Universidade Federal do Pará
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ALTERAÇÕES ELETROCORTICOGRÁFICAS, ELETROCARDIOGRÁFICAS E DE CÁLCIO SÉRICO CAUSADAS POR DOSE SUPRAFISIOLÓGICA DE VITAMINA A

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Dissertação apresentada em formato de Artigo ao Programa de Pós-Graduação em Neurociências e Comportamento do Núcleo de Teoria e Pesquisa do Comportamento da Universidade Federal do Pará como parte dos requisitos para obtenção do título de Mestre em Neurociências e Comportamento

Orientadora: Profa. Dra. Silene Maria Araújo de Lima
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RESUMO

A vitamina A e seus derivados são amplamente utilizados terapeuticamente, principalmente para pele e cabelos, devido à sua atividade antioxidante que protege as células contra os danos causados pelo excesso de radicais livres. A vitamina A atua em receptores nucleares e, como resultado, aumenta a expressão gênica. Altas doses de vitamina A e seus derivados podem levar a alterações nas funções dos órgãos, como atividade osteoclástica e aumento dos níveis séricos de cálcio, que podem precipitar osteopenia e osteoporose. Pouco se sabe sobre sua atividade no sistema nervoso central; no entanto, há relatos de alterações comportamentais e possibilidade de exacerbação de sintomas depressivos com aumento do risco de suicídio. Foram utilizados 45 ratos Wistar machos para este estudo, aos quais foram implantados eletrodos de prata no córtex motor (coordenada estereotáxica de -0,96 do bregma). Para os exames de ECG foi utilizada a derivação D2 e, posteriormente, foram coletadas amostras de sangue dos animais para dosagem do cálcio sérico. Os ratos foram tratados com 50.000 UI/kg i.p. a cada 24 horas por 3, 7 e 14 dias. Cada grupo teve sua atividade eletrocorticográfica (ECoG), atividade eletrocardiográfica (ECG) e níveis séricos de cálcio avaliados. Demonstrou-se que houve aumento gradativo do cálcio sérico, mas manteve-se dentro da normalidade para a espécie. O ECoG revelou aumento da atividade nas bandas de oscilação cerebral de baixa frequência (delta, teta e alfa). A atividade cardíaca estava próxima do normal com ritmo sinusal e diminuição dos intervalos RR e QT.

Palavras-chave: Vitamina A, Eletrocorticograma, Oscilações cerebrais, Ondas cerebrais, Eletrocardiograma.

ABSTRACT

Vitamin A and its derivatives are widely used therapeutically, primarily for skin and hair, due to its antioxidant activity that protects cells against damage caused by excess free radicals. Vitamin A acts on nuclear receptors and, as a result, increases gene expression. High doses of vitamin A and its derivatives can lead to changes in organ functions, such as osteoclastic activity and increased levels of serum calcium, which can precipitate osteopenia and osteoporosis. Little is known about its activity in the central nervous system; however, there have been reports of behavioral changes and the possibility of exacerbating depressive symptoms with an increased risk of suicide. There were used 45 male Wistar rats for this study, to which silver electrodes were implanted in the motor cortex (stereotactic coordinate of -0.96 from bregma). For the ECG examinations, the D2 lead was used, and subsequently, blood samples were collected from the animals for serum calcium measurement. The rats were treated with 50,000 IU/kg i.p. every 24 hours for 3, 7, and 14 days. Each group had its electrocorticographic (ECoG) activity, electrocardiographic (ECG) activity, and serum calcium levels evaluated. It was demonstrated that there was a gradual increase in serum calcium, but it remained within the normal range for the species. The ECoG revealed increased activity in low-frequency brain oscillation bands (delta, theta, and alpha). The cardiac activity was close to normal with a sinus rhythm and a decrease in the RR and QT intervals.

Keywords: Vitamin A, Eletrocorticogram, Brain Oscillations, Brain Waves, Eletrocardiogram.

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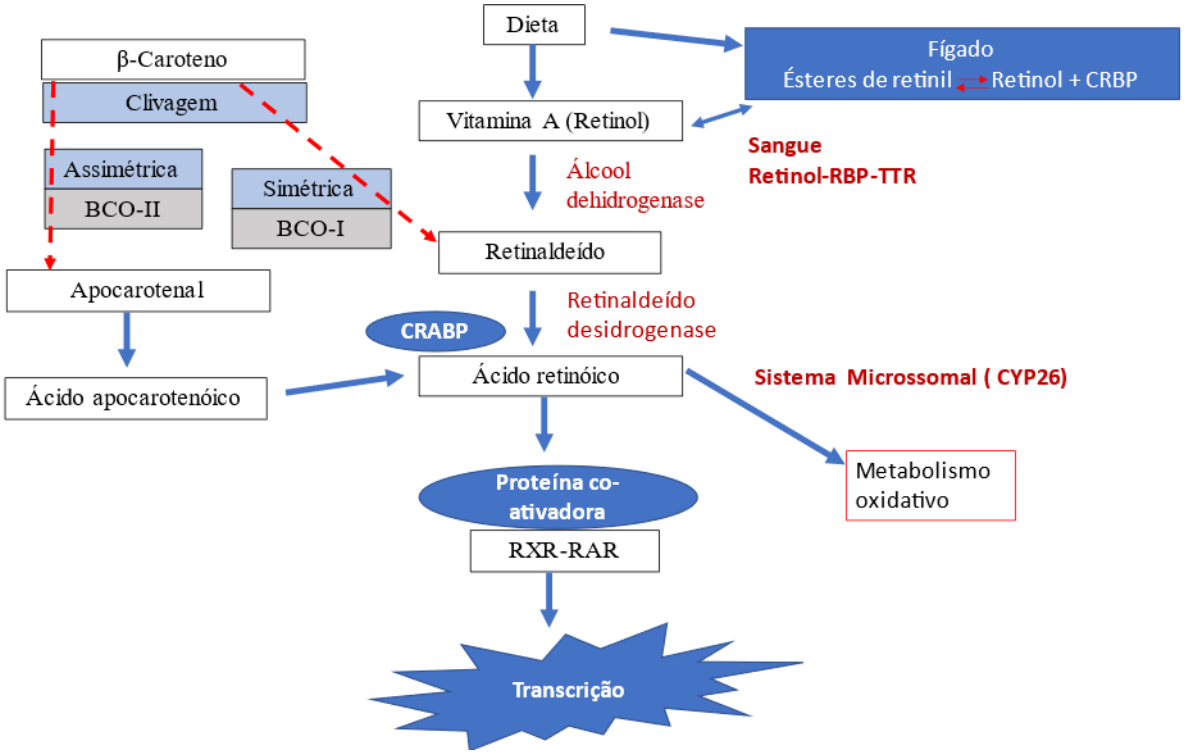
INTRODUÇÃO GERAL

Nutrientes são substâncias químicas encontradas nos alimentos que são essenciais para o funcionamento adequado do corpo humano. Eles são classificados em macro e micronutrientes, de acordo com a quantidade que o corpo precisa de cada um deles (Tiffon, 2018). Os macronutrientes são aqueles que o corpo precisa em grandes quantidades e fornecem energia ao organismo. São macronutrientes: carboidratos, proteínas e gorduras. Os micronutrientes, por outro lado, são aqueles que o corpo precisa em pequenas quantidades, não fornecem energia ao organismo, mas que são essenciais para a manutenção de processos biológicos importantes. São micronutrientes: minerais e vitaminas. As vitaminas são compostos orgânicos essenciais para o funcionamento adequado do corpo humano. Elas são necessárias em pequenas quantidades e devem ser obtidas por meio da alimentação ou suplementação, já que o organismo humano não consegue produzi-las em quantidade suficiente (Guilland, 2013).Segundo o estudo de Berger et al. (2022), sabe-se o que são e quais os benefícios dos micronutrientes, porém muito ainda se tem para estudar, pois os oligoelementos desses nutrientes ainda são desconhecidos, com isso há duas preocupações: saúde pública e saúde individual.As funções das vitaminas são diversas no organismo, importantes por atuar como coenzimas em reações metabólicas, auxiliar na produção de energia, fortalecer o sistema imunológico, manter a saúde dos ossos, dos dentes, dos cabelos e das unhas, entre outras (Guilland, 2013; Eveleens et al, 2021). Elas podem ser divididas em dois grupos: solúveis em água (hidrossolúveis) e solúveis em gordura (lipossolúveis). As vitaminas solúveis em água são as vitaminas C e o complexo B. As vitaminas solúveis em gordura, por outro lado, são as vitaminas K, E, D e A (Guilland, 2013).

