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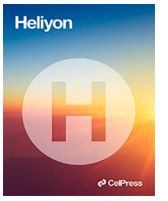

Research article

FNDC5/irisin mediates the protective effects of Innovative theta-shaking exercise on mouse memory

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ABSTRACT

As a passive motion and non-invasive treatment, theta-shaking exercise is considered an alternative to traditional active exercise for slowing down brain ageing. Here, we studied the influence of theta-shaking exercise on fibronectin type III domain containing 5/irisin (FNDC5/irisin) in the anterior nucleus of the thalamus, hippocampus, and medial prefrontal cortex (ATN–HPC–MPFC). Further, we assessed memory in senescence-accelerated prone mice (SAMP-10 mice) using a behavioural test to confirm the protective effect of theta-shaking exercise against age-related memory decline. SAMP-10 mice were subjected to theta-shaking exercise for 9–30 weeks. Mice then performed the T-maze test and passive avoidance task. Immunohistochemical analysis and ELISA were used to assess FNDC5/irisin, nerve growth factor (NGF), and neurotrophin 4/5 (NT4/5) expression in the ATN–HPC–MPFC. In the shaking group, FNDC5 was locally upregulated within the hippocampus and MPFC area rather than exhibiting even distribution throughout brain tissue. Irisin levels were generally higher in the control group. Meanwhile, hippocampal NGF levels were significantly higher in the shaking group, with no differences noted in neurotrophin levels. Theta-shaking preserved normal neurons in certain sub-regions. However, no beneficial changes in neuronal density were noted in the ATN. Theta-shaking exercise positively affects memory function in SAMP-10 mice. FNDC5 upregulation and higher levels of NGF, along with the potential involvement of irisin, may have contributed to the preservation of normal neuronal density in the hippocampus and MPFC subregions.

1. Introduction

Neuronal network fidelity within and between brain regions is disturbed during brain ageing, manifesting as mild short-term

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Abbreviations

ANOVA	Analyses of Variance
ATN	Anterior Thalamic Nuclei
CA1	Cornu Ammonis Field 1
CA3	Cornu Ammonis Field 3
DG	Dentate Gyrus
DR	Dorsal Rostral
FNDC5	Fibronectin Type III Domain Containing 5
HPC	Hippocampus
MPFC	Medial Prefrontal Cortex
NGF	Nerve Growth Factor
NT4/5	Neurotrophin 4/5
SAMP-10 mice:	Senescence-Accelerated Prone Mouse
VR	Ventral Rostral

memory impairment that inevitably progresses to severe deficits in most cognitive domains. Exercise plays an important role in preventing age-related memory impairment [1].

Mounting evidence suggests that anterior thalamic nuclei (ATN) projections exhibit substantial distal influence on cortical and hippocampal interactions [2,3]. ATN participate in memory function, particularly in encoding and retrieval [4,5]. The hippocampus (HPC) is central to memory formation [6]. Exercise increases neurogenesis and synaptic plasticity in the hippocampus to preserve memory [7–9]. Moreover, the medial prefrontal cortex (MPFC) [10], through its complex interconnectivity with subcortical regions, including the thalamus and hippocampus [11], is believed to be involved in memory consolidation [12]. Physical exercise can have beneficial effects on MPFC, improving cognitive function and overall brain health [13,14]. Exercise-induced [15] myokine fibronectin type III domain containing 5/irisin (FNDC5/irisin) can enhance neurotrophin expression in the hippocampus and MPFC [16], which is crucial for neuronal growth, differentiation, and survival associated with memory. Long-term exercise can increase neurotrophin expression in the brain, particularly in the hippocampus and MPFC [17]. Although brain health can be bolstered by exercise, traditional exercise interventions may have a limited effect on older adults [18].

Accordingly, we are committed to developing a shaking simulation exercise [19,20] as a passive motion and non-invasive treatment to prevent brain ageing. Accumulating data suggest that shaking exercise can reduce the accumulation of non-functional proteins [21,22] and support receptor function, lowering the risk of memory decline [23]. Recently, theta peripheral stimulation was proposed as a new approach that effectively restores theta rhythm [24,25]. While the role of exercise in memory enhancement and the involvement of neurotrophins have been investigated previously, the unique effects of theta-shaking exercise on FNDC5/irisin in specific brain regions are unknown. Accordingly, we hypothesised that theta-shaking slows the decline in ATN–HPC–MPFC circuit function to preserve memory. To test this hypothesis, we evaluated the influence of theta-shaking exercise on FNDC5/irisin in ATN–HPC–MPFC and assessed memory using a behavioural test in senescence-accelerated prone mice (SAMP-10 mice).

2. Materials and methods

2.1. Animals

All animal experiments were performed in accordance with the guidelines of the National Institutes of Health (Bethesda, MD, USA). The study protocol was approved by the Institutional Animal Care and Use Committee of Fujita Health University (Toyoake, Japan; approval number: APU19120). Eight-week-old male SAMP-10 (TaSlc; SLC, Shizuoka, Japan) were used. The SAMP-10 mice were randomly assigned to one of the two groups: shaking (n = 14) or control (n = 14).

