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VITAMINA C E DESENVOLVIMENTO CEREBRAL: EFEITOS SOBRE A DEPRESSÃO ALASTRANTE CORTICAL EM RATOS JOVENS EM DISTINTOS ESTADOS NUTRICIONAIS

CINTHIA KARLA RODRIGUES DO MONTE GUEDES

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Dissertação apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco.

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RESUMO

O Ácido Ascórbico (AA) é uma molécula antioxidante, muito concentrada no cérebro e que pode provocar tanto efeitos anticonvulsivantes quanto proconvulsivantes em diferentes modelos de epilepsia experimental. Neste trabalho nós exploramos se a administração crônica de AA altera a excitabilidade neural avaliada pela Depressão Alastrante Cortical (DAC). Ratos Nutridos (N) e Desnutridos (D) foram tratados por gavagem com 60 mg/kg/dia de ácido L-ascórbico do 7º ao 28º dia pós-natal e a DAC foi analisada entre os 30-40 dias de vida. Comparados ao grupo N, os ratos D apresentaram maiores ($p<0.05$) velocidades de propagação da DAC, confirmando achados anteriores. Comparados aos controles tratados com solução salina (Sal), os ratos tratados com AA tiveram maiores velocidades ($p<0.05$) de propagação da DAC nas condições N e D. Um outro grupo controle "ingênuo" (I; que não recebeu gavagem) não diferiu do controle Sal. A média±DP da velocidade de propagação da DAC (em mm/min) para os grupos Sal, AA e I foram, respectivamente 3.75±0.03, 4.26±0.08 e 3.81±0.04 para os animais N e 4.29±0.08, 4.51±0.04 e 4.30±0.04 para os animais D. Os resultados demonstraram uma facilitação da DAC pelo AA, independentemente do estado nutricional. Eles ainda sugerem que em doses de 60 mg/Kg/dia administradas cronicamente durante o desenvolvimento cerebral, o AA parece atuar como um pró-oxidante no cérebro, em vistas ao efeito contrário, em comparação com outros antioxidantes, que reduzem a propagação da DAC.

Palavras-chaves: Desenvolvimento cerebral; Desnutrição; Ácido ascórbico; Depressão alastrante cortical; Antioxidantes; Pró-oxidantes.

ABSTRACT

Ascorbic acid (AA) is an antioxidant molecule that is highly concentrated in the brain and can exert both anticonvulsant and proconvulsant effects in distinct models of experimental seizures. Herein, we explore the question of whether AA chronic administration alters cortical excitability as indexed by the cortical spreading depression (CSD). Well-nourished (W) and malnourished (M) rats were treated, by gavage, with 60 mg/kg/day of L-ascorbic acid from postnatal days 7-28, and CSD propagation was analyzed at 30-40 days. Compared to the W groups, M rats presented higher ($p<0.05$) CSD velocities of propagation, confirming previous reports. Compared to non-treated ('naïve'; Nv) and saline-treated (Sal) controls, AA-treated rats displayed higher CSD velocities ($p<0.05$) in the W and M conditions. The mean±sd CSD velocities of propagation (in mm/min) for the Sal, AA and Nv groups were respectively 3.75±0.03, 4.26±0.08 and 3.81±0.04 for the W condition and 4.29±0.08, 4.51±0.04 and 4.30±0.04 for the M groups. The results demonstrate a CSD-facilitation by AA regardless of nutritional status. They also suggest that at the dose of 60 mg/Kg/day chronically administered during brain development, AA may act as a prooxidant in brain, in view of the contrasting effect as compared with other antioxidants, which reduce CSD propagation.

Keywords: Brain development; Malnutrition; Ascorbic Acid; Cortical spreading depression, Antioxidants, Prooxidants.

INTRODUÇÃO

O estado nutricional exerce um profundo impacto sob crescimento e desenvolvimento do sistema nervoso central e de suas estruturas e funções (Schweigert et al., 2009). No sistema nervoso embrionário, os processos de neurogênese, gliogênese e migração neuronal são viabilizados pelos fenômenos da hiperplasia, hipertrofia e mielinização. Esses processos estão intensificados no que se chama de "período de crescimento rápido do cérebro" ou simplesmente o "período crítico". Essa fase acha-se compreendida entre o terceiro trimestre gestacional e o segundo ano de vida, no homem, ou durante o período de aleitamento, no rato (Fig. 1; Morgane et al.; 1993). Ela é considerada crítica para o perfeito desenvolvimento e funcionamento neurológico, sendo uma etapa de grande vulnerabilidade a agressões internas e/ou externas, a exemplo da nutricional (Dobbing, 1968)

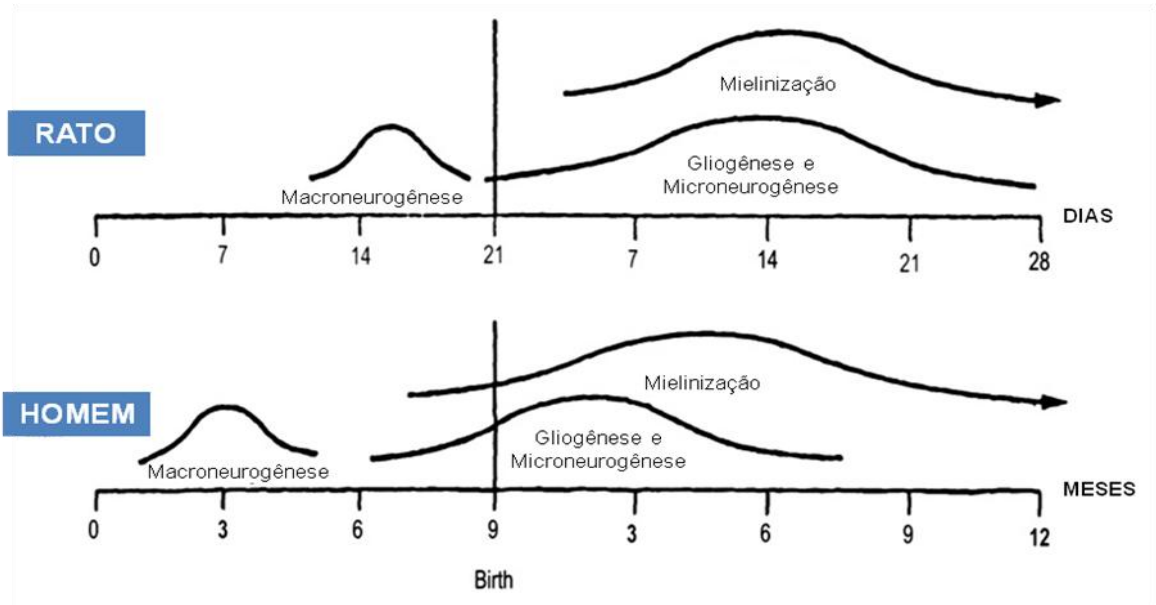

Figura 01: Comparação entre as etapas de desenvolvimento do sistema nervoso no homem e no rato. Adaptada de Morgane et al., 1993.

Estudos clássicos vêm evidenciando que a desnutrição, durante o período crítico de desenvolvimento provoca alterações bioquímicas, anatômicas e fisiológicas no encéfalo (Morgane et al., 1992; 1993; 2002). Isso tem sido evidenciado tanto em animais de laboratório (Morgane et al., 1978; 1993; Chen et al., 1997) quanto em humanos (Nwuga, 1977; Granthan-McGregor, 1995; Levitsky e Strupp, 1995; Galler et al., 1998). Tais alterações são refletidas em prejuízos cognitivos e comportamentais, repercutindo na capacidade de memória e motivação do indivíduo (Hack et al., 1991; Strupp e Levitsky, 1995; Ranade et al.; 2008). Sob dependência da intensidade e do tipo de desnutrição causados, os efeitos deletérios podem persistir por longo-prazo ou tornarem-se permanentes (Guedes et al., 1996; Borba et al., 2000).

Reconhecida mundialmente como um importante problema de saúde pública, a desnutrição infantil ainda atinge uma importante parcela da população, seja ela subdesenvolvida ou de primeiro mundo (De Onis et al., 2004). As conseqüências desta desnutrição para o sistema nervoso vêm sendo extensamente estudadas (Udani, 1992; Odebode e Odebode, 2005; Benton, 2008), tendo-se evidenciado uma relação direta entre a desnutrição naquela etapa da vida e o risco para o desenvolvimento de doenças neurológicas e psicológicas, tais como a esquizofrenia e epilepsia (Susser et al., 1996; Hackett e Iype, 2001; Diop et al., 2003)

Em vistas ao exposto, o impacto da desnutrição nos diversos sistemas orgânicos vem sendo estudado (Valadares e Almeida, 2005; Kar et al., 2008; Pereira-da-Silva et al., 2009; De Frias et al., 2010). O uso de modelos experimentais que mimetizem a desnutrição, como no caso do uso de dietas experimentais, é uma maneira eficiente para a realização de novas investigações que visem aprofundar o estudo da desnutrição.

A “Dieta Básica Regional” (DBR) é considerada um modelo experimental importante para estudos sobre a desnutrição humana, uma vez que foi desenvolvida com base nos alimentos regionais consumidos pela população do nordeste brasileiro na década de 60, época em que a desnutrição, nesta região, acometia boa parte de população (Batista-Filho, 1968; Teodósio et al., 1990). Sua validade tem sido reiterada em diversos estudos envolvendo os sistemas cardio-vascular (Monteiro et al., 2001), renal (Vieira-Filho et al., 2009) e nervoso (Farias-Santos el al., 2009).

Condições experimentais relacionadas à desnutrição e seus efeitos sob o sistema nervoso central tem sido extensamente estudadas através do fenômeno da Depressão Alastrante Cortical (DAC). Esse fenômeno foi descrito inicialmente no coelho por Leão (1944), que observou uma diminuição acentuada na atividade elétrica cerebral (espontânea ou evocada) em resposta à estimulação elétrica, mecânica ou química em um ponto do tecido cortical. A propagação da onda de depressão da atividade eletroencefalográfica ocorre simultaneamente a uma variação lenta de voltagem (VLV) tecidual. Essa propagação se dá de forma concêntrica e reversível, a partir do ponto estimulado, numa velocidade entre 2 e 5 mm/min, sendo sua latência de reversão de 10 a 15min (Martins-Ferreira, 1983). A Fig. 2 ilustra o fenômeno.

