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Omega-3 effects on electrocorticographic patterns of adult Wistar rats exposed to ionizing radiation

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ABSTRACT

This study aimed to assess the effect of supplementation with omega-3 in Wistar rats exposed to ionizing radiation in a dose of 18 Gy on the cortical electrical activity, using mathematical methods such as the power spectrum (PS) and the detrended fluctuation analysis (DFA) in the evaluation of the electrocorticogram (ECoG) record. The PS analysis showed that in non-irradiated animals but supplemented with omega-3 there was a decrease in the power of the beta rhythm, while the DFA applied to different frequency ranges of the ECoG showed a significant increase in the long-range correlation only for the theta wave when compared with non-supplemented animals. In the evaluation of the radiation effect through the PS, an increase in the power of the theta rhythm was observed in both groups (non-supplemented and supplemented animals) only when they were evaluated one week after irradiation. The DFA method also showed difference in this wave. The PS and DFA methods applied to the ECoG record allowed a quantitative analysis of the cortical electrical activity in rats in response to the omega-3 effects, ionizing radiation, or both.

1. Introduction

Ionizing radiation (IR) has multiple effects on brain functions, acting directly on the nervous system and indirectly on other systems through the reactivity of the central nervous system (CNS) [1]. In studies conducted with individuals who worked during the Chernobyl accident, changes in brain wave activity were observed, suggesting that exposure to ionizing radiation may interfere with brain electrical activity causing neuropsychophysiological disorders [2].

The electrical behavior of brain tissue can also be influenced by its lipid composition, corresponding to up to 70% of its dry weight [3]. Polyunsaturated fatty acids (PUFA) are the most abundant in this tissue and are related to the neuronal cell membrane fluidity and, consequently, to the cell signaling processes. Thus, changes in the qualitative and quantitative characteristics of these PUFA can result in brain electrical activity modifications [4].

Omega-3 PUFA is considered the type of fat preferred by the brain and nervous system, having a unique, important, and non-replaceable contribution in their functions [5]. This PUFA is essential for some animal species, such as humans, who are unable to synthesize or convert them into their derivatives due to the absence of desaturases and elongases enzymes [6].

Fish-derived omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) can be synthesized from alpha-linolenic acid (ALA), an important precursor to this family [5]. Among the several benefits related to these PUFA, DHA is considered fundamental for the brain and visual system development, while EPA is mainly related to cardiovascular health protection [7].

Therefore, this study aimed to investigate the omega-3 supplementation influence on brain electrical activity of animals exposed to IR and its possible action as a neuroprotector. For this, mathematical methods such as the Power Spectrum (PS) and the Detrended Fluctuation Analysis (DFA) were applied to analyze the electrocorticogram (ECoG).

2. Material and methods

Adult male Wistar rats (*Rattus novergicus*, var. *albinus*) were housed at controlled temperature (23 ± 2 °C), humidity (50%), a 12-h light-dark

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cycle and had free access to food and water. They were divided into two groups (n = 8/group). The supplemented group (SG) received omega-3 (1 ml/p. o./day) while non-supplemented group (NSG) animals received 0.9% sodium chloride solution (1 ml/p. o./day) from 8th to 17th postnatal week.

Committee for the Care and Ethical Use of Animals in Research: Approbation protocol number 97/2018 CEUA-UFRPE.

2.1. Electrodes implant

In the 17th postnatal week, the animals were submitted to a surgical procedure to implant electrodes on the right cerebral hemisphere, one inserted in the parietal bone over the region of the sensorimotor cortex and another one anterior to the bregma [8].

2.2. Ionizing radiation exposure

In the 15th postoperative day (19th week) the animals were exposed to IR at the Institute of Radiotherapy Waldemir Miranda (IRWAM - Recife/PE). The irradiation was performed in animals under anesthesia with ketamine 75 mg/kg and xylazine 10 mg/kg IP (19th week). 18 Gy of X-ray radiation was applied: 9 Gy on the top and another 9 Gy on the bottom of the head [9]. The Clinac 600C linear accelerator (Varian Medical System, Palo Alto, CA, USA) was used. The dose rate used was 2.4 Gy by minute at 1.5 cm deep, for a total exposure time of 5.02 min.

