neurodegenerative disorders such as dementia. Specifically, a reduction in pattern separation, which is the process by which similar, but not identical experiences are distinguished from one another,[3] is frequently observed in healthy older individuals, and is considered a feature of age-associated mild cognitive impairment,[4] which can present during middle age.[5]

In the absence of effective treatments, it is important that research is directed toward novel approaches of mitigating age-related cognitive impairment. Studies in both animals and humans have demonstrated that dietary interventions can ameliorate cognitive impairment.[6,7] Specifically, dietary modifications have been shown to improve the cellular process of adult hippocampal neurogenesis (AHN) and associated memory,[8] and it is now established that AHN is a key process in pattern separation.[9]

A previous study has demonstrated that a marine mineral blend (MMB) (a marine algae-derived multimineral dietary supplement that is rich in bioavailable calcium[10] and a seawater-derived bioavailable magnesium[11]) improved the diversity of the gastrointestinal microbiome in adult rats.[12] Magnesium can cross the blood–brain barrier[13] and is reported to enhance learning and memory in both young and aged rats[14] and reverse cognitive impairment in an animal mouse model of Alzheimer's disease.[15]

As middle age is emerging as a critical period in which cognitive decline can be both detected, and targeted with various interventions, this study was designed to evaluate whether supplementation of MMB to middle-aged rats influenced age-related cognitive impairment.

Adult male Sprague Dawley rats (young cohort aged 12 weeks, middle-aged cohort aged 16 months) (Envigo,

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USA)[16] were maintained on a 12 h:12 h light:dark cycle (lights on at 08.00 h) under regulated temperature (21°C ± 2°C) and humidity (30–50%), with food and water available *ad libitum*. Experimental chow was formulated as previously described[12] to give an additional 1 g magnesium/kg diet (Envigo), which equates to 50 mg magnesium/kg body weight per day in an average 400 g rat.[12] The term MMB is used to indicate the experimental diet (Fig. 1A). Animals were randomly divided into four groups: young/control (standard chow), young/MMB, middle-aged/control, and middle-aged/MMB ($n = 7–10$) and were pair-housed with free access to standard or MMB-supplemented chow for 4 weeks before behavioral assessments (Fig. 1B). Animals remained on experimental chow for the duration of the study (6 weeks in total) to align with a previously published study that demonstrated that 6 weeks of MMB significantly enhanced the gut microbial diversity in adult male rats.[12] All experiments were conducted in accordance with the European Directive 2010/63/EU, under an authorization issued by the Healthcare Products Regulatory Authority Ireland (AE19130/P021) and approved by the Animal Ethics Committee of University College Cork.

For the open field test, animals were placed in a white round arena (90 cm in diameter) and their behavior was recorded for 10 min. Velocity and distance traveled were measured using Ethovision XT software (Noldus, USA) (Fig. 1E). Novel object recognition was assessed as described previously[17] (Fig. 2B) and working memory was measured using spontaneous alternations in the Y maze.[18] Pattern separation was assessed using the modified spontaneous location recognition task, as previously described.[19,20] The times each animal spent exploring the object in the novel location and the familiar location in a large separation (Fig. 2C) or small separation (Fig. 2D) paradigm were recorded and a discrimination ratio was calculated (Novel Exploration/(Novel + Familiar Exploration). All data were analyzed using Statistica (Statsoft) and graphed using GraphPad Prism. Data are expressed as means ± the standard error of the mean. Statistical analysis was carried out using a two-way ANOVA to determine diet or age effect or interaction between both factors. Statistical tests were two-tailed with a significance level of $\alpha \leq 0.05$.