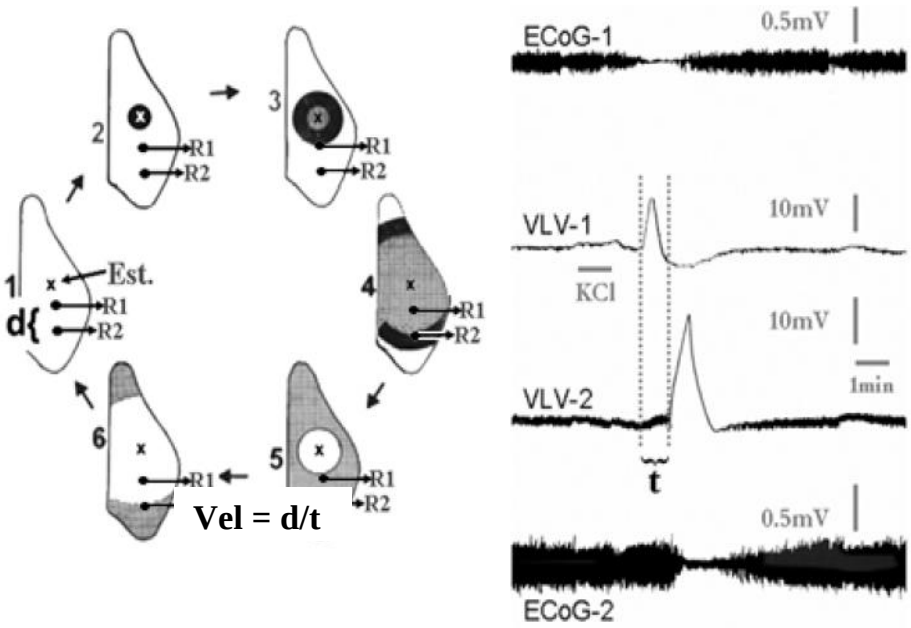

Figura 02: À esquerda observa-se os eventos ocorridos durante a propagação da DAC em seqüência temporal cíclica. R1 e R2 indicam pontos de registro. Um estímulo provocado (x) deu início ao fenômeno (etapa 1) que se propaga de forma concêntrica (etapas 2-4). As áreas escuras (etapas 2, 3 e 4) representam áreas corticais na vigência do fenômeno enquanto que as áreas quadriculadas (etapas 3 a 6) indicam o princípio da recuperação tissular. As áreas claras indicam o tecido recuperado (etapas 5-6), o que também ocorre de forma concêntrica, retornando à condição inicial (etapa 1). À direita observa-se o eletrocorticograma (ECoG) e a variação lenta de voltagem (VLV), esta última presente durante a DAC, quando o ECoG diminui sua amplitude. Tais registros, obtidos em nosso laboratório foram feitos simultaneamente no pontos R1 e R2. Observe a recuperação do ECoG após a passagem do fenômeno (Guedes et al., 2004).

Evidências experimentais indicam que o tecido nervoso apresenta naturalmente uma resistência à passagem da DAC (Guedes e Do-Carmo, 1980), e que esta resistência pode diminuir ou aumentar na vigência de alguns tratamentos, modificando assim a sua velocidade de propagação (Abadie-Guedes et al., 2008). Diversas modificações de condições sistêmicas, a exemplo da desnutrição, podem alterar a propagação da DAC (Guedes, 1984; Guedes et al., 1987; Andrade et al., 1990; Guedes et al., 1992; Rocha-de-Melo e Guedes, 1997). Tratamentos locais do tecido cortical podem também modificar a sua propagação (Richter et al., 2005; Guedes et al, 1987; Amâncio-dos-Santos et al., 2006).

As Tabelas 01 e 02 apresentam diversas condições, já estudadas, que podem dificultar ou facilitar a propagação da DAC.

Tabela 01: Algumas condições que dificultam a propagação da DAC

Condição experimental	Autor/Ano
Tratamento dietético com lítio	Guedes et al., 1989
Hiperglicemia	Ximenes-da-Silva e Guedes,1991; Costa-Cruz et al., 2006
Anestésicos	Guedes e Barreto, 1992
Hipotireoidismo	Guedes e Pereira-da-Silva, 1993
Envelhecimento	Guedes et al., 1996
Dieta hiperlipídica	Paixão et al., 2007
Epilepsia crônica provocada pela pilocarpina	Guedes e Cavalheiro, 1997; Costa-Cruz et al., 2006
Estimulação ambiental	Santos-Monteiro et al., 2000
Ativação do Sistema Serotoninérgico	Guedes et al., 2002; Amâncio-dos-Santos et al., 2006
Estimulação Elétrica Cerebral direta e trans-craniana	Fregni et al., 2005; 2007
Condições favoráveis de aleitamento	Rocha-de-Melo et al., 2006

Tabela 02: Algumas condições que facilitam a propagação da DAC

Condição experimental	Autor/Ano
Redução do Cloreto extracelular	Guedes e Do Carmo, 1980
Privação do sono paradoxal	Vasconcelos et al, 2004
Diazepam	Guedes et al., 1992
Etanol	Guedes e Frade, 1993; Bezerra et al, 2005
Deficiência nutricional pela DBR	Rocha-de-Melo e Guedes,1997
Hipertireoidismo	Santos, 2000
Hipoglicemia	Costa-Cruz e Guedes, 2001
Privação sensorial	Tenório et al., 2009
Arginina durante o desenvolvimento	Maia et al, 2009
Hipertermia ambiental	Farias-Santos et al., 2009
Glutamina durante o desenvolvimento	Lima et al, 2009
Uso de dipirona no início da vida	Amaral et al., 2009

Importantes estudos (Guedes et al., 1996; El-Bachá et al., 1998) sugerem que a baixa oferta dietética de substâncias antioxidantes leve à produção excessiva de radicais livres no tecido cerebral. Isso é demonstrado pelo favorecimento da propagação da Depressão Alastrante Cortical (DAC). Estudos recentes, com o uso de carotenóides, incrementam essa hipótese (Bezerra et al., 2005; Abadie-Guedes et al., 2008).

O ácido ascórbico (AA) ou simplesmente vitamina C foi isolado primeiramente pelo cientista húngaro Szent-Györgyi em 1928, durante experimentos com a glândula adrenal, onde a vitamina encontra-se em níveis bastante elevados em relação a outros órgãos. Estudos posteriores evidenciaram que o cérebro também apresenta altos teores desta vitamina. A concentração de vitamina c em alguns órgãos está mostrada na Figura 03 (Schenk et al., 1982; Miele e Fillenz, 1996).

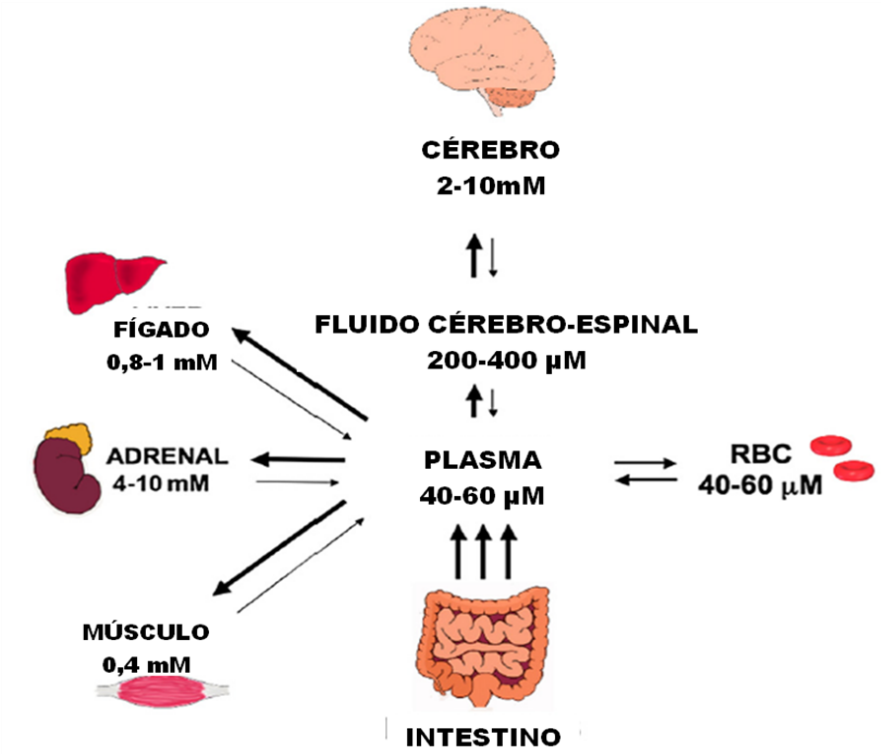

Figura 03: Concentração de Vitamina C no plasma e em alguns órgãos (Harrison e May, 2009)

Estudos sobre o escorbuto, doença caracterizada pela deficiência da vitamina C (Lind, 1772) em porquinhos-da-Índia (*Cavia porcellus*), mostrou uma manutenção de 25% do nível de AA no tecido cerebral, diante de uma total depleção orgânica da vitamina (Hughes et al., 1971). Esta evidência sugere a importância vital do AA para o sistema

nervoso (Arrigoni e De Tullio, 2002). Depleções nos níveis de dopamina e norepinefrina (catecolaminas com biossíntese dependente do AA) na vigência do escorbuto também foram demonstradas (Hoehn e Kanfer, 1980)

Segundo Lane e Lawen (2009), o AA acumula-se no cérebro tanto através dos transportadores de vitamina C dependentes de sódio (SVCT2), que transportam a vitamina, em sua forma reduzida, para dentro do sistema nervoso central, quanto de transportadores da família GLUT, que transportam a forma oxidada do AA (ácido deidroascórbico; Figuras 4 e 5)

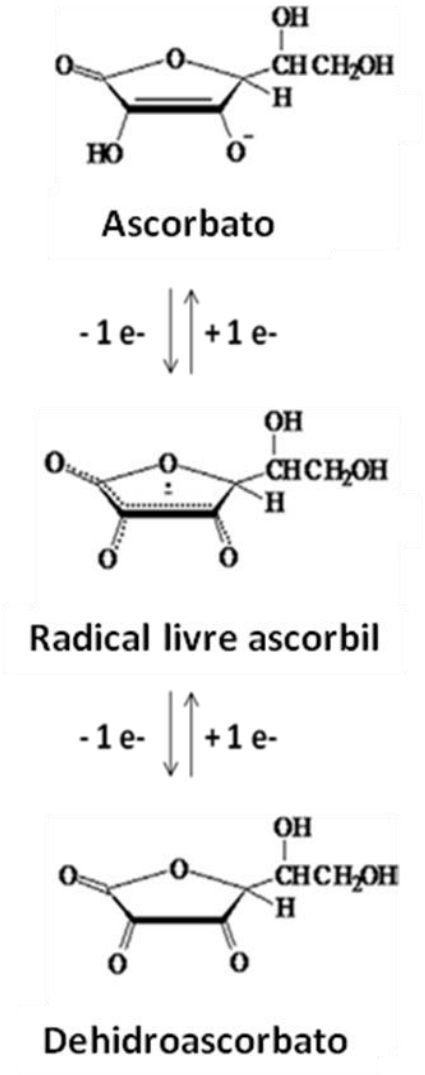

Figura 04: Formas químicas da Vitamina C (Corti et al., 2010)

