The Effect of Omega-3 Fatty Acid Supplementation and Exercise on Locomotor Activity, Exploratory Activity, and Anxiety-Like Behavior in Adult and Aged Rats

Lívia GAJDOŠOVÁ[1], Barbora KATRENČÍKOVÁ[1], Veronika BORBÉLYOVÁ[2], Jana MUCHOVÁ[1]

[1]Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic, [2]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

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Summary

Aging is an inevitable and complex biological process that is associated with a gradual decline in physiological functions and a higher disease susceptibility. Omega-3 fatty acids, particularly docosahexaenoic acid, play a crucial role in maintaining brain health and their deficiency is linked to age-related cognitive decline. Combining omega-3-rich diets with exercise may enhance cognitive function more effectively, as both share overlapping neurobiological and physiological effects. This study aimed to evaluate the effect of exercise and omega-3 fatty acid (FA) supplementation in two different doses (160 mg/kg and 320 mg/kg) on anxiety-like behavior and cognitive abilities in both adult and aged rats. Male Wistar rats (4-5- and 23-24-month-old) were randomly divided into seven groups: 3-week control supplemented with placebo without exercise, low-dose omega-3 FAs, high-dose omega-3 FAs, 7-week control supplemented with placebo without exercise, exercise-only, low-dose omega-3 FAs with exercise, and high-dose omega-3 FAs with exercise. The administered oil contained omega-3 FAs with DHA:EPA in a ratio of 1.5:1. Our results indicate that aging negatively impacts the locomotor and exploratory activity of rats. In adult rats, a low dose of omega-3 FAs reduces locomotor activity when combined with exercise while high dose of omega-3 FAs reduces anxiety-like behavior and improves recognition memory when combined with exercise. The combination of omega-3 FAs and exercise had varying impacts on behavior, suggesting a need for further research in this area to fully understand their therapeutic efficacy in the context of cognitive changes associated with aging.

Key words

Aging • Physical activity • Omega-3 fatty acids • Locomotion • Anxiety

Corresponding author

J. Muchová, Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Sasinkova 2, 811 08 Bratislava, Slovak Republic. E-mail: jana.muchova@fmed.uniba.sk

Introduction

The process of physiological aging is described as the slow change in the body's functioning over time [1]. It has been shown that physical activity contributes to a healthy lifestyle [2-5] and promotes neurogenesis, angiogenesis, and synaptic plasticity [6-8]. During exercise, cerebral blood flow increases, which facilitates the removal of metabolic waste [6,9]. Exercise has also been shown to have antidepressant and anxiolytic effects [10-12]. In addition, besides improving mental health, emotional, psychological, and social well-being, regular physical activity also enhances cognitive function [13]. As outlined in WHO´s 'Global Recommendations on Physical Activity for Health', adults aged 65 and older should engage in at least 150 min of moderate-intensity or 75 min of vigorous-intensity aerobic exercise [14]. However, the current guidelines for physical activity are rarely met, particularly by older adults [15]. The amount of time spent exercising among older adults remains

below the recommended 150 min per week despite its health benefits [16].

The two main omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic (EPA) and docosahexaenoic (DHA), are present in fish and some plants [17]. Aging is associated with a reduction in cerebral long-chain PUFAs levels because of lower absorption, reduced PUFAs capacity to cross the blood-brain barrier [18], and lower conversion of PUFAs into long-chain fatty acids (FAs) in the brain. DHA is the main component of the neuronal membrane regulating neurogenesis, synaptogenesis, and the fluidity of the membrane. The proportion of EPA in the total brain long-chain PUFAs is relatively low when compared with DHA. However, EPA inhibits proinflammatory metabolism and promotes blood flow to the brain [19]. Omega-3 FAs are well known to serve as precursors for the synthesis of specialized lipid mediators, such as resolvins, protectins, and maresins, which possess strong anti-inflammatory and pro-resolving properties. These lipid mediators have been suggested to contribute to the beneficial effects of omega-3 FAs in protecting against various health conditions [20]. Low levels of omega-3 FAs in the brain and plasma are linked to age-related cognitive decline [21,22]. Based on recent studies with aged rats [23] and mice [24], EPA and/or DHA supplementation results in a decrease in microglial activation and neuroinflammation, which in turn contributes to improved memory and cognitive abilities [25].

There is an inadequate intake of EPA and DHA across the entire European Union (EU); in 74 % of the EU countries, the intake was found to be lower than the 250 mg recommended intake by the European Food Safety Agency [26-28]. Globally, a large study identified the mean individual consumption of EPA and DHA in adults to be 163 mg/day [29]. As a result, a sufficient dietary intake of omega-3 FAs may delay cognitive decline and lessen the symptoms of aging-related physiological disorders [17]. There is increasing evidence that omega-3 FAs can promote mental health [30], reduce anxiety [31] and can also be effective in preventing cognitive impairment associated with aging [25]. Even though the underlying mechanisms are unclear, omega-3 FAs have been shown to exert pleiotropic effects on neural structure and function. Therefore, omega-3 FAs play a fundamental role in neural functions involved in mood regulation. Omega-3 FAs exert a range of positive effects, such as anti-inflammatory, anti-oxidative and anti-neurodegenerative [32]. They have the potential to enhance brain plasticity, neuronal protection, and cognitive performance, thereby counteracting the mental decline associated with aging [33-35].

Omega-3 FAs and exercise share overlapping neurobiological and physiological effects, and each has been independently associated with better cognitive performance [36,37]. As a result, FA supplementation may modulate the neurocognitive effects of exercise [36,38]. The addition of exercise could potentially complement the effect of omega-3 FA intake on cognition, providing a synergistic enhancement to various aspects of cognitive performance [39]. This suggests that combining an omega-3-FAs-rich diet with an active way of life could be more beneficial for cognitive function than treatment with omega-3 FAs alone [37].

The present study aimed to examine the possible beneficial effect of omega-3 FAs and exercise on locomotor activity and anxiety-like behavior using the open-field test, as well as exploratory activity and recognition memory using the novel object recognition test in both adult and aged rats.

Methods

Animals

Male Wistar rats (n=82) were obtained from the Centre of Experimental Medicine, Department of Toxicology and Laboratory Animal Breeding, Dobrá Voda, Slovak Republic. The rats were housed in groups of three per polycarbonate cage (50×36×19 cm) and were provided with *ad libitum* access to water and a pelleted standard rat laboratory chow (energy content 3.62 kcal/g). The composition of the diet per 100 g of pellets is as follows: moisture 14.5 g; coarse fiber 5 g; fat 4.5 g; ash 8.1 g; starch 37.6 g; total sugars 4.8 g; reducing sugars 3.6 g; protein 25.5 g; calcium 1.7 g; phosphorus 0.9 g; magnesium 0.2 g; sodium 0.2 g; potassium 0.9 g; vitamin A 2.000 IU; vitamin D3 200 IU; vitamin E 7 mg; DL-methionine 120 mg; L-lysine 80 mg. The rats were acclimatized to the new environment for 10 days before the start of the experiment. During this period, the rats were handled daily to minimize stress and to familiarize them with the experimenters. The rats were divided into two age categories: adult (4-5 months) and aged (23-24 months). Adult rats (n=42) weighed between 250-400 g and aged rats (n=40) weighed between 300-700 g. The rats were kept at 23±2 °C and 55±5 % humidity on a 12-hour light/12-hour dark cycle. Both adult and aged rats, respectively, were randomized into

seven groups (Fig. 1). In the experimental design, each group initially consisted of six rats. However, during the experiment, one rat from both aged C7 and EX groups died of natural causes.