2.2. Exercise protocol and theta-shaking exercise

The shaking exercises began at 9 weeks and were terminated at 30 weeks (Fig. 1). The theta-shaking group exercised on a shaking machine (NR-3; TAITEC Co. Ltd., Saitama, Japan) with a horizontal rotation movement (movement distance: 50 mm, 5 times/s) on a

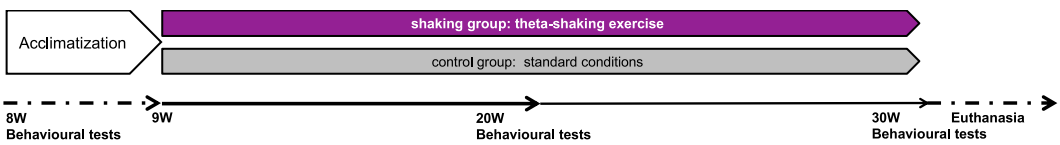

Fig. 1. Study timeline. All the mice were acclimated for 1 week. The theta-shaking exercise was initiated when the mice were 9 weeks old. Behavioural tests were performed at 8, 20, and 30 weeks. Brain tissue was obtained after the last behavioural test.

variable axis uniformly applied in all directions at 360°. The 14 mice were placed in separate spaces on the shaking machine. They remained separated during shaking without interfering with each other. The shaking exercise was applied for 30 min/day, 3 times/week for 21 consecutive weeks.

2.3. Behavioural tests

2.3.1. T-maze test

Short-term memory was assessed via the spontaneous alternation T-maze test ([width] 300 mm × [diameter] 60 mm × [height] 150 mm; LE843D-S, BRC Co., Nagoya, Japan) in 8-week (8W), 20-week (20W), and 30-week (30W) old mice. The mice had free access to food and water. No pretraining was performed before formal testing. A mouse began at the base of the T and selected one of the goal arms, abutting the other end of the stem, where it was confined for 30 s before returning to the starting arm and being confined for 5 s. Subsequently, the mice selected an arm again to reflect their memory of the first choice. A score of 0 was assigned if the mice selected the same arm, and a score of 1 was assigned if they alternated arms. The time required to make the choice was also recorded. This procedure was based on the natural preference of rodents toward exploring a novel arm over a familiar one, causing them to alternate the goal arm over repeated trials.

2.3.2. Passive avoidance task

The passive avoidance task (STC-001 M and SGS-003DX; Muromachi Kikai Co., Ltd., Tokyo, Japan) was performed according to a standard protocol. Each mouse was placed in an illuminated space on Day 1 (memory acquisition). After 1 min, the sliding door was opened, and access to a closed dark compartment was provided via a narrow hole. Following their first encounter with the open door, the latency to enter the dark compartment was measured. When all four paws entered the dark room, the door was closed, and a foot shock (3 s, 0.25 mA) was delivered. On the next day (memory regeneration), each mouse was placed in the illuminated room. The latency before entering the dark room (up to 300 s) was measured.

2.4. Tissue preparation

After completing the final behavioural test, all mice underwent thoracotomy under general anaesthesia (isoflurane), followed by perfusion with phosphate-buffered saline and subsequent removal of the brain. Tissues intended for biochemical analysis were temporarily stored at −80 °C. Tissues designated for histological analysis were fixed in 10 % neutral-buffered formalin and embedded in paraffin using an automated embedding device. From each group of 14 mice, seven were used for immunohistochemistry and seven for ELISA.

2.5. Immunohistochemistry

Sagittal brain sections (20 μm), including the central zone of the ATN, HPC, and MPFC (interaural coordinates = 1.00 mm), were obtained using a microtome. The sections were first treated with an anti-FNDC5 antibody (ab181884; 1:100; Abcam PLC, Cambridge, UK) and then with a biotin-conjugated goat anti-rabbit IgG (H + L) secondary antibody (BA-1000; 1:1000; Vector Laboratories, Burlingame, CA, USA). An enzyme substrate solution (PK-6100; VECTASTAIN Elite ABC Standard Kit; Vector Laboratories) and diaminobenzidine (cat. no. 40651; Muto Pure Chemicals Co., Ltd., Tokyo, Japan) were used for colorimetric detection. The tissue processing and immunohistochemical steps were performed according to standard protocols.