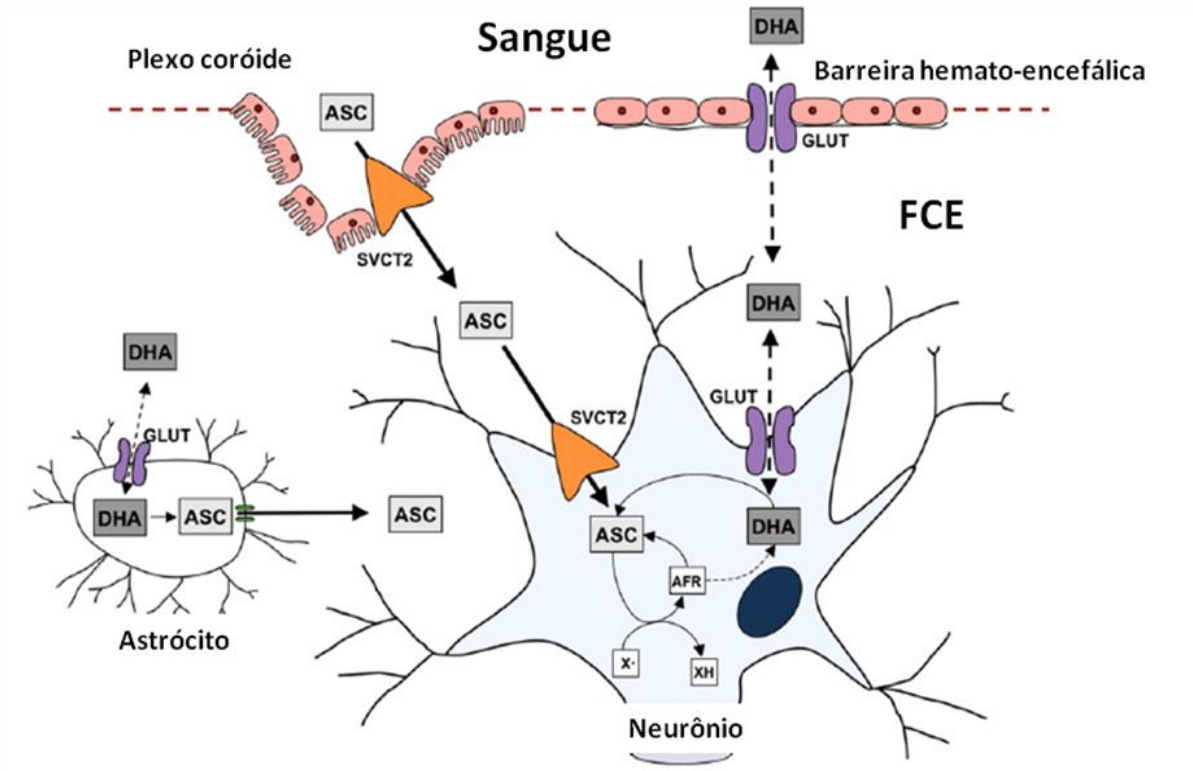

Figura 05: Transportadores de Vitamina C no sistema nervoso. Asc: Ascorbato; SVCT2: Transportador de vitamina c dependente de sódio 2; AFR: radical livre ascorbil, X-XH: Indica perda de um elétron; GLUT: transportador de glicose; DHA: Ácido dehidroascórbico. (Harrison e May, 2009)

A maioria dos mamíferos têm a capacidade de sintetizar o AA. Os primatas e as cobaias são uma exceção, visto que não possuem a enzima L-gulonolactona oxidase, o que impede a síntese hepática do ácido L-ascórbico a partir da D-glicose (Nishikimi et al., 1994; Linster e Van Schaftingen, 2007). Esta impotência foi extremamente discutida pelo renomado pesquisador Linus Pauling, defensor irredutível do consumo diário de megadoses de vitamina C (Ferreira, 2004). A crença girava em torno da hipótese de que o ácido ascórbico atuava em quase todas as reações químicas do organismo, sendo imprescindível em muitas delas.

O AA tem funções plenamente reconhecidas como a de prevenir o escorbuto, a de ser cofator enzimático de diversas reações, como as de síntese de carnitina, aminoácidos, catecolaminas, colesterol, e até mesmo alguns hormônios peptídeos (Chatterjee et al., 1975). Ele é essencial para a formação das fibras colágenas, além de atuar como um potente antioxidante. Atua na defesa do organismo contra infecções, sendo eficaz na captação de radicais livres e de espécies reativas de oxigênio, protegendo os lipídios e as lipoproteínas de baixa densidade (LDL) de danos oxidativos (Pinnel et al., 1987; Retsky et al., 1993; Martin e Frei, 1997).

No sistema nervoso, a vitamina C exerce tanto funções antioxidantes, como não-oxidantes. Dentre as funções antioxidantes, podemos ressaltar sua atuação como de varredora de espécies reativas de oxigênio e nitrogênio, como o superóxido (Jackson et al., 1998). Também tem papel fundamental na reciclagem do radical α-tocoferoxil, reduzindo-o em α-tocoferol (vitamina E) na superfície da bi-camada lipídica das células (Niki et al., 1995), sendo assim, também está inteiramente ligada à prevenção da peroxidação lipídica (Seregi et al., 1978).

A neurotransmissão é uma das atividades não antioxidantes exercidas pelo AA. A vitamina tem sido apontada como neuromodulador nos processos de neurotransmissão mediada por glutamato, acetilcolina e dopamina (Kuo et al., 1979; Rebec e Pierce, 1994; Rice, 2000). Os mecanismos, entretanto, não estão plenamente esclarecidos, mas sabe-se que em todas as suas funções, o AA atua como doador de elétrons (Harrison e May, 2009).

Um dos papéis mais importantes da vitamina C é sua atuação como cofator na síntese de colágeno, uma vez que este é essencial para os processos de mielinização e diferenciação das células de Schwann (Eldridge et al., 1987). Estudos recentes têm sugerido ação importante do AA a nível comportamental, em que ele tem sido identificado tanto

como possível mediador dos processos de aprendizagem e memória (Shahidi el al., 2008), quanto como agente restaurador dos danos de memória provocados pela idade (Parle e Dhingra, 2003).

É interessante a evidência de que o teor de AA no cérebro, ao final do período gestacional, no rato, chega a duplicar (Kratzing e Kelly, 1982; Kratzing et al., 1985), sugerindo sua atuação na diferenciação de células tronco-embrionárias (Lee et al., 2000) e no aumento da expressão dos genes ligados à neurogênese, à maturação e à neurotransmissão (Shin et al., 2004).

Apesar das evidências da ação benéfica do AA sob o sistema nervoso, a vitamina C também tem sido descrita como pró-oxidante em certas condições experimentais (in vivo e in vitro), podendo induzir à morte celular (Sakagami e Satoh, 1997). Há uma hipótese de que a vitamina perderia sua capacidade antioxidante quando em altas concentrações (Young e Lowe, 2001).

Frente ao exposto, o presente trabalho se propôs a estudar o efeito da suplementação de AA no rato, durante o período do desenvolvimento cerebral, e constatar se esta suplementação seria capaz de alterar o fenômeno da DAC em ratos jovens (de 30-40 dias de vida).

OBJETIVOS

Geral

Avaliar os possíveis efeitos eletrofisiológicos da administração oral de vitamina C sobre o sistema nervoso em desenvolvimento, em diferentes condições nutricionais.

Específicos

- Analisar em ratos jovens, com 30-40 dias de vida, os efeitos da administração de vitamina C, durante o período de aleitamento, sobre o fenômeno da Depressão Alastrante Cortical (DAC);
- Investigar se a desnutrição durante o aleitamento influencia esse efeito, comparando-se a propagação da DAC, nas condições acima, com a propagação em animais previamente desnutridos.

ARTIGO CIENTÍFICO

Title: **Chronic treatment with ascorbic acid enhances cortical spreading depression in developing well-nourished and malnourished rats**

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Abstract

Ascorbic acid (AA) is an antioxidant molecule that is highly concentrated in the brain and can exert both anticonvulsant and proconvulsant effects in distinct models of experimental seizures. Herein, we explore the question of whether AA chronic administration alters cortical excitability as indexed by the cortical spreading depression (CSD). Well-nourished (W) and malnourished (M) rats were treated, by gavage, with 60 mg/kg/day of L-ascorbic acid from postnatal days 7-28, and CSD propagation was analyzed at 30-40 days. Compared to the W groups, M rats presented higher ($p<0.05$) CSD velocities of propagation, confirming previous reports. Compared to non-treated ('naïve'; Nv) and saline-treated (Sal) controls, AA-treated rats displayed higher CSD velocities ($p<0.05$) in the W and M conditions. The mean±sd CSD velocities of propagation (in mm/min) for the Sal, AA and Nv groups were respectively 3.75±0.03, 4.26±0.08 and 3.81±0.04 for the W condition and 4.29±0.08, 4.51±0.04 and 4.30±0.04 for the M groups. The results demonstrate a CSD-facilitation by AA regardless of nutritional status. They also suggest that at the dose of 60 mg/Kg/day chronically administered during brain development, AA may act as a prooxidant in brain, in view of the contrasting effect as compared with other antioxidants, which reduce CSD propagation.

Keywords: Brain development; Malnutrition; Ascorbic Acid; Cortical spreading depression; antioxidants; prooxidants

Abbreviations

AA – Ascorbic acid

CSD – Cortical spreading depression

DC – direct current

ECoG – Electrocorticogram

EEG – electroencephalogram

i.p – intraperitoneal

M – Malnourished

Nv – Naïve

RBD – Regional Basic Diet

Sal – Saline

SD – Standard Deviation

W – Well-nourished

Introduction

The postulation that active oxygen free radicals participate in the mechanisms of excitability-related brain disturbances [8, 35] certainly stimulated the use of antioxidants in protecting the brain against excitability-induced damage [36]. Ascorbic acid (AA) is an important antioxidant which acts as an enzyme cofactor and water-soluble reducing agent [37]. It is highly concentrated in the adrenal gland and central nervous system, [32]. It has been suggested that AA may be important for brain development [52]. Under physiological conditions, the important antioxidant action of AA can protect the brain against reactive oxygen species and damage associated with neurodegenerative disorders such as Alzheimer's disease [22]. However, under certain conditions AA has also been shown to exert biphasical modulating action on excitability-dependent brain phenomena [5, 39].