The administered oil was a mixture of two fish oils (provided by Cultech Ltd., Port Talbot, UK), DHA Finest Pure Fish Oil (Pharmax Ltd, UK) and Finest Pure Fish Oil (Vega Nutritionals®, UK), which were combined in a 1:1 ratio and contained omega-3 FAs in a ratio of DHA:EPA – 1.5:1. The control groups were given sunflower oil as a placebo daily for 3 and 7 weeks, respectively. All groups of rats were supplemented directly into their mouths by an automatic micropipette using disposable tips (Thermo Scientific™, #94300220). Body weight of rats was evaluated at the beginning and at the end of the experiment. All groups of rats were evaluated for locomotor activity and anxiety-like behavior using the open-field test, as well as exploratory activity and memory using novel object recognition test at the end of the experiment. All behavioral tests were conducted during the light phase of the light/dark cycle. One day after completion of the behavioral tests, the rats were sacrificed by decapitation and blood samples were kept on ice and centrifuged immediately at 4 °C (1200× g for 10 min) to separate plasma, which was stored at -80 °C until analyzed.

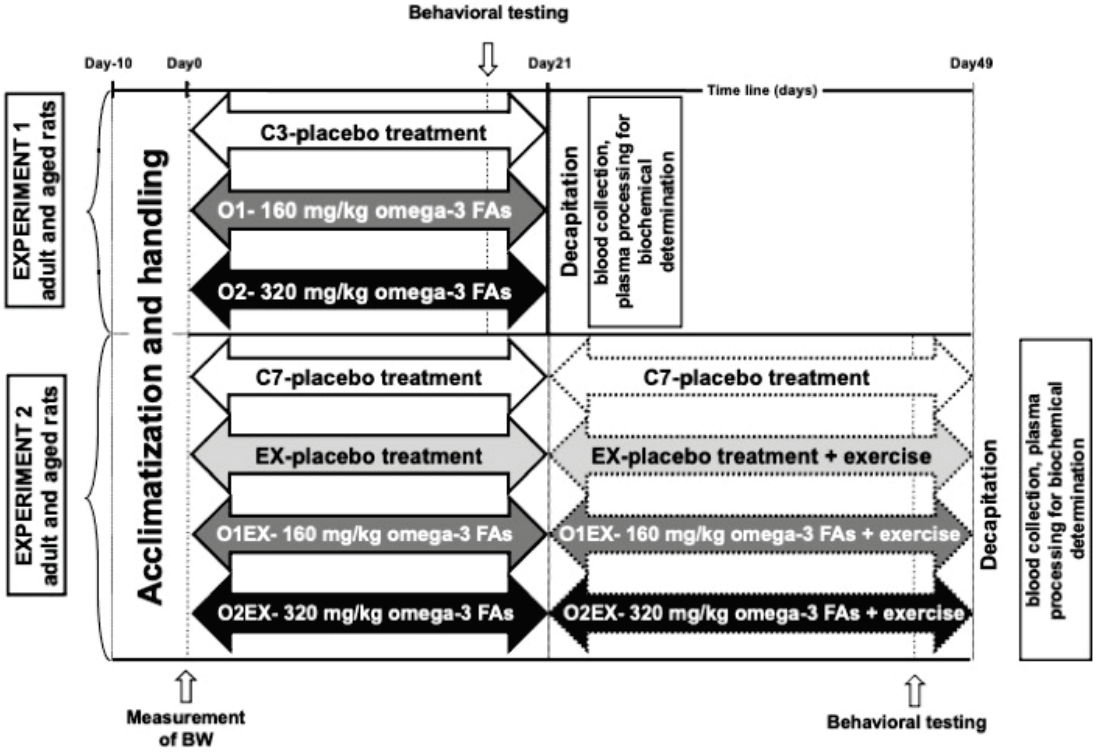

Fig. 1. Schematic diagram of experimental design.

Treadmill exercise protocol

The exercise intervention involved forced exercise on a rodent motorized treadmill (Ugo Basile, Italy) for 4 weeks, with low to moderate intensity of the exercise increasing gradually. The treadmill lanes were separated by non-transparent walls. The exercise was carried out in a dimly lit room to account for the fact that rats are more active in the dark. The exercise protocol consisted of three phases: In the first phase – familiarization phase (1 week), rats were initially adapted to a plane with no inclination, running at a speed of 10 m/min. Rats unable to complete this phase due to noncompliance and/or injuries preventing exercise were excluded from the study. In the second phase (1 week), the exercise load was increased by tilting the plane to an inclination of 5° at a speed of 12 m/min. In the third phase (2 weeks), the exercise load was further increased to 10° while maintaining a speed of 12 m/min. This protocol was modified using a protocol published by Roya *et al.* [40]. Each individual lane, including the walls and treadmill belt, was wiped out with Incidur spray (Ecolab, Dusseldorf, Germany) after each treadmill exercise training session.

Open-field test

The open-field test was performed using an apparatus consisting of a standardized black plexiglass box with square area (100 cm×100 cm), divided into two main virtual zones: a center zone, defined as a square within the larger open-field arena (40 cm×40 cm) slightly illuminated with white light (25 lx), and an encompassing border zone, surrounded by a 50 cm high wall. Testing was conducted between 08:00 and 11:00. At the beginning of the test, the rats were initially positioned within the center zone of the arena and allowed to explore the arena freely for 5 min without any interference. The open-field arena was cleaned with Incidur spray (Ecolab, Dusseldorf, Germany) between trials. Animal behavior was recorded and analyzed using the Noldus EthoVision XT 10 video tracking system (Noldus Information Technology, Wageningen, The Netherlands) using a camera mounted above the apparatus. The center point of the animal was used for the evaluation of center zone entries in the arena which was automatically evaluated by the EthoVision software. The open-field test was performed to measure locomotor and anxiety-like behavior in rats. Locomotor activity of rats was evaluated by determining the total distance moved, as well as the average velocity of movement. Anti-anxiety-like behavior was assessed by the frequency and amount of time spent in the center zone. In addition, vertical activity of rat – rearing (frequency) and grooming (duration) was also assessed [41]. Rearing behavior was manually quantified by counting the number of times when the animal stood on its hind limbs with its front limbs either against a wall or freely in the air during the open field test. Grooming behavior was assessed by measuring the duration of time spent on body cleaning or body scratching [42].

Novel object recognition test

The novel object recognition (NOR) test was performed using a slightly modified version of the object recognition test described by Havranek *et al.* [43]. The test was performed in the open-field arena (100 cm×100 cm) between 08:00-12:00. The open-field test was conducted one day prior to NOR and was considered a habituation trial. The novel object recognition test consisted of two trials – trial 1 and trial 2, each lasting for a duration of 5 min, separated by a retention interval of 1 h. Two different kinds of objects were used in the task: a standard 750 ml plastic bottle and a 1 l glass bottle, both filled with water. The task involved the use of three identical copies of each object to minimize potential odor cues during testing. In trial 1, the animals were placed in the middle of the arena with two identical objects (2 plastic bottles) placed at opposite corners of the arena (27 cm from the wall and 55 cm apart from each other). Time spent exploring object 1 or 2 was marked as “a1” or “a2”, respectively. In trial 2, one plastic bottle was left in the arena (time “a3”) and one plastic bottle was replaced with a glass bottle that served as a novel object (time “b”). Throughout both trials, animals were allowed to freely explore the arena and objects without any interference. NOR was recorded using the Noldus EthoVision XT 10 video tracking system (Noldus Information Technology, Wageningen, The Netherlands) using a camera mounted above the apparatus and analyzed manually by an experimenter blinded to the treatment condition of the animals. Object exploration was characterized by actions such as sniffing, licking, or touching and rearing on the object while facing it. The arena and objects were cleaned with Incidur spray (Ecolab, Düsseldorf, Germany) between each animal to eliminate olfactory disturbance. NOR test was performed to evaluate exploratory activity and recognition memory in rats. The recorded parameters included the total time exploring both objects in trial 1 and trial 2 (trial 1: e1 = a1 + a2, trial 2: e2 = a3 + b) for determining exploratory activity, and the absolute time difference between investigating the sample and the novel object (trial 2: d1 = b - a3) for determining recognition memory. These parameters were determined using a protocol published by Borbélyová *et al.* [41].