During ATN observation, the hippocampus was divided into seven portions: dentate gyrus (upstream DG and downstream DG), cornu ammonis field 3 (proximal CA3 and distal CA3), cornu ammonis field 1 (proximal CA1 and distal CA1), and subiculum. The

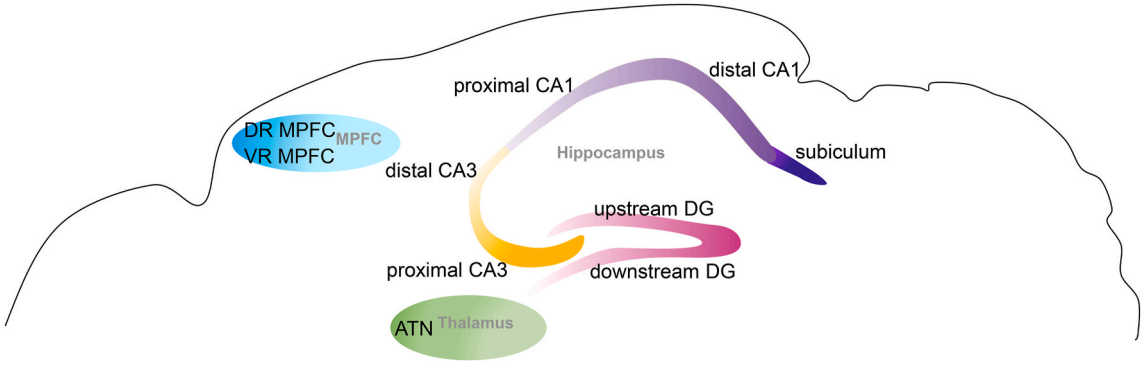

Fig. 2. ATN–HPC–MPFC diagram. The anterior nucleus of the dorsal thalamus is also observed. The hippocampus is divided into seven parts: the dentate gyrus (DG: upstream and downstream), cornu ammonis field 3 (CA3: proximal and distal), cornu ammonis field 1 (CA1: proximal and middle distal), and the subiculum. The medial prefrontal cortex (MPFC) is divided into two parts (the dorsal and ventral rostral). ATN, anterior thalamic nuclei, DG, dentate gyrus; CA3, cornu ammonis field 3; CA1, cornu ammonis field1; DR, dorsal rostral; VR, ventral rostra.

rostral MPFC was classified as the dorsal rostral (DR MPFC) and ventral rostral (VR MPFC) [26] (Fig. 2). ATN–HPC–MPFC neurons were manually counted and quantified using ImageJ software (1.52a, National Institutes of Health, Bethesda, MD, USA) [27,28]. ImageJ software was also used to assess the proportion of FNDC5-positive neurons (%), abnormal neurons (n), and the total number of neurons (n).

2.6. ELISA

For the analysis of wide-area brain tissue (excluding the hippocampus) and hippocampus tissue (monomer), samples were immersed in liquid nitrogen, crushed in a mortar, and subjected to tissue lysis using a protein extraction buffer (RIPA Lysis and Extraction Buffer; Thermo Fisher Scientific, Waltham, Massachusetts, USA) supplemented with a protease inhibitor cocktail (Sigma-Aldrich, Milan, Italy). The tissue lysate was centrifuged, and the supernatants were collected for analysis. Following measurement and adjustment of protein concentration to a standardised level, quantification of irisin, NGF, and NT4/5 protein levels was carried out using a commercially available Irisin-ELISA Kit (BP3-08118; Novus Biologicals, LLC., Minnesota, USA) and Neurotrophin-ELISA Kit (BEK-2231; Biosensis Pty Ltd., Thebarton, AUS). Sample dilution optimisation was performed; all procedures were conducted following the manufacturer's instructions.

2.7. Statistical analysis

The T-maze tests were analysed using Pearson's chi-squared test to compare groups. To assess the latency of the passive avoidance task and T-maze tests, repeated measures analysis of variance (ANOVA) followed by Bonferroni post-hoc tests were employed. Immunohistochemistry and ELISA data were analysed using independent *t*-tests for normally distributed data or Mann-Whitney U-tests for data that were not normally distributed. Data were presented as the mean ± standard error of the mean (SEM); P-values <0.05 indicated statistical significance. SPSS software (version 25.0; SPSS, Chicago, IL, USA) was used for all statistical analyses.

3. Results

3.1. T-maze

In the T-maze test, the accuracy rate of the control group was lower than that of the shaking group, with no significant advantage in memory of the first choice for either group at each test node. However, at 30W, the shaking group approached significance, suggesting a weak difference (Table 1): 8W [χ^2 (1) = 0.243, p = 0.622, φ = 0.093], 20W [χ^2 (1) = 1.474, p = 0.225, φ = −0.229] and 30W [χ^2 (1) = 3.743, p = 0.053, φ = −0.366]. A significant difference was observed in the time spent between the shaking and control groups at 20W (p = 0.017). The theta-shaking group mice were less hesitant in selecting a direction, which may reflect better short-term memory and faster decision-making (Fig. 3).

3.2. Passive avoidance task

On the first day of the passive avoidance task (memory acquisition), all mice entered the dark room based on instinct without hesitation, with no significant difference between the two groups (16.857 ± 1.901 s vs. 23.143 ± 1.481 s, t (16.14) = 0.68, n.s.). However, on the next day (memory regeneration), some mice restlessly explored the border, walked back and forth, probed the situation in the dark room with one or sometimes both forepaws and repeatedly stuck out their heads sniffing the air. They learned to avoid stepping through borders. After initial hesitation, some mice crossed the border into the dark room. Two-way ANOVA (Fig. 4) of the shaking and control groups showed a significant effect (week: F (2,52) = 10.789, p < 0.001; group × week: F (2,52) = 0.806, p = 0.419). Both groups showed age-related long-term memory decline. For 8W and 30W mice, the control group (p < 0.001) had a shorter latency than the shaking group (p = 0.027). Following theta-shaking, the latency of the shaking group was significantly longer than the control group (p = 0.001). Further, the latencies in the shaking group at 20W (p = 0.018) and 30W (p = 0.024) were significantly longer than that at 8W.