Supplementation with MMB for 4 weeks did not affect bodyweight ($F[1, 32] = 0.01$, $P = .91$; Fig. 1C) or food intake ($F[1, 11] = 0.08$, $P = .93$; Fig. 1D) in either young or middle-aged animals. However, middle-aged animals displayed a significant reduction in body weight ($F[1, 32] = 65$, $P < .001$) compared with young animals irrespective of diet, and this was not associated with food intake ($F[1, 11] = 0.63$, $P = .44$; Fig. 1C). In the open field test, middle age was associated with a significant decline in locomotor activity (distance traveled $F[1, 32] = 14.84$, $P < .001$) and velocity ($F[1, 32] = 14.77$, $P < .001$) in both control and MMB-supplemented rats (Fig. 1E). MMB supplementation did not affect locomotor activity (distance traveled ($F[1, 32] = 0.30$, $P = .58$) and velocity ($F[1, 32] = 0.29$, $P = .59$) (Fig. 1E). Neither MMB supplementation ($F[1, 31] = 2.1$, $P = .15$) nor middle age ($F[1, 31] = 0.22$, $P = .64$) had an effect on the percentage of spontaneous alternations in the Y maze (Fig. 2A) or on the discrimination ratio in a novel object recognition task (diet $F[1, 31] = 0.04$, $P = .84$; age $F[1, 31] = 1.72$, $P = .19$; Fig. 2B). However, middle age was associated with a decreased number of arm entries ($F[1, 31] = 24.1$, $P < .001$) in the Y-maze task (Fig. 2A). In the modified spontaneous location recognition task, there was a significant decrease in the discrimination ratio in the large separation paradigm performed by both young and middle-aged animals supplemented with the MMB compared with their counterparts on control chow ($F[1, 31] = 4.87$, $P = .035$; Fig. 2C). In the small separation paradigm (pattern separation), two-way ANOVA showed a significant interaction effect (age × diet $F[1, 30] = 4.92$, $P = .03$; Fig. 2D). *Post hoc* analysis revealed that MMB supplementation significantly enhanced pattern separation performance in middle-aged animals ($P = .047$).

This study was designed to evaluate whether supplementation of an MMB to middle-aged rats could ameliorate age-related cognitive impairment. The results of the study show that incorporation of the MMB supplement into standard chow had no overall effects on body weight gain, food intake, or general locomotor activity. Furthermore, the MMB did not affect performance in the hippocampal-associated behavioral tasks that assessed working memory and recognition memory. However, MMB supplementation alleviated a middle-age-associated deterioration in the pattern separation task.

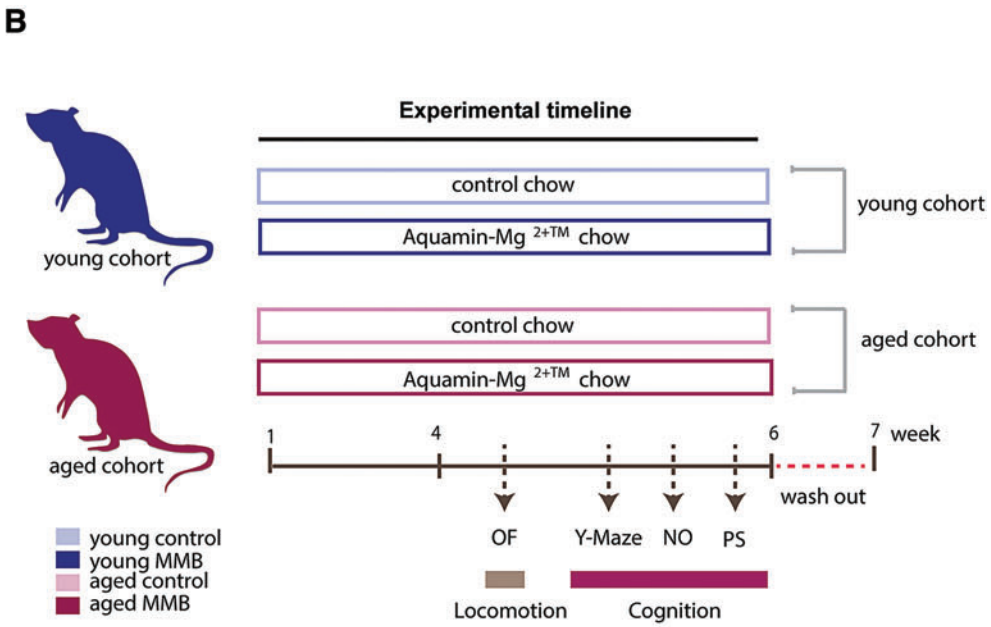
Consistent with previous findings, MMB supplementation was not associated with changes in body weight or food intake.[12] There was a decrease in body weight gain over the course of the study in middle-aged compared with young animals that was not associated with a concurrent change in food intake. This is most likely due to the fact that the younger rats were still growing and gained weight over the course of the experiment. Consistent with previous results, MMB supplementation had no effect on activity in the open field paradigm.[12] However, middle-aged animals displayed reduced locomotor activity (both distance moved and velocity) compared with their younger counterparts, which is widely recognized as a natural consequence of aging.[21]