Em 1913 a vitamina A foi a primeira vitamina lipossolúvel a ser reconhecida (Roncada, 1998). Especificamente, a vitamina A é um termo genérico e é de uma família de retinóides que se refere a três compostos pré-formados que exibem atividades metabólica: álcool (retinol), o aldeído (retinal) e o ácido (ácido retinóico - AR) e como provitamina A (carotenoides) (Amaral et al., 2014; Zinder et al, 2019). Entre os anos de 1940 e 1950, foi determinado estrutura química da vitamina A (retinoides e carotenoides), juntamente com estudos realizados para descrever sua função biológica e a síntese comercial foram rapidamente desenvolvidos (Bitecouurt, 2013). A provitamina A é o precursor do retinol, o metabólito mais ativo. Sendo essa um micronutriente lipossolúvel essencial que não pode ser sintetizado pelo corpo humano e deve ser obtido da dieta, onde a cocção moderada de vegetais aumenta a liberação de carotenoides, permitindo uma maior absorção (Blaner, 2019). O

ácido retinóico, composto ativo, nas células e nos tecidos devem ser controlados através de um equilíbrio entre sua síntese e catabolismo (Samarut; Rochette-Egly, 2012).

Figure 1. A via metabólica dos retinóides. ADH, álcool desidrogenase; BCO-I, β,β-caroteno-15,15'-monooxigenase; BCO-II, β,β-caroteno-9',10'-dioxigenase; CRABP, proteína celular de ligação do ácido retinóico; CRBP, proteína celular de ligação ao retinol; CYP26, família 26 do citocromo P450; HAT, histona acetiltransferase; HDAC, histona desacetilase; Raldh, retinaldeído desidrogenase; RAR, receptor do ácido retinóico; RBP, proteína de ligação ao retinol; RXR, receptor de retinóide X; SDR, desidrogenases/redutases de cadeia curta; TTR, transtirretina.



A vitamina A desempenha papel importante no corpo humano, sendo essencial para a visão, principalmente no processo visual, participando do grupo prostético das opsinas - proteínas sensíveis à luz da retina (Cuppari, 2019; Polcz MD; Barbul MD, 2019), o crescimento e desenvolvimento dos ossos, o sistema imunológico e a saúde da pele e cabelos (Blaner, 2019; Eveleens et al, 2021; Yee et al, 2021). Ela também é um antioxidante que ajuda a proteger as células contra danos dos radicais livres. (Yee et al, 2021).

A deficiência de vitamina A pode levar a uma série de problemas de saúde, incluindo: cegueira noturna, xeroftalmia, infecções, problemas de crescimento e desenvolvimento, problemas

de pele, anemia e comprometimento da função imunológica. O excesso de Vitamina A também é maléfico: pode causar uma condição chamada hipervitaminose A. Alguns dos sintomas são: náuseas e vômitos, tontura e dor de cabeça, pele seca e coceira, problemas ósseos e articulares, perda de cabelo, visão turva ou distorcida, fadiga e fraqueza muscular, dificuldade em dormir (Olson; Ameer; Goyal, 2022). A absorção da vitamina A pode ser prejudicada pela deficiência de zinco, consumo de álcool, óleo mineral, neomicina e colestiramina. Uma vez na digestão, a vitamina A é empacotada em quilomícrons pelas células intestinais. O quilomícron é metabolizado pela lipoproteína lipase no duodeno e absorvido pelas células da mucosa do intestino delgado. Ele viaja através do plasma até o fígado, onde cerca de 70% da vitamina A é armazenada em excesso como éster retinílico no células estreladas hepáticas (Zinder et al, 2019).

Em solo brasileiro a deficiência de Vitamina A é combatida desde 2005 através do Programa Nacional de Suplementação de Vitamina A (PNSVA) cujo objetivo é a redução da morbidade e mortalidade em crianças menores de cinco anos de idade. A oferta da suplementação é ofertada pelo Sistema Único de Saúde (SUS) por meio de nove megadoses a cada seis meses de vida da criança residente no Norte, Nordeste e diversos municípios das demais regiões (Brasil, 2023).Por conta dos prejuízos, que tanto a deficiência quanto o excesso de Vitamina A podem causar no organismo humano, é recomendado, em caso de dúvidas, consultar profissionais da área de saúde para que se faça monitoramento de dosagem, visando reduzir o consumo em casos de excesso e aumentar o consumo em casos de deficiência (Soares et al, 2019). A toxidade da Vitamina A é identificada por meio de exames clínicos e físicos, sendo que quando presente durante a gravidez pode causar má formação fetal, entre outros. Exceto em casos de ingestão acidental por parte de crianças, quando se dá a intoxicação aguda, os casos de Hipervitaminose ocorrem devido o uso além do recomendado diariamente. A hipervitaminose natural é improvável, mesmo que haja o consumo de alimentos ricos em carotenoides em excesso, uma vez que o organismo converte e absorve o micronutriente de forma lenta. No entanto, é possível o aparecimento da carotenemia, normalmente assintomática, porém, pode provocar o amarelamento da pele (carotenose). Germano e Brazaca (2004) definem a biodisponibilidade de Vitamina A como a quantidade que é ingerida e a quantidade que de fato é absorvida pelo organismo do indivíduo, sendo que fatores genéticos, o estado nutricional, o tipo e a quantidade de carotenoides e a presença de fibras em maior ou menor volume interferem diretamente no resultado da absorção, bem como o consumo de alimentos crus ou cozidos, manipulados e processados, de origem vegetal ou animal. Johnson (2022) afirma que a confirmação do quadro de hipervitaminose ocorre com os seguintes resultados de exames laboratoriais

considerando o estado de jejum e que o exame clínico pode ser confundido com outras condições clínicas, já que a carotenose se mostra presente no hipotireoidismo grave e na anorexia nervosa. É possível observar que vários pesquisadores defendem que a utilização correta do nutriente é aliado na prevenção de certos tipos de câncer, ao passo que a ingestão indevida pode provocar o aparecimento de tumores malignos, além de doenças cardiovasculares.

Além disso, distúrbios relacionados às funções do sistema nervoso foram descritos e parecem estar associados à ingestão excessiva de vitamina A ou seus derivados, como a isotretinoína. Dentre esses efeitos colaterais, os mais comumente observados são confusão, irritabilidade, ansiedade, depressão e ideação suicida (Snodgrass,1992). Aumento da pressão no líquido cefalorraquidiano também foi relatado (Pilorget, 1995), bem como teratogênese e aborto (Szymonski, 2020). Os derivados da vitamina A também foram aplicados com sucesso em uma variedade de condições dermatológicas, incluindo câncer de pele, psoríase, acne e ictiose (Ghyselinck et al, 2019).

O ácido retinóico, um metabólito do retinol, funciona como um ligante para receptores RA nucleares (RARs) e receptores X retinóides (RXRs), que regulam o desenvolvimento de animais cordados e podem ativar ou reprimir a transcrição de genes-chave do desenvolvimento, incluindo a medula espinhal, membros anteriores, coração, olho e trato reprodutivo (Ni et al, 2019; Ghyselinck et al, 2019). Ambos RAR e RXR são membros de uma grande superfamília de proteínas de ligação ao DNA chamadas de receptores hormonais nucleares que estão presentes em todos os animais multicelulares. Além de RAR e RXR, esta superfamília inclui receptores para hormônios tireoidianos, esteroides (estrogênio, glicocorticóide, mineralocorticóide, progesterona e andrógeno) e vitamina D (Schubert; Germanin, 2023). Casos de bloqueio atrioventricular causados por derivados da vitamina A, como o ácido all-trans-retinóico, têm sido relatados, demonstrando possíveis alterações diretas no funcionamento do sistema cardiovascular (Shih; Wu, 2014).

Estudos revelaram consequências deletérias da falha na sinalização da vitamina A em comportamentos motores relacionados à dopamina, bem como melhora nos movimentos voluntários devido à suplementação de nutrientes (Marie et al, 2022). Adicionalmente, foram observadas alterações no eletroencefalograma de pacientes em uso de isotretinoína, mostrando picos anormais nos exames de pacientes suscetíveis (Hermando-Requejo et al, 2021).

Os distúrbios relacionados às funções nervosas foram relatados e aparecem na lista de efeitos colaterais resultantes da ingestão excessiva de vitamina A. A ingestão de vitamina A em

níveis moderados a altas doses (25.000 - 150.000 UI/kg) resultaram em uma variedade de efeitos, por exemplo, intoxicação gastrointestinal aguda e crônica, pseudotumor cerebral, irritabilidade, ansiedade e, mais severamente, depressão (O'reilly et al., 2008; Oliveira et al., 2012).

Para identificação dos efeitos neurológicos, existe o eletroencefalograma (EEG), o qual corresponde a um mecanismo complexo de registro da atividade elétrica cerebral, responsável pelo fornecimento de informações sobre atividades neurais subjacentes no cérebro, e nesse caso, representa um papel significativo para as pesquisas na área da saúde (Machado et al., 2010; Ahirwal e Kumar, 2016). Assim, a eletroencefalocorticografia é uma metodologia que utiliza o registro da atividade elétrica cerebral, podendo ser utilizada para identificar o funcionamento das mais diferentes áreas cerebrais. Seus resultados identificam as diferentes ondas cerebrais geradas pela atividade elétrica, tais como delta, gama, alpha, theta e beta. (Sanei e Chambers, 2007).