2.3. Cortical electrical activity record and signal processing

Animals were placed in a box, inside the Faraday cage to minimize noise from the local electrical network and the ECoG signals obtained during 30 min of spacial exploration were amplified and recorded using an EMG 410C device (EMG System do Brasil, São José dos Campos, SP, Brazil). These records were obtained at three distinct moments: 24 h before, 24 h and one week after IR exposure (M1, M2 and M3, respectively).

Records were segmented in 5-min windows. The signal filtering and the ECoG analysis were performed using Phyton programming language to apply the math methods.

2.4. Fourier transform and power spectrum

The Fourier Transform (FT) allows us to know the contribution of each frequency component present in a time series. The ECoG record is a complex signal that can be broken down into sub-rhythms and represented in the frequency domain. After the decomposition of the signal, the energy for each frequency range can be calculated. The FT square of ECoG generates its PS. The average power obtained in the spectrum allows estimating the contribution of different brain rhythms to the ECoG signal [10].

2.5. Detrended fluctuations analysis

The DFA is a non-linear method based on fluctuation analysis of the data after the removal of trends in an integrated time series [11]. This series integrated is described as follows equation (1):

$$y(k) = \sum_{i=1}^{k} (y(i) - M) \quad (1)$$

where M is the mean value of the original series y(i), with i = 1, 2, …, N and k is an integer number. The series integrated y(k) is divided into intervals of length n. Each interval is set by using polynomial functions, representing the trend in each interval. The function that characterizes the length of the fluctuations for a length n of the intervals used to remove the trend is shown by the following expression:

$$F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^{N} [y(k) - y_n(k)]^2} \quad (2)$$

The calculation is repeated at various interval lengths n to determine the relationship between fluctuations (F (n)) and the length of interval n. For a process self-similar, F (n) increases with n by the power law, as shown in equation: $F(n) \approx n^{\alpha}$. The self-similarity exponent α can be calculated by using the slope obtained by linear regression of graph log F (n) versus log n. If $\alpha = 0.5$, is a random event; $\alpha > 0.5$ has a persistent correlation; $\alpha < 0.5$ has an anti-persistent correlation; $\alpha = 1$ is a 1/f noise.

2.6. Statistical analysis

Shapiro-Wilks test was applied to verify the normal distribution of data obtained. Comparison between groups was performed by the unpaired *t*-test. For the same group, the data whose values followed a Gaussian distribution the ANOVA and Tukey's post hoc tests were used, while those with non-Gaussian distribution were analyzed by Kruskal-Wallis and Dunn's post hoc tests. p-values < 0.05 were considered statistically significant.

3. Results

3.1. Evaluation of omega-3 effect

Omega-3 supplementation promoted changes in the cortical electrical activity of animals when evaluated before exposure to radiation (M1), with a significant increase in beta rhythm power to the SG in comparison with NSG ($p = 0.0184$). For the other rhythms, no significant changes were identified ($p > 0.05$). This data can be seen in Table 1.

In total ECoG, no significant change was identified in the long-range correlation (α-DFA) associated with the omega-3 supplementation ($p = 0.2124$) as can be seen in Fig. 1. On the other hand, when applied to the different frequency bands (delta, theta, alpha, and beta) the DFA technique showed a significant difference only for the theta wave ($p = 0.0003$) when the SG is compared to the NSG.

3.2. Evaluation of ionizing radiation effect

In ECoG of animals that were not supplemented with omega-3 (NSG), it can be observed that the exposure of the head and neck region to X radiation at a dose of 18 Gy did not produce an immediate effect (in the evaluation performed 24 h after exposure - M2) in the cortical electrical activity ($p = 0.8033$). However, there was a significant increase in the power of the theta rhythm ($p = 0.0453$) in the evaluation carried out one week after irradiation (M3) (Table 2).

The α-DFA analysis of the total ECoG record and for the different brain rhythms (theta, delta, alpha, and beta) evaluated 24 h before radiation exposure did not show a significant change in this parameter in relation to evaluations performed 24 h ($p = 0.7730$) and one week ($p = 0.7372$) after exposure to IR (M2 and M3, respectively) as can be seen in Fig. 2.