Biochemical parameters

The determination of plasma biochemical parameters took place in an accredited laboratory of Synlab Slovakia s.r.o. The diagnostic instrument Alinity ci-series (Abbott, USA) was used for the determination of basic biochemical parameters (glucose, albumin, urea, total cholesterol and triglycerides). The principle of spectrophotometry was employed for these biochemical examinations.

Statistical analysis

Statistical analysis of the data was conducted using GraphPad Prism version 10.0.0 (GraphPad Software, Inc., CA, USA). The normality of the data was initially assessed using the Shapiro-Wilk test. The effects of age, diet, and combined intervention were analyzed

within groups. Normally distributed data were analyzed using the *t*-test and are presented as mean ± SEM, while non-normally distributed data were analyzed using the Kruskal-Wallis test with a Conover-Iman *post hoc* analysis and presented as medians and ranges (minimum and maximum). A p-value less than 0.05 was considered statistically significant.

Ethics statement

All procedures have been conducted in accordance with the guidelines for the care and use of experimental animals and EU regulations (2010/63/EEA) and were approved by the State Veterinary and Food Administration of the Slovak Republic (Nr. 3665/18-221/3).

Results

Experiment 1: Effect of omega-3 FAs on the behavior of Wistar rats

Body weight

The rats were weighed at the beginning and on the final day of the experiment, and the average weight values before and after the intervention are summarized in Table 1. Prior to the intervention, all groups of aged rats exhibited significantly higher body weight compared to the adult rats (aged vs. adult; C3: p=0.0001; O1: p=0.0004; O2: p=0.0006). Post-intervention, aged rats in groups O1 (p=0.0207) and O2 (p=0.0155) still maintained a significantly higher body weight compared to the adult rats. While the adult rats in all groups showed a significant weight gain during the intervention, body weight of aged rats remained relatively unchanged.

Table 1. Effect of supplementation with omega-3 FAs on body weight of rats.

Experimental groups		Before intervention	After intervention	p	Weight difference (%)
Adult rats	C3	436.50 ± 5.25	504.67 ± 8.31	<0.001	+15.63
	O1	448.17 ± 8.18	509.17 ± 13.51	0.003	+13.62
	O2	384.50 ± 8.20	451.00 ± 9.50	<0.001	+17.30
Aged rats	C3	575.50 ± 20.00$^{###}$	541.50 ± 41.74	ns	-5.90
	O1	649.00 ± 37.48$^{###}$	635.83 ± 44.13$^{#}$	ns	-2.02
	O2	558.50 ± 34.10$^{###}$	564.50 ± 37.81$^{#}$	ns	+1.07

The data are presented as the average ± SEM (standard error mean). C3 – control group, O1 – 160 mg/kg omega-3 FA, O2 – 320 mg/kg omega-3 FA. Effect of age (aged vs. adult within the same intervention): $^{#}$ p<0.05, $^{###}$ p<0.001, ns – non-significant.

Open-field test

Locomotor activity

The Kruskal-Wallis H test indicated that there is a significant difference in locomotor activity measured as mean velocity of the movement ($\chi^2(5)=21.83$, p<0.001) and total distance moved ($\chi^2(5)=21.83$, p<0.001) between the groups of rats: aged rats exhibited a significantly lower average velocity of movement (aged vs. adult; C3: p=0.0005; O1: p=0.0020; O2: p<0.0001; Fig. 2) and moved significantly less compared to adult rats (aged vs. adult; C3: p=0.0005; O1: p=0.0020; O2: p<0.0001; Fig. 2). However, no significant effect of any treatment was found on locomotor activity in both adult and aged rats.

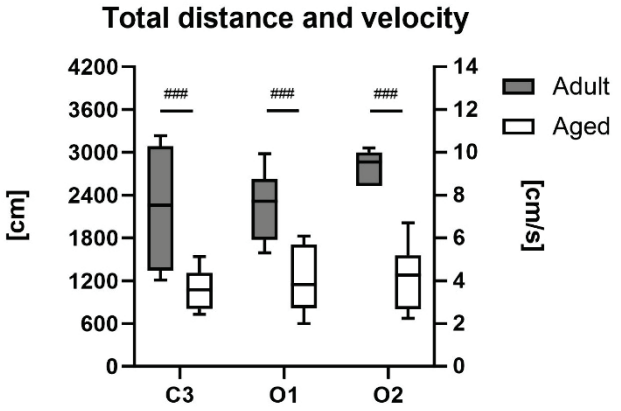

Fig. 2. Locomotor activity of rats in the open field test. Horizontal locomotor activity – total distance (left Y axis) and velocity of the movement (right Y axis). Adult rats (grey bar) and aged rats (white bar). The data are presented as medians (min.-max.). C3 – control group, O1 – 160 mg/kg omega-3 FA, O2 – 320 mg/kg omega-3 FA. Effect of age (aged vs. adult within the same intervention): $^{###}$ p<0.001.

Anxiety-like behavior

There was a significant difference between groups in frequency of entering the center zone (Kruskal-Wallis; $\chi^2(5)=13.58$, p=0.018). However, no significant differences were observed between adult and aged rats. In terms of intervention, adult O2 group entered the center zone of the arena more frequently compared to adult C3 group (p=0.0029). In addition, a significant difference between groups was found in the cumulative duration spent in the center zone (Kruskal-Wallis; $\chi^2(5)=15.39$, p=0.009). Adult O2 group spent significantly more time in the center zone compared to adult O1 and C3 group (p=0.0364 and p=0.0008), suggesting lower anxiety-like behavior in O2 group of rats. In terms of rearing behavior, a significant difference between groups was found (Kruskal-Wallis; $\chi^2(5)=16.65$, p=0.005): rearing frequency was significantly lower in all aged groups compared to adults (aged vs. adult; C3: p=0.0401, O1: p=0.0450, O2: p=0.0027). The grooming duration did not show significant differences among the groups (Kruskal-Wallis; $\chi^2(5)=4.61$, p=0.466) (Table 2).

Table 2. Effect of supplementation with omega-3 FAs on anxiety-like behavior.

Parameter	Adult rats			Aged rats		
	C3	O1	O2	C3	O1	O2
Center-zone frequency (nr. of entries)	0.50 (0.00-1.00)	2.00 (0.25-5.25)	5.00$^{••}$ (3.25-6.00)	0.00 (0.00-1.00)	1.00 (1.00-1.00)	1.50 (1.00-2.00)
Time in center-zone (s)	0.10 (0.00-0.23)	1.82 (0.04-4.59)	5.96$^{•••,+}$ (3.78-9.07)	0.00 (0.00-0.08)	0.70 (0.23-1.20)	1.22 (0.27-4.06)
Rearing (nr. of rears)	19.00 (3.0-21.0)	14.50 (6.0-37.0)	17.00 (12.0-26.0)	4.00$^{#}$ (2.0-11.0)	5.00$^{#}$ (1.00-10.00)	5.00$^{##}$ (1.00-15.00)
Grooming (s)	13.50 (8.3-36.20)	13.04 (2.1-23.45)	16.42 (3.34-28.12)	20.24 (7.26-33.0)	22.10 (10.3-41.97)	17.12 (0.11-30.08)

The data are presented as medians and range (min.-max.). C3 – control group, O1 – 160 mg/kg omega-3 FA, O2 – 320 mg/kg omega-3 FA. Effect of age (aged vs. adult within the same intervention): $^{#}$p<0.05, $^{##}$p<0.01. Effect of treatment (within the same age group): $^{••}$p<0.01, $^{•••}$p<0.001 vs. C3, $^{+}$p<0.05 vs. O1.