A onda Delta ocorre durante o sono profundo. As ondas Theta, estão presentes em crianças, mas podem ocorrer em adultos com algum tipo de estresse emocional. Já as ondas Alpha, indicam uma condição de consciência relaxada ou sem estímulo externo. As ondas Betas, são associadas à atividade mental intensa, à atenção concentrada em um estímulo externo e à resolução de problemas concretos (Webster e Nimunkar, 2020).

Existem relados de que a ingestão crônica de vitamina A até níveis de 10 a 20 vezes maiores que a dose diária recomendada pode levar à hipervitaminose A, porém a dose tóxica efetiva depende da idade, dosagem e duração do tratamento. Em adultos, a ingestão crônica de mais de 30 mg por dia de retinol (equivalentes a 100.000 UI/dia) frequentemente leva à hipervitaminose A, entretanto, sintomas leves podem aparecer com a ingestão crônica, em níveis baixos como 10mg por dia (equivalentes a 33.000 UI/dia). Em crianças a intoxicação pode ocorrer com doses ainda mais baixas de retinol (Penniston, 2006).

Além disso, a suplementação aguda e crônica de vitamina A na dose terapêutica (1000 UI /kg dia ou 2500 UI / kg dia) ou doses altas (4500 UI /kg dia ou de 9000 UI / kg dia) induzem insulto oxidativo no córtex cerebral e cerebelo de rato adulto (Oliveira et al., 2008). Esses mesmos autores observaram que a suplementação de palmitato de retinol (1.000 a 9.000 UI/ dia) por 28 dias diminuiu o fator neurotrófico derivado do cérebro (BDNF) no hipocampo de ratos (Oliveira et al., 2012).

ARTIGO

Electrocorticographic, electrocardiographic, and serum calcium Alterations caused by a supraphysiological dose of vitamin A.

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Vitamin A is a vital nutrient that plays a crucial role in numerous functions within the human body , its indiscriminate use has increased and contributed to hypervitaminosis. This condition is rarely described in terms of electrophysiology. Silver electrodes were implanted in the motor cortex Rats Wistar for the ECG examinations, the D2 lead was used, and subsequently, blood samples were collected from the animals for serum calcium measurement. The animals recive 50.000 IU/kg i.p. every 24 hours for 3, 7, and 14 days. Electrocorticographic (ECoG) electrocardiographic (ECG) activities and serum calcium levels evaluated. The gradual increase in serum calcium was observed , but it remained within the normal range for the species. The ECoG revealed increased activity in low-frequency brain oscillation bands (delta, theta, and alpha). The ECG express a decrease in the RR and QT intervals. These findings contribute to the elucidation of electrophysiological changes in hypervitaminosis.

Keywords: Vitamin A, hypervitaminosis, Eletrocorticogram, Brain Waves-Oscillations, Eletrocardiogram, Calcium.

1. Introduction

Vitamin A is a vital nutrient that plays a crucial role in numerous functions within the human body. It is involved in various aspects, including vision, bone growth and development, immune system support, skin and hair health, reproductive health, cognitive function, and acting as an antioxidant to shield cells against harm caused by free radicals (1,2). Moreover, this vitamin aids in wound healing, assists in the pancreatic regulation of blood glucose, and stimulates tissue proliferation, including the growth of epithelial tissue, collagen formation, and fibroblast activity. Furthermore, Vitamin A contributes to significant activities in the body such as gene expression, reproduction, embryonic development, and immune function (3,4).

It can be found in the body in two main forms, which are retinol and its derivatives, such as retinoic acid (RA) and retinaldehyde, or carotenoids, such as beta-carotene. It is obtained through diet and supplementation, either through clinical use or inadvertently (2).

The status of vitamin A is monitored as part of health programs to prevent the occurrence of subclinical diseases due to deficiency, as well as the effects of toxicity. The detrimental outcomes of vitamin A deficiency are well-known. However, further studies are needed to determine if subclinical toxicity exists or not, and if it does, what are the effects on health and overall well-being (5,6).

In this regard, research on the toxicity of vitamin A has mainly been conducted in animals, and most studies have been short-term and focused on acute effects (7,8). Therefore, the most common parameters analyzed in cases of vitamin A toxicity include the observation of acute signs and symptoms such as nausea, vomiting, diarrhea, headache, bulging fontanelle in infants, fever (9), and teratogenesis in animal and human models (8).

Furthermore, disorders related to nervous system functions have been described and appear to be associated with excessive intake of vitamin A or its derivatives, such as isotretinoin. Among these side effects, the most commonly observed are confusion, irritability, anxiety, depression, and suicidal ideation (10). Increased pressure in the cerebrospinal fluid has also been reported (11) as well as teratogenesis and abortion

(12). Vitamin A derivatives have also been successfully applied in a variety of dermatological conditions, including skin cancer, psoriasis, acne, and ichthyosis (13).

Retinoic acid, a metabolite of retinol, functions as a ligand for nuclear RA receptors (RARs) and retinoid X receptors (RXRs), which regulate the development of chordate animals and can activate or repress the transcription of key developmental genes, including the spinal cord, forelimbs, heart, eye, and reproductive tract (14,15). Both RAR and RXR are members of a large superfamily of DNA-binding proteins called nuclear hormone receptors that are present in all multicellular animals. In addition to RAR and RXR, this superfamily includes receptors for thyroid hormones, steroids (estrogen, glucocorticoid, mineralocorticoid, progesterone, and androgen), and vitamin D (16). Cases of atrioventricular blockage caused by derivatives of vitamin A, such as all-trans-retinoic acid, have been reported, demonstrating potential direct alterations in the functioning of the cardiovascular system (17).

Studies have revealed deleterious consequences of vitamin A signaling failure in dopamine-related motor behaviors, as well as improvement in voluntary movements due to nutrient supplementation (18). Additionally, alterations in the electroencephalogram of patients undergoing isotretinoin use have been observed, showing abnormal spikes in the exams of susceptible patients (19).

The relationship between the outcomes of administering supraphysiological doses of vitamin A and subsequent neurological effects is a field of knowledge that needs further clarification, as there are few reports regarding the impact of vitamin A use on the power of ECoG bands, as well as cardiac function and plasma calcium levels.

2. Methods

2.1. Animals.

A total of 45 Wistar strain rats (Rattus norvegicus) aged between 8 and 10 weeks, weighing between 180 to 200g, were used for the experiments conducted at the Laboratory of Pharmacology and Toxicology of Natural Products - UFPA. The animals were kept in an environment with a temperature of 22 ± 2°C, appropriate humidity of around 55 ± 10% relative air humidity, artificial light with a photoperiod of 12 hours light and 12 hours dark, considering the light period from 6:00 am to 6:00 pm, with

controlled noise. They were housed in cages with filtered water and ad libitum access to food throughout the study. The cages were cleaned three times a week. The recordings were made between 8:00 am and 11:00 am.

The study was conducted after approval by the Ethics Committee for Research with Experimental Animals of UFPA (CEPAE-UFPA), protocol number 2252220321.

2.2. Drugs.

The following chemical substances were used for the execution of the study: Ketamine Hydrochloride (Köing Laboratory, Santana de Parnaíba, SP, Brazil); Xylazine Hydrochloride (Vallée Laboratory, Montes Claros, MG, Brazil); Lidocaine Hydrochloride (Hipolabor Laboratory, Sabará, MG, Brazil); Diazepam 10mg/2ml (União Química, Embu-Guaçu, SP, Brazil); Vitamin A (Monovin A) 100,000 IU/ml (Bravet LTDA Laboratory, Rio de Janeiro, Brazil).

2.3. Experimental Design.

The animals were treated with vitamin A at a dose of 50,000 IU/kg via intraperitoneal injection (i.p.). The treatment was administered once daily for a duration of 14 days, according to experiments 1 and 2. The parameters evaluated included electrocorticogram (ECoG) and electrocardiogram (ECG).

2.4. Experiment 1: Electrocorticogram (ECoG).

For this experiment, electrode implants were performed in the motor cortex region two days before starting the treatment. Each group consisted of nine animals, distributed as follows: a) Control group: This group did not receive any drug. b) SHAM control group: This group received physiological saline solution for 14 days at a volume of 0.15 ml via intraperitoneal injection (i.p.). c) Group treated with vitamin A 50,000 IU/kg i.p. for three days. d) Group treated with vitamin A 50,000 IU/kg i.p. for seven days. e) Group treated with vitamin A 50,000 IU/kg i.p. for 15 days.