Cortical spreading depression (CSD) is an excitability-related neural phenomenon that was first reported as a reduction of spontaneous electrical activity of the cerebral cortex in response to mechanical, electrical or chemical stimulation of one point on the cortical surface [26]. During CSD, cerebrovascular alterations [27, 25], as well as a slow negative potential change [28] have been described. Also, water- and ion transmembrane flow occur concomitantly with the EEG depression [24]. The brain susceptibility to CSD can be easily estimated by measuring CSD velocity of propagation along the cortical tissue. CSD changes have been previously demonstrated in rats under conditions of environmental, pharmacological, and nutritional manipulations [1-3, 30].

Although the mechanisms underlying CSD are not yet fully clarified, several pieces of evidence suggest the participation of ionic processes [17, 45], as well as neurotransmitters [3] and the production of reactive oxygen species in the brain [13]. The

brain is particularly vulnerable to oxidative lipid damage, because it has a high rate of oxidative metabolism [5], and its constitution includes a high content of polyunsaturated fatty acids. This is especially critical in the immature brain [9].

Malnutrition can cause structural, neurochemical and functional changes of organs and tissues, especially in the nervous system, both in laboratory animals [10, 33, 34, 50] and in humans [15, 16, 29]. It has been well established that early malnutrition [43], as well as enteral administration of the amino acid L-arginine [14] and glutamine [30] increase CSD propagation, but no information is available regarding systemic AA effects on CSD in vivo.

In the present work, we used electrophysiological recording of CSD, in order to address the following two questions in the brain of weaned young rats, subjected to malnutrition during lactation followed by nutritional recovery: (i) How does daily enteral administration of AA during the brain development affect CSD propagation, and (ii) if so, how would this effect be influenced by the previous brain nutritional condition. We were able to demonstrate a facilitation effect of AA that is not appreciably modified by malnutrition.

Materials and methods

Animals

Wistar rats (n=65) from the colony of Departamento de Nutrição of Universidade Federal de Pernambuco (Brazil) born from several dams were pooled at birth and then randomly distributed to form litters with six pups per nurse and assigned to two nutritional groups according to the mother's dietary conditions: Well-nourished group (W, n=33), suckled by dams fed a commercial laboratory chow diet (Purina do Brasil LTDA), containing 23% protein, and Malnourished group (M, n=32), suckled by dams fed a Regional Basic Diet (RBD), containing 7,87% protein, that mimics the low-income human populations diet of Northeastern Brazil [48]. Both nutritional groups were subdivided in three other groups according to the daily treatment during lactation days 7-28: (i) group sal and (ii) group AA, receiving respectively, per gavage, saline and 60mg/kg/d AA (Sigma-Aldrich), as previously described [5]; (iii) group "naïve" (Nv) receiving no gavage. The handling procedures involving the animals were in accordance with the Institution's guidelines, which comply with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA). Animals were reared in polypropylene cages (51 cm X 35,5 cm X 18,5 cm) in a room maintained at 22+ 1°C with a 12h light/ 12h dark cycle (lights on at 7:00 a.m.) with free access to water and food.

Surgical procedure

The surgical procedure was performed as previously described [1] with little modification. Briefly, under anesthesia (1 g⁄kg urethane plus 40 mg⁄kg chloralose, i.p.) three trephine holes with 2-3mm in diameter were made on the right side of the skull,

parallel to the midline. The first hole (on the frontal bone) was used to apply the stimulus to elicit CSD. The other two holes (on the parietal bone) were used to record the propagating CSD wave. Rectal temperature was continuously monitored and maintained at 37 ± 1ºC by a heating blanket.

CSD elicitation and recording

CSD was elicited at 20 min intervals by applying, for 1 min, a cotton ball (1–2 mm diameter), soaked in 2% KCl solution (approximately 0.27 M) to the anterior hole drilled at the frontal region. The electrocorticogram (ECoG) depression and the DC-potential change typical of CSD were recorded simultaneously at the two parietal points on the cortical surface by using a pair of Ag-AgCl agar-Ringer electrodes. A common reference electrode, of the same type, was placed on the nasal bones. The velocity of CSD propagation was calculated based on the time required for a CSD wave to cross the distance between the 2 recording electrodes. In the measurement of CSD velocities, the initial point of each DC negative rising phase was used as the reference point. After finishing the recording session, the animal, while anesthetized, was submitted to euthanasia by bulbar injury. This was carried out by introducing a sharp needle into the cisterna magna, provoking immediate cardio-respiratory arrest.

Statistics

CSD- propagation rates were compared between groups by ANOVA, followed by a post-hoc (Tukey–Kramer) test when indicated. Differences were considered significant when $p \leq 0.05$. All values are presented in the text as means ± standard deviations.

Results

At the day of the CSD recording, animals of the M-groups presented lower ($p<0.05$) body weights as compared with those of the W-groups. The mean±sd body weights (in g) in the well-nourished and the malnourished animals were respectively 98.0 ± 4.0 (group sal), 100.6 ± 5.5 (AA) and 84.3 ± 4.10 (Nv) for the well-nourished condition and 23.8 $\pm$ 1.1 (Sal), 25.4 $\pm$ 1.0 (AA) and 27.8 $\pm$ 1.8 (Nv) for the malnourished groups. Data are presented in Fig 1.

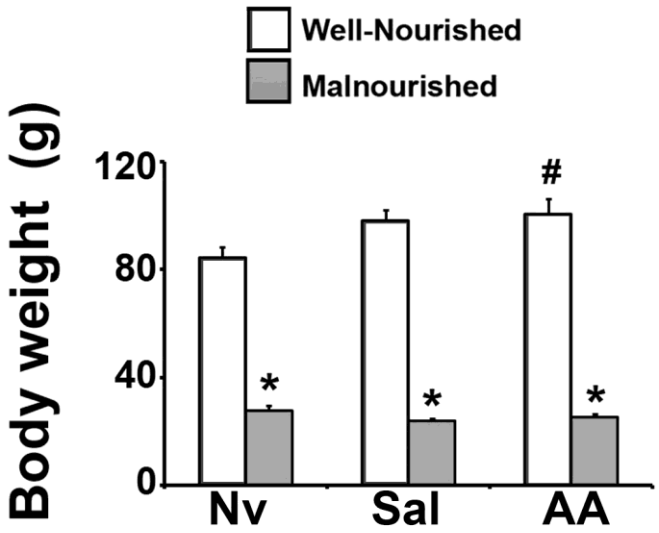

Figure 1: Body weights (mean±SD) of well-nourished (W; white columns) and malnourished (M; gray columns) Wistar rats treated per gavage from the 7th to the 28th postnatal days with 60mg/Kg/day of ascorbic acid (AA group) or saline (Sal group) or non treated (Naïve; NV group). Body weights were measured at postnatal day 32. Asterisks (*) indicate that all M values are significantly different from the corresponding W values ($p<0.05$). The symbol # signifies that W rats treated with AA presented higher body weight ($p<0.05$) as compared with the W-NV group.

In all groups, topical application of 2% KCl for 1 min at the frontal cortex reproducibly elicited a single CSD wave, which propagated and was recorded at two points in the parieto-occipital region of the stimulated hemisphere. Examples of CSD episodes in

3 well-nourished and 3 malnourished rats (one Sal, one AA and one Nv animal from each nutritional condition) can be seen in the ECoG- and DC-potential change recordings presented in Fig 2.

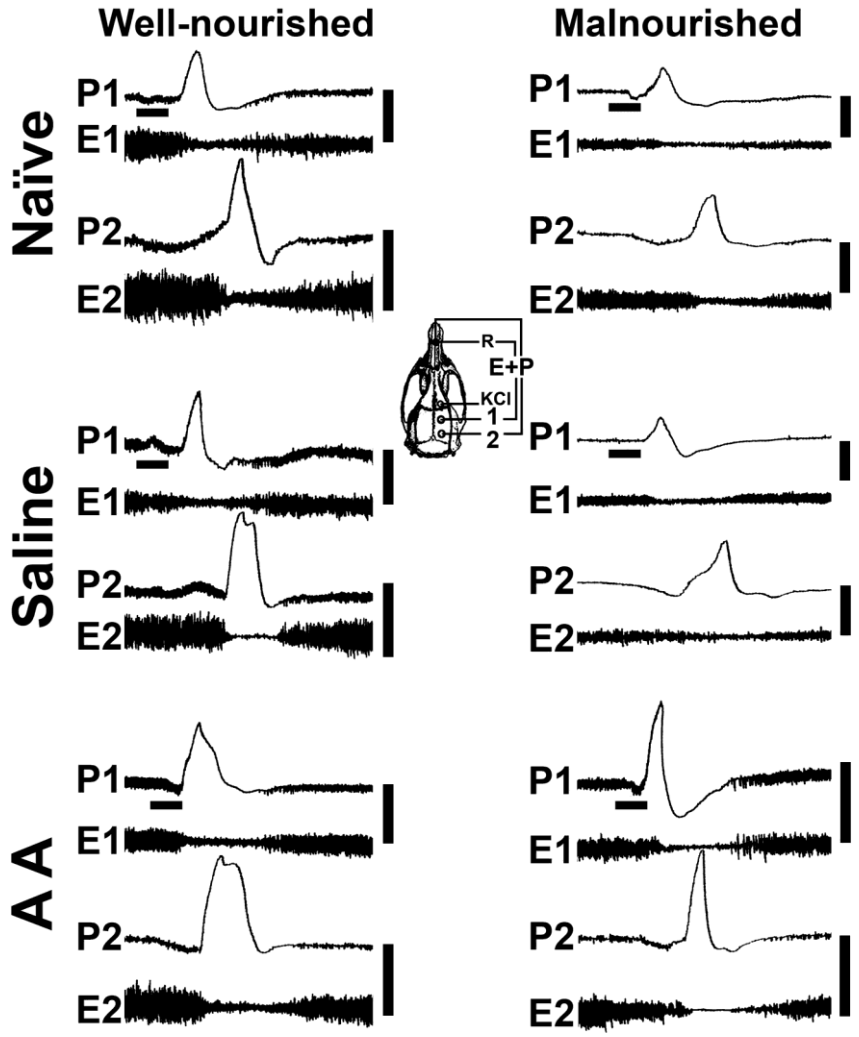

Figure 2: Electrophysiological recordings: ECoG (E) and slow potential change (P) recorded during cortical spreading depression (CSD) in the right hemisphere of 30–40 days-old well-nourished and malnourished rats which received per gavage, from the 7th to th 28th postnatal days, 60mg/Kg/day of ascorbic acid (AA group) or saline (Sal group) or no gavage (Naïve; NV group). The horizontal bars (P1-trace) show the period (1 min) of stimulation with 2% KCl on frontal region of the right hemisphere, to elicit CSD. The vertical bars correspond to 10 mV in P and 1 mV in ECoG (negative upwards). The inset shows the place of KCl application and the recording positions 1 and 2, from which the traces marked with the same numbers were obtained. The interelectrode distance was kept constant for each animal (range: 2.8-5.9 mm).

