



RESEARCH ARTICLE

Modulation of Spatial Learning and Memory of Obese Mice by Germinated Sang-Yod Rice

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Abstract

Background: In this work, an association between chronic consumption of high fat diet (HFD) and changes of cognitive ability in C57BL/6J mice was ascertained. The Morris water maze was employed to determine effects of germinated Sang-Yod rice (GR) supplement on hippocampal-dependent memory tasks in regard to spatial learning and memory.

Methods: Eighteen male C57/BL/6J mice were randomly divided and allocated into 3 groups (n = 6), including (i) CONTROL, mice that fed with normal diet; (ii) HFD, mice that fed with high fat diet (HFD); and (iii) HFD-GR, mice that fed with HFD plus 0.25% germinated Sang-Yod rice per kilogram body weight per day. Measurement of body weight was carried out every week. Learning ability and memory of mice were investigated by using Morris water maze (MWM) test.

Results: Improved cognitive impairment was demonstrated when germinated Sang-Yod rice at a dose of 0.25% per kilogram body weight per day was consumed by HFD-induced mice for 12 weeks. The rice could be served as a body weight control feed, since the body weights of mice fed by normal diet, HFD, and HFD + 0.25% rice for 12 weeks were in similar ranges. The positive influences on cognition and obesity might be attributed to anthocyanins and γ -amino butyric acid presenting in the germinated brown rice, which confer anti-oxidant and anti-inflammatory properties, respectively.

Conclusion: These health effects suggest the utility of germinated Sang-Yod rice as food alternative or supplement

in order to ameliorate obesity-induced brain dysfunctions, such as learning and memory loss. Further studies are warranted to localize areas in the brain that would be influenced by long terms consumption of germinated Sang-Yod rice.

Keywords

Hippocampal-dependent memory task, Spatial learning and memory, High fat diet-induced mice model, Morris water maze

Abbreviations

HFD: High fat diet; GR: Germinated Sang-yod rice; MWM: Morris water maze

Introduction

According to The World Health Organization (WHO), obesity is clinically recognized as body mass index (BMI) of $\geq 30 \text{ kg/m}^2$. The global rate of obesity is developing to epidemic proportions in both adults and children. Also, a myriad of diseases is associated with obesity such as type 2 diabetes, cardiovascular disease, gastrointestinal and respiratory difficulties, and many types of cancer. These health problems are considerably realized, resulting in the establishment of national policy for prevention of obesity in combined with the searching of dietary supports for body weight control [1].

Disruption of vascular systems as a result of obesity

Table 1: Compositions of normal diet and HFD used for feeding mice*.

On a caloric basis (per 100 g)	Normal diet (C1090-10) (weight per 10% energy from fat)	HFD (C1090-60) (weight per 60% energy from fat)
Moisture	7.9%	2.9%
Crude Ash	4.3%	3.2%
Crude Fiber	3.1%	4.7%
Crude Fat	4.0%	35.0%
Crude Protein	20.7%	21.0%
Nitrogen free extractives	60.0%	33.2%
Total calories	351 kcal	523 kcal

*Altromin Spezial futter GmbH & Co. KG (Germany) was the diets' supplier.

is critical to normal brain functions that majorly lead to cognitive impairment [2]. Literatures have demonstrated that the hippocampus is fully associated with learning ability and memory of mammals [3], and is susceptible to damages induced by high fat diets [4]. The Morris Water Maze (MWM) test is widely used as a tool for determining impaired hippocampal-dependent memory tasks in preclinical animal model [5]. In this project, spatial learning and memory of high fat diet-induced C57BL/6J mice was measured by using the MWM test following these mice had received 0.25% of germinated Sang-Yod rice per kilogram body weight per day as an intervention throughout the entire duration of 12 weeks.