Pattern separation is a key component of episodic memory,[22] and the hippocampus is instrumental in formation of these memories.[4,22] Although spontaneous alternations[23] and novel object recognition[17] are both hippocampal-dependent behaviors, pattern separation has been shown to be dependent on AHN.[3,9] This unique form of brain plasticity is necessary for pattern separation, which can be modulated through a variety of measures such as exercise and environmental enrichment.[20,24] Recently, dietary modification in the form of energy restriction has been reported to enhance pattern separation in an adult human cross-sectional study.[8] Conversely, high sucrose diets and early malnutrition are reported to impair pattern separation in rats.[25,26] In this study, middle-aged animals demonstrated an impairment in a modified spontaneous location recognition task, a means of assessing pattern separation,[3] which is in line with several other studies that show a marked decrease in the ability to pattern separate with increasing age.[27–30] However,

A

Aquamin-Mg $^{2+TM}$ chow

(>5 ppm)	ppm	(<5 ppm)	ppm	(<1 ppm)	(<0.2 ppm)
Calcium	285.00	Iodine	4.75	Hafnium	Rhodium
Magnesium	214.650	Barium	4.63	Cadmium	Tin
Carbon	106.500	Chromium	4.33	Antimony	Gallium
Sulfur	3174	Copper	3.73	Bismuth	Europium
Sodium	2835	Fluoride	2.97	Gold	Holmium
Chloride	1451	Zinc	2.77	Lithium	Terbium
Strontium	1262	Cerium	2.13	Selenium	Lutetium
Iron	975.50	Silver	2.07	Tellurium	Thulium
Silicon	380.00	Neodymium	1.92	Thallium	Rubidium
Aluminium	270.00	Lanthanum	1.66	Dysprosium	Tantalum
Manganese	265.35	Molybdenum	1.62	Praseodymium	Germanium
Potassium	142.60	Arsenic	1.47	Gadolinium	Cesium
Boron	110.8	Scandium	1.37	Erbium	Mercury
Phosphorus	96.65	Cobalt	1.24	Palladium	Platinum
Titanium	23.65	Nickel	1.19	Samarium	Iridium
Zirconium	10.45	Beryllium	1.10	Lead	Osmium
Vanadium	9.64	Ruthenium	1.10	Ytterbium	Rhenium
Thorium	9.08				Indium
Niobium	6.26				
Tungsten	5.57				
Yttrium	5.47				



