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ANALYSIS OF SPATIAL WORKING MEMORY USING THE Y-MAZE ON RODENTS TREATED WITH HIGH-CALORIE DIET AND MODERATE-INTENSITY EXERCISE

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ABSTRACT

Spatial working memory (SWM) in humans and animal models with impaired cognitive functions has been analyzed through a number of methods. However, this is still understudied in animal models treated with a high-calorie diet (HCD) and moderate-intensity exercise (MIE). The Y-maze was utilized as the assessment method in this study. A 40 x 9 x 9 cm^3 Y-maze was employed to observe the animal models' spontaneous alternation (SA) as the representation of their SWM. This was done by calculating the total alternation percentage divided by total entry minus two. A total of 17 female *Mus musculus* mice aged 8 weeks were tested in the Y-maze to investigate their SWM using SA calculations. Each mouse was analyzed for eight minutes and recorded in a dark and quiet room to minimize bias due to environmental noise and lighting. Comparing the treatment group's (HCD+MIE) SA to the control group's SA revealed no statistically significant difference (p=0.451). Seven mice in the treatment group performed similarly to the mice in the control group in the Y-maze test, with no significant difference in their ability to complete the task. The mice in the treatment group exhibited no motor impairment, as indicated by complete movements of all their extremities while exploring the Y-maze within the allotted time. In conclusion, the Y-maze can be used as a reliable method to analyze SWM in overweight/obese *Mus musculus* animal models treated with moderate-intensity physical exercise.

Keywords: Spatial working memory; Y-maze; cognition; mice; obesity

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Highlights:

1. This article reports the use of the Y-maze as a simple yet effective method to measure spatial working memory in mice.
2. The Y-maze method can be used safely without exposing the animals to additional stressors, as evidenced by the absence of mortality following the test.

INTRODUCTION

The global death and disability-adjusted life years attributable to obesity have increased, indicating that it continues to be a major health concern (Dai et al. 2020). Obesity caused by a high-calorie diet (HCD) has been associated with early cognitive decline (i.e., early-onset Alzheimer's disease with unclear pathophysiology). Neurodegeneration processes, including neuroinflammation, synaptic disruption, and neuronal cell death have been observed to be similar to those observed in Alzheimer's disease (AD) patients who are typically over 65 years old (Lam et al. 2013, Tellechea et al. 2018, Mendez 2019, Eugenia et al. 2022). Reportedly, certain physical exercise regimens, including moderate-intensity exercise (MIE), aid in the recovery of impaired tissue and cells caused by HCD-related obesity (Dye et al. 2017, Tellechea et al. 2018, Coll-Padrós et al. 2019). Physical exercise in general also has a positive influence on subjects with stroke and cognitive function impairment (Aisyah et al. 2020).

Spatial working memory (SWM) is a type of neurocognitive function that correlates with the hippocampal and prefrontal cortex nerve pathways. Neuronal proliferation and apoptosis have been reported to be significantly affected by exercise in obese rodents (Laing et al. 2016, Wirt & Hyman 2017). Overweight and obese individuals have been reported to suffer from cognitive impairment with varying degrees and morphological changes related to cognitive function (Cook et al. 2017, Sack et al. 2017, Meo et al. 2019). In general, HCD-treated animal models exhibited the pathophysiology of overweight and obesity. It has been reported that moderate-intensity exercise (MIE) mitigated the adverse effects of HCD, and that it improved cognitive functions (Rebelo et al. 2020, de Sousa et al. 2021). Several studies reported the use of Y-maze as a tool to analyze the SWM in various animal models (Woo et al. 2018, Faradila et al. 2020). The aim of this study was to investigate if the Y-maze is reliable as a tool to measure SWM in overweight and/ or obese animal models treated with MIE, since this has not been widely explored in a lot of other studies.

MATERIALS AND METHODS

Seventeen female *Mus musculus* mice, aged 8 weeks, were tested using the Y-maze (Figure 1) to study the SWM using the spontaneous alternation (SA) calculation. Each mouse was analyzed for eight minutes and recorded in a dark and quiet room to reduce the influence of environmental noise and lighting on the nocturnal animals. The SA of the treatment group (HCD+MIE) (n=7) was then compared to those of the control group (n=10). Similar to a study by Herawati et al. (2020) and Kumalasari et al. (2021), the treatment group received 0.013 g/g body weight of 40% dextrose (D40) as the HCD. As suggested by Rahayu et al. (2021), the MIE for the treatment group consisted of swimming three times per week with a 6% body weight tail-attached load, and the duration was increased every week (5 m in week-1, 8 m in week-2, 11 m in week-3, 15 m in week-4). The body weight (g) of each mouse was measured using a digital scale (Idealife, Indonesia) prior to the experiment and after four weeks of treatment.