Table 1
Alternate choice results of the T-maze test.

Week	Group	Incorrect	Correct	Total	Incorrect (%)	Correct (%)	Total (%)
8	Control	2	12	14	14.30 %	85.70 %	100.00 %
	Shaking	3	11	14	21.40 %	78.60 %	100.00 %
20	Control	6	8	14	42.90 %	57.10 %	100.00 %
	Shaking	3	11	14	21.40 %	78.60 %	100.00 %
30	Control	8	6	14	57.10 %	42.90 %	100.00 %
	Shaking	3	11	14	21.40 %	78.60 %	100.00 %
Total	Control	16	26	14	38.10 %	61.90 %	100.00 %
	Shaking	9	33	14	21.40 %	78.60 %	100.00 %

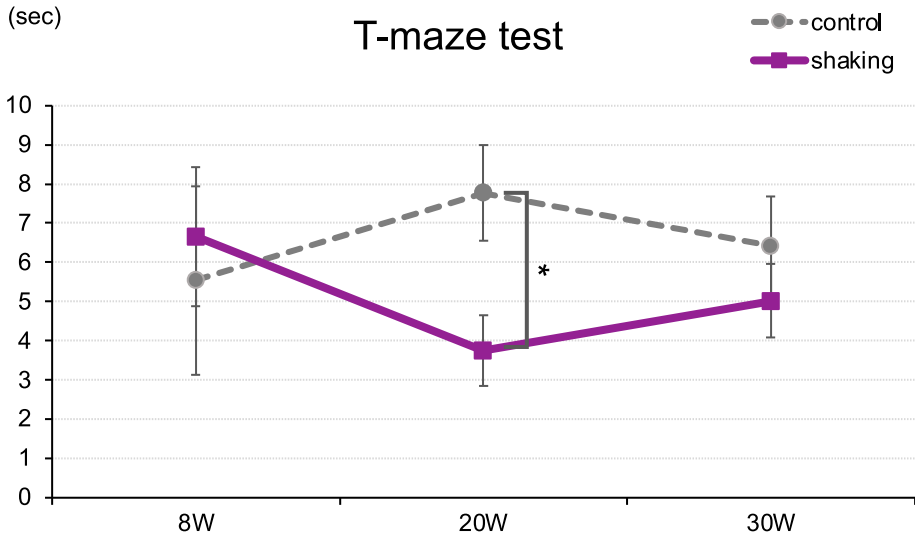

Fig. 3. Short-term memory assessment through a spontaneous alternation T-maze test. The shaking group (20W) differed significantly from the control. No significant differences were observed at 30W. Grey line: control group, purple line: theta-shaking group (*p < 0.05 and **p < 0.01).

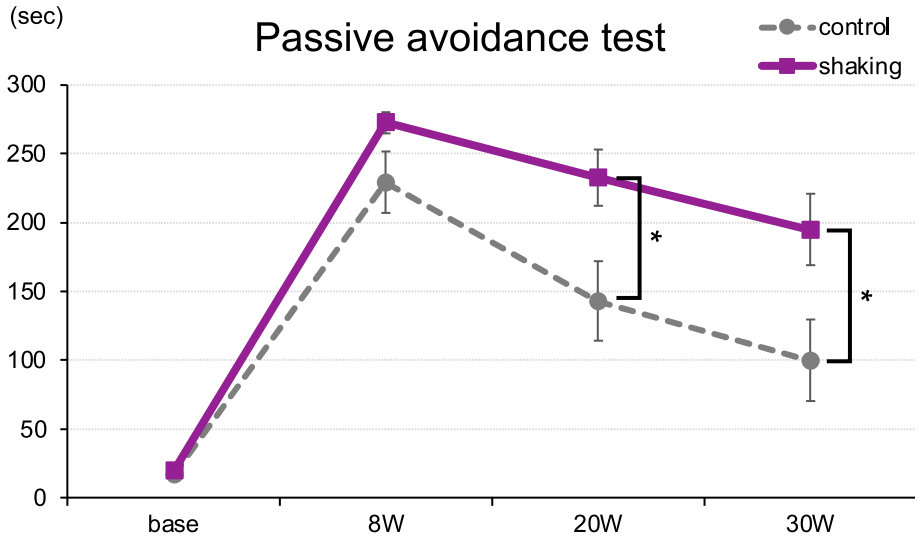

Fig. 4. Passive avoidance task. Both groups exhibited age-related long-term memory decline. Grey line: control group, purple line: theta-shaking group (*p < 0.05 and **p < 0.01).