2.5. Experiment 2: Electrocardiogram (ECG).

For this experiment, electrodes were implanted to capture the D2 lead, using the right axillary region (third intercostal space) as the reference for the reference

electrode. The recording electrode was placed on the left side, 2 cm lateral to the xiphoid cartilage, above the thirteenth intercostal space. ECG recordings were made 30 minutes after the ECoG recordings. The groups followed the same distribution as in Experiment 1.

Each recording lasted for 2 minutes, and the following data were analyzed: Heart rate (BPM), Amplitude (mV), R-R interval (ms), P-Q interval (ms), QRS duration (ms), QT interval (ms).

2.6. Surgery for electrode placemaent.

Two days before the start of vitamin A application, the animals in the groups were anesthetized by intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (5 mg/kg). After the abolition of the interdigital reflex, the animals were positioned in a stereotaxic apparatus. The surgical procedure involved exposing the skull. Activated silver electrodes (0.5 mm in diameter) were positioned on the dura mater above the motor cortex at the coordinates of bregma - 0.96 mm and ± 1.0 mm lateral (20). A screw was fixed in the occipital skull, and the electrodes were secured with self-polymerizing acrylic cement.

2.7. Electroencephalographic (EEG) recordingsa.

After surgery, the animals were kept in individual cages. The recording sessions were conducted on days 3, 7, and 14 of vitamin A treatment. The electrodes were connected to a digital data acquisition system consisting of a high-impedance amplifier (Grass Technologies, P511), an oscilloscope (Protek, 6510), and a data acquisition and digitization board (National Instruments, Austin, TX). The data were continuously sampled at 1 kHz with a low-pass filter of 3 kHz and a high-pass filter of 0.3 Hz. During the recordings, the animals were confined in acrylic boxes with restricted spacing (20 x 45 x 15 cm). For all treatments, the ECoG recordings followed a standard protocol: 10 minutes of accommodation, followed by a 2-minute recording session.

2.8. Serum Calcium Levels.

For the evaluation of serum calcium levels, the rats were anesthetized with ketamine and xylazine. Once an appropriate anesthetic depth was reached, blood collection was performed through cardiac puncture and kept refrigerated. Serum calcium levels were measured using a quantitative colorimetric assay (Calcium Liquiform, Labtest, MG, Brazil).

2.9. Data Analysis.

The offline analysis was performed using a tool created in the Python programming language (version 2.7). The "Numpy" and "Scipy" libraries were used for mathematical processing, and the "matplotlib" library for generating graphs and plots. A graphical interface was developed using the PyQt4 library. Spectrograms were calculated using a Hamming window with 256 points (256/1000 s). For power spectral density (PSD), each frame was generated with a 128-point overlap per window. For each frame, the PSD was calculated using the Welch's averaged periodogram method. Frequency histograms were obtained by calculating the PSD of the signal using a Hamming window with 256 points without overlap, resulting in a resolution of 1 Hz per box. Each wave displayed in the PSD represents an average of a set of experiments. The PSDs were calculated for each group, and the means are displayed in individual boxes. The analyses were performed up to a frequency of 50 Hz and divided into bands according to Jalilifare et al. (2017) as Beta (1-4 Hz), Theta (4-8 Hz), Alpha (8-12 Hz), Beta (12-28 Hz), and Gamma (28-40 Hz), to interpret the dynamics during the development of intoxication.

2.10. Euthanasia of the animals.

After the recording period, under the effect of supraphysiological doses of vitamin A, the animals were euthanized using high doses of ketamine hydrochloride (300 mg/kg i.p.) and xylazine hydrochloride (30 mg/kg i.p.) administered intraperitoneally to prevent future issues, following institutional requirements for the euthanasia of these animals.

2.11. Statistical Analysis.

Normality and homogeneity of variances were assessed using the Kolmogorov-Smirnov and Levene tests, respectively. Since the residuals were normally distributed and the variances were equal, comparisons between the average amplitude of the traces and the control values were performed using ANOVA followed by Tukey's test. Mean values were presented with their respective standard deviations (mean ± SD). The significance level was set as * $p<0.01$ ** $p<0.001$ *** $p<0.0001$. GraphPad® Prism 5 software was used for the statistical tests.

3. Results

3.1. Supraphysiological doses of vitamin A increased serum calcium levels in rats.

To assess the ability of vitamin A to induce hypercalcemia, we analyzed serum calcium levels during the treatment. The control animals (8.774 ± 0.47 mg/dL) showed no significant difference compared to the SHAM groups (8.922 ± 0.429 mg/dL) ($p=0.999$) and the group treated for three days (9.83 ± 0.681 mg/dL) ($p=0.0527$). The group treated for seven days had a mean of 10.67 ± 0.728 mg/dL, which was higher than the control and SHAM groups but similar to the group treated for three days ($p=0.185$). The group treated for 14 days (11.23 ± 1.316 mg/dL) showed higher levels than the control, SHAM, and three-day treated groups. However, it did not maintain statistical difference compared to the seven-day treated group ($p=0.576$) (Figure 1).

Figure S1.

3.2. High doses of vitamin A increased the power band strength of brain waves.

During the study, animals treated with vitamin A did not show behavioral changes and maintained their food and water intake similar to the control and vehicle groups (data not shown). The ECoG recordings of the control and SHAM groups showed amplitudes below 0.1 mV (typically low amplitude, Figure 2A and B), as demonstrated in the 1 second amplification (Figure 2A and B, center), and the spectrogram showed the highest energy intensity below 10 Hz (Figure 2A and B, right). The ECoG recordings for the group treated with vitamin A for three days showed a greater

distribution of power in frequencies above 10 Hz when compared to the control group (Figure 2C). In the group that received vitamin A treatment for seven days, alterations in the ECoG traces were observed, increasing the power intensity in frequencies below 15 Hz (Figure 2D), as shown in the spectrogram (Figure 2D, right). The group that received treatment for 14 days showed changes in the ECoG traces with higher intensity in frequencies up to 40 Hz (Figure 2E), as demonstrated in the spectrogram on the right. However, the amplitude of the traces remained below 0.1 mV for the groups treated with vitamin A (Figure 2C, D, and E).

The decomposition of the total spectral power distribution revealed changes in the power tracking in the oscillations up to 40 Hz in animals treated with vitamin A for 3, 7, and 14 days (Figure 3A).

Figure S2.

Significant variation between the vitamin A treatment and the other groups was found in the analysis of the distribution of linear frequencies up to 40 Hz ($F(4, 40) = 150.7$, $p < 0.0001$). In this case, the vitamin A group treated for fourteen days had higher total spectral power than the control, SHAM, three-day treatment, and seven-day treatment groups (control: 0.08484 ± 0.007803 mV^2/Hz x 10^-3; SHAM: 0.08981 ± 0.004983 mV^2/Hz x 10^-3; three-day treatment: 0.1138 ± 0.01167 mV^2/Hz x 10^-3; seven-day treatment: 0.1962 ± 0.01862 mV^2/Hz x 10^-3; fourteen-day treatment: 0.2212 ± 0.02501 mV^2/Hz x 10^-3; $p < 0.001$ for all comparisons; Figure 3A). The decomposition of brain waves was also analyzed regarding the distribution of power levels in the delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-28 Hz), and gamma (28-40 Hz) frequencies. Significant variation was observed in the delta wave frequencies ($F(4, 40) = 19.16$; $p < 0.0001$), with a significant increase observed in animals treated with vitamin A for three days (0.04382 ± 0.003594 mV^2/Hz x 10^-3), seven days (0.04039 ± 0.005225 mV^2/Hz x 10^-3), and fourteen days (0.04145 ± 0.003594 mV^2/Hz x 10^-3) compared to the control (0.03240 ± 0.004734 mV^2/Hz x 10^-3, $p < 0.0001$) and SHAM group (0.02943 ± 0.003276 mV^2/Hz x 10^-3, $p < 0.001$). No differences were observed between the vitamin A treatment groups (Figure 3B). For the theta waves, differences were also observed between the groups ($F(4, 40)$

= 68.10; $p < 0.0001$). The animals in the vitamin A group had higher average theta power: for the three-day treatment group (0.02799 ± 0.002529 mV^2/Hz x 10^-3), seven-day treatment group (0.02984 ± 0.004494 mV^2/Hz x 10^-3), and fourteen-day treatment group (0.03563 ± 0.002879 mV^2/Hz x 10^-3) compared to the control group (0.01596 ± 0.002157 mV^2/Hz x 10^-3, $p < 0.0001$), SHAM group (0.01808 ± 0.002383 mV^2/Hz x 10^-3, $p < 0.0001$), and no difference was observed between the three-day treatment group and the seven-day treatment group ($p = 0.1224$; Figure 3C).

Figure S3.