Materials and Methods

Preparation of germinated Sang-Yod rice

Sang-Yod brown rice was obtained from a farm in Phatthalung province, Thailand. The rice of about 5 kg was rinsed 3 times with tap water, and any incomplete seeds were removed. Then, it was soaked in excess water for 12 hours in the dark at room temperature with changes of soaking water every 4 h. After draining off the soaked water, the rice was wrapped in cotton canvas and placed in moist environment with good ventilation for 24 hours at room temperature. The germinated rice being obtained was dried in a hot air oven at 45 °C until 8% moisture was reached. The germinated rice was ground to fine powder and kept in an air-tight container at 4 °C until use.

Animal preparation

The animal experiment was done in regard to the ethical guidelines of a laboratory animal at Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia. Eight-teen male C57BL/6J mice, aged 6-weeks-old, were purchased from Monash University, Kuala Lumpur, Malaysia. They were housed 4 per cage under climate-controlled conditions, i.e., 22 ± 2 °C with a 12 h light-dark cycle (lighted on at 07.00 am) to acclimate the housing conditions for 2 weeks and freely accessed to standard laboratory chow and tap water. These mice were weighed and allocated to a group by maintaining an equal weight per group since the beginning. Three groups of mice were

prepared (n = 6) as follows: (i) CONTROL, mice that fed with normal diet; (ii) HFD, mice that fed with highfat diet (HFD); and (iii) HFD-GR, mice that fed with HFD plus 0.25% of germinated Sang-Yod rice per kilogram body weight per day. Compositions of normal diet and HFD were detailed in Table 1. These diets are nutritionally complete based on the Guidelines of Lab Animal Information of Germany. The mice body weight and the amount of feed intake were recorded entire the study.

Setting up apparatuses for the MWM test

A round pool with 100 cm in diameter was arranged at a room's center. It was filled with water kept constantly at 25 ± 2 °C. A square platform measuring 3 × 3 inches was movable to a desired location in the pool. On the first day of pre-training, the platform was raised 1 inch above the water surface. During 4 days of acquisition trial, the platform was submerged 1 inch below the water surface. For the probe trial, the platform was removed from the pool. The room was sound proof and exposed to homogenous illumination. A video camera was fixed to the ceiling to record behaviors of a mouse. Its swimming paths were tracked by SMART Video Tracking Software version 3 (Panlab, Harvard Apparatus). A spatial cue was distantly deposited on the north wall of the room. Locations of the platform and starting points for releasing a mouse were shown in Figure 1 and Table 2.

The MWM test

The MWM procedures previously described were used to assess spatial learning and memory of the mice as follows [5]. Mice were transferred to the prepared room by 30 min before starting. The test was conducted on 6 consecutive days, consisting of 1 day of pre-training, 4 days of acquisition trial, and 1 day of probe trial. On the pre-training day, a mouse was provided 60 sec to find the platform. If it did not do so or the time it spent was more than 60 sec, it was gently guided back to the platform and allowed to sit on the platform for 15 sec before removed to home cage. This was called 1 cycle of the pre-training. Five cycles with 5 min interval of the pre-training were completed within 1 day. For the acquisition trial, a mouse was released at the desired site