The Y-maze used was a black triangular maze made of polyvinyl chloride (PVC). As shown in Figure 1, the maze consisted of three arms (A, B, and C), each measuring 9 cm in width, 9 cm in height, and 40 cm in length. Prior to the experimentation, the mice were acclimatized in the laboratory for at least seven days. The Y-maze had to be cleaned using 70% alcohol and paper towels to ensure the absence of an odorous smell that might interfere with the experiment. After the maze was completely dry, the camera (Oppo, China) was placed above the maze in a way that all of its arms were fully recorded.

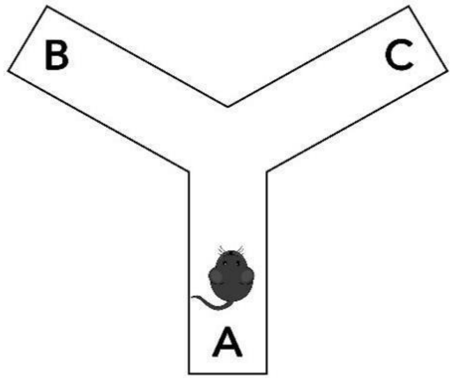

Figure 1. The Y-maze used in this study, with a dimension of 40 x 9 x 9 cm^3 and 120° between the two legs, made of polyvinyl chloride (PVC) and assembled using PVC glue.

The experiment was conducted in an environment that simulated the nighttime. All materials were prepared before the experimentation. After the Y-maze settings had been completed, the mice were placed on arm A. The recording began simultaneously with the placement of the mice. The mice were allowed to move freely in all of the arms for 8 minutes. Afterwards, the recording was stopped and the mice were taken out from the maze. The maze should also be wiped down with 70% alcohol and paper towels after each task (Kraeuter et al 2019). The total entry and total alternation were counted from the recordings. Each time all four legs of the mouse completely entered an arm of the Y-maze, it was considered an entry. If the mouse could enter all three arms (A, B, and C) consecutively, it was considered an alternation. Spontaneous alternation (SA) is an indicator of spatial working memory that is calculated by dividing actual alternation (total alternation) by total alternation possibilities (total entry minus two) multiplied by 100 percent, as shown in the equation (Kraeuter et al. 2019).

$$Spontaneous\ alternation = \frac{Total\ alternation}{Total\ entry - 2} \times 100\%$$

The data from the observation were presented as mean±SD. Normality test and homogeneity test using Shapiro-Wilk and Levene's tests, respectively, were performed prior to the independent t-test to compare the SA between the two groups, where a p-value<0.05 was considered significant. The statistical analysis was performed with the help of SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, Ill., USA).

RESULTS

Table 1 displays the characteristics of each group's body weight. Although not statistically significant (p=0.957), the treatment group had a higher body weight than the control group prior to the experiment. After four weeks, the average body weight of the treatment group was lower when compared to those of the control group (p=0.379).

Table 1. Average body weight of treatment and control groups showed paradoxical modulation during the four-week experiment.

	Mean±SD		
Body weight	Control group (n=10)	Treatment group (n=7)	p-value
Pre (g)	23.29±0.81	24.00±0.95	0.957
Post (g)	23.80±0.74	22.75±1.18	0.379

Seven mice in the treatment group performed in the Y-maze test with results comparable to those of the control group. All animals showed no significant difference in their ability to complete the task. Comparing the treatment group to the control group, no indication of motor impairment was observed. All extremities of the mice exhibited complete movements when exploring the arms of the Y-maze in the allotted time.

Table 2. Comparison of spontaneous alternation (SA) between control and treatment groups.