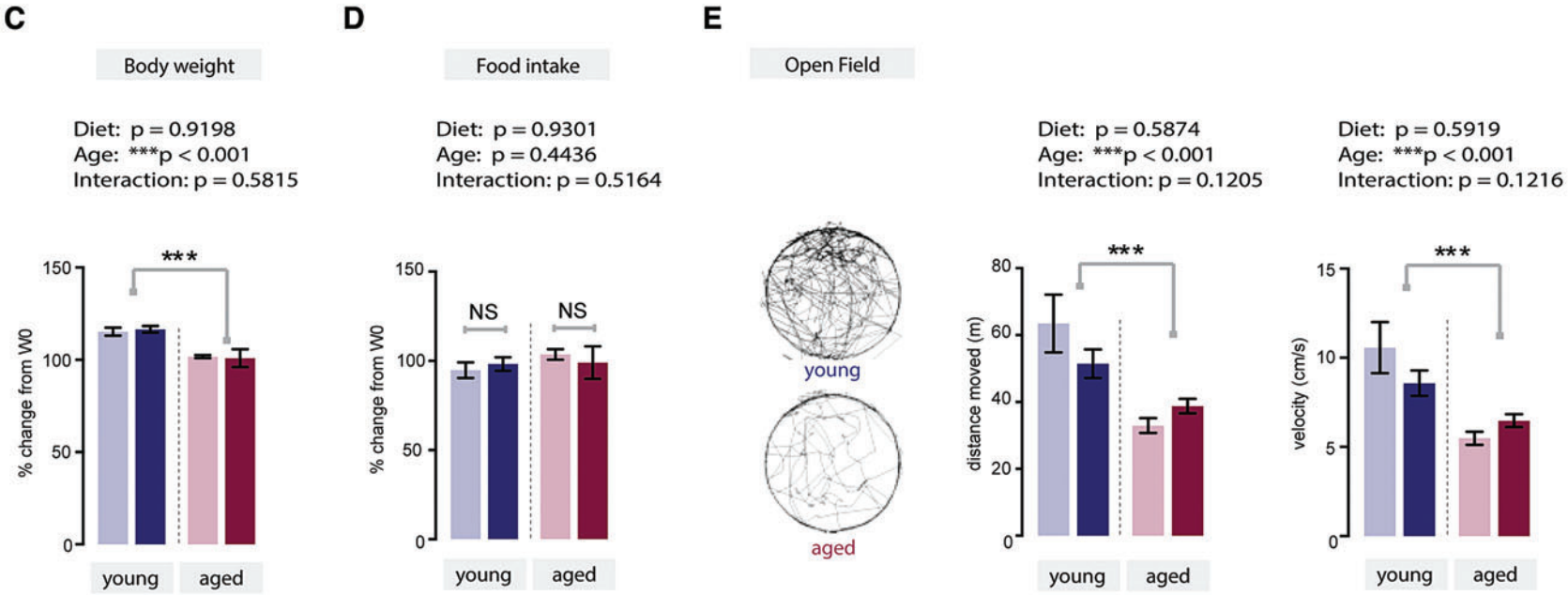


FIG. 1. **(A)** Mineral composition of marine mineral blend (ppm). **(B)** Timeline and study design. **(C)** Body weight gain. **(D)** Percentage change in food intake. **(E)** Distance traveled and velocity in the open field test. All data are expressed as mean ± SEM, $n = 7–10$, ***$P < .001$ compared with younger counterparts. NO, novel object; NS, not significant; OF, open field; ppm, parts per million; PS, pattern separation; SEM, standard error of the mean; W0, week 0. Color images are available online.

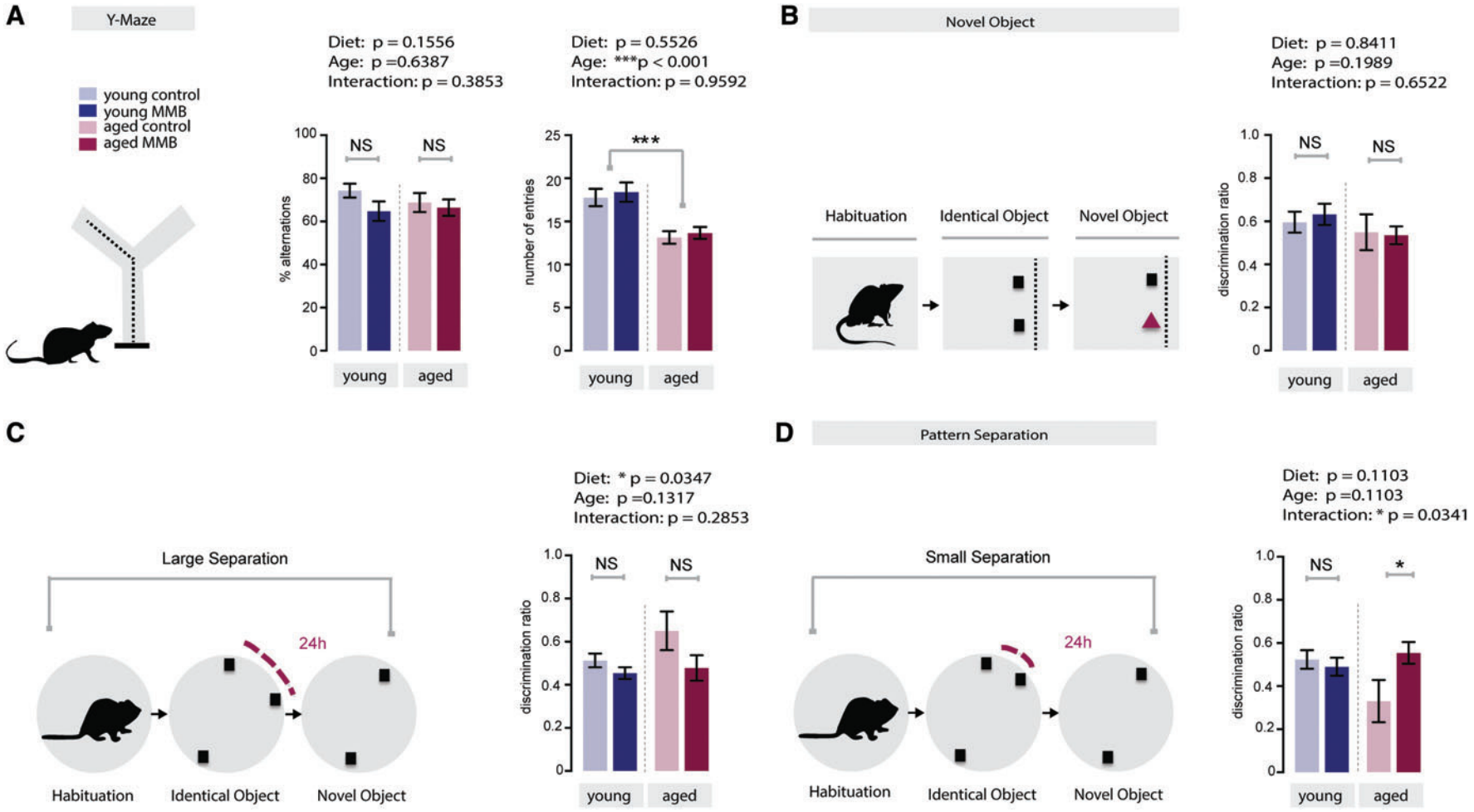


FIG. 2. **(A)** Percentage of alternations in the Y-maze test. **(B)** Novel object recognition depicted as DR between novel and familiar objects. DR in a modified spontaneous location recognition task using **(C)** a large separation paradigm and **(D)** a small separation paradigm. All data are expressed as mean ± SEM, $n = 7–10$, $^{*}P < .05$ compared with control diet counterpart. $^{***}p < 0.001$ compared with younger counterparts. DR, discrimination ratio. Color images are available online.

supplementation with MMB significantly attenuated this reduction in the middle-aged rats. Interestingly, magnesium supplementation has been reported to enhance pattern separation in adult rats,[31] whereas magnesium deficiency is associated with impaired hippocampal-dependent memory formation in adult mice.[32] Restoration of brain magnesium levels has also been reported to reverse memory deficits associated with chemotherapeutic agents in adult rats.[33]

It has previously been reported that magnesium derived from this MMB product is bioavailable to a significantly greater degree than MgO and displays a similar bioavailability profile as $MgCl_2$ *in vitro*.[11] In this *in vivo* study, the concentration of magnesium in the MMB is in line with that used in a study that showed increased brain bioavailability of magnesium without any adverse effects[14] MMB is rich in magnesium but also an array of other trace minerals and it has been suggested that mineral supplementation in concert with other complementary minerals as is the case for MMB, enhances health benefits.[34] We have shown that MMB enhances gut microbial diversity,[12] and magnesium-deficient diets have been shown to adversely affect gut microbial composition.[35] In line with the role of gut microbiota in metabolism and energy homeostasis, supplementation with magnesium decreases serum glucose and insulin levels, visceral fat, as well as a wide variety of hormones.[36] The effects of MMB on metabolic parameters have yet to be assessed.

Middle age has been identified as a critical time for detecting subtle changes in episodic memory, and specifically in pattern separation. It is a potentially crucial time period for interventions designed to delay or minimize cognitive deterioration.[28] The potential protective impact of MMB supplementation on cognitive function warrants further investigation, with particular emphasis on the role of magnesium.

AUTHORS' CONTRIBUTIONS

E.K.C., C.M.L.-S., and Y.M.N. conceived and designed the experiments; E.K.C. performed the experiments; E.K.C. and S.G. processed and analyzed the data; E.K.C., S.G., A.S., and Y.M.N. wrote the article; Y.M.N. and E.K.C. edited the article. D.M.O'G. provided MMB.

AUTHOR DISCLOSURE STATEMENT

D.M.O'G. and A.S. are employees of Marigot Ltd. For all other authors, no competing financial interests exist.

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