3.3. Histomorphological effects

3.3.1. FNDC5-positive neurons

FNDC5 expression was analysed in the relevant brain regions (Fig. 5A–a, B). Significant differences were detected in the distal CA1 ($p = 0.017$), subiculum ($p = 0.026$), DR MPFC ($p = 0.004$), and VR MPFC [$t(12) = -3.785$, $p = 0.003$]. However, no significant differences were observed in the ATN subregion, DG region, CA3 region, and proximal CA1. When compared to control rats, those from the shaking group exhibited more FNDC5-positive neurons in the distal CA1 and subiculum subregions as well as in the rostral MPFC.

3.3.2. Abnormal neurons

Compared to the shaking group, abnormal neurons were significantly enriched in the following subregions in controls (Fig. 5A–b, B): upstream DG [$t(12) = 3.312$, $p = 0.006$], proximal CA3 [$t(12) = 2.585$, $p = 0.024$], distal CA1 [$p = 0.004$] and DR MPFC [$p = 0.001$]. Further, a significant trend was observed in the downstream DG [$t(12) = 2.137$, $p = 0.054$] and VR MPFC [$p = 0.053$]. The ATN, distal CA3, proximal CA1, and subiculum subregions did not exhibit significant differences in abnormal neuron counts. Taken together, shaking exercise preserved normal neurons in the DG, proximal CA3, distal CA1, and MPFC subregions.

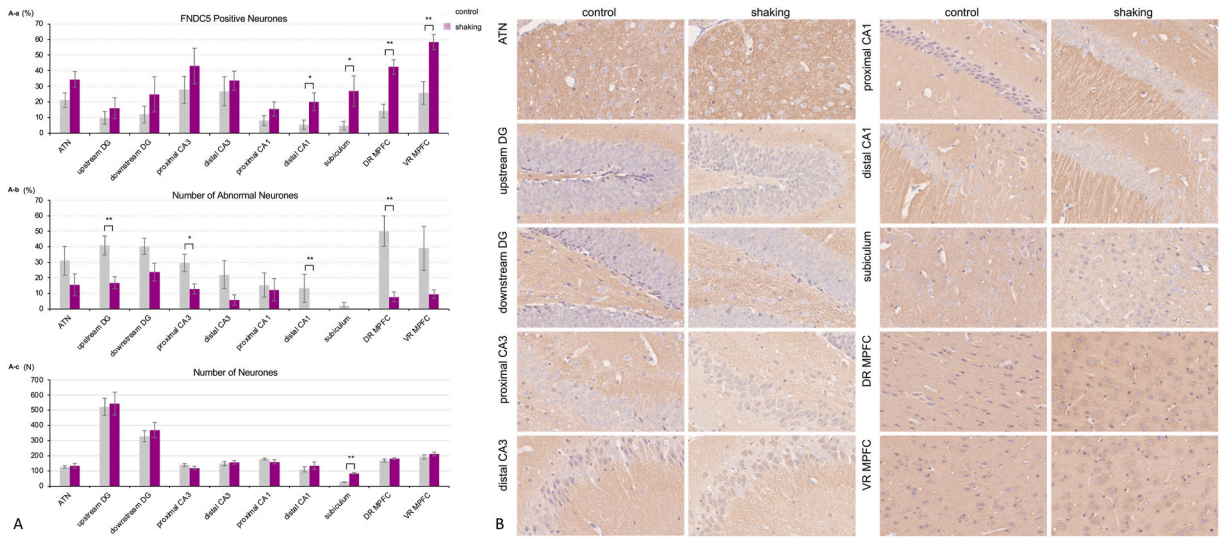

Fig. 5. Histomorphological analysis of the ATN–HPC–MPFC circuit. (A) Quantification of FNDC5 staining in the ATN–HPC–MPFC circuit. (A-a) FNDC5-positive neurons are present in sagittal brain sections. (A-b) Abnormal neuron counts in sagittal brain sections. (A-c) Neuron counts in sagittal brain sections. Grey bars: control group, purple bars: theta-shaking group (*$p < 0.05$ and **$p < 0.01$; paired Student's *t*-test; two-sided). (B) Photomicrographs of FNDC5 staining in the ATN–HPC–MPFC circuit. Neuronal morphology, staining patterns, nucleus positioning, and structural integrity were assessed to identify and characterise abnormal neurons. ATN: anterior thalamic nuclei; HPC: hippocampus; MPFC: medial prefrontal cortex; FNDC5: fibronectin type III domain containing 5; DG: dentate gyrus; CA3: cornu ammonis field 3; CA1: cornu ammonis field 1; DR: dorsal rostral; VR: ventral rostral.

3.3.3. *Number of neurons*

Assessment of neuron counts revealed a significant difference between the two groups in the subiculum subregions [t (8.781) = −14.345, $p < 0.01$]; Fig. 5A–c, B). No significant differences were detected between conditions in the ATN, DG, CA3, CA1, and MPFC regions. Neuronal density in the subiculum was higher in the shaking group than in the controls.