Subject	Spontaneous Alternation (%)	
	Control Group (n=10)	Treatment Group (n=7)
Animal 1	60.92	62.04
Animal 2	68.29	70.59
Animal 3	73.68	56.21
Animal 4	67.27	67.9
Animal 5	51.25	71.43
Animal 6	55.81	64.52
Animal 7	66.23	54.1
Animal 8	79.31	-
Animal 9	53.85	-
Animal 10	66.67	-
Mean±SD	64.33±8.89	63.83±6.79
p-value between the groups’standard deviation (SD)	0.451	

The mean of the SA was 63.83±6.79 (%) in the treatment group and 64.33±8.89 (%) in the control group. The values of the treatment and control groups’ SA were not significantly different (p=0.451), as shown in Table 2.

DISCUSSION

This study reveals that the treatment group experienced a decrease in body weight, while the control group exhibited a contrast pattern. Although the difference in body weight between these two groups before and after the experiment was not statistically significant, the decrease pattern in the treatment group might indicate a response to the physical stressors compared to the control group. In overweight and obese animal models, such as those treated with HCD, physical exercise may improve altered metabolic and cognitive functions. A study on rodents treated with a high-glucose diet and physical exercises reported improved long-term memory and glucose tolerance. Other research reported that the brain cortical capillary volume and surface area of rodents with a high-fructose diet and physical exercise were significantly greater than those of the control group (Wang et al. 2015, Rebelo et al. 2020).

By calculating the SA in animal models treated with HCD and MIE, the Y-maze proved to be a reliable tool for measuring SWM in this study. Comparable SA between treated and control groups demonstrated this. MIE can exert neuroprotective properties by preserving cellular function, possessing anti-inflammatory properties, and enhancing the release of neurotrophic factor, although this may be influenced by the type, duration, and intensity of the physical exercise (Kim et al. 2019, Cerqueira et al. 2020).

Dopamine plays an important role in the modulation of spatial working memory. Environmental stimulation induces the firing rate of dopaminergic neurons in the ventral tegmental area, resulting in dopamine release (Bäckman et al. 2017). Increased dopamine will activate neurons in the prefrontal cortex. These neurons will process the information collected from the cortex in the form of working memory. Several forms of dopamine exist, including dopamine-1 (D1) and dopamine-2 (D2), which have dynamics related to the decision-making process. The activation of D1 receptors will be enhanced with Ca^{2+}, K^{+}, and Na^{+}, while the activation of D2 receptors decreases with L-type Ca^{2+} through induction after depolarization. This mechanism was found to be the basis of spatial working memory, in which hippocampus-processed spatial memory undergoes additional analysis to form new memories (Puig et al. 2014).

There are various methods to assess the working memory in mice other than the Y-maze, such as the Morris water maze (Maramis et al. 2021). Xu et al. (2015) reported the use of the Morris water maze to study spatial learning and memory in HCD-induced obese mice. Another study by Tian et al. (2017) reported the use of T-maze in post-surgery mice as a simple behavioral task to assess spatial working memory. The Y-maze had been utilized as a method that can be constructed by researchers with simple materials, but when used correctly, could be used to analyze spatial working memory. The interpretation of spontaneous alternation as an indicator of spatial working memory is based on the instinct of a mouse to explore new territory. A higher alternation represented superior performance in spatial working memory as it implied that the mouse remembered the arm it entered (Heredia-López et al. 2016, Kraeuter et al. 2019).

Strength and limitations

In this study, other biomarkers from the research animals were not analyzed. However, this study can help to determine the Y-maze as a tool for measuring SWM in overweight and/ or obese animal models treated with MIE, which has not been fully reported previously.

CONCLUSION

Y-maze can be used to analyze the spatial working memory in *Mus musculus* as the animal models in an experiment. More precisely, this model has proven effective in experiments in which physical exercise is applied to treat overweight and/ or obese *Mus musculus*. The Y-maze method offers a simple and effective, yet safe way of studying this variable, thus helping further studies in elucidating neurocognitive impairment prevention and management.

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Conflict of interest

None.

Ethical consideration

Ethical clearance of this study was granted by the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (no. 259/EC/KEPK/FKUA/2021 on 22/11/2021).

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Author contribution

RRM collected, analyzed, and interpreted the data, as well as drafted, revised, and approved the final manuscript. VPK conceptualized the study design, analyzed and interpreted the data, as well as drafted, revised, and approved the final manuscript. LH conceptualized the study design, interpreted the data, as well as revised and approved the final manuscript. YS drafted, revised, and approved the final manuscript. ZO checked and approved the final manuscript.

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