The CSD propagation velocities are presented in Fig. 3. In well-nourished animals the mean±sd CSD velocities were (in mm/min) 3.75 ± 0.03, 4.26 ± 0.08 and 3.81 ± 0.04 for the Sal, AA and Nv groups, respectively. In the malnourished rats the CSD velocities were 4.29 ± 0.08, 4.51 ± 0.04 and 4.30 ± 0.04 for the Sal, AA and NV groups, respectively. ANOVA revealed a main effect of the nutrition condition and the Tukey test indicated that CSD velocities were higher in the malnourished rats, as compared to the corresponding well-nourished controls ($p<0.05$).

Independent of the nutritional status, a main effect of the gavage condition was also detected. The Tukey test revealed that the treatment with AA significantly increased the CSD propagation velocities ($p<0.05$), as compared with the two control groups (Nv and Sal), which presented comparable CSD values (Fig 3).

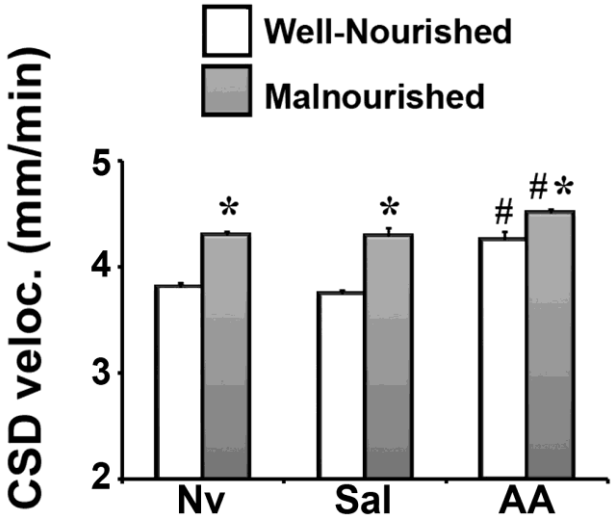

Figure 3: Propagation velocity of cortical spreading depression (CSD; mean ± SEM) of Well-nourished (W) and Malnourished (M) 30–40-day-old rats, which received, from the 7th to the 28th postnatal days 60mg/Kg/day of ascorbic acid (AA group) or saline (Sal group) or non treated rats (Naïve – NV group). Asterisks indicate that the CSD velocities in the malnourished groups are significantly higher ($P<0.05$) than their corresponding well-nourished groups. The symbol # shows that W and M rats treated with AA presented higher CSD velocities ($p<0.05$) as compared with the corresponding NV and Sal groups.

Discussion

The present findings constitute the first description regarding the effects of systemic application of AA on CSD in the developing rat brain. Data indicate an increase of CSD susceptibility in both well-nourished (W) and malnourished (M) conditions, as shown by the higher CSD velocities (Fig. 3), as compared to the saline- and naïve controls. Our data suggest that brain developmental changes are produced by early chronic AA administration and this is causally associated, at least in part, with the facilitation of CSD propagation. Such effect is unlikely to be caused by the stress associated to the gavage procedure, since no effect was observed in the control groups that have been equally submitted to the gavage. Interestingly, malnutrition did not seem to modulate it.

In the present study, malnutrition was indicated by the significantly lower body weights in the M groups, when compared to their respective W controls. It confirms that nutritional restriction by receiving the "regional basic diet" (RBD, with 8% protein) early in life, is able to provoke malnutrition in the pups during the suckling period, as previously reported [42]. Malnutrition is known to impair the organism's development, with repercussions on vital organs, including the brain. It may lead to a deficiency in processes like synapse formation, dendritic development and myelination [33, 34, 40]. These processes may be involved in the present facilitating CSD effects associated to malnutrition, which are in accordance with previous reports demonstrating that malnutrition facilitates CSD in the rat brain [4, 18, 30, 42, 43]. Alteration in the action of synaptic neurotransmitters, increase in the brain cell packing density, and reduction in the brain myelination are factors that have been postulated to be involved in the CSD facilitation in the malnourished brain [11, 42]. Concerning the relationship between myelination and

CSD, an inverse correlation has been recently demonstrated in animal models in which the myelination pattern has been altered by means of genetic (hypermyelinated transgenic animals), immunological (cortical hypomyelination produced by immunization against myelin) and dietary approaches [31]. These authors have shown that the more myelinated the cortex was, the lower the CSD velocity of propagation, and vice-versa, indicating a dichotomous modulation of the myelin participation in the CSD effects. In overfed and malnourished rats, a similar diet-dependent dichotomous modulation has also been found, with CSD deceleration in overfed rats and acceleration in malnourished rats, as compared to the well-nourished controls [43].

Early malnourished animals can present diminished [20, 51], equal [3, 20], or enhanced [49] changes in brain CSD reaction in response to the administration of distinct substances, such as, respectively, glucose and diazepam (diminished CSD responses in the malnourished brain), citalopram and fluoxetine (equal CSD responses) and pilocarpine (enhanced CSD responses). In the present study we found that early malnutrition did not change the AA effect on CSD. Taken together, these findings collectively indicate that the malnourished brain may have distinct responsiveness of the CSD to different pharmacological agents. If this could be extrapolated, in the human species, to other neural actions of distinct drugs, the possible clinical implication would be that one such drug could have different effectiveness, as a function of the early nutritional status of the patient. This possibility should be diligently investigated in the medical clinic.

Concerning the CSD-antioxidants relationship, previous studies demonstrated that the production of reactive oxygen species can elicit CSD in the isolated chicken retina [38] and in the rat cortex [13]. Also, the antioxidant astaxanthin impairs the chronic ethanol facilitating effect on CSD propagation [1]. In contrast to that findings, in the present study

animals treated with AA show an increase in CSD velocities. AA is a strong water soluble reducing agent known to act normally as an antioxidant in vivo and in vitro [7]. It is effective in scavenging free radicals including reactive oxygen species (ROS), protecting the lipids from oxidation [41]. Antioxidant molecules are also important in protecting the brain against free radicals, particularly in neurodegenerative diseases [47]. However, the in vivo [5] as well as in vitro [23, 46] evidence suggest that, depending on the conditions under which it is acting, AA can also have prooxidant action, and thus can cause neurotoxicity. Furthermore, AA can exert both anticonvulsant and proconvulsant effects in the brain [6, 39]. Both AA and its oxidized form, the dehidroascorbic acid are speculated as being cell division regulators [12]. According to Halliwell [21] various non-radical compounds including AA can produce reactive radical species in the presence of transition metal ions in the brain. Based on the above reports, and also considering that in the present study the daily AA treatment per gavage was long-lasting (22 days), it is tempting to speculate that this body of evidence would constitute a possible explanation for our present findings on CSD facilitation by AA. It is possible that, at the dose of 60 mg/Kg/day, administered chronically during an intense period of neural development, as in the present study, AA may act as prooxidant in the brain. The measurement of blood- and brain reactive oxygen species levels, as well as treating developing rats with lower AA doses, or treating already developed animals, shall confirm this hypothesis in future experiments.

In conclusion, our data have demonstrated, for the first time, that chronic AA treatment of developing animals facilitates CSD propagation, and this effect is not modulated by early malnutrition. These novel findings are consistent with the hypothesis that, at the dose of 60 mg/k/d, AA can have a prooxidant effect, as previously postulated [5].

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References

[1] Abadie-Guedes, R., Santos, S.D., Cahú, T.B., Guedes, R.C.A., Bezerra, R.S. Dose-dependent effects of astaxanthin on cortical spreading depression in chronically ethanol-treated adult rats. Alcohol. Clin. Exp. Res. 32 (2008) 1417-1421.

[2] Aguiar, M. J. L., Alves-de-Aguiar, C. R. R., Guedes, R.C.A. Caffeine/nutrition interaction in the rat brain: Influence on latent inhibition and cortical spreading depression. Europ. J. Pharmacol. 650 (2011) 268-274.

[3] Amâncio-dos-Santos A. , Pinheiro, P. C. F., Lima, D. S. C., Ozias, M. G., Oliveira, M. B., Guimaraes, N. X., Guedes, R. C. A. Fluoxetine inhibits cortical spreading depression in weaned and adult rats suckled under favorable and unfavorable lactation conditions. Exp. Neurol. 200 (2006) 275-282.

[4] Andrade, A. F. D., Guedes, R. C. A., Teodósio, N. R. Enhanced rate of cortical spreading depression due to malnutrition: Prevention by dietary protein suplementation. Braz. J. Med. Biol. Res. 23 (1990) 889-893.

[5] Aydoğan, M., Korkmaz, A., Barlas, N., Kolankaya, D. The effect of vitamin C on bisphenol A, nonylphenol and octylphenol induced brain damages of male rats. Toxicology 249 (2008) 35-39.

[6] Ayyildiz M, Coskun s, Yildirim M, Agar E. The effect of ascorbic acid on penicillin-induced epileptiform activity in rats. Epilepsia 48 (2007) 1388–1395.

[7] Bendich, A., Gabriel, E., Machlin, L. J. Dietary vitamin E requirement for optimum immune responses in the rat. J. Nutr. 116 (1986) 675-681.

[8] Braugher, J.M, Hall, E.D. Central nervous system trauma and stroke: I. biochemical considerations for oxygen radical formation and lipid peroxidation. Free Rad. Biol. Med. 6 (1989) 289–301.

[9] Buonocore, G., Groenendaal F. Anti-oxidant strategies. Semin Fetal Neonatal Med. 12 (2007) 287–295.

[10] Chen J.C., Turiak G., Galler J., Volicer L., Postnatal changes of brain monoamine levels in prenatally malnourished and control rats. Int. J. Dev. Neurosci. 2 (1997), 257–263.

[11] De Luca B., Cioffi L.A., Bures J. Cortical and caudate spreading depression as an indicator of neural changes induced by early malnutrition in rats. Activ. Nerv. Sup. 19 (1977) 130–131.

[12] Edgar, J. A. Dehydroascorbic acid and cell division. Nature, 227 (1970) 24.

[13] El-Bachá, R.S., Lima-filho, J.L., Guedes, R.C.A. Dietary antioxidant deficiency facilitates cortical spreading depression induced by photo-activated riboflavin. Nutr. Neurosci. 1 (1998) 205–212.

[14] Frazão, M. F., Maia, L. M. S. S., Guedes R. C. A. Early malnutrition, but not age, modulates in the rat the l-Arginine facilitating effect on cortical spreading depression. Neurosci. Lett. 447 (2008) 26–30.

[15] Galler, J.R., Ramsey, F.C., Harrison, R.H., Brooks, R., Weiskopf-Bock, S. Infant feeding practices in Barbados predict later growth. J Nutr. 128 (1998) 1328-1335.