3.4. *Effects of theta-shaking on the levels of neurotrophic factors*

Significantly higher irisin levels were noted in the wide-area brain ($p = 0.022$) and hippocampus ($p < 0.01$) of control mice

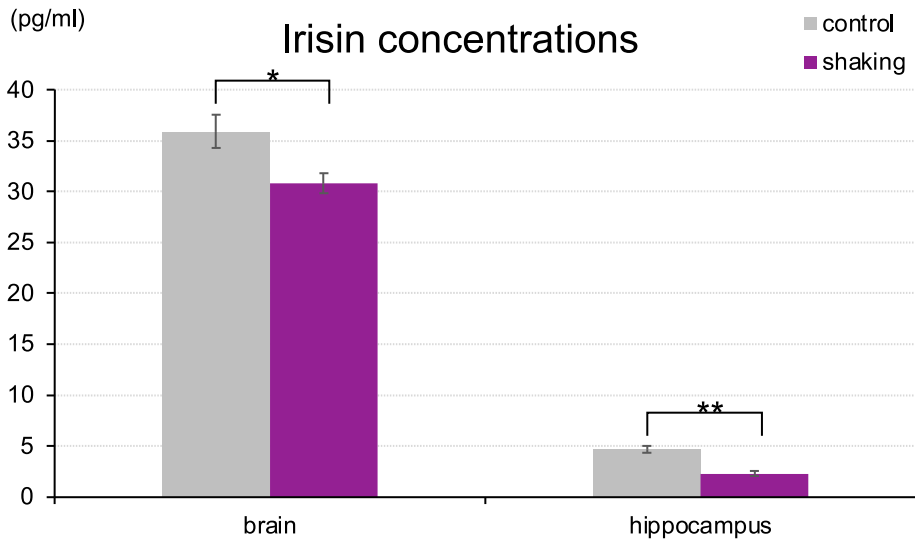

Fig. 6. Effects of theta-shaking exercise on irisin protein levels in the hippocampus and wide-area brain. A significantly higher expression was present in the wide-area brain ($p = 0.022$) and local hippocampus control group ($p < 0.01$). Grey bars: control group, purple bars: theta-shaking group. (*$p < 0.05$ and **$p < 0.01$; paired Student's *t*-test; two-sided, $n = 7$ control mice, 7 shaking mice). The experiments were repeated twice with similar results.

compared to mice in the shaking group (Fig. 6). Significantly higher NGF levels were noted in the hippocampus of shaking mice ($p = 0.038$; Fig. 7). However, no significant differences were observed in the wide-area brain. No differences in NT4/5 levels were detected in the wide-area brain or hippocampus. Our findings indicate that the theta-shaking exercise did not affect NT4/5 levels in the wide-area brain (Fig. 8).

4. Discussion

In this study, we found that mice subjected to theta-shaking exercise and control mice exhibited age-related memory decline. In the T-maze test, the effect of shaking exercise was reflected primarily at 20W, with no difference at 30W. Hence, while theta-shaking affected the short-term memory of mice, the advantage did not extend to 30W. Meanwhile, the passive avoidance test results revealed significant differences between the 20W and 30W treatments. The shaking group demonstrated an absolute advantage in long-term, rather than short-term, memory in middle- and old-age mice. It is speculated that there is a mechanism at play over a prolonged period that mediates the beneficial effects of theta-shaking on memory.

FNDC5 was found to be expressed in the mouse hippocampus and cortex. Interestingly, when compared with the control group mice, FNDC5-positive neurons were present in a specific portion of the hippocampus and MPFC of mice from the shaking group, with uneven distribution throughout the brain. FNDC5-positive neurons were particularly maintained in the distal CA1 and subiculum subregions, as well as the rostral MPFC. Recent anatomical evidence suggests a functionally significant back-projection pathway from the subiculum to CA1 [29,30]. Importantly, the functional role of this pathway has only been considered in the context of theta frequency oscillations, which temporally organise most hippocampal spiking dynamics [31]. It is hypothesised that the oscillations generated by the theta-shaking exercise target CA1, while the subiculum strictly controls the firing of projection neurons and transmits relevant information to downstream regions, such as the MPFC. Gene expression studies have revealed the role of the MPFC [32], located in the downstream region of information [33], in long-term memory [34,35]. The frontal midline theta rhythm is a distinct theta activity of the EEG in the frontal midline area that controls activity in the parietal cortex associated with memory maintenance [36]. Following theta-shaking exercise, FNDC5 was highly expressed in the MPFC, strengthening cortical theta oscillations and allowing neurons to compute and communicate top-down control in a wide range of networks. Although the ATN are a subcortical structure that mediates memory and rhythm generation [37], notable changes were not observed in the ATN in the current study. This suggests that theta-shaking exercise has a smaller impact on the ATN relative to the hippocampus and MPFC. Since ATN have unique connectivity patterns within the brain circuitry [38], they receive input from multiple brain regions and project them to various targets. The specific connections and network interactions of the ATN may influence their response to external stimuli such as theta-shaking exercise. It is therefore plausible that the theta-shaking exercise selectively influences FNDC5 expression in specific brain regions, such as the CA1, subiculum, and rostral MPFC. This regional specificity underpins the clinical relevance and efficacy of peripheral theta-shaking exercise.