Novel object recognition test

Exploratory activity and memory

The Kruskal-Wallis H test indicated that there is a significant difference in total exploratory activity between the groups, in trial 1: ($\chi^2(5)=18.79$, p=0.002) and trial 2: ($\chi^2(5)=18.92$, p=0.002). The results show that aging reduces total exploration activity (total time spent interacting with each individual object) in both trial 1 (aged vs. adult; C3: p=0.0185; O1: p=0.0091; O2: p=0.0003; Fig. 3A) and trial 2 (aged vs. adult; O1: p=0.008; O2: p<0.0001; Fig. 3B). The ability of rats to distinguish a novel object from the familiar one (the absolute time difference between investigating the sample and the novel object) was not significantly different between groups (Kruskal-Wallis; $\chi^2(5)=8.67$, p=0.123; Fig. 3C).

Biochemical parameters

The results of the determination of basic biochemical parameters are presented in Table 3. Both adult and aged rats showed significantly higher glucose concentrations in the O2 group compared to the C3 group (p=0.0360 and p=0.0246). Aged rats had significantly lower albumin levels than adult rats, but only in the O2 group (p=0.0001). In adult rats, albumin levels were higher in the O1 and O2 groups compared to the C3 group (p=0.0245 and p=0.0002). Among aged rats, the albumin levels in the O1 group were significantly higher than those in the C3 group (p=0.0044). However, the albumin levels in the O2 group were lower compared to those in the O1 group (p=0.0009). Aged rats in the O1 and O2 groups displayed significantly lower urea concentrations than adult rats (p=0.0001 and p=0.001). Aged rats showed higher total cholesterol levels than adult rats in the C3 and O1 groups (p=0.0004 and p=0.0001). Adult rats in the O2 group had significantly lower total cholesterol levels than those in the C3 group (p=0.0304). Similar results were observed in aged rats, where the O2 group showed significantly lower concentration of total cholesterol than the C3 (p=0.0001)

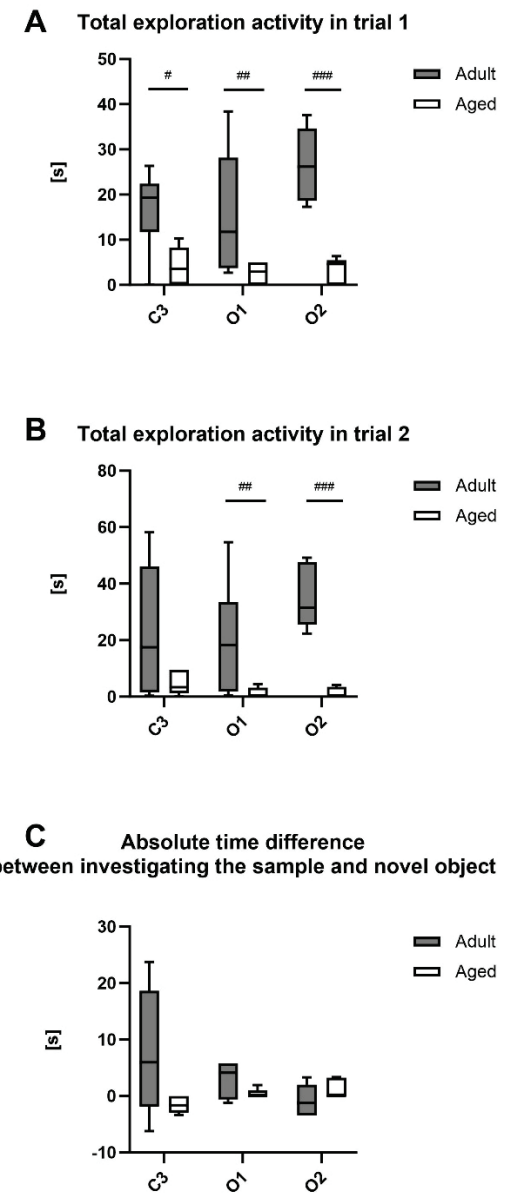

Fig. 3. (**A**) Total time spent interacting with each individual object in trial 1 and (**B**) in trial 2. (**C**) Absolute time difference between investigating the sample and novel object. Adult rats (grey bar) and aged rats (white bar). The data are presented as medians (min.-max.). C3 – control group, O1 – 160 mg/kg omega-3 FA, O2 – 320 mg/kg omega-3 FA. Effect of age (aged vs. adult within the same intervention): $^{\#}$ p<0.05, $^{\#\#}$ p<0.01, $^{\#\#\#}$ p<0.001.

Table 3. Effect of supplementation with omega-3 FAs on biochemical parameters.

Parameter	Adult rats			Aged rats		
	C3	O1	O2	C3	O1	O2
Glucose (mmol/l)	6.65±0.14	6.93±0.11	7.07±0.10$^{\bullet}$	6.47±0.11	6.70±0.30	6.77±0.02$^{\bullet}$
Albumin (g/l)	30.47±0.41	32.15±0.38$^{\bullet}$	33.53±0.55$^{\bullet\bullet\bullet}$	30.77±0.74	32.97±0.64$^{\bullet\bullet}$	30.32±0.64$^{\#\#\#,++}$
Urea (mmol/l)	8.45 (7.73-10.48)	9.40 (7.78-10.20)	9.40 (8.40-10.10)	7.55 (6.20-9.90)	6.65$^{\#\#\#}$ (5.83-8.41)	7.20$^{\#\#}$ (6.50-7.85)
TCh (mmol/l)	2.03 (1.72-2.47)	1.85 (1.61-2.17)	1.77$^{\bullet}$ (1.53-2.06)	2.60$^{\#\#\#}$ (2.44-2.86)	2.78$^{\#\#\#}$ (2.28-4.04)	1.97$^{\bullet\bullet\bullet,+++}$ (1.69-2.35)
TG (mmol/l)	1.08±0.12	1.13±0.07	0.94±0.08	1.13±0.09	2.09±0.44$^{\#\#,\bullet\bullet}$	1.05±0.10^{+++}

The data are presented as the average ± SEM (standard error mean) or median (min.-max.). TCh – total cholesterol, TG – triglycerides, C3 – control group, O1 – 160 mg/kg omega-3 FA, O2 – 320 mg/kg omega-3 FA. Effect of age (aged vs. adult within the same intervention): $^{\#\#}$ p<0.01, $^{\#\#\#}$ p<0.001. Effect of treatment (within the same age group): $^{\bullet}$ p<0.05, $^{\bullet\bullet}$ p<0.01, $^{\bullet\bullet\bullet}$ p<0.001 vs. C3, $^{+++}$ p<0.001 vs. O1.

and O1 group (p=0.0001). Triglyceride concentrations were significantly higher in the aged rat group O1 than adult rats (p=0.0017). Within the aged rat group, the O1 group showed significantly higher triglyceride concentrations than the C3 (p=0.0018), and the O2 group had lower concentrations than the O1 group (p=0.0008).

Experiment 2: Effect of physical activity and combined intervention (physical activity with omega-3 FAs) on the behavior of Wistar rats

Body weight

The rats were weighed at the beginning and on the final day of the experiment, and the average weight values before and after the intervention are summarized in Table 4. Prior to the intervention, each group exhibited significantly higher body weight in aged rats compared to adult rats (aged vs. adult; C7: p=0.0043; EX: p=0.0057; O1EX: p<0.001; O2EX: p<0.001). Post-intervention, a significant increase in body weight was noted in the adult rats across all groups, while the weights of the aged rats remained stable. However, the aged rats in the O1EX group still showed a significantly higher body weight compared to the adult rats (p=0.0051).