Similar to delta and theta waves, it showed significant variation between groups for alpha waves ($F (4, 40) = 60.51$; $p <0.0001$). Again, the vitamin A treated animals showed higher power in the alpha oscillations: for the group treated for three days (0.01156 ± 0.001069 mV^2 / Hz x 10-3); for the group treated for seven days (0.021180 ± 0.003307 mV^2 / Hz x 10-3); for the group treated for fourteen days (0.02031 ± 0. 004797 mV^2 / Hz x 10-3), when compared to the control group (0.006683 ± 0.001319 mV^2 / Hz x 10-3) and SHAM (0.007497 ± 0.0007985 mV^2 / Hz x 10-3), with the highest elevations in alpha obtained between the groups treated for seven and fourteen days ($p = 0.3132$), but maintained a difference for the group treated for three days ($p < 0.0001$) and control and SHAM groups ($p < 0.0001$). The control and SHAM groups maintained no differences between them ($p=0.1326$) (Figure 4 A). In beta oscillations, only for the group treated for fourteen days obtained difference in high dose (0.01501 ± 0.002150 mV^2 / Hz x 10-3) when compared to control (0.01157 ± 0.001291 mV^2 / Hz x 10-3) ($p=0.0008$) and SHAM (0.01111 ± 0.001107 mV^2 / Hz x 10-3) groups ($p=0.0002$). The three-day treated (0.01286 ± 0.002386 mV^2 / Hz x 10-3) and seven-day treated (0.01395 ± 0.0034161 mV^2 / Hz x 10-3) groups showed no statistical difference to the control, SHAM and fourteen-day treated groups ($F (4, 40) = 4. 787$, $p = 0.003$) (Figure 4 B); For the gamma oscillations, there was an increase for the vitamin A treated group over the fourteen day period (0.002409 ± 0. 0003110 mV^2 / Hz x 10-3); when compared to control group (0.001885 ± 0.0001849 mV^2 / Hz x 10-3) ($p=0.0005$); SHAM group (0.001849 ± 0.0003132 mV^2 / Hz x 10-3)($p=0.0015$) and

group treated for three days (0.001806 ± 0.0002147 mV^2 / Hz x 10-3) (p= 0.0002), but showed no difference for the seven-day treated group (0.002142 ± 0.0002687 mV^2 / Hz x 10-3) (p=0.0689) ($F(4, 40) = 8.421$; $p < 0.0001$)(Figure 4 C).

Figure S4.

3.3. Electrocardiographic changes induced by high-dose vitamin A.

Figure 5A shows the cardiac activity of the control group, showing the amplitude of the recordings and the sinus rhythm (Figure 5A). When amplified, all cardiac deflagrations could be observed, with atrial activity represented by the P wave, ventricular activity represented by the QRS complex, and the T wave of ventricular repolarization (Figure 5B). The animals in the SHAM group showed similar characteristics to the control group, demonstrating cardiac activity in sinus rhythm with all deflagrations of cardiac functioning (Figure 5C-D). Regarding the groups treated with high doses of vitamin A in periods of three days (Figures 6 A and B), seven days (Figures 6 C and D) and fourteen days (Figures 6 E and F), all showed sinus rhythm with maintenance of cardiac electrophysiological characteristics.

Figure S5.

Figure S6.

Cardiac activity was evaluated for each group of animals, for heart rate a statistical difference was observed between groups ($F(4,40) = 16.12$; $p < 0.0001$). The animals receiving vitamin A for fourteen days showed an increase in mean heart rate of 412.0 ± 19.52 bpm compared to the control (360.0 ± 18.28 bpm; $p < 0.0001$); SHAM group (359.8 ± 15.86 bpm; $p < 0.0001$); animals treated for three days (362.0 ± 18.52 bpm; $p<0.0001$) and animals treated for seven days (387.6 ± 13.67 bpm; $p<0.01$). The animals treated with vitamin A for seven days showed statistical difference to the control, SHAM and three-day treated groups ($p< 0.01$) (Figure 7A).

The electrocardiogram amplitude showed a difference between the groups treated with vitamin A for fourteen days (0.7507± 0.06040 mV) and the SHAM group (0.6505± 0.04417 mV; $p < 0.01$). For the other groups: control (0.6782± 0.04417 mV), group treated for three days (0.6797± 0.06748 mV) and group treated for seven days

(0.7201± 0.06748 mV) no statistical difference was found ($p>0.01$) ($F(4, 40) = 3.662$; $p=0.0124$) (Figure 7 B).

The animals that received vitamin A showed a decrease in the R-R interval, a statistical difference was observed ($F (4,40) = 14.49$; $p <0.0001$). The animals treated with vitamin A for 14 days had a mean R-R interval of 145.3 ± 7.018 ms compared to the control group (166.6 ± 8.308 ms; $p <0.0001$); SHAM group (166.4 ± 7.601 ms; $p <0.0001$); 3-day group (165.6 ± 8.502 ms; $p <0.0001$) and 7-day group (154.7 ± 5.477 ms; $p <0.01$). The seven-day treated group showed statistical difference to the control, SHAM and three-day treated groups ($p<0.01$) (Figure 7 C).

Figure S7.

During the PQ interval ($F (4, 40) = 2.368$; $p = 0.0688$) and QRS interval ($F (4, 40) = 0.9462$; $p = 0.4474$) no difference was observed (Figure 8 A and B). Significant difference was observed between the groups in QT interval ($F (4, 40) = 8.550$; $p <0.0001$), with the animals treated with vitamin for fourteen days (63.02 ± 4.876 ms;) when compared with the control group (70.01 ± 3.892 ms; $p<0.001$); SHAM group (71.47 ± 4.125 ms; $p<0.001$) and group treated for three days (73.83 ± 1.876 ms; $p<0.0001$). For the group treated for seven days (67.30 ± 5.599 ms), a statistical difference was observed to the group treated for three days ($p<0.01$) (Figure 8 C).

Figure S8.

4. Discussion

The effects of excess vitamin A (hypervitaminosis A) in vivo increase the number of osteoclasts, causing an increase in the amount of serum calcium. In this way, vitamin A can increase the activity of osteoclasts, allowing for an elevation of plasma calcium (21, 22, 23).

Serum calcium levels increased gradually and the differences were observed starting on the seventh day of treatment, with maintenance at fourteen days, but remained within the normal range for the species according to Santos et al., 2004 (24).

Studies have shown that the molecular components necessary for vitamin A signaling are expressed in the adult brain, and the overlapping of brain areas involved in the function of vitamin A and stress and depression suggests that retinoids may play a role in affective disorders (25).

Disorders related to nervous system functions have been reported and appear on the list of side effects resulting from excessive intake of vitamin A. The ingestion of vitamin A at moderate to high doses (25,000 - 150,000 IU/kg) has resulted in a variety of effects, such as acute and chronic gastrointestinal intoxication, pseudotumor cerebri, irritability, anxiety, and more severely, depression (26,2).

Brain waves are influenced by various factors such as mental activity, stress, sleep, and other physiological conditions. Although retinoic acid can affect neuronal activity under certain circumstances, there are no studies indicating an increase in low-frequency brain waves as a direct consequence of excessive vitamin A use (6).

In this study, vitamin A in high doses increased the power of low-frequency brain oscillations, with rapid responses in the elevation of power in delta, theta, and alpha oscillations. However, beta and gamma oscillations showed more stable powers during the treatment.

According to Gulluni et al. 2018 (28), the application of Cannabis essential oil showed a significant increase in the average frequency of alpha (8-13 Hz), theta (4-8 Hz), and delta (0.5-4 Hz) waves, and the relative power was recorded in the posterior region of the brain. These data demonstrate similar alterations to those found in electrocorticograms after supraphysiological application of vitamin A.

On the other hand, an increase in delta, theta, and alpha waves may indicate brain dysfunction (29). However, alpha wave activity is more frequently increased (30), but in our study, the increase in power occurred more rapidly with oscillations in delta and theta.

According to Gaubert et al. 2019 (31), an increase in the power of beta and gamma oscillations and a decrease in low-frequency oscillations (delta and theta power) are linked to degenerative processes. After the application of vitamin A, an early increase in low-frequency power (delta and theta) can be observed, which would be contrary to the prevalence of power observed during degenerative processes as proven by EEG. On the other hand, Endres et al. 2017 (32) indicate that an increase in

delta and theta power is observed in the EEG of adult patients with attention deficit and hyperactivity.

Some drugs, such as tricyclic antidepressants, increased activity in the delta, theta, and beta bands in spontaneous EEG. Ketamine, an antagonist of the N-methyl-D-aspartate receptor, showed an increase in the theta band (33). Intravenous pentazocine decreased the EEG power in the theta, alpha, and beta bands (34). Opioids generally induce a slowing down of spontaneous EEG, increasing the power in the delta band (33).

According to Olejniczak (2006) – (35), theta activity can represent the inhibitory action of GABAergic interneurons in the corticothalamic network. In addition, theta oscillations can be generated by the cortex through the activation of glutamatergic pathways or dopamine release, causing a slowing down of delta rhythm oscillations, which reflects in the electrical activity generated by the cortex when no sensory input is being processed. Our data demonstrated that during the treatment with vitamin A, there was an increase in delta and theta oscillations in the motor cortex.