The contradictory results obtained in the present study regarding FNDC5 and irisin levels in the hippocampus and brain are intriguing. Irisin expression may be more widespread in the brain relative to the localised distribution of FNDC5, resulting in lower hippocampal expression or dilution of irisin. However, quantitative ELISA failed to reflect the possible regional distribution of irisin. Moreover, we speculated that FNDC5 exhibits tissue-specific expression as well as differential release and distribution of irisin. Another possible explanation could be the dynamic regulation and kinetics of FNDC5 and irisin expression following shaking exercise. The kinetics of FNDC5 cleavage and subsequent irisin release [16,39] might have differed between the shaking and control groups. In the shaking group, more FNDC5 protein was produced locally, with the conversion of FNDC5 to irisin and its subsequent utilisation being more rapid, leading to lower detectable levels throughout the brain and in the hippocampal regions of mice in the shaking group. Conversely, irisin expression was higher in the control group in the absence of theta-shaking interference. Current research is limited to the relationship between theta oscillations and irisin levels in the brain. FNDC5/irisin may exhibit dynamic changes in response to theta-shaking with temporal variations in expression [40,41]. The observed differences in FNDC5/irisin expression may be specific to the time points assessed in this study. Examining their expression at different intervals during and after the shaking exercise could provide further insights into the temporal dynamics and potential relationships between these factors. In summary, the differences between FNDC5 and irisin levels in the hippocampus and whole brain in the shaking and control groups can be attributed to various factors, including irisin differential release and distribution patterns, dynamic regulation, and kinetics.

We noted a significant increase in NGF within the hippocampus, without changes in the whole brain of the shaking group mice. Several factors may have contributed to this. First, NGF may have a specific local role within the hippocampus. The hippocampus—a key region involved in exercise-induced neuroplasticity—exhibits higher sensitivity to NGF than other brain regions. The advantage observed in the hippocampus could be related to signalling pathways that promote cytoskeletal reorganisation as well as neuronal survival, growth, and differentiation [42]. Second, NGF regulation and expression are influenced by a complex network. Few studies have demonstrated interactions between neurotrophins NGF and FNDC5/irisin [43–45]. Our study showed that theta shaking enhanced the local expression of FNDC5, with different trends in NGF levels noted in the hippocampus. This suggests that the potential influence of FNDC5 cannot be excluded. In addition, NGF does not pass through the blood–brain barrier, further highlighting the potential of the theta-shaking intervention to cause neural recruitment, directly or indirectly inducing an increase in endogenous NGF. Thus, NGF and FNDC5/irisin have complementary and redundant roles in promoting neuronal health and memory.

Several factors could potentially contribute to the absence of changes in NT4/5 levels in response to shaking. First, it is important to consider the study timeframe. The experimental protocol involved the administration of shaking exercises for 9–30 weeks. Hence, the duration or frequency of the shaking exercise might have been insufficient to induce significant alterations in NT4/5 expression. Neurotrophins exhibit complex temporal and spatial patterns of expression, and their levels may vary depending on the specific stage

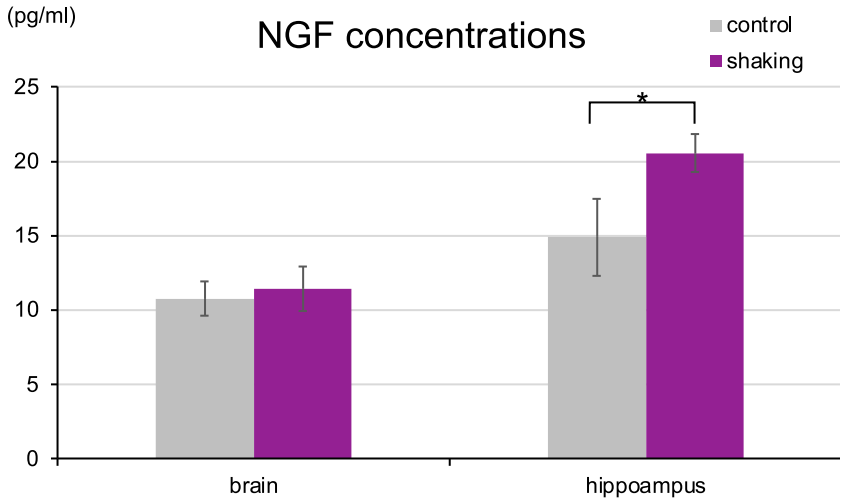

Fig. 7. Effects of theta-shaking exercise on NGF levels in the hippocampus and wide-area brain. A significant increase occurred in NGF in the theta-shaking group ($p = 0.038$). Grey bars: control group, purple bars: theta-shaking group (*$p < 0.05$, paired Student's *t*-test; two-sided, $n = 7$ control mice, 7 shaking mice). The experiments were repeated twice with similar results. NGF, nerve growth factor.