[16] Grantham-McGregor S. A review of studies of the effect of severe malnutrition on mental development, J. Nutr. 125 (1995), 2233S–2238S.

[17] Guedes R.C.A., Do Carmo R.J. Influence of ionic disturbances produced by gastric washing on cortical spreading depression, Exp. Brain Res. 39 (1980) 341–349.

[18] Guedes, R.C.A., Andrade, A.F.D., Cabral-Filho, J.E. Propagation of cortical spreading depression in malnourished rats: facilitatory effect of dietary protein deficiency. Braz. J. Med. Biol. Res. 20 (1987) 639-642.

[19] Guedes, R.C.A., Santos, A.A., Manhães-de-Castro R., Cruz, R.R.G.C. Citalopram has an antagonistic action on cortical spreading depression in well-nourished and early-malnourished adult rats. Nutr. Neurosci. 5 (2002) 115-123.

[20] Guedes, R.C.A., Cabral-Filho, J.E., Teodósio, N.R. Gabaergic mechanisms involved in cortical spreading depression in normal and malnourished rats. In: Do Carmo, R.J. (Ed.). Spreading Depression. (Experimental Brain Research Series No. 23). Berlin: Springer, 23 (1992) 17-26.

[21] Halliwell B. Reactive oxygen species and the central nervous system. J. Neurochem. 59 (1992) 1609–1623.

[22] Halliwell, B. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 97 (2006)1634–1658.

[23] Hisanaga K., Sagar S.M., Sharp F.R. Ascorbate neurotoxicity in cortical cell culture. Ann. Neurol. 31 (1992), 562–565.

[24] Kraig, R.P., Nicholson, C. Extracellular ionic variation during spreading depression. Neuroscience 3 (1978) 1045–1059.

[25] Lauritzen, M. Cortical spreading depression as a putative migraine mechanism. Trends Neurosci. 10 (1987) 8–13

[26] Leão, A.A.P. Spreading depression of activity in the cerebral cortex. J. Neurophysiol. 7, (1944a) 359–390.

[27] Leão, A.A.P. Pial circulation and spreading depression of activity in the cerebral cortex. J. Neurophysiol. 7 (1944b) 391-396.

[28] Leão, A.A.P. Futher observations on the spreading depression of activity in the cerebral cortex. J. Neurophysiol. 10 (1947) 409-414.

[29] Levitsky, D.A., Strupp, B.J. Malnutrition and the brain: changing concepts, changing concerns. J. Nutr. 125 (1995) 2212S-2220S

[30] Lima, D.S.C., Maia, L.M.S.S., Barboza, E.A., Duarte, R.A., Souza, L.S., Guedes, R.C.A. L-glutamine supplementation during the lactation period facilitates cortical spreading depression in well-nourished and early-malnourished rats. Life Sci. 85 (2009) 241-247.

[31] Merkler, D., Klinker, F., Jürgens, T., Glaser, R., Paulus, W., Brinkmann, B.G., Sereda, M.W., Stadelmann-Nessler, C., Guedes, R.C.A., Brück, W., Liebetanz, D. Propagation of spreading depression inversely correlates with cortical myelin content. Ann. Neurol. 66 (2009) 355-365.

[32] Miura, S., Ishida-Nakajima, W., Ishida, A., Kawamura, M., Ohmura, A., Oguma, R. Sato, Y., Takahashi, T. Ascorbic acid protects the newborn rat brain from hypoxic-ischemia. Brain & Devel. 31(4) (2008) 307-317.

[33] Morgane, P.J., Austin-La France, R., Bronzino, J., Tonkiss, J., Díaz-Cintra, S., Cintra, L., Kemper, T. and Galler, J.R. Prenatal malnutrition and development of the brain. Neurosci. Biobehav. Rev. 17 (1993) 91–128.

[34] Morgane, P.J., Miller, M., Kemper, T., Stern, W., Forbes, W., Hall, R., Bronzino, J., Kissane, J. Hawrylewicz, E., Resnick, O. The effects of protein malnutrition on

the developing nervous system in the rat. Neurosci. Biobehav. Rev. 2 (1978) 137–230.

[35] Mori, A., Hiramatsu M., Yokoi, I., Edamatsu, R. Biochemical pathogenesis of posttraumatic epilepsy. Pav. J. Biol. Sci. 25 (1990) 54–62.

[36] Murashima Y.L., Kasamo K., Suzuki, J. Antiepileptic effects of allopurinol on EL mice associated with changes in SOD isoenzyme activities. Epilepsy Res. 32 (1998) 254–265.

[37] Muthuvel, R., Venkataraman, P., Krishnamoorthy, G., Gunadharini, D.N., Kanagaraj, P., Jone Stanley, A., Srinivasan, N., Balasubramanian, K., Aruldhas, M.M., Arunakaran, J. Antioxidant effect of ascorbic acid on PCB (Aroclor 1254) induced oxidative stress in hypothalamus of albino rats. Clin. Chim. Acta 365, (2006) 297–303.

[38] Netto, M., Martins-Ferreira, H. Elicitation of spreading depression by rose bengal photodynamic action. Photochem. Photobiol. 50 (1989). 229-234.

[39] Oliveira, M.S., Furian, A.F., Royes, L.F.F., Fighera, M.R., De Carvalho J., Fiorenza, N.G. Ascorbate modulates pentylenetetrazol-induced convulsions biphasically. Neuroscience 128 (2004) 721–728

[40] Picanço-Diniz, C.W., Borba, J.M.C., Araújo, M.S., Guedes, R.C.A. Nadph-diaphorase containing neurons and biocytin-labelled axon terminals in the visual cortex of adult rats malnourished during development. Nutr. Neurosci. 1 (1998) 35-48.

[41] Retsky, K.L., Freeman, M.W., Frei, B. Ascorbic acid oxidation product(s) protect human low density lipoprotein against atherogenic modification. J. Biol. Chem. 268 (1993) 1304-1309.

[42] Rocha-de-Melo, A.P., Guedes, R.C.A. Spreading depression is facilitated in adult rats previously submitted to short episodes of malnutrition within the lactation period. Braz. J. Med. Biol. Res. 30 (1997) 663-670.

[43] Rocha-de-Melo, A.P., Cavalcanti, J.B., Barros, A.S., Guedes, R.C.A. Manipulation of rat litter size during suckling influences cortical spreading depression after weaning and at adulthood. Nutr. Neurosci. 9 (2006) 155-160.

[44] Santos, A.A., Pinheiro, P.C.F., Lima, D.S.C., Ozias, M.G., Oliveira, M.B., Guimaraes, N.X., Guedes, R.C.A. Fluoxetine inhibits cortical spreading depression in weaned and adult rats suckled under favorable and unfavorable lactation conditions. Exp. Neurol., 200 (2006) 275-282.

[45] Siesjö, B.K., Bengtsson, F. Calcium fluxes, calcium antagonists and calcium-related pathology in brain ischemia, hypoglycemia and spreading depression: a unifying hypothesis. J. Cereb. Blood Flow Metabol. 9 (1989).127–140.

[46] Song J.H., Shin S.H., Ross G.M., Oxidative stress induced by ascorbate causes neuronal damage in an in vitro system, Brain Res. 895 (2001) 66–72.

[47] Tapiero H., Townsend, D.M., Tew, K.D. The role of carotenoids in the prevention of human pathologies. Biomed. Pharmacother. 58 (2004) 100–110.

[48] Teodósio, N.R., Lago, E.S., Romani, S.A.M., Guedes, R.C.A. A regional basic diet from Northeast Brazil as a dietary model of experimental malnutrition. Arch. Latinoam. Nutr. 40 (1990) 533-547.

[49] Vasconcelos, C.A.C., Oliveira, J.A.F., Costa, L.A.O., Guedes, R.C.A. Malnutrition and REM-sleep deprivation modulate in rats the impairment of spreading depression by a single sub-convulsing dose of pilocarpine. Nutr. Neurosci. 7 (2004) 163-170.

[50] Winick, M., Brasel, J.A., Rosso, P. Nutrition and cell growth. Nutr. Devel. (1972) 49-98.

[51] Ximenes-da-Silva, A., Guedes, R.C.A. Differential effect of changes in blood glucose levels on the velocity of propagation of cortical spreading depression in normal and malnourished rats. Braz. J. Med. Biol. Res. 24 (1991) 1277-1281.

[52] Zalani, S., Rajalakshmi, R., Parekh, L.J. Ascorbic acid concentration of human fetal tissues in relation to fetal size and gestational age. Br. J. Nutr. 61 (1989) 601-606.

CONCLUSÕES

A análise dos resultados desta dissertação permite as seguintes conclusões:

- A administração de ácido ascórbico, durante o período crítico do desenvolvimento cerebral, na dose de 60 mg/Kg/dia, facilitou a DAC, a julgar pelo aumento na sua velocidade de propagação;

- Esse efeito facilitador sobre a DAC ocorreu também no cérebro previamente desnutrido, indicando que a desnutrição não inibe tal efeito;

- O déficit ponderal apresentado pelos animais que receberam a Dieta Básica Regional indica a eficácia da dieta em promover a desnutrição energético-protéica, confirmando estudos anteriores.

PERSPECTIVAS

Como perspectiva de continuidade desta linha de pesquisa, sugere-se a investigação das seguintes condições:

- Efeito da administração de Ácido Áscórbico em animais adultos, com o sistema nervoso já desenvolvido;

- Efeito da aplicação tópica de Ácido Áscórbico sobre o córtex cerebral;

- Efeito da suplementação de distintas dosagens de Ácido Áscórbico, com o propósito de construção de uma "curva dose-resposta" da relação Ácido Áscórbico X Depressão Alastrante Cortical;

- Correlacionar os efeitos eletrofisiológicos com dosagens séricas e neuroquímicas de AA.

- Testar a hipótese de que a ação do ácido ascórbico na dose de 60mg/Kg/dia atua como pró-oxidante.

REFERÊNCIAS BIBLIOGRÁFICAS

Abadie-Guedes, R.; Santos, S.D.; Cahú, T.B.; Guedes, R.C.A.; Bezerra, R.S. Dose-dependent effects of astaxanthin on cortical spreading depression in chronically ethanol-treated adult rats. Alcohol. Clin. Exp. Res. 2008;32: 1417-1421.

Aguiar, M. J. L., Alves-de-Aguiar, C. R. R., Guedes, R.C.A. Caffeine/nutrition interaction in the rat brain: Influence on latent inhibition and cortical spreading depression. Europ. J. Pharmacol. 2011;650 268-274.

Amâncio-dos-Santos A.; Pinheiro, P.C.F.; Lima, D.S.C.; Ozias, M.G.; Oliveira, M.B.; Guimaraes, N.X.; Guedes, R.C.A. Fluoxetine inhibits cortical spreading depression in weaned and adult rats suckled under favorable and unfavorable lactation conditions. Exp. Neurol. 2006; 200: 275-282.