According to the study by Marie et al. (2021) – (36), an enzyme expressed by dopaminergic neurons known as Aldehyde dehydrogenase (ALDH1A1), which allows detoxification of these neurons, is under the control of retinoic acid. Our results demonstrated a slowing down of the delta waves in the motor cortex, leading to an increase in low-frequency oscillations power, which supports the possibility that high doses of vitamin A may be beneficial in Parkinson's disease.

Vitamin A is associated with antioxidant activities, with experimental evidence supporting the efficacy of vitamin A and carotenoids in reducing cardiovascular diseases (37, 38). However, studies have shown that high doses of vitamin A can lead to hypercholesterolemia (39).

In the study conducted by Ay et al. (2019) and Karadag et al. (2012) – (40, 41), after the use of vitamin A derivatives for acne treatment, ECG parameters were evaluated, including the QRS wave duration and QT interval. After one month of treatment, it was observed that there were no significant changes in the baseline ECG parameters. Dursun et al. (2011) – (42) evaluated isotretinoin and found no changes in the QT interval in the ECG. Our data showed an increase in heart rate, amplitude, a decrease in the QT interval, and the RR interval. However, parameters such as the PQ

interval and QRS duration did not show differences compared to the control group, and sinus rhythm was observed in all recordings. Although there are reports of coronary thrombus formation, which is often the cause of myocardial infarction in young patients using isotretinoin, a potent derivative of vitamin A (43).

Thus, supraphysiological doses of vitamin A can increase the power of low-frequency oscillations in delta, theta, and alpha, maintaining their predominance during the experiment. The ECG recordings showed changes such as a decrease in the RR and QT intervals. However, cardiac activity was close to normal with sinus rhythm in all recordings. Further studies are needed to evaluate the parameters of electrocorticographic and electrocardiographic alterations mediated by high doses of vitamin A.

5. Author Contributions

We strongly encourage authors to include author contributions and recommend using CRediT for standardised contribution descriptions. Please refer to our general author guidelines for more information about authorship.

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8. Availability of data and materials

Acessible.

9. Authors Declarations:

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Oliveira de Araújo, Maria Clara Otake Hamoy, Juliana Mendes Coelho contributed to the research design, conduction of experiments (animal experiments) data collection, interpretation, and manuscript preparation; Vanessa Jóia de Mello and Allane Patricia Santos da Paz contributed to the research design, conduction of experiments (analyses) , data interpretation, manuscript revision, Deise de Lima Cardoso, Tays Mata Câmara1 Murilo Farias dos Santos, Anthony Lucas Gurge do Amaral contributed to data interpretation and manuscript revision; Moises Hamoy contributed to the research design, study supervision, funding acquisition, data interpretation, manuscript revision and funding support. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

10. Ethics approval and consent to participate:

The study was conducted after approval by the Ethics Committee for Research with Experimental Animals of UFPA (CEPAE-UFPA), protocol number 2252220321.

11. Patient consent for publication

Not applicable.

12. Conflicts of interest

There are no conflicts to declare.

13. Use of artificial intelligence tools (to be included only when AI tools are used):

Not applicable.

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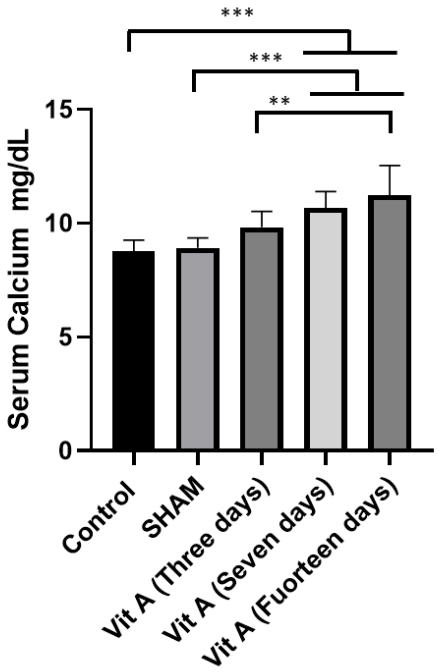

*Figure S1. Results of the analysis of serum calcium levels in animals treated or not with vitamin A by one-way ANOVA test. Data are expressed as mean ± SD (n = 9 animals per group, **p < 0.01 and ***p < 0.001).*

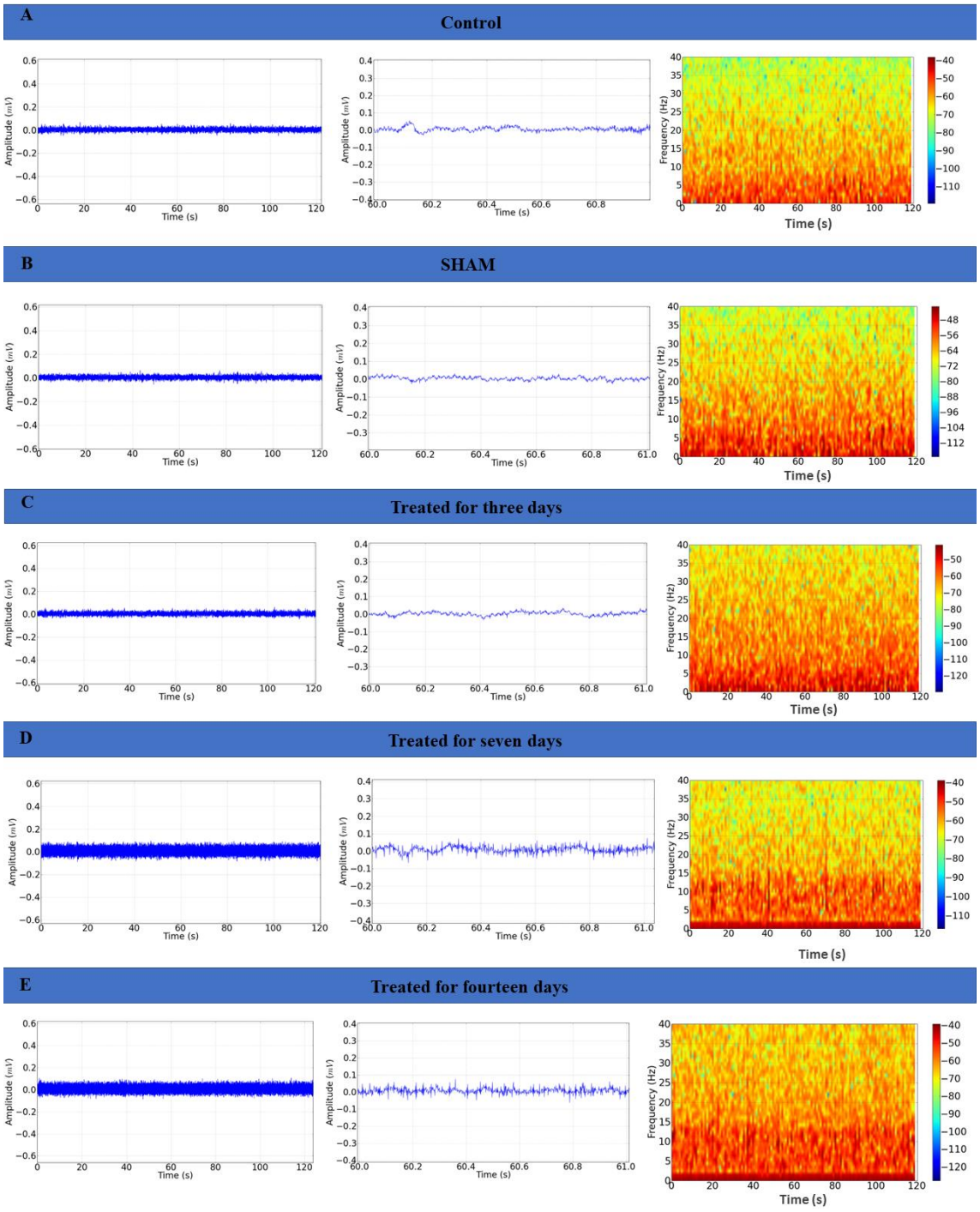

Figure S2. Illustrations of electrocorticographic (ECoG) recordings with a duration of 2 minutes. ECoG trace for the control group (left), 1 second amplification of the trace (center), and energy distribution spectrogram (right) (A); ECoG trace for the SHAM vehicle group (left), 1 second amplification of the recording (center), and corresponding spectrogram with power distribution up to 40 Hz (right) (B); ECoG trace for the vitamin A group on the third day of treatment (left), 1 second amplification of the recording (center), and spectrogram (right) (C); ECoG recording for the vitamin A group on the seventh day of treatment (left), 1 second amplification of

the recording (center), and corresponding spectrogram (right) (D); Recording for the vitamin A group on the fourteenth day of treatment (left), 1 second amplification (center), and spectrogram (right).