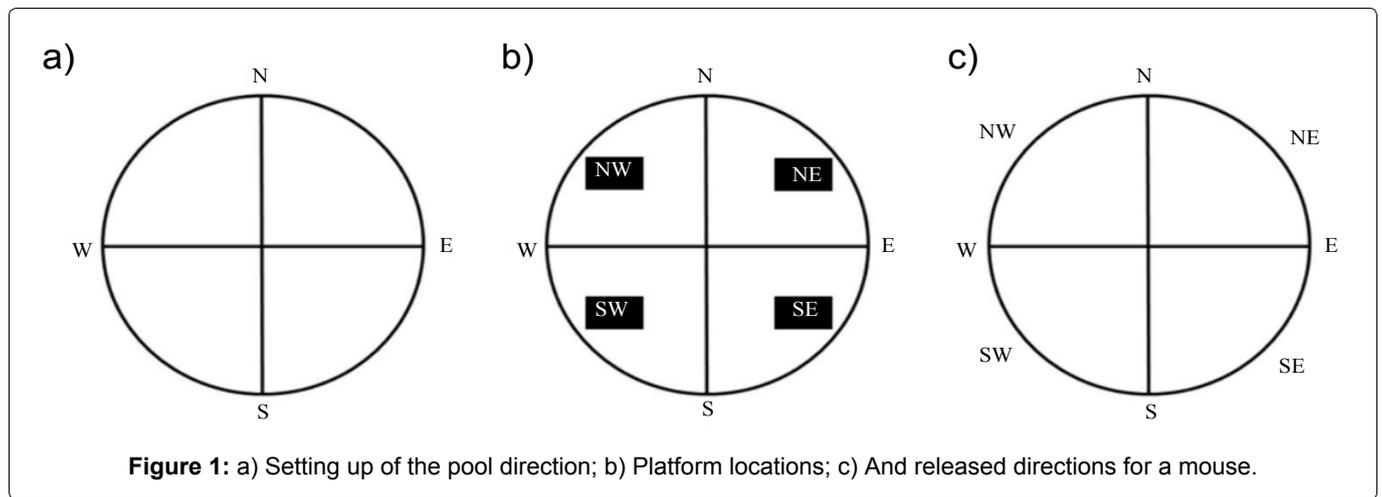


Figure 1: a) Setting up of the pool direction; b) Platform locations; c) And released directions for a mouse.

Table 2: Platform locations and starting directions for a mouse by the MWM test.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	
	Pre-training	Acquisition				Probe	
Platform set up	1 inch above the water surface	1 inch below the water surface				No platform	
	Platform location	Starting direction					
Trial 1	SW	S	W	NW	NW	SE	N
Trial 2	NW	W	S	W	SE	S	
Trial 3	NE	S	NW	SE	W	W	
Trial 4	SW	W	SE	W	S	SE	
Trial 5	SE	S	S	S	NW	NW	

Table 3: Records of mice body weight for 12 weeks of the feeding programs.

Week No.	Body Weight (g)		
	CONTROL ¹	HFD ²	HFD-GR ³
0	20.38 ± 0.66 ^{a,*}	24.06 ± 0.60 ^a	24.18 ± 0.88 ^a
1	21.63 ± 0.72 ^{a,*}	24.57 ± 0.45 ^a	25.49 ± 0.77 ^a
2	22.29 ± 0.66 ^a	24.90 ± 1.23 ^a	26.57 ± 0.87 ^a
3	23.39 ± 0.74 ^a	25.87 ± 0.94 ^a	27.41 ± 0.91 ^a
4	24.17 ± 0.89 ^a	26.47 ± 0.85 ^a	27.88 ± 0.87 ^a
5	24.72 ± 0.72 ^b	27.51 ± 0.97 ^a	28.59 ± 0.89 ^a
6	25.23 ± 1.00 ^b	28.30 ± 1.03 ^a	29.82 ± 1.03 ^a
7	25.95 ± 1.03 ^b	28.61 ± 1.09 ^a	30.75 ± 1.33 ^b
8	26.42 ± 0.96 ^b	28.79 ± 1.01 ^b	30.90 ± 1.41 ^b
9	26.80 ± 0.91 ^b	28.44 ± 1.00 ^a	31.39 ± 1.47 ^b
10	26.47 ± 1.11 ^b	29.06 ± 0.89 ^b	32.01 ± 1.63 ^b
11	27.60 ± 1.06 ^b	29.91 ± 1.07 ^b	32.46 ± 1.64 ^b
12	27.82 ± 0.89 ^b	30.22 ± 1.00 ^b	32.42 ± 1.62 ^b
Body weightchange (g)	7.44	6.16	8.24

¹Mice fed with normal diet, ²Mice fed with HFD, ³Mice fed with HFD plus 0.25% of germinated Sang-Yod rice per kilogram body weight per day, Data were mean ± SEM, The means within the respective columns followed by similar superscript were not significantly different at $p \leq 0.05$, Analyzed by ANOVA followed by Tukey's post hoc test for multiple comparison, a, b, *, $p < 0.05$ compared with HFD.

and allowed for 60 sec to find the platform. This mouse was acquired 5 cycles with 5 min interval per day. These 5 cycles were continued for 4 consecutive days. For the probe trial, the mouse was released on the north loca-

tion only. Parameters, including swimming path length, escape latency, and the number of entries were recorded and measured.