Open-field test

Locomotor activity

The Kruskal-Wallis H test indicated that there is a significant difference in locomotor activity measured as mean velocity of the movement ($\chi^2(7)=21.59$, p=0.003) and total distance moved ($\chi^2(7)=21.69$, p=0.003) between the groups of rats: aged EX and O2EX rats displayed significantly lower average velocity of movement compared to adult EX and O2EX rats (p=0.0009 and p=0.0031) and moved significantly less compared to adult rats in both EX and O2EX (p=0.0021 and p=0.0062). There was a significantly lower average velocity of the movement in O1EX compared to EX in adult rats (p=0.0113). Adult O1EX rats moved significantly less compared to EX rats (p=0.0463) (Fig. 4). There were no other significant differences observed across the treatments.

Anxiety-like behavior

The Kruskal-Wallis H test revealed no significant differences among the groups in terms of the frequency of entering the center zone ($\chi^2(7)=11.7$, p=0.111), the total time spent in the center ($\chi^2(7)=8.35$, p=0.303), or grooming ($\chi^2(7)=8.4$, p=0.299). Age did not significantly affect the center zone entries, time spent in the center zone, or grooming. However, significant difference was found in rearing frequency ($\chi^2(7)=24.79$, p<0.001): aged rats reared significantly less compared to adult rats (C7: p=0.0032, EX: p=0.0008, O2EX: p=0.0250) and among adult rats, the O1EX group exhibited significantly fewer instances of rearing behavior compared to the EX rats (p=0.0331) (Table 5).

Novel object recognition test

Exploratory activity and memory

In both trials, there were significant differences between groups in exploratory activity (Kruskal-Wallis; trial 1: $\chi^2(7)=24.73$, p<0.001 and trial 2: $\chi^2(7)=26.37$, p<0.001). The results demonstrate that aging reduces the total exploratory activity (total time spent interacting with each individual object) in trial 1 (aged vs. adult; C7: p=0.0003; O1EX: p=0.0189; O2EX: p=0.009; Fig. 5A) and trial 2 (aged vs. adult; C7: p=0.0054; EX: p=0.0435; O1EX: p=0.0003; O2EX: p=0.0003; Fig. 5B). No significant effect of any treatment on the exploratory behavior of rats was found. There was a significant difference between groups in the absolute time difference between investigating the sample and the novel object (Kruskal-Wallis; $\chi^2(7)=17.69$, p=0.013). Time difference was found to be significantly lower in aged rats compared to adult rats in C7 (p=0.0068) and O2EX (p=0.0367) groups (Fig. 5C). In addition, adult C7 rats were observed to investigate the novel object significantly longer than the sample object compared to adult EX (p=0.0038) and O1EX (p=0.0087). Adult O2EX rats spent significantly more time investigating the novel object than adult EX (p=0.004) and O1EX rats (p=0.009) (Fig. 5C).

Biochemical parameters

The results of the determination of basic biochemical parameters are presented in Table 6. A significant change in plasma glucose concentration was observed only in adult rats, with the O1EX group showing a significantly lower concentration compared to the EX group (p=0.0266). Aged rats displayed significantly lower plasma level of albumin only in the C7 group compared to adults (p=0.0059). When comparing treatment groups, the adult O1EX group showed a significantly lower albumin level compared to the EX group (p=0.0031). In comparison to the C7 group of adult rats, both the O1EX (p=0.0016) and O2EX (p=0.0272) groups exhibited significantly lower levels of plasma albumin. Across all groups of aged rats, urea concentration values were significantly lower than those

Table 4. Effect of physical activity and combined intervention (omega-3 FAs + exercise) on body weight of rats.

Experimental groups		Before intervention	After intervention	p	Weight difference (%)
Adult rats	C7	412.83 ± 12.53	507.50 ± 24.83	0.007	+22.93
	EX	435.00 ± 5.74	525.17 ± 14.42	<0.001	+20.73
	O1EX	397.17 ± 7.69	464.67 ± 13.78	0.002	+17.00
	O2EX	424.00 ± 7.27	539.33 ± 20.99	<0.001	+27.20
Aged rats	C7	556.80 ± 39.23$^{\#\#}$	573.00 ± 39.32	ns	+2.91
	EX	539.00 ± 29.10$^{\#\#}$	510.60 ± 27.06	ns	-5.27
	O1EX	559.50 ± 12.86$^{\#\#\#}$	539.33 ± 15.73$^{\#\#}$	ns	-3.61
	O2EX	572.33 ± 7.14$^{\#\#\#}$	569.33 ± 10.44	ns	-0.52

The data are presented as the average ± SEM (standard error mean). C7 – control, EX – exercise, O1EX – 160 mg/kg omega-3 FA with exercise, O2EX – 320 mg/kg omega-3 FA with exercise. Effect of age (aged vs. adult within the same intervention): $^{\#\#}$ p<0.01, $^{\#\#\#}$ p<0.001, ns – non-significant.

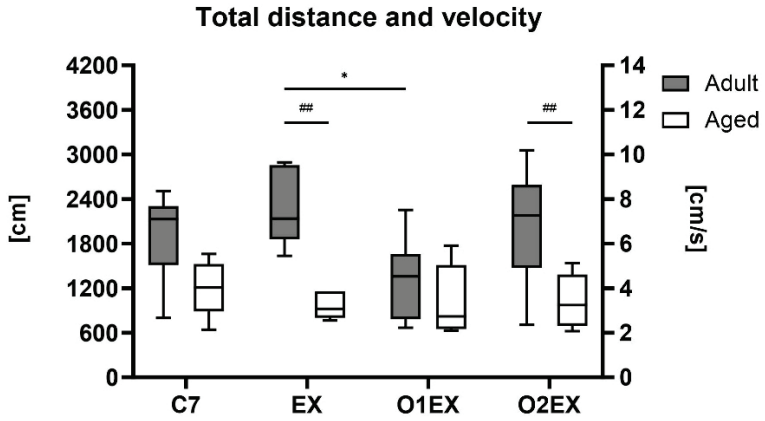

Fig. 4. Locomotor activity of rats in the open field test. Horizontal locomotor activity – total distance (left Y axis) and velocity of the movement (right Y axis). Adult rats (grey bar) and aged rats (white bar). The data are presented as medians (min.-max.). C7 – control group, EX – exercise, O1EX – 160 mg/kg omega-3 FA with exercise, O2EX – 320 mg/kg omega-3 FA with exercise. Effect of age (aged vs. adult within the same intervention): $^{\#\#}$ p<0.01. Effect of treatment (within the same age group): * p<0.05.

Table 5. Effect of physical activity and combined intervention (omega-3 FAs + exercise) on anxiety-like behavior.

Parameter	Adult rats				Aged rats			
	C7	EX	O1EX	O2EX	C7	EX	O1EX	O2EX
Center-zone frequency (nr. of entries)	0.00 (0.00-0.75)	1.00 (1.00-1.00)	0.00 (0.00-0.75)	1.00 (1.00-2.50)	0.00 (0.00-1.00)	0.00 (0.00-1.00)	0.00 (0.00-0.75)	1.00 (0.25-1.00)
Time in center-zone (s)	0.00 (0.00-2.16)	2.28 (1.10-2.44)	0.00 (0.00-0.42)	0.56 (0.10-1.86)	0.00 (0.00-1.32)	0.00 (0.00-4.28)	0.00 (0.00-1.47)	1.42 (0.21-2.57)
Rearing (nr. of rears)	16.00 (14.00-21.00)	20.50 (13.00-30.00)	10.50$^{\Delta}$ (2.00-23.00)	14.00 (4.00-30.00)	6.00$^{\#\#}$ (1.00-14.00)	3.00$^{\#\#\#}$ (2.00-10.00)	4.50 (3.00-14.00)	3.00$^{\#}$ (1.00-11.00)
Grooming (s)	16.44 (10.02-24.12)	11.50 (2.25-35.11)	22.30 (0.00-37.10)	19.25 (2.09-45.40)	10.12 (2.20-11.46)	15.50 (0.00-25.30)	24.00 (7.10-48.70)	23.10 (12.00-27.30)

The data are presented as medians and range (min.-max.). C7 – control group, EX – exercise, O1EX – 160 mg/kg omega-3 FA with exercise, O2EX – 320 mg/kg omega-3 FA with exercise. Effect of age (aged vs. adult within the same intervention): $^{\#}$ p<0.05, $^{\#\#}$ p<0.01, $^{\#\#\#}$ p<0.001. Effect of treatment (within the same age group): $^{\Delta}$ p<0.05 vs. EX.