Table 1
Power values (mean ± standard deviation) of the ECoG rhythms of supplemented (SG) and non-supplemented (NSG) groups.

Group	Delta	Theta	Alpha	Beta
SG	11.88 ± 0.79a	5.36 ± 0.39a	2.76 ± 0.33a	1.25 ± 0.11a
NSG	13.02 ± 0.57a	6.11 ± 0.56a	2.84 ± 0.20a	0.90 ± 0.06b

Different letters in the same column represent a statistically significant difference ($p < 0.05$). Unpaired *t*-test.

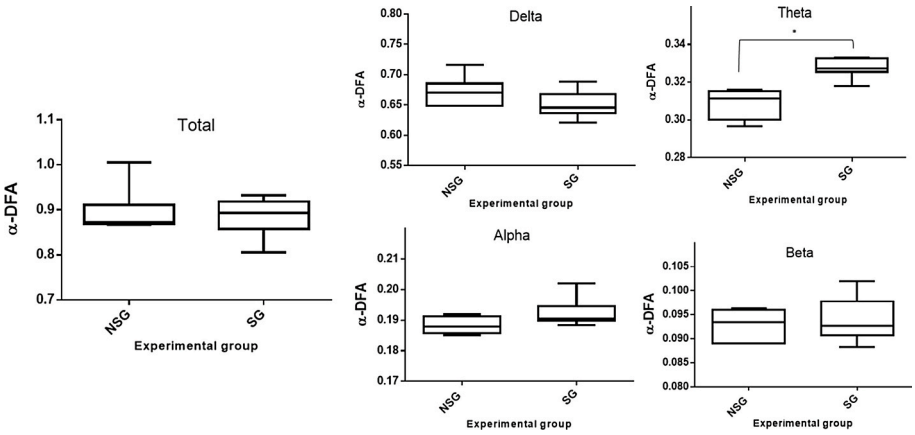

Fig. 1. α-DFA values obtained to the total ECoG and to different brain rhythms: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (16–30 Hz) of the non-supplemented (NSG) and supplemented (SG) groups evaluated 24 h before radiation exposure. Values with (*) represent a significant difference between groups ($p < 0.05$). Unpaired *t*-test.

Table 2
Power of the ECoG delta, theta, alpha, and beta rhythms (mean ± standard deviation) of the non-supplemented group (NSG) 24 h before, 24 h, and one week after irradiation (M1, M2, and M3, respectively).

Evaluation time	Delta	Theta	Alpha*	Beta
M1	11.88 ± 1.94a	5.36 ± 0.97a	2.76 ± 0.83a	1.25 ± 0.27a
M2	11.27 ± 2.24a	5.79 ± 1.30 ab	2.73 ± 0.55a	1.30 ± 0.18a
M3	11.79 ± 1.74a	7.32 ± 1.34b	3.00 ± 0.36a	1.27 ± 0.19a

Different letters in the same column represent significant difference ($p < 0.05$). ANOVA and Tukey's multiple comparisons tests. *Kruskal-Wallis and Dunn's multiple comparisons tests.

3.3. Evaluation of ionizing radiation effect in animals supplemented with omega-3

In the animals that received omega-3 supplementation and were subsequently irradiated, no immediate effect on the power spectrum of the different brain rhythms ($p = 0.8328$) was observed (24 h after irradiation - M2). However, there was a significant increase in the power of the theta rhythm of the ECoG ($p = 0.0220$) of these animals, when evaluated one week after irradiation (M3) (Table 3).

The values obtained for the α-DFA parameter in the total ECoG record did not show significant changes ($p < 0.05$) between the times of evaluation (M1, M2, or M3), as can be seen in Fig. 3. The DFA technique applied to the different frequency ranges showed a statistically significant difference only for the theta wave ($p = 0.0053$) in the SG when evaluated one week after exposure to ionizing radiation (M3).

Comparison between supplemented and non-supplemented groups after irradiation.