Statistical analysis

Data were analyzed by using SPSS 19.0 statistical software package and expressed as means \pm standard errors. Repeated measures in the determinations of body weight, swimming path length, and escape latency, as well as 1-way between groups ANOVA for the measurement of the number of entries were performed, followed by Tukey's honest significant difference (HSD) test for post-hoc analysis when there was a significant interaction effect. Differences were accepted as statistically significant at $p < 0.05$.

Results

Effects of diets on the body weight of mice

Changes of the mice body weight were according to what the diet they consumed (Table 3). In CONTROL group, the ended bodyweight was raised by 7.44 g. The total body weight change for HFD mice was 6.16 g. Instead, HFD-GR mice exhibited the body weight change by 8.24 g. Although increased body weight of mice in a particular group could be observed, there was no significant difference of this parameter among the three animal groups at the final.

The spatial learning and memory of mice

Results of spatial learning and memory according to the MWM test of mice fed with different diets were shown in Figure 2. Escape latency is defined as time duration an animal used to find the platform, and path length is total distance traveled by an animal from a starting site to platform location. In Figure 2a and Figure 2b, mice in all groups showed decrease of escape latency and path length on every next consecutive day during the acquisition trial. It was significant in reducing path length for the HFD-GR mice on day 4 in compared to day 1. Mice of HFD group presented the highest escape latency and the longest path length although not significantly different among other two groups. The sums of escape latency and path length in the acquisition trial for mice of HFD group were thus highest. For the probe trial, mice in HFD groups significantly indicated the lowest number of entries to platform location. In contrast, the HFD-GR mice demonstrated the highest number of entries to the target quadrant (Figure 2c).

Discussion

In this project, data obtained from male C57BL/6J mice consuming normal diet, HFD, or HFD plus 0.25% of germinated Sang-Yod rice per kilogram body weight per day were described. It was possible to consume this rice as a weight control diet, because changes of the body weight were not significantly varied by feeding mice with diets that differed in fat contents (Table 3). In addition, experiments were designed to evaluate if this germinated rice could alter cognitive ability *in vivo* by using the Morris water maze (MWM) test. This behavioral tool is versatile for testing cognitive processes

such as spatial learning and memory in laboratory rodent models [6]. Whether intervention by 0.25% germinated Sang-Yod rice per kilogram body weight per day could counteract cognitive detracting effect of HFD was examined. We found that the escape latency and path length performed by HFD mice was longer (Figure 2a and Figure 2b), and the number of entries in the target quadrant was lower (Figure 2c) than those of CONTROL mice, consisting with previous report [7]. Thus, spatial learning and memory of HFD mice was reduced following at least 12 weeks of high fat diet access, suggesting a consequence of HFD-induced impairment on the hippocampus [8]. Partial recovery of the cognitive dysfunctions was observed when the diet high in fat was supplemented with 0.25% germinated Sang-Yod rice per kilogram body weight per day, resulting in a shorter escape latency and path length and greater numbers in the target quadrant of HFD-GR mice in compared to HFD mice. It could say that mice of all three groups were able to learn the location of the hidden platform as evidenced by the decrease of latency and distance traveled to reach the platform. This was true, in particular, for the mice being intervened by 0.25% germinated Sang-Yod rice per kilogram body weight per day. Data from the probe trial additionally indicated that the group of HFD-GR mice exhibited the most preference to the target quadrant (Figure 2c). Recently, the mechanisms by which germinated Sang-Yod rice influences cognitive functions are not yet clear, but may be attributed to properties such as anti-oxidation of anthocyanins present in the rice pericarp [9] and anti-inflammation of GABA as stimulated to produce by germination [10,11]. However, further investigations are required to determine the localized effects of germinated Sang-Yod rice in the brain when it is regularly consumed to mediate changes of cognitive performance.

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Statement of Equal Authors' Contribution

All authors contributed equally.