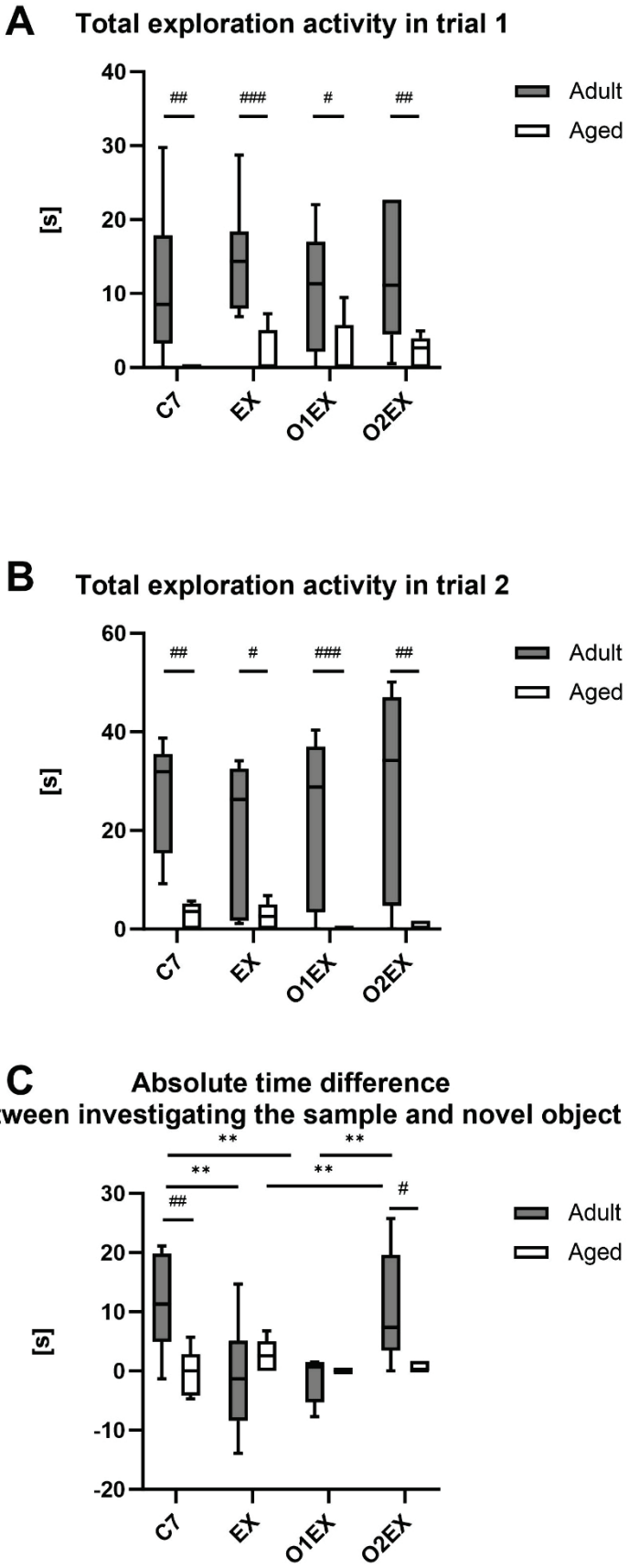

Fig. 5. (**A**) Total time spent interacting with each individual object in trial 1 and (**B**) in trial 2. (**C**) Absolute time difference between investigating the sample and novel object. The data are presented as medians (min.-max.). C7 – control group, EX – exercise, O1EX – 160 mg/kg omega-3 FA with exercise, O2EX – 320 mg/kg omega-3 FA with exercise. Effect of age (aged vs. adult within the same intervention): $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$. Effect of treatment (within the same age group): ** $p<0.01$.

Table 6. Effect of physical activity and combined intervention (omega-3 FAs + exercise) on biochemical parameters.

Parameter	Adult rats				Aged rats			
	C7	EX	O1EX	O2EX	C7	EX	O1EX	O2EX
Glucose (mmol/l)	6.67±0.13	6.87±0.10	6.28±0.12$^{\Delta}$	6.82±0.14	6.60±0.20	6.84±0.11	6.52±0.20	6.82±0.18
Albumin (g/l)	33.98±0.77	32.67±0.50	30.48±0.27$^{**\Delta\Delta}$	31.47±0.60*	30.80±0.29$^{\#\#}$	31.40±1.06	31.57±0.79	30.43±0.18
Urea (mmol/l)	8.85 (7.23-9.50)	8.35 (7.50-8.93)	8.35 (7.31-9.58)	8.95 (8.40-9.44)	6.90$^{\#\#}$ (6.4-7.30)	6.70$^{\#}$ (6.20-7.57)	6.80$^{\#\#}$ (6.63-7.32)	7.25$^{\#\#}$ (6.52-9.31)
TCh (mmol/l)	2.19±0.09	1.94±0.14	1.75±0.12	1.71±0.07*	3.13±0.43$^{\#}$	2.77±0.12$^{\#}$	2.53±0.15$^{\#\#}$	2.47±0.09$^{\#}$
TG (mmol/l)	1.06±0.13	1.18±0.13	1.20±0.20	1.18±0.14	1.64±0.19	1.16±0.10	1.39±0.28	1.27±0.10

The data are presented as the average ± SEM (standard error mean) or median (min.-max.). TCh – total cholesterol, TG – triglycerides, C7 – control group, EX – exercise, O1EX – 160 mg/kg omega-3 FA with exercise, O2EX – 320 mg/kg omega-3 FA with exercise. Effect of age (aged vs. adult within the same intervention): $^{\#}$ p<0.05, $^{\#\#}$ p<0.01. Effect of treatment (within the same age group): * p<0.05, ** p<0.01 vs. C7, $^{\Delta}$ p<0.05, $^{\Delta\Delta}$ p<0.01 vs. EX.

in adult rats (aged vs. adult; C7: p=0.0027; EX: p=0.0109; O1EX: p=0.007; O2EX: p=0.0091). All aged groups of rats had significantly higher total cholesterol levels in comparison to adult rats (aged vs. adult; C7: p=0.0424; EX: p=0.0115; O1EX: p=0.0075; O2EX: p=0.0199). Among adult rats, the O2EX group had significantly lower total cholesterol levels than the C7 group (p=0.049). There were no significant differences in either adult or aged rats in plasma triglyceride levels.

Discussion

In this study, the effect of 3-omega FAs and its combination with exercise on behavior in both adult and aged Wistar rats was investigated focusing on locomotor activity, anxiety-like behavior, exploratory activity, and memory. Our initial hypothesis was that the interplay between omega-3 FAs and physical activity could have a synergistic effect on cognitive performance. This was based on previous studies showing that each factor independently contributes to cognitive enhancement [36,37]. We suggested that their combined effect could potentially offer a greater benefit. The findings from our study enrich the existing knowledge regarding the effect of omega-3 FAs and exercise on the behavior of adult and aged Wistar rats, which are currently among the most popular rat strains used in research.