No significant difference ($p > 0.05$) was observed in the ECoG power spectrum between SG (Table 2) and NSG (Table 3) groups when evaluated 24 h (M2) and one week after irradiation (M3).

The α-DFA indexes obtained to total ECoG of SG and NSG did not

Table 3
Power of the ECoG delta, theta, alpha, and beta rhythms (mean ± standard deviation) of the supplemented group (SG) 24 h before, 24 h, and one week after irradiation (M1, M2 and M3, respectively).

Evaluation time	Delta	Theta	Alpha	Beta
M1	13.02 ± 1.51a	6.11 ± 1.48a	2.84 ± 0.55a	0.90 ± 0.17a
M2	12.66 ± 2.45a	6.53 ± 1.73ab	2.41 ± 0.52a	1.09 ± 0.27a
M3	11.66 ± 1.31a	8.49 ± 0.77b	3.02 ± 0.86a	1.21 ± 0.20a

Different letters in the same column represent a statistically significant difference ($p < 0.05$). ANOVA and Tukey's tests.

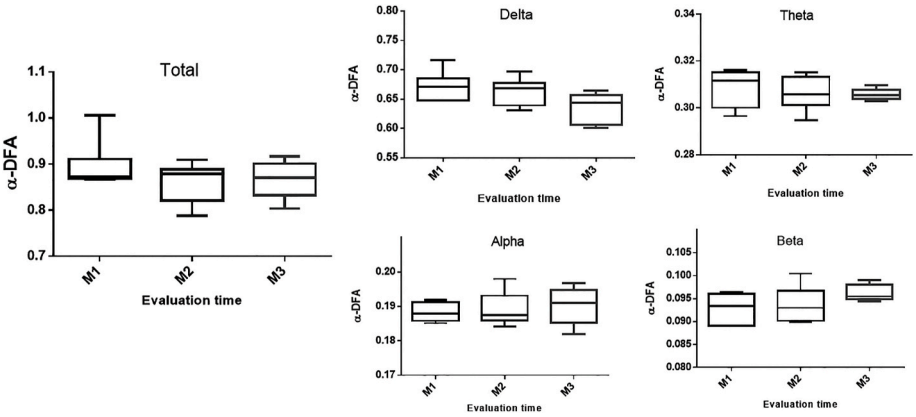

Fig. 2. α-DFA values obtained to the total ECoG and different brain rhythms: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (16–30 Hz) of the non-supplemented group (NSG) evaluated at 24 h before irradiation (M1), 24 h (M2) and one week (M3) after exposure to ionizing radiation. No significant difference was observed ($p > 0.05$). ANOVA test.

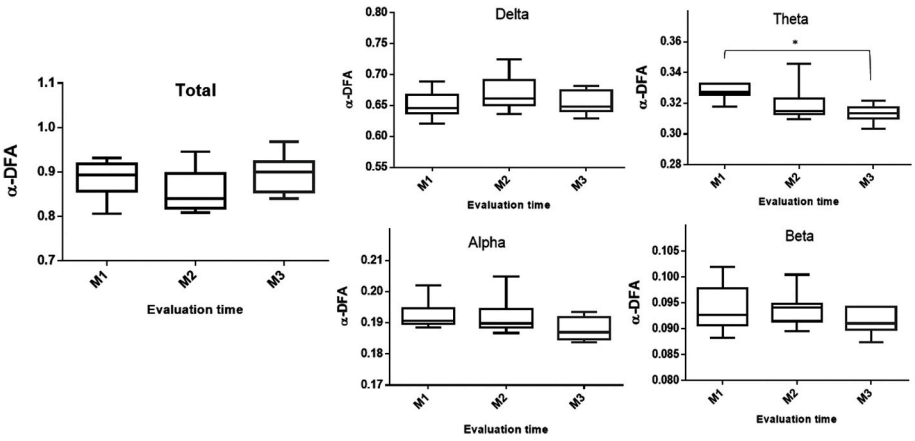

Fig. 3. α-DFA values obtained to the total ECoG and to different brain rhythms: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (16–30 Hz) from the supplement group (SG) evaluated 24 h before, 24 h, and one week after irradiation (M1, M2 and M3, respectively). Values with (*) represent a statistically significant difference in the group concerning the moment of evaluation ($p < 0.05$). Kruskal-Wallis and Dunn's tests to total ECoG, alpha, and theta rhythms. ANOVA with Tukey's tests to delta and beta rhythms.

differ significantly from each other ($p > 0.05$) when the analysis was performed at 24 h and one week after IR exposure. On the other hand, significant differences were observed at M2 to the theta rhythm ($p = 0.0055$) and at M3 for both theta ($p = 0.0252$) and beta rhythms ($p = 0.0067$) when the analysis were performed to each ECoG wave (Fig. 4).