Amaral, A.P.B.; Barbosa, M.S.S.; Souza, V.C.; Ramos, I.L.T.; Guedes, R.C.A. Drug/nutrition interaction in the developing brain: dipyrone enhances spreading depression in rats. Exp. Neur. 2009;219: 492-498.

Andrade, A.F.D.; Guedes, R.C.A.; Teodósio, N.R. Enhanced rate of cortical spreading depression due to malnutrition: Prevention by dietary protein suplementation. Braz. J. Med. Biol. Res. 1990;23: 889-893.

Arrigoni, O.; De Tullio, M.C. Ascorbic acid: much more than just an antioxidant. Biochim. Biophys. Acta Gen. Subj. 2002;1569:1–9.

Aydoğan, M., Korkmaz, A., Barlas, N., Kolankaya, D. The effect of vitamin C on bisphenol A, nonylphenol and octylphenol induced brain damages of male rats. Toxicology 2008;249: 35-39.

Ayyildiz M, Coskun s, Yildirim M, Agar E. The effect of ascorbic acid on penicillin-induced epileptiform activity in rats. Epilepsia 2007;48:1388–1395.

Batista-Filho, M. Pesquisa Nutricional na Zona da Mata. Erecife, Universidade Federal de Pernambuco, 1968, 133p.

Bendich, A., Gabriel, E., Machlin, L. J. Dietary vitamin E requirement for optimum immune responses in the rat. J. Nutr. 1986;116: 675-681.

Benton, D. The influence of children's diet on their cognition and behavior. Europ. J. Nut. 2008;47(3): 25S-37S

Bezerra, R.S.; Abadie-Guedes, R.; Melo, F.R.M.; Paiva, A.M.A.; Santos, A.A.; Guedes, R.C.A. Shrimp carotenoids protect the developing rat cerebral cortex agains. Neurosc. Let. 2005; 391(1-2): 51-55.

Borba, J.M.C.; Araújo, M.S.; Diniz, C.W.P.; Manhães-de-Castro R.; Guedes, R.C.A. Permanent and transitory morphometric changes of NADPH-diaphorase-containing neurons in the rat visual cortex after early malnutrition. Brain Research Bulletin 2000; 53:193-201.

Braugher, J.M, Hall, E.D. Central nervous system trauma and stroke: I. biochemical considerations for oxygen radical formation and lipid peroxidation. Free Rad. Biol. Med. 1989;6: 289–301.

Buonocore, G., Groenendaal F. Anti-oxidant strategies. Semin Fetal Neonatal Med. 2007;12: 287–295.

Chatterjee, I.B.; Majumder, A.K.; Nandi, B.K.; Subramanian, N. Synthesis and some major functions of vitamin C in animals. Ann. N. Y. Acad. Sci. 1975;258:24–47.

Chen J.C.; Turiak G.; Galler J.; Volicer L.; Postnatal changes of brain monoamine levels in prenatally malnourished and control rats. Int. J. Dev. Neurosci. 1997; 2: 257–263.

Corti, A.; Casini, A.F.; Pompella, A. Cellular pathways for transport and efflux of ascorbate and dehydroascorbate. Arch. Bioche. Biophy. 2010; 500: 107–115.

Costa-Cruz, R.R.G.; Guedes, R.C.A. Cortical spreading depression during streptozotocin-induced hyperglycaemia in nutritionally normal and early-malnourished rats. Neurosc. Let. 2001;303(3):177-180.

Costa-Cruz, R.R.G.; Santos, A.A.; Guedes, R.C.A. Characterization of cortical spreading depression in adult well-nourished and malnourished rats submitted to the association of pilocarpine-induced epilepsy plus streptozotocin-induced hyperglycemia.. Neurosc. Let. 2006; 401(3): 271-275.

De Frías, V.; Varela, O.; Oropeza, J.J.; Bisiacchi, B., Alvarez, A. Effects of prenatal protein malnutrition on the electrical cerebral activity during development. Neurosci Lett. 2010; 482(3):203-207.

De Luca B., Cioffi L.A., Bures J. Cortical and caudate spreading depression as an indicator of neural changes induced by early malnutrition in rats. Activ. Nerv. Sup. 1977;19: 130–131.

De Onis, M.; Blössner, M.; Borghi, E.; Frongillo, E.A.; Morris, R. Estimates of global prevalence of childhood underweight in 1990 and 2015. JAMA. 2004; 291(21):2600-2606.

Diop, A.G.; Boer, H.M.; Mandlhate, C.; Prilipko, L.; Meinardi, H. The global campaign against epilepsy in Afrika. Acta Trop. 2003; 87(1):149-159.

Dobbing J. Vulnerable periods in developing brain. In: A.N. Davison and J. Dobbing, Editors, Applied Neurochemistry, Blackwell, Oxford 1968: 287–316.

Edgar, J. A. Dehydroascorbic acid and cell division. Nature, 1970;227: 24.

El-Bachá, R.S.; Lima-Filho, J.L.; Guedes, R.C.A. dietary antioxidant deficiency facilitates cortical spreading depression induced by photoactivated riboflavin. Nut. Neurosc. 1998;1(3): 205-212.

Eldridge, C.F.; Bunge, M.B.; Bunge, R.P.; Wood, P.M. Differentiation of axonrelated Schwann cells in vitro. I. Ascorbic acid regulates basal lamina assembly and myelin formation. J. Cell Biol. 1987;105:1023–1034.

Farias-Santos, R.C.; Lira, M.C.A.; Pereira, D.E.S.; Sá, I.R.; Pimentel, M.R.F.; Araújo, L.L.; Guedes, R.C.A. Exposure of developing well-nourished and malnourished rats to environmental heating facilitates cortical spreading depression propagation at adulthood. Neuroscience Letters 2009; 454: 218-222.

Ferreira, R. Linus Pauling: por que Vitamina C?. Quím. Nova 2004;27(2): 356-357.

Frazão, M. F., Maia, L. M. S. S., Guedes R. C. A. Early malnutrition, but not age, modulates in the rat the l-Arginine facilitating effect on cortical spreading depression. Neurosci. Lett. 2008;447: 26–30.

Fregni, F.; Liebetanz, D.; Monte-Silva, K.K.; Oliveira, M.B.; Amancio-dos-Santos, A.; Nitsche, M.A.; Pascual-Leone, A.; Guedes, R.C.A. Effects of transcranial direct current stimulation coupled with repetitive electrical stimulation on cortical spreading depression. Exp. Neurol. 2007; 204: 462-466.

Fregni, F.; Monte-Silva, K.K.; Oliveira, M.B.; Freedman, S.; Pascual-Leone, A.; Guedes, R.C.A. Lasting accelerative effects of 1 Hz and 20 Hz electrical stimulation on cortical spreading depression: relevance for clinical applications of brain stimulation.. Europ. J. Neurosc. 2005; 21(8): 2278-2284.

Galler, J.R., Cervera, M.D., Harrison, R.H. A preliminary study of temperament among malnourished Mayan children. Nutritional Neuroscience, 1998;1: 141-149.

Grantham-McGregor, S.; A review of studies of the effect of severe malnutrition on mental development, J. Nutr.1995; 125: 2233S–2238S.

Guedes R.C.A., Do Carmo R.J. Influence of ionic disturbances produced by gastric washing on cortical spreading depression, Exp. Brain Res. 1980;39: 341–349.

Guedes, R.C.A. On Some Conditions That Influence Cortical Spreading Depression. Anais da Academia Brasileira de Ciências 1984; 56(4): 445-455.

Guedes, R.C.A., Andrade, A.F.D., Cabral-Filho, J.E. Propagation of cortical spreading depression in malnourished rats: facilitatory effect of dietary protein deficiency. Braz. J. Med. Biol. Res. 1987;20: 639-642.

Guedes, R.C.A., Cabral-Filho, J.E., Teodósio, N.R. Gabaergic mechanisms involved in cortical spreading depression in normal and malnourished rats. In: Do Carmo, R.J. (Ed.). Spreading Depression. (Experimental Brain Research Series No. 23). Berlin: Springer, 1992; 23: 17-26.

Guedes, R.C.A., Santos, A.A., Manhães-de-Castro R., Cruz, R.R.G.C. Citalopram has an antagonistic action on cortical spreading depression in well-nourished and early-malnourished adult rats. Nutr. Neurosci. 2002; 5:115-123.

Guedes, R.C.A.; Amorim, L.F.; Teodósio, N.R. Effect Of Aging On Cortical Spreading depression. Braz. J. Med. Biol. Res.1996; 29(12): 1407-1412.

Guedes, R.C.A.; Andrade, A.F.D.; Cabral-Filho, J.E. Propagation of cortical spreading depression in malnourished rats: facilitatory effect of dietary protein deficiency. Braz. J. Med. Biol. Res. 1987; 20: 639-642.

Guedes, R.C.A.; Barreto, J. Effect Of Anesthesia On The Propagation Of Cortical Spreading Depression. Braz. J. Med. Biol. Res. 1992; 25(4): 393-397.

Guedes, R.C.A.; Cabral-Filho, J.E.; Teodósio, N.R. GABAergic mechanisms involved in cortical spreading depression in normal and early malnourished rats. In do Carmo, R.J. (Ed.), Experimental Brain Research Series. Spreading Depression. Springer, Berlin, 1992;23: 17–26.

Guedes, R.C.A.; Cavalheiro, E. Blockade Of Spreading Depression In Chronic Epileptic Rats: Reversion By Diazepam. Epilep. Res. 1997; 27: 33-40.

Guedes, R.C.A.; Do Carmo, R.J. Influence of ionic disturbances produced by gastric washing on cortical spreading depression. Exp. Brain Res. 1980;39: 341–349.

Guedes, R.C.A.; Frade, S.F. Effect Of Ethanol On Cortical Spreading Depression. Braz. J. Med. Biol. Res. 1993; 26:1241-1244.

Guedes, R.C.A.; Pereira-da-Silva, M. Effect Of Pre- And Postnatal Propylthiouracil Administration On The Propagation Of Cortical Spreading Depression Of Adult Rats.. Braz. J. Med. Biol. Res. 1993; 26: 1123-1128.

Guedes, R.C.A.; Silva, A. T.; Teodósio, N.R.; Amorim, L.F. Effect Of Dietary Lithium On Cortical Spreading Depression. Brazilian Journal of Medical and Biological Research. 1989; 22: 923-925.

Guedes, RCA, Rocha-de-Melo, A.P.; Teodósio, N.R. Nutrição adequada: a base do funcionamento cerebral. Ciência e Cultura. 2004; 56(1): 32-35.