Effect of age and intervention on locomotor activity of rats

In terms of the effects of aging on behavioral characteristics of rats, there is a correlation between aging and a decline in physical performance, such as locomotor activity [44]. Arnold *et al.* [45] has shown that aging-related locomotor decline begins between 12 and 18 months of age in rats. Our study showed that increasing age reduces locomotor activity in rats, which is further supported by the results of other experimental studies performed on rats [44-46] or mice [47,48]. With age, morphological changes occur in skeletal muscle [49], which leads to a significant decrease in muscle strength and endurance, primarily due to diminished muscle mass and protein production [50,51]. These changes are linked to age-related alterations in the central and peripheral nervous systems [52], including neurochemical changes within the nigrostriatal dopaminergic system [53], a gradual loss of motoneurons, and degeneration of neuromuscular junctions [54,55]. The results of experiment 1 suggest that omega-3 FA supplementation did not have a significant effect on locomotor activity, regardless of the dose, which is consistent with previous studies [17,56]. Another study that looked at the effect of different doses (10, 15, 30, and 60 mg/kg of body weight) of EPA and DHA in a ratio of 1:1 has also reported no significant differences in locomotor activity of adult rats [57]. However, Lange *et al.* [58] have reported that chronic consumption of an omega-3 FA-enriched diet results in lower locomotor activity in adult rats. These findings highlight the importance of further research in this area.

In experiment 2, the negative effect of aging on the locomotor activity of rats was significant in the

exercise group and the high-dose omega-3 FAs combined with the exercise group of rats. When considering the effect of combined intervention of omega-3 FAs and exercise, we found that the combination of a lower dose of omega-3 FAs and exercise led to lower locomotor activity compared to exercise alone, but only in adult rats, indicating that this combination may have a negative effect on locomotor activity in adult rats. Regular, low-intensity exercise has been shown to counteract age-induced oxidative changes and improve physical performance in rats [59], while omega-3 FAs may play a role in reducing exercise-induced muscle damage [60,61]. Contrary to our expectations, combination of omega-3 FAs with exercise did not lead to increased locomotor activity in our study. Interestingly, the impact of omega-3 FAs on locomotor activity differs between aged rats and adult rats. This underscores the complex interplay between age, omega-3 FAs, and behavioral outcomes. At present, the exact mechanism behind the attenuating effect of the combination of lower dose of omega-3 FA and exercise remains unclear. Further research is needed to elucidate the complex relationship and potential age-related differences in the response to omega-3 FA supplementation and exercise in rats.

Effect of age and intervention on anxiety-like behavior of rats

Results of experiment 1 indicate that aging did not significantly affect the number of entries and time spent in the center zone, or the duration of grooming behavior, suggesting that rats aged 23-24 months, in our study, did not demonstrate a significant change in anxiety-like behavior. The center zone in an open field test is typically associated with anti-anxiety-like behavior in rats [62], and the lack of significant change in the frequency of entering this zone suggests that the anxiety levels of the rats did not increase with age. Similarly, grooming is a common behavior in rats that can be indicative of their emotional state [63] and the lack of change in grooming duration suggests that the emotional well-being of the rats was not significantly affected by age.

Our results contradict previous findings reporting either higher anxiety-like behavior [46,64-68], or lower anxiety-like behavior in aged rats [69]. For instance, Sotoudeh *et al.* [66], observed higher anxiety-like behavior in Wistar male rats aged 18-20 months compared to young rats aged 2-3 months, using the elevated plus maze test (EPM), which differs from the methodology employed in the present study. Conversely, Torres-Garcia *et al.* [69] reported a reduction in anxiety-like behavior in aged (24 months old) male Wistar rats compared to young (3 months old) rats, with middle-aged (17 months old) rats exhibiting intermediate values between those of the young and aged.

However, we observed significantly lower rearing behavior in aged rats, suggesting a reduced exploratory behavior or curiosity. This observation aligns with the findings of Adelöf *et al.* [70] who reported diminished rearing behavior in 22-month-old male mice. The decline in rearing behavior could be attributed to several factors. One potential factor could be physical limitations stemming from age-related neuronal changes, which can result in a gradual decline in neurophysiological functions [71]. Additionally, as cognitive function tends to deteriorate with age [72] this could induce changes in motivation and perception, subsequently impacting rearing behavior.

In adult rats, supplementation with high-dose omega-3 FAs resulted in higher number of entries and more time spent in the center zone when compared to the control and low-dose omega-3 FAs groups. These findings suggest that a high-dose of omega-3 FAs might be more effective in reducing anxiety-like behavior than lower dose. This effect could be attributed to the anti-inflammatory and neuroprotective properties of omega-3 FAs, which may help to modulate anxiety-like behavior [73-76]. Although exercise improves mood [77], its impact on anxiety-like behavior in experimental animal models has been a subject of inconsistent reports. Some studies have demonstrated lower anxiety-like behavior in rats [78] and mice [79], others have reported higher anxiety-like behavior in rats [80] or no effect of exercise on anxiety [81,82]. These discrepancies may be attributed to variations in methodological details, including the type (voluntary vs. forced exercise), intensity (low, medium or high), and duration of exercise, as well as animals (mouse vs. rat) or age of the animals used. In addition, behavioral tests used to evaluate anxiety differ in mentioned studies (OF vs. EPM) may also affect the outcomes. For instance, studies by Pietrelli *et al.* [78] and Uysal *et al.* [79] found that chronic aerobic exercise reduced anxiety in rats and mice, respectively. Conversely, Jaehne *et al.* [83] found that chronic running wheel exercise led to higher anxiety in adult male Sprague-Dawley rats. Similarly, Burghardt *et al.* [81] found that unrestricted access to running wheels resulted in higher anxiety in adult male Harlan Sprague-Dawley rats, although chronic treadmill running did not affect anxiety. These findings underscore the importance of considering

various factors, particularly the type and duration of exercise may be an important factor in determining the impact of exercise on anxiety-like behavior.

In experiment 2, while most measures of anxiety-like behavior did not differ significantly with age, rearing behavior did show significant decline in aged rats. In terms of intervention, our results revealed lower number of rears in adult rats subjected to exercise combined with a low dose of omega-3 FAs compared to exercise only. It is plausible that the low dose of omega-3 FAs may have interfered with the beneficial effects of exercise on anxiety. However, since other parameters evaluating anxiety-like behavior were not significantly changed, we cannot assume that the intervention had an effect on anxiety-like behavior based solely on the significant difference in rearing behavior. Gokdemir *et al.* [67] have reported that multiple exercise sessions may lead to a reduction in anxiety-like behavior and that chronic anxiety could have been reduced by exercise. This suggests that the effects of exercise on reducing anxiety-like behavior accumulate over time and that constant exercise is necessary for long-term anxiety reduction. These findings highlight the complex interplay between exercise and omega-3 FAs in modulating anxiety-like behavior and underscore the need for further research to elucidate the underlying mechanisms.

Effect of age and intervention on exploratory activity and recognition memory of rats

In the context of exploratory activity, our results from experiment 1 indicate that aged rats exhibit lower exploratory activity than adult rats. While aged rats showed no significant difference in preferring the novel object over the familiar object in comparison to adult rats in experiment 1, in experiment 2, the aged control group and the aged group with the high dose of omega-3 FAs and exercise interacted less with the novel object compared to adult rats. These results suggest that aging may have an effect on exploratory behavior which may also impact the interpretation of experimental results of NOR test. In addition, while aged rats may exhibit lower exploratory activity, their ability to recognize and differentiate between novel and familiar objects is controversial. Aktoprak *et al.* [84] reported that there was no difference in the time spent exploring the novel object between young and aged rats (10 months vs. 28 months old), confirming the lack of age-related differences in the novel object recognition test. However, Taoro-González *et al.* [85], reported that the ability to distinguish a novel object from a familiar one declines with age, which is contradictory to our findings in experiment 1. Additionally, Teather and Wurtman [86] showed that learning memory assessed by the water-maze test is impaired in aging rats (17 months old) compared to adults (5 months old). This finding is supported by Barcelo-Coblijn *et al.* [87], who found that 24-month-old rats had impaired learning memory compared to 2-month-old rats. It is important to consider the potential influence of the minimal exploratory activity of aged rats on our findings. Reduced exploratory activity in aged rats may impact their engagement with NOR test, potentially influencing the results. Aged rats may spend less time exploring both the novel and familiar objects due to lower motivation or physical limitations, which could mask potential differences in recognition memory.