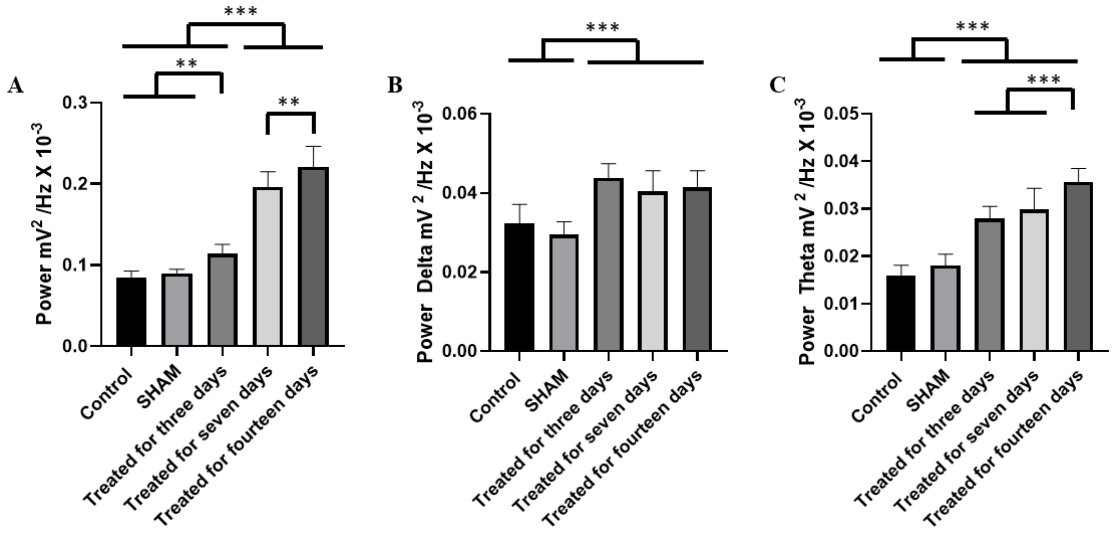

*Figure S3: Graph of linear power distribution between groups with frequency up to 40 Hz (A); Graph of mean force distribution in delta frequency (1-4 Hz) (B), Mean force distribution in theta frequency (4-8 Hz) (C).(After ANOVA followed by Tukey, * p<0.01 ** p<0.001 ***p<0.0001, n=9).*

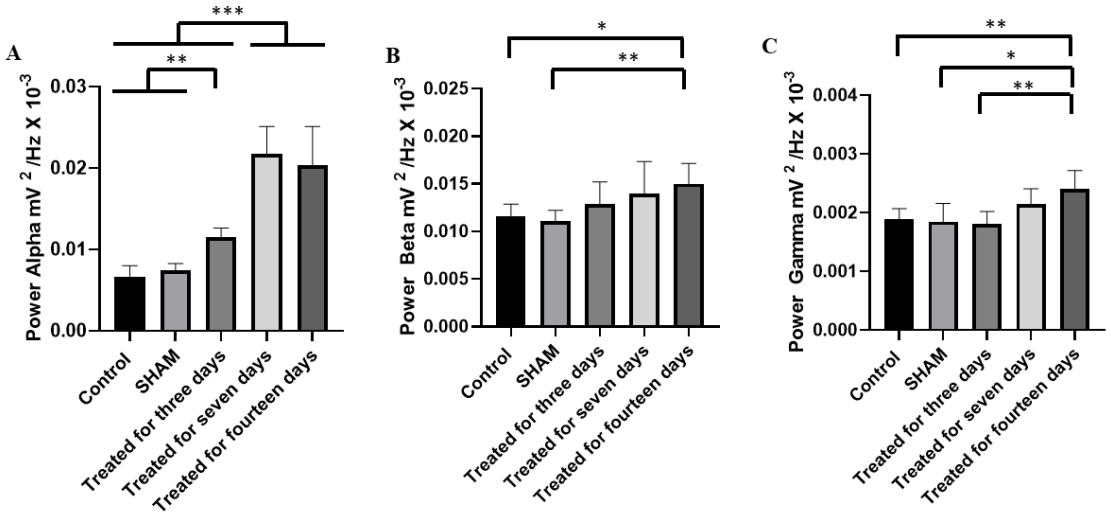

*Figure S4. Linear power distribution graph for Alpha oscillations (8-12 Hz) (A), Linear power distribution graph for Beta oscillations (12-28 Hz) (B), Linear power distribution graph for Gamma (28-40 Hz (C).. (After ANOVA followed by Tukey, * p<0.01 ** p<0.001 ***p<0.0001, n=9).*

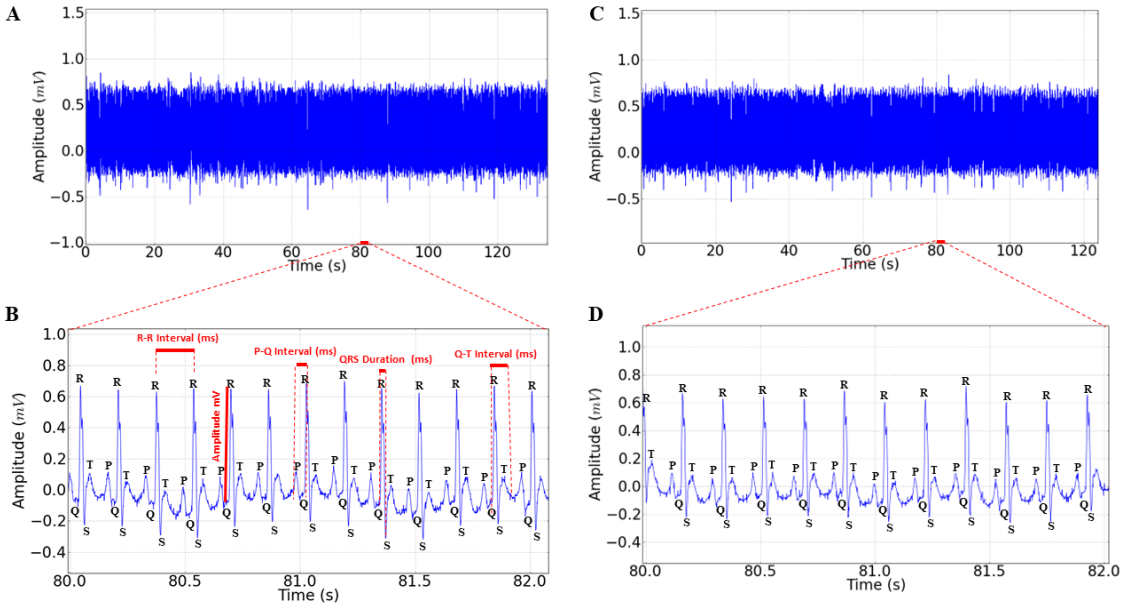

Figure S5 - Control electrocardiogram in D-II lead with duration of 2 minutes (A), Amplification of the recording in the time of 2 seconds in red traces represents the intervals to be analyzed: Amplitude (mV), R-R interval (ms), P-Q interval (ms), Q-T interval (ms), QRS complex duration (ms) (B). Electrocardiographic recording of the SHAM group lasting 2 minutes (C); Amplification of 2 seconds of the recording demonstrating the components related to cardiac deflagrations (D).

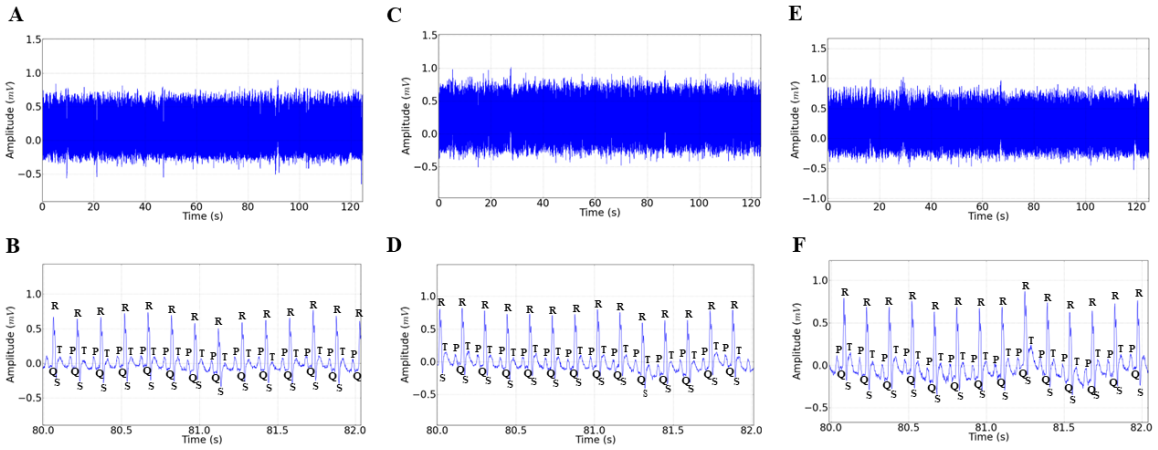

Figure S6 - Electrocardiogram in D-II lead lasting 2 minutes for animals treated for three days (A); Amplification of recording in 2 s (recording period 80 to 82 s) with presence of P, QRS complex and T deflagrations for the group treated for three days with vitamin A (B). ECG recording represented in the 2-min tracing for the group treated with vitamin A for seven days (C); Amplification of 2 s recording demonstrating sinus rhythm after seven days treatment with vitamin A (D); Electrocardiographic tracing of the animals submitted to treatment with vitamin A for

14 days (E); Amplification of 2 s recording demonstrating the electrocardiographic components after 14 days treatment with vitamin A 50,000 IU/Kg i.p.