4. Discussion

The omega-3 supplementation has been recommended for a variety of acute and chronic diseases. However, the clinical and experimental results about the efficiency of these long-chain PUFAs are still conflicting [12].

Our results suggest that omega-3 can modify brain dynamics, promoting a slowing down in brain electrical activity, since it reduced the contribution of the beta rhythm.

Supplementation with PUFAs and their metabolites can decrease neuronal excitability through modulation of sodium and calcium channels [13]. Since the PUFAs are capable to reduce neuronal excitability, their consumption can prevent nervous system disorders, such as epilepsy [14].

Rats supplemented with omega-3 showed an increase in the power of the beta and a decrease of the delta waves, indicating that this lipid increased brain activity [8]. This observation can result from an increase in membrane fluidity, which could provide an adequate environment for ion channels to perform their functions [15].

On the other hand, rats supplemented with omega-3 from the intrauterine phase to adulthood, showed a reduction in the power of the beta rhythm [16], corroborating our results. The authors suggest that oils rich in PUFA are highly prone to oxidation of lipid peroxides and other secondary oxidation products, which in turn could promote changes in brain dynamics. There are indications that more unsaturations in the chain increase the susceptibility of PUFAs to oxidation, therefore EPA and DHA become susceptible to this phenomenon, while saturated and monounsaturated fatty acids do not undergo peroxidation [17,18].

The exposure of rats to IR in the head and neck regions at doses of 18 Gy promoted significant changes in the power of theta wave when the animals were evaluated 24 h and 90 days after exposure, demonstrating changes in the animals cortical activity resulting from the effects of radiation on brain tissue [11]. According to these authors, the theta wave can be considered as a possible exposure biomarker for IR, due to the greater sensitivity of this brain rhythm in relation to the others. In the present study, a change in this brain rhythm could be observed one week after the animals' IR exposure, corroborating what was previously reported.

IR exposure can have different consequences on the brain structure and function, as they influence the CNS and behavior, resulting from direct and indirect effects by CNS reactivity to radiation damage from other systems [20]. Molecular studies of brain irradiation effects in animals and humans provide evidence of different abnormalities such as apoptosis, neuroinflammation, loss of myelin sheaths and irreversible damage to neural trunk compartments with long-term impairment of adult neurogenesis [20–22].