Hack, M.; Breslau, N.; Weissman, B.; Aram, D.; Klein, N.; Borawski, E. Effect of very low birth weight and subnormal head size on cognitive abilities at school age. N Engl J Med. 1991; 325(4):231-237.

Hackett, R.; Iype, T. Malnutrition and childhood epilepsy in developing countries. Seizure. 2001; 10(8): 554-558.

Halliwell B. Reactive oxygen species and the central nervous system. J. Neurochem.1992; 59:1609–1623.

Halliwell, B. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 2006;97:1634–1658.

Harrison, F.E.; May, J.M. Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2 Free Radical Biology & Medicine 2009;46 719–730.

Hisanaga K., Sagar S.M., Sharp F.R. Ascorbate neurotoxicity in cortical cell culture. Ann. Neurol. 1992;31: 562–565.

Hoehn, S.K.; Kanfer, J.N. Effects of chronic ascorbic acid deficiency on guinea pig lysosomal hydrolase activities. J. Nutr. 1980;110:2085–2094.

Hughes, R.E.; Hurley R.J; Jones P.R. The retention of ascorbic acid by guinea-pig tissues. Br. J. Nutr. 1971;26: 433–438.

Jackson, T.S.; Xu, A.M.; Vita, J.A.; Keaney, J.F.Jr. Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. Circ. Res. 1998;83:916–922.

Kar, B.R.; Rao, S.L.; Chandramouli, B.A. Cognitive development in children with chronic protein energy malnutrition. Behav Brain Funct. 2008;24(4):31.

Kraig, R.P., Nicholson, C. Extracellular ionic variation during spreading depression. Neuroscience 1978; 3:1045–1059.

Kratzing, C.C.; Kelly, J.D. Tissue levels of ascorbic acid during rat gestation. Int. J. Vitam. Nutr. Res. 1982;52:326–332.

Kratzing, C.C.; Kelly, J.D.; Kratzing, J.E. Ascorbic acid in fetal rat brain. J. Neurochem. 1985;44:1623–1624.

Kuo, C.H.; Hata, F.; Yoshida, H.; Yamatodani, A.; Wada, H. Effect of ascorbic acid on release of acetylcholine from synaptic vesicles prepared from different species of animals and release of noradrenaline from synaptic vesicles of rat brain. Life Sci. 1979;24:911–915.

Lane, D.J.R.; Lawen, A.. Ascorbate and plasma membrane electron transport—Enzymes vs efflux. Free Radical Biology & Medicine 2009;47 485–495.

Lauritzen, M. Cortical spreading depression as a putative migraine mechanism. Trends Neurosci. 1987;10:8–13

Leão, A.A.P. Futher observations on the spreading depression of activity in the cerebral cortex. J. Neurophysiol. 1947;10: 409-414.

Leão, A.A.P. Pial circulation and spreading depression of activity in the cerebral cortex. J. Neurophysiol. 1944b;7:391-396.

Leão, A.A.P. Spreading depression of activity in the cerebral cortex. J. Neurophysiol. 1944a;7: 359–390.

Leão, A.A.P. Spreading depression of activity in the cerebral cortex. J. Neurophysiol. 1944;7: 359–390.

Lee, S.H.; Lumelsky, N.; Studer, L.; Auerbach, J.M.; McKay, R.D. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. Nat. Biotechnol. 2000;18:675–679.

Levitsky, D.A.; Strupp, B.J. Malnutrition and the brain: changing concepts, changing concerns. Journal of Nutrition, 1995;125: 2212S-2220S.

Lima, D. S. C.; Maia, L. M. S. S. ; Barboza, E. A.; Duarte, R.A. ; Souza, L.S.; Guedes, R. C. A. L-glutamine supplementation during the lactation period facilitates cortical

spreading depression in well-nourished and early-malnourished rats. Life Sciences, 2009;85: 241-247.

Lind,J.A. Treatise on the Scurvy. The Classics Med. Lib.. Gryphon Ed. Birmingham, 1772

Linster C.L.; Van Schaftingen, E. Vitamin C. Biosynthesis, recycling and degradation in mammals. FEBS J. 2007 ;274(1):1-22.

Maia, L.M.S.S.; Amancio-dos-Santos, A.; Duda-de-Oliveira, D.; Angelim, M.K.C.; Germano, P.C.P.; Santos, S.F.; Guedes, R.C.A.. L-Arginine administration during rat brain development facilitates spreading depression propagation: evidence for a dose- and nutrition-dependent effect. Nut. Neurosc. 2009; 12:73-80.

Martin, A.; Frei, B. Both intracellular and extracellular vitamin C inhibit atherogenic modification of LDL by human vascular endothelial cells, Arterioscler Thromb Vasc Biol 1997;17:1583–1590.

Martins-Ferreira, H. Spreading depression in chick retina. In Ookawa, T. (Ed.) The Brain and Behavior of the Fowl. Tokyo: Japan Scientific Societies Press, 1983:317-333

Merkler, D., Klinker, F., Jürgens, T., Glaser, R., Paulus, W., Brinkmann, B.G., Sereda, M.W., Stadelmann-Nessler, C., Guedes, R.C.A., Brück, W., Liebetanz, D. Propagation of spreading depression inversely correlates with cortical myelin content. Ann. Neurol. 2009;66: 355-365.

Miele, M.; Fillenz, M. In vivo determination of extracellular brain ascorbate. J. Neurosci. Methods 1996;70:15–19.

Miura, S., Ishida-Nakajima, W., Ishida, A., Kawamura, M., Ohmura, A., Oguma, R. Sato, Y., Takahashi, T. Ascorbic acid protects the newborn rat brain from hypoxic-ischemia. Brain & Devel. 2008;31(4): 307-317.

Monteiro, F.M.; Lahlou, S.; Albuquerque, J.A.; Cabral, A.M. Influence of a multideficient diet from northeastern Brazil on resting blood pressure and baroreflex sensitivity in conscious, freely moving rats. Braz J Med Biol Res. 2001; 34(2):271-280.

Monteiro, J.S.; Teodósio, N.R.; Guedes, R.C.A. Long-lasting effects of early environmental stimulation on Cortical Spreading Depression in normal and malnourished rats. Nut. Neurosc. 2000; 3(1): 29-40.

Morgane, P.J.; Austin-La France, R.; Bronzino, J.; Tonkiss, J.; Díaz-Cintra, S.; Cintra, L.; Kemper, T.; Galler, J.R. Prenatal malnutrition and development of the brain. Neurosci. Biobehav. Rev. 1993;17: 91–128.

Morgane, P.J.; Austin-La France, R.; Bronzino, J.; Tonkiss, J.; Galler, J.R. Malnutrition and Developing central nervous system. The vulnerable brain and environmental risks (1) Hezard Assessment Plenun Press, New York, 1992; 1:3-44.

Morgane, P.J.; Miller, M.; Kemper, T.; Stern, W.; Forbes, W.; Hall, R.; Bronzino, J.; Kissane, J.; Hawrylewicz, E.; Resnick, O. The effects of protein malnutrition on the developing nervous system in the rat. Neurosci. Biobehav. Rev.1978 2: 137–230.

Morgane, P.J.; Mokler, D.J.; Galler, J.R. Effects of prenatal protein malnutrition on the hippocampal formation. Neurosci Biobehav Rev. 2002 Jun;26(4):471-83.

Mori, A., Hiramatsu M., Yokoi, I., Edamatsu, R. Biochemical pathogenesis of posttraumatic epilepsy. Pav. J. Biol. Sci. 1990;25: 54–62.

Murashima Y.L.; Kasamo K.; Suzuki, J. Antiepileptic effects of allopurinol on EL mice associated with changes in SOD isoenzyme activities. Epilepsy Res. 1998;32:254-65.

Muthuvel, R., Venkataraman, P., Krishnamoorthy, G., Gunadharini, D.N., Kanagaraj, P., Jone Stanley, A., Srinivasan, N., Balasubramanian, K., Aruldhas, M.M., Arunakaran,

J. Antioxidant effect of ascorbic acid on PCB (Aroclor 1254) induced oxidative stress in hypothalamus of albino rats. Clin. Chim. Acta 2006; 365: 297–303.

Netto, M., Martins-Ferreira, H. Elicitation of spreading depression by rose bengal photodynamic action. Photochem. Photobiol. 1989;50: 229-234.

Niki, E.; Noguchi, N.; Tsuchihashi, H.; Gotoh, N. Interaction among vitamin C, vitamin E, and β-carotene. Am. J. Clin. Nutr. 1995;62:1322S–1326S.

Nishikimi M.R.; Fukuyama, S.; Minoshima, N.; Shimizu; Yagi, K. Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. J Biol Chem, 1994; 269(18):13685-13688.

Nwuga, V.C. Effect of severe kwashiorkor on intellectual development among Nigerian children. Am J Clin Nutr 1977;30(9):1423–30.

Odebode, T.O.; Odebode S.O. Protein Energy Malnutrition and the Nervous System: the Impact of Socioeconomic Condition, Weaning Practice,Infection and Food Intake, an Experience in Nigeria. Pakistan J.Nut. 2005;4 (5): 304-309.

Oliveira, M.S., Furian, A.F., Royes, L.F.F., Fighera, M.R., De Carvalho J., Fiorenza, N.G. Ascorbate modulates pentylenetetrazol-induced convulsions biphasically. Neuroscience 2004;128:721–728.

Paixão, A.D.O.; Trindade, A.S.; Dantas, A.C.; Barreto, I.S.S.; Vieira-Filho, L.D.; Medeiros M.C.; Teodósio N.R.; Guedes, R.C.A. Impact of two early malnutrition models on renal and neural functions in rats. In: Vesler LW (Ed) Malnutrition in the 21st Century [ISBN 978-1-60021-788-3] Nova Science Publishers, Inc., N.York, Chapter 13, 2007;239-263.

Parle, M.; Dhingra, D. Ascorbic acid: a promising memory-enhancer in mice. J. Pharmacol. Sci. 2003;93:129–135.

Pereira-da-Silva, M.S.; Cabral-Filho, J.E.; De-Oliveira, L.M. Effect of early malnutrition and environmental stimulation in the performance of rats in the elevated plus maze. Behav Brain Res. 2009; 205(1):286-289.

Picanço-Diniz, C.W., Borba, J.M.C., Araújo, M.S., Guedes, R.C.A. Nadph-diaphorase containing neurons and biocytin-labelled axon terminals in the visual cortex of adult rats malnourished during development. Nutr. Neurosci. 1998;1: 35-48.

Pinnel, S.R.; Murad, S.; Darr, D., Induction of collagen synthesis by ascorbic acid. A possible mechanism. Arch Dermatol 1987;23(12):1684-1686.

Ranade, S.C.; Rose, A.; Rao, M.; Gallego, J.; Gressens, P. Mani Different types of nutritional deficiencies affect different domains of