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Research report

Fish oil supplementation and physical exercise program: Distinct effects on different memory tasks

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HIGHLIGHTS

- Cognitive effects of fish oil concomitantly or not with exercise were investigated.
- Fish oil facilitated the long-term habituation and recognition memories.
- Physical exercise facilitated the discriminative avoidance memory.
- Both treatments improved cognitive function in mature rats with no synergic effects.
- The cognitive effect was critically dependent on the type of memory evaluated.

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ABSTRACT

Both fish oil supplementation and physical exercise are able to induce benefits to mental health by providing an improvement in cognitive performance and enhancing neuroplasticity and protection against neurological lesions. The aim of the present study was to investigate the cognitive effects in rats of the: (1) a diary and prolonged fish oil supplementation (85 mg/kg/day) initiated from prenatal period to the midlife (300 day/old); (2) moderate physical exercise in treadmill initiated from adolescent period to midlife and (3) association of fish oil supplementation and moderate physical exercise protocol during the same period. Animals were submitted to the habituation in the open-field, object recognition and to the plus-maze discriminative avoidance tasks. Our results demonstrated that a diary and prolonged fish oil supplementation can facilitate the persistence of the long-term habituation and recognition memories without, however, affecting the discriminative avoidance memory. Conversely, although the program of physical exercise exerted no effects on habituation or objects recognition, it was able to potentiate the persistence of the discriminative avoidance memory. Such promnestic effects (induced by both fish oil supplementation and physical exercise) were not accompanied by alterations in emotionality or locomotor activity. Our findings suggest that fish oil supplementation, initiated from prenatal period to midlife, and physical exercise program applied throughout the life induced distinctly a better cognitive performance.

1. Introduction

A rich diet in omega-3 fatty acids is critical for maintaining brain function and structure, especially during development and aging [1]. One of the most important omega-3 fatty acid is docosahexaenoic acid (DHA), which is obtained from diet rich in fish and it may play an essential role in brain functioning [2–4]. In this regard, evidences demonstrate DHA involvement in regulating emotions, exploratory activity and cognitive functions both in animals and humans (for review, see [4]. Additionally, increasing evidences demonstrate that omega-3 supplementation can enhance learning and memory [5–7], in a variety of task both in humans [8–13] and in laboratory animals [6,14]. Moreover, omega-3 enhances cognitive function and promotes neuroplasticity and protection against neurologic lesions [15–17]. On the other hand, eicosapentaenoic acid (EPA) is usually less found in the central

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nervous system (CNS) [18,19], but has an important role in cardiovascular and immune systems [20].

Physical exercise has the capability of influencing brain plasticity and function under homeostatic and challenging conditions. Specifically, physical exercise modulates hippocampal neurogenesis [21–23], reduces oxidative stress [24,25], increases brain-derived neurotrophic factor levels [26–29] and brain vascularization [30]. In human, exercise can decreases cognitive impairment associated with aging [31] and reduces the risk for several neurological diseases [32,33]. In laboratory animals, exercise improves the cognitive function at different ages [34,35] and in several tasks such as the Morris water maze [23,36–40], the inhibitory avoidance [24,25,41,42] and context fear conditioning [43,44]. Within this context, it is well accepted that both omega-3 supplementation and physical exercise may affect several cognitive parameters and brain plasticity [34,35,40,45]. Since diet and exercise are routine, it could be postulate that their effect could be complementary. Thus, recent studies have demonstrated a synergism between diet and exercise [46].

Concerning the possible association of omega-3 and exercise effects, from the best of our knowledge, only Wu and colleagues [15] have systematically investigated this interaction. It was observed that a short treatment with DHA supplementation for 12 days enhanced learning and this promnestic effect was potentiated by a concomitant voluntary physical exercise protocol. However, other functions as attention or emotional levels and the effects of a chronic supplementation throughout life and aging remain overlooked. In this scenario, the objectives of the present study were to investigate the effects of fish oil (rich in omega-3) supplementation, initiated at the prenatal period until the maturity, associated or not with physical exercise throughout development until the maturity, on the cognitive performance (associative and non-associative memory, learning, emotionally and exploratory activity) of middle-aged rats.

2. Materials and methods

2.1. Subjects

The experimental procedures were approved by the Institutional Animal Care and Use Committee under the protocol #0252/09. Forty-five adults Wistar male rats (outbred, raised, and maintained in the Centre for Development of Experimental Models in Medicine and Biology of Universidade Federal de São Paulo) were used. Animals were housed under environmentally controlled conditions (22–23 °C, light/dark cycle) with free access to food and water.

2.2. Fish oil supplementation

The fish oil capsules (PROEPA – Ache®), containing the polyunsaturated fatty acids DHA (120 mg/1 g) and EPA (180 mg/1 g) were dissolved in Cremophor (Sigma®) 0.009% in distilled water and administered by oral gavage using a stainless steel curved feeding needle. Vehicle solution (V) consisted of the same amount of Cremophor and water.