Although the mechanisms by which ionizing radiation affects brain

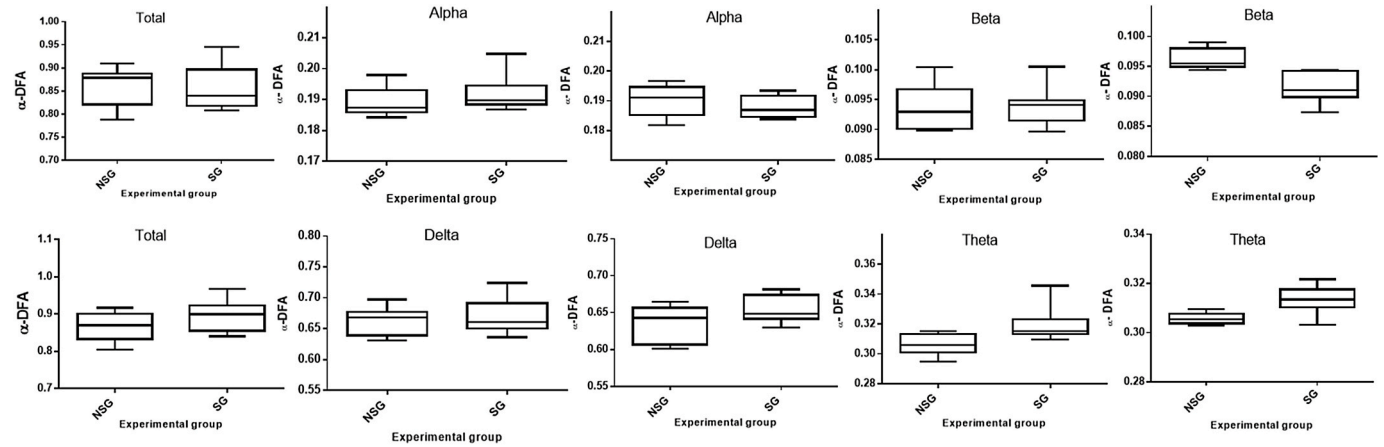

Fig. 4. α-DFA values obtained to the total ECoG and to different brain rhythms: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), and beta (16–30 Hz) of the non-supplemented (NSG) and supplemented (SG) groups evaluated 24 h (M2) and one week after radiation exposure (M3). Values with (*) represent a significant difference between groups ($p < 0.05$). Unpaired *t*-test.

electrical activity are not yet fully elucidated, it is suggested that these changes may be due to the formation of reactive oxygen species (ROS) which can result in membrane phospholipids peroxidation and changes in their conductivity and structural integrity [23].

The administration of an antioxidant substance before irradiation could reduce the radiation effect because it absorbs free radicals, protects tissues against toxic oxygen derivatives (superoxide anions and hydroxyl radicals), inhibits enzymes involved in initiation reaction [24] and stops chain reactions by donating hydrogen atoms to peroxide radical [25]. However, radiation has an action on fatty acids promoting the destruction of natural antioxidants and allowing peroxide formation [19].

The brain has a low capacity to react against ROS formation because of its limited antioxidant defenses, high oxygen consumption, and high content of unsaturated fatty acids, compared to other tissues, making this organ more vulnerable to the increase of reactive species [26,27].

A study performed by Ref. [27] identified radio-induced lipid changes in brain tissue of rats that suggest several possibilities of direct DNA damage, ROS formation that could lead to rupture of intermolecular connections in DNA, lipid oxidation, changes in signaling mechanisms for cellular and molecular repair and intracellular microstructural changes. However, according to the authors, further studies to unravel the radio induced physiological and molecular mechanisms are necessary.

The cortical electrical activity characteristics of self-similarity and chaoticity allow its behavior to be evaluated using techniques such as DFA. The values obtained for the α-DFA index allow us to distinguish the absence or presence of correlation of the signal over time and whether the referred correlation, when present, is persistent or anti-persistent. Through this parameter, then, it would be possible to assess changes in the behavior of brain electrical activity in individuals exposed to radiation with or without omega-3 supplementation.

In the present study, the α-DFA index for the total ECoG of the animals from both experimental groups at the different moments of the evaluation showed persistent behavior. The same behavior was observed in the delta wave. On the other hand, the other brain rhythms (theta, alpha, and beta) showed a long-term anti-persistent correlation. In the persistent correlation, over time, higher values are expected to occur for a given frequency range in the ECoG while the anti-persistent correlation, observed for the faster frequencies indicates that over time higher frequencies are more likely to be followed by lower frequencies, and vice versa [11].

Additionally, the DFA method can be applied to electrophysiological signals as a sensitive tool to distinguish normal and pathological conditions in dynamic systems, including brain electrical activity [11]. Despite the distinct correlation behavior for delta, alpha, theta, and beta rhythms, both omega-3 supplementation and IR exposure or a combination of both did not change the brain dynamics correlation in the conditions used in our experiments.

5. Conclusion

Omega-3 can act as a modulator of the cortical activity, promoting a decrease in the power of the beta rhythm and an increase in the long-range correlation of theta wave. The radiation promoted an increase in the power of theta rhythm as a late effect independent of omega-3 supplementation. The PS and DFA methods applied to the ECoG record allowed a quantitative analysis of the cortical electrical activity in rats in response to the omega-3 effects, ionizing radiation, or both.

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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