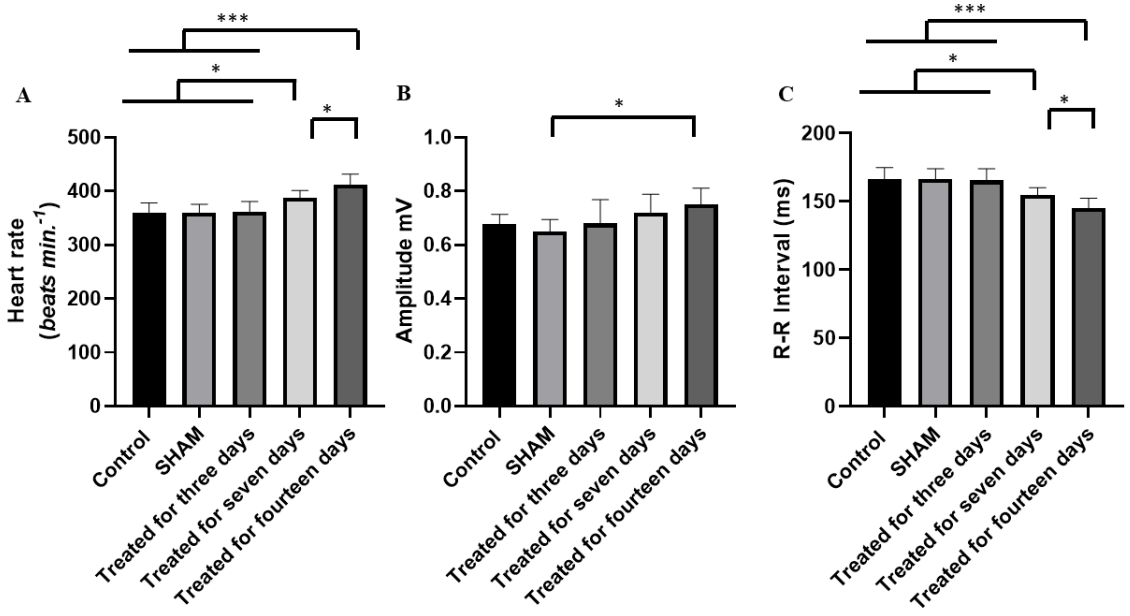

*Figure S7. Heart rate averages (bpm) recorded in the control group, SHAM group, after three days, after seven days and after 14 days of vitamin A treatment (A); Evaluation of the amplitude averages (mV) of electrocardiograms for the groups (B); Evaluation of the R-R interval averages (ms) for the groups (C). (After ANOVA followed by Tukey, * p<0.01 ** p<0.001 ***p<0.0001, n=9).*

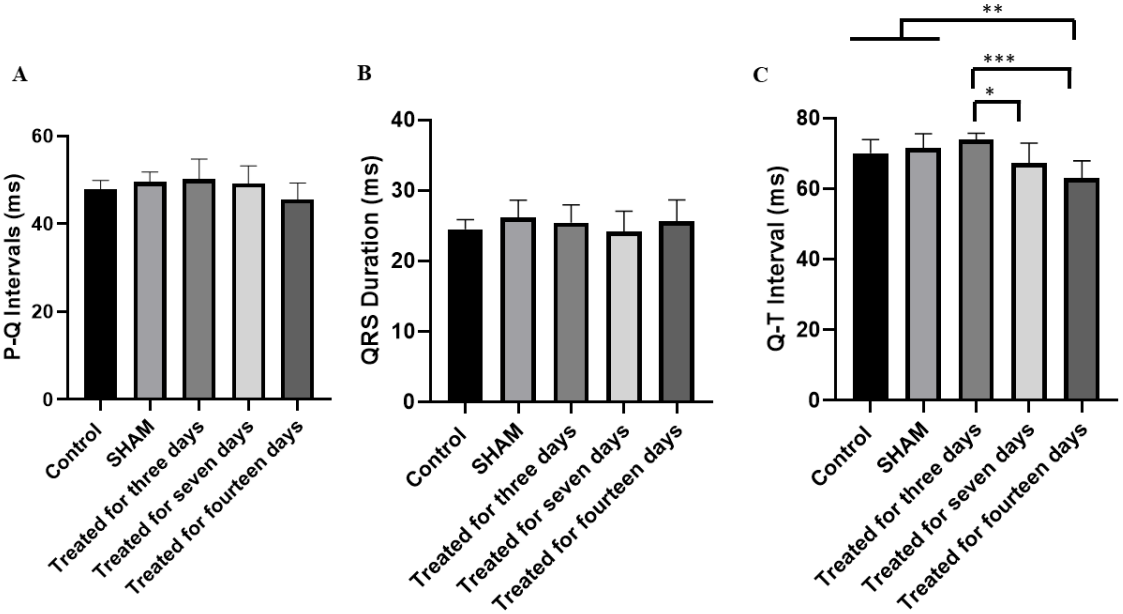

Figure S8.
*P-Q interval averages (ms) recorded in the control, SHAM, after three days of treatment, after seven days of treatment and after 14 days of vitamin A treatment groups (A); Evaluation of QRS complex duration averages (ms) for the groups (B); Evaluation of Q-T interval averages (ms) for the groups (C). (After ANOVA followed by Tukey, * $p<0.01$ ** $p<0.001$ ***$p<0.0001$, $n=9$).*

CONSIDERAÇÕES FINAIS

A vitamina A faz parte dos micronutrientes que precisam ser obtidos da alimentação, onde se faz necessário para diversas funções do corpo. Devido sua importância a população faz uso indiscriminado, principalmente para queda de cabelo, unhas fracas e prevenção de acne. Com isso mostrou – se necessário a pesquisa realizada em dosagem aproximadas a que um ser humano suplementa.

A relação entre os resultados da administração de doses suprafisiológicas de Vitamina A e os efeitos neurológicos subsequentes é um campo de conhecimento a ser melhor elucidado, visto que existem poucos relatos acerca do uso indiscriminado da vitamina A na potência de bandas do EEG. As ondas cerebrais são influenciadas por vários fatores, como atividade mental, estresse, sono e outras condições fisiológicas. Embora o ácido retinóico possa afetar a atividade neuronal em algumas circunstâncias, não há estudos que indiquem um aumento das ondas cerebrais de baixa frequência como consequência direta do seu excesso.

Dessa maneira, neste estudo foi constatado que a vitamina A em altas doses aumentou a força de banda das ondas cerebrais, conforme foi observado nos traçados eletrocorticográficos. Foi verificado que o grupo tratado com vitamina A, durante três dias, teve maior distribuição de força nas frequências acima de 10Hz, em relação ao grupo controle e veículo. Da mesma maneira, no grupo que recebeu vitamina A, durante sete dias, também foi observada alteração no traçado ECoG, mediante aumento de intensidade de potência nas frequências abaixo de 15 Hz. Para o grupo que recebeu tratamento durante 14 dias, houve alterações de traçado ECoG com maior intensidade nas frequências até 40 Hz, no entanto, a amplitude se apresentou abaixo de 0,1 mV. Foi relatado de forma similar aos nossos resultados, que bandas de ondas cerebrais são geralmente aumentadas em portadores de esquizofrenia quando comparado a indivíduos saudáveis. Outros autores também demostram que a atividade de ondas delta, theta, beta aumentam (Grin, 2017), já as atividades de ondas alfa é mais frequentemente mais aumentada (Newson, 2019).

Com isso, na atualidade, muitos jovens fazem o uso de um produto que é substrato da vitamina A (isotretionína), para o tratamento de acnes, que mesmo melhorando o aspecto da pele tem uma ingestão aumentada de acido retinóico. Portanto considerando a prevalência do uso da medicação, este estudo analisou alterações que podem ser altamente prejudiciais para esta faixa etária, assim a importância de sugerir outros estudos para fortalecer a dissertação em estudo.

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