The oral fish oil supplementation was made daily with 85 mg/kg/day on female rats (which were the progenitors of the rats that receive fish oil throughout the study) started 15 days before mating until lactation and continued after weaning. In parallel, other female rats (which were the progenitors of the rats that receive vehicle solution throughout the study) were administered with vehicle solution for the same period. Only parental females were treated. At first, it was select females with regular estrous cycle. Fifteen females were mated, twelve females became pregnant, and half of them received fish oil supplementation. It was obtained 12 litters which produced a total of 108 puppies. Litter size range from 9 to 11 pups. Four days after delivery, all litters were reduced to 8 pups to avoid the risk of malnutrition. At postnatal day 21, 3–4 male animals of each litter were randomly chosen and distributed in the groups. The groups were supplemented with daily fish oil (85 mg/kg/day) or V (1 mL/250 g body weight) until 10-month-old.

2.3. Animal groups

Rats administrated with fish oil or V were submitted to physical exercise (EX) or maintained in their home cages (control condition – C): control-vehicle- (CV, $n = 11$), control-fish oil (CF, $n = 11$), exercise-vehicle-(EXV, $n = 11$) and exercise-fish oil (EXF, $n = 12$). Another group ($n = 12$, obtained from 3 litters) was also used as second control: animal at 90-days-old administered with vehicle throughout the life.

2.4. Physical exercise program

At postnatal day 21 (P21) animals of the exercise group were familiarized with the apparatus for 3 days by placing them on a treadmill (Columbus instruments) for 5 min/day at speed of 8 m/min at 0% degree incline. Electric shocks were used sparingly to motivate the rats to run. To provide a measure of trainability, we rated each animal's treadmill performance on scale of 1–5 according to the following anchors: 1, refused to run; 2, below average runner (sporadic, stop and go, wrong direction); 3, average runner; 4, above average runner (consistent runner occasionally fell back on the treadmill); 5, good runner (consistently stayed at the front of the treadmill) [47]. Animals with a mean rating of 3 or higher were included to the exercise group. This procedure was used to exclude possible different levels of stress among animals. Subsequently, selected animals were submitted to a physical exercise program performed between P21 and P60, 6 days per week. Each training session started with a 5 min warm-up at 8–10 m/min. Running time and speed gradually increased during the subsequent days, until reaching 18 m/min during 60 min. Animals of the control group were transferred to the experimental room and handled in the same way as animals of the exercise group (water and food deprivation during treadmill exercise). After this period animals started to run 5 days/week at 20 m/min during 30 min until 270-day-old.

2.5. Behavioral tests

2.5.1. Open-field habituation test

The open-field apparatus was designed as described by Broadhurst [48] and consisted of a circular arena (96 cm diameter) enclosed by matte white walls (circumference 40 cm) with an open top and floor divided into 19 squares. Each animal was placed in the center of the apparatus and observed for 10 min. Hand-operated counters were used to score total locomotion (number of any floor unit entered). The procedure was repeated after seven days. The observer was always unaware of the experimental design and the apparatus was cleaned with a 5% alcohol solution after each behavioral session.

2.5.2. Object recognition test

The object recognition test was carried out in the same arena used for the open-field test, as described above. Before training, all animals were habituated to the experimental arena. During habituation sessions animals were free to explore the open-field arena in the absence of any specific behavioral stimulus. The animals were placed in the arena containing two different objects and left to explore them freely for 10 min. The test occurred 180 min later, in order to evaluate short-term memory. Seven days later, long-term memory was investigated. In the tests, one of the objects was changed for a new object and the rat was introduced in the arena for more 3 min. The positions of the objects (familiar or novel) were randomly permuted for each experimental animal and the arena was cleaned between trials. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on or turning around the object was not considered exploratory behavior. The time spent to explore each object was recorded by an observer blind to the treatment. The objects and the apparatus were cleaned with a 5% alcohol solution after each behavioral session. The objects used were a transport module for rodents (Habitrail OVO®, Hagen), a rubber ball for pets supported on a T-form tube (Habitrail®, Hagen), a house and a seesaw both made of wood for rodents (Animalíssimo®).

2.5.3. Plus-maze discriminative avoidance task (PM-DAT)

The apparatus employed was a modified plus-maze, elevated 63 cm from the floor, made of wood, containing two enclosed arms with side-walls, and no top (50 cm × 40 cm × 12 cm: $l \times h \times w$, 03 lx at floor level) opposite to two open arms (50 cm × 12 cm, 09 lx at floor level). A 100-W lamp was placed exactly over the middle of one of the enclosed arms (aversive enclosed arms, 660 lx at floor level). Still, the floor of this arm was covered with black rubber, being a spatial cue to the animals. In the training session, each rat was placed in the center of the apparatus (facing the space between both open arms) and, over a period of 10 min, every time the animal entered with the four paws in the enclosed arm containing the lamp, an aversive situation was produced until the animal left the arm. The aversive stimuli were the 100-W light and a frontal cold-air blow produced by a 700-W hair drier placed above the end of the aversive enclosed arm. Both sessions (training and test) occurred in the same room with a controlled intensity of light throughout the behavioral sessions (09 lx). On each side of the plus-maze discriminative avoidance apparatus, there were different extra-maze visual cues (door, window, cupboard and observer) that rats could use to distinguish the location of the different arms of the maze. In the test session (performed in the same room with the observer in the same position), the rats were again placed in the apparatus for 3 min without receiving any aversive stimulation. In all experiments, the animals were observed in a random order and in a blind manner, and the apparatus was cleaned with a 5% alcohol solution after each behavioral session. Total number of entries in any of the arms and percent time spent in open arms (time spent in open arms/time spent in both open and enclosed arms) were calculated. Learning and memory were evaluated by the comparison between the time spent in the aversive enclosed arm and in