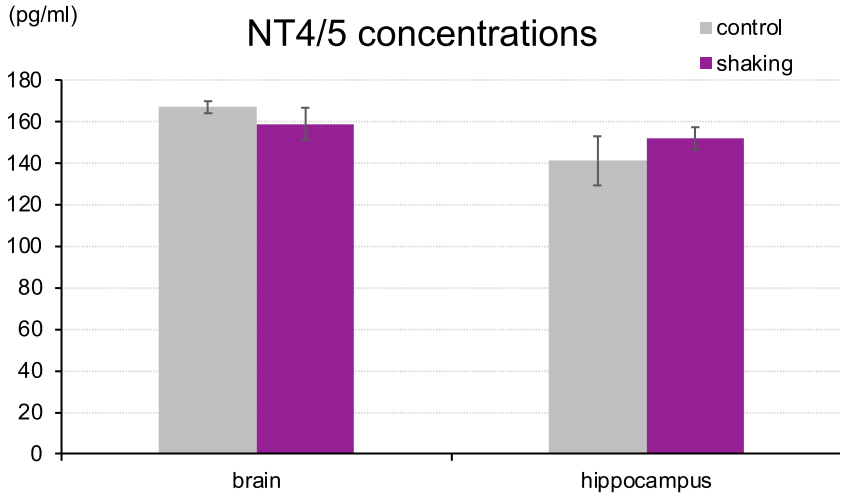

Fig. 8. Effects of the theta-shaking exercise on NT4/5 levels in the hippocampus and wide-area brain. No changes in NT4/5 were detected in the wide-area brain or hippocampus in either group. Grey bars: control group, purple bars: theta-shaking group (paired Student's *t*-test; two-sided, $n = 7$ control, 7 shaking). The experiments were repeated twice with similar results. NT4/5: neurotrophin 4/5.

of neurodevelopment or the experimental time point. Another possibility is that the shaking exercise may have a more specific or localised impact on certain neurotrophic factors or signalling pathways.

In light of our previous findings [21–23], our focus remains on the effects of shaking exercise on neuronal morphology and memory. Notably, in the present study we employed a theta shaking frequency (5Hz), as opposed to previous studies where primarily 2.5Hz was used. Additionally, differences in shaking duration and the anatomical planes studied may contribute to the nuanced variation in outcomes. While previous studies have shown a significant impact on specific brain regions (e.g., upstream DG and boundary areas of CA subregions), we observed a more pronounced effect elicited by shaking in the distal CA1 and subiculum subregions. This phenomenon was further supported by the distal CA1 and subiculum subregions retaining more normal neurons and the subiculum having a greater neuronal density. Our findings further underscore the susceptibility of the CA1 and subiculum regions, which are crucial for information output. These differences and relationships provide us with a more comprehensive perspective for understanding the impact of shaking exercise on the brain.

5. Conclusions

Our study provides evidence that theta-shaking exercise positively affects memory function, especially long-term memory, in

SAMP-10 mice. The increased expression of FNDC5 and higher levels of NGF in the HPC–MPFC circuit, along with the potential involvement of irisin, may have contributed to the maintenance of normal neuron density in the hippocampus and MPFC subregions. These findings suggest that theta-shaking exercise-induced effects, such as FNDC5 and NGF upregulation in specific regions, may have potential therapeutic benefits for cognitive disorders related to ageing. As such, theta-shaking exercise represents a promising strategy for maintaining memory function and delaying age-related decline. Notably, the present study provides valuable insights into the regional distribution of FNDC5 in the brain and highlights the potential involvement of the hippocampus in mediating the effects of exercise on memory. The contradictory expression patterns observed for FNDC5, irisin, NGF, and NT4/5 in this study may be attributed to the intricate regulation and interplay among these factors, regional specificity, differential release and distribution, temporal dynamics, as well as complex interactions with other signalling pathways. Further investigation, including more comprehensive experimental designs and additional time points, could help unravel the underlying mechanisms and provide a clearer understanding of these contradictory findings in other brain regions and their contributions to memory processes.

Ethical statement

All animal experiments were performed in accordance with the guidelines of the National Institutes of Health (Bethesda, MD, USA). The study protocol was approved by the Institutional Animal Care and Use Committee of Fujita Health University (Toyoake, Japan; approval number: APU19120).

Declaration about publishing

The work has not been published previously.

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Data availability statement

The data supporting the findings of this study are available in the Mendeley Data repository with the https://doi.org/10.17632/xcm2tv4hnv.3.

CRediT authorship contribution statement

Kouji Yamada: Writing – review & editing, Validation, Supervision, Investigation. **Sho Izawa:** Validation, Software, Methodology, Investigation. **Takumi Kito:** Resources, Project administration, Methodology, Data curation. **Hirohide Sawada:** Visualization, Validation, Resources, Methodology. **Takeshi Chihara:** Visualization, Validation, Supervision, Methodology. **Naoki Aizu:** Validation, Software, Resources, Methodology. **Daiki Iwata:** Resources, Investigation, Formal analysis, Data curation. **Kazuhiro Nishii:** Writing – original draft, Validation, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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