Our results show that omega-3 FAs did not exert a positive effect on improving recognition memory in both age groups. Experimental studies describing the effects of omega-3 FAs on memory are conflicting. For instance, Ferraz *et al.* [74] did not show significant impact of 4-month omega-3 FAs supplementation on the working memory of adult rats in Morris water-maze test. Conversely, Gamoh *et al.* [88] demonstrated an improved learning memory in aged rats following administration of 300 mg of DHA/kg/day for 5 weeks, as assessed in the radial-maze test. Similarly, Taoro-González *et al.* [85] demonstrated that an omega-3 FA-enriched diet led to the reversal of age-induced impairment of recognition memory. Sidhu *et al.* [89] reported improved working memory in elderly mice after a two-month omega-3 FAs treatment, as tested in the Y-maze test.

In comparison to the control group, we observed that exercise alone and in combination with a lower dose of omega-3 FAs appeared to reduce interaction with the novel object in adult rats. Conversely, the higher dose of omega-3 FAs combined with exercise showed higher exploration of novel object although this effect was not evident in aged rats. It appears that high doses of omega-3 FAs along with exercise may interact and modulate recognition memory. According to Chytrova *et al.* [38], exercise plays a crucial role in modulating the effects of dietary factors on brain function. Earlier research has demonstrated that omega-3 FAs has positive effects on learning and memory. For instance, fish oil supplementation (3.0 g/kg of body weight, containing lipid emulsions with 12 % EPA and 18 % DHA) was found to prevent memory deficits and learning impairments in rats subjected to restraint stress [74].

Aged rats typically exhibit a less effective response to exercise [90] suggesting that the combination of omega-3 FAs with exercise may not be as efficacious in aged rats as it is in younger animals.

Effect of age and intervention on body weight of rats

The significantly higher body weight observed in all groups of aged rats compared to the adult rats suggests an age-related effect on body weight. This finding aligns with previous research by Ništiar *et al.* [91], which observed that rats aged 4-5 months weigh less than rats aged 24-25 months. The significant increases in body weight observed in each group of adult rats throughout the intervention, despite the omega-3 FA acid intervention, suggest that other factors may have contributed to the observed changes. The study by Altun *et al.* [92] reported that rats that are fed *ad libitum* experience an increase in body weight throughout their lifespan, reaching a peak at approximately 18-24 months of age. After this period, body weight no longer increases and may even decrease slightly, which could be associated with the aging process of the organism. However, the lack of significant change in body weight in the aged rats following the supplementation indicates that omega-3 FA intervention did not have a discernible impact on body weight in any of the age groups. Although physical activity has been shown to reduce body weight [93-95], we only observed a non-significant weight loss in the exercise groups, which is consistent with the results of some studies [96,97]. However, in contrast to our results, the study by Silva *et al.* [98], where they observed a lower weight gain in exercising rats compared to non-exercising rats, but in this study the physical activity intervention lasted for 60 min/day for 54 weeks. Therefore, we can assume that the intensity and duration of exercise in our study was not sufficient to cause weight loss.

Effect of age and intervention on biochemical parameters of rats

When monitoring the biochemical parameters, we found decreased urea concentration and increased cholesterol in aged rats compared to adults. Kidney aging is associated with a significant decrease in the expression of aquaporin and urea transporters in the inner medulla, as well as a decrease in papillary osmolality, resulting in impaired urine-concentrating ability and a decreased plasma urea concentration [99,100]. Plasma cholesterol levels increase during the normal aging process in both rodents and humans. This is connected with a decreased clearance of plasma LDL through receptor-mediated mechanisms, as well as a reduction in the elimination of cholesterol as bile acids [101]. No significant changes were found on any of the parameters following interventions, however, a noticeable rise in triglycerides was observed solely in aged rats within the low dose of omega-3 FA group as compared to the adult and control group. We assume that this finding may be independent of the interventions and could be attributed to other factors, such as age-related metabolic changes.

The present study offers a thorough investigation into the effects of omega-3 FAs and exercise on anxiety-like behavior and cognitive abilities in Wistar rats. However, the findings from our study did not support our initial hypothesis. Despite this, they enrich the existing knowledge regarding the effect of omega-3 FAs and exercise on the behavior of adult and aged Wistar rats. A notable strength of this study lies in its inclusion of both adult and aged rats, thereby offering a more comprehensive understanding of the effects of these interventions across different life stages. Moreover, the study encompassed seven different treatment groups, including control groups, exercise-only groups, omega-3 FA-only groups, and combinations of the two, thereby providing a thorough evaluation of the effects of these interventions. Additionally, the study considered dose-response effects by evaluating the impacts of different doses of omega-3 FAs, thereby enabling the identification of an optimal dose and a more complete understanding of the effects of these interventions.

However, there are several inherent limitations of our study, including a small sample size within individual groups and a disparate duration of supplementation between experiment 1 (3 weeks) and experiment 2 (7 weeks). Additionally, our study observed large differences in variance observed in the measured values, which could potentially be attributed to the heterogeneity and size of our sample. In NOR test, the minimal exploratory activity of rats may have biased the results. Therefore, future experiments should consider modifying the NOR test protocol to account for reduced exploratory activity in aged animals or consider alternative behavioral tests to assess recognition memory. Furthermore, our study relied exclusively on the open-field test for assessing anxiety-related behavior in rodents. This test may not capture the full spectrum of such behaviors, as highlighted by La-Vu *et al.* [102]. Therefore, in further studies should consider using

multiple behavioral assays in parallel to provide a more comprehensive assessment of anxiety-related behavior.

Further studies should aim to investigate the effect of omega-3 FAs deficiency on the behavior of both adult and aged rats as consumption of omega-3 FAs in the human population is generally below recommended levels. To gain a deeper understanding of the complex relationship between omega-3 FAs, exercise, and their effects on the aforementioned behavioral parameters, it is important to conduct further studies using diverse experimental designs, dosages, and durations of treatment. Additionally, incorporating both male and female rats in these studies would be essential to understand potential sex differences. This would help to verify the current findings and provide a more reliable and comprehensive understanding of the potential therapeutic benefits of omega-3 FAs and exercise in the context of aging, anxiety, and cognitive abilities.

Based on our results, aging has a negative impact on both locomotion and exploration in rats but does not seem to affect anxiety-like behavior. Furthermore, supplementation with a low dose of omega-3 FAs in adult rats appears to mitigate the positive effects of exercise on locomotor activity. In addition, a higher dose of omega-3 FAs exhibits a positive impact on reducing anxiety in adult rats. Additionally, a higher dose of omega-3 FAs combined with exercise appear to improve recognition memory in adult rats, but not in aged rats. These findings suggest that omega-3 FAs can positively influence anxiety and memory in rats, while the effects may be dose and age-dependent. Furthermore, combining omega-3 FAs with exercise could potentially further enhance the effects on recognition memory in adult rats. Taken together, these findings underscore the potential of omega-3 FAs as a promising area of research for improving memory and reducing anxiety. However, further studies investigating the effects of various doses, age groups, and the combined administration of omega-3 FAs and exercise are needed to gain a more comprehensive understanding of their potential therapeutic applications.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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