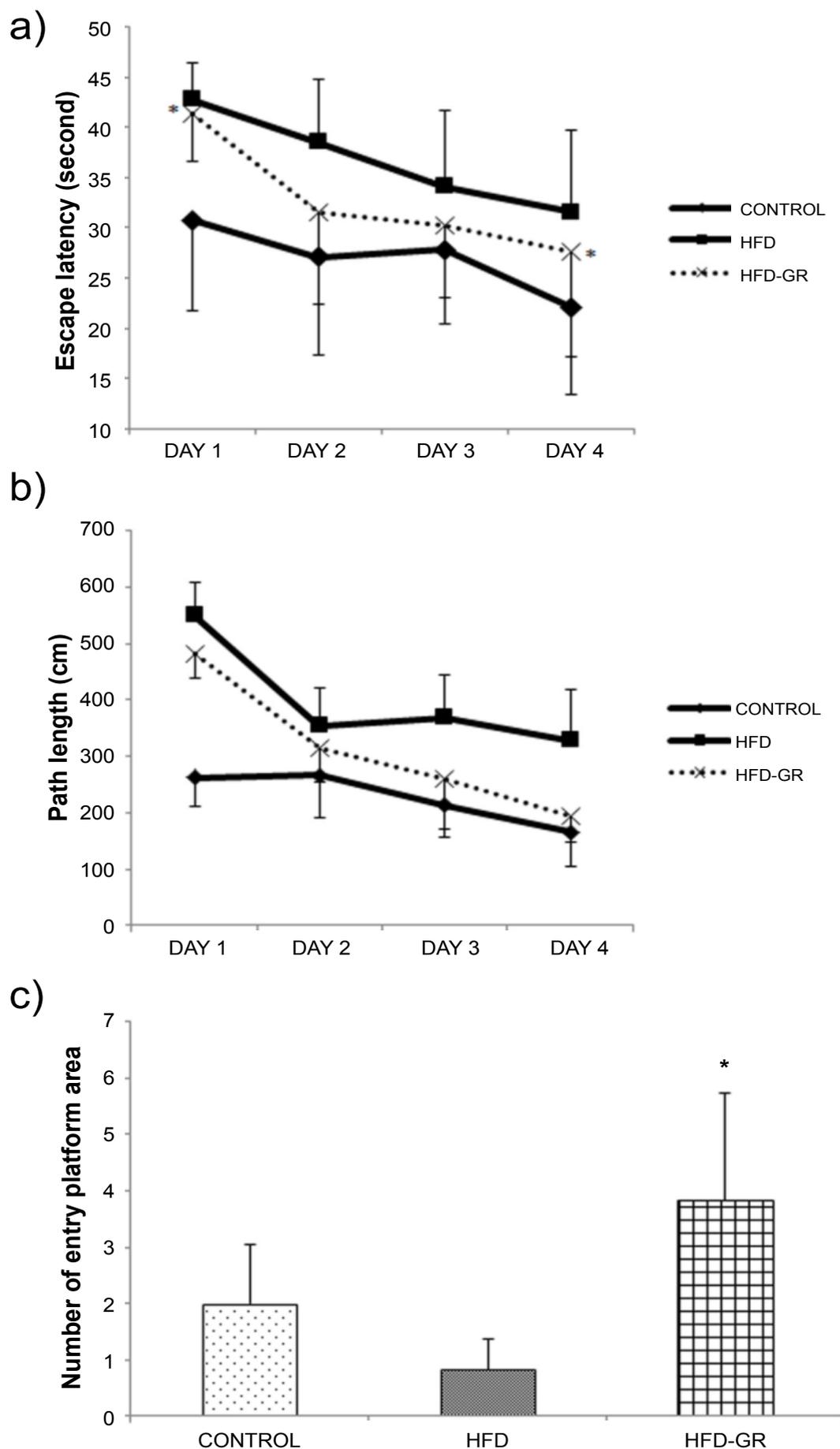


Figure 2: a) Means \pm SEM of escape latency for 4 days of the acquisition trial; b) Means \pm SEM of path length for 4 days of the acquisition trial; c) Means \pm SEM of the number of entries to the platform arena of the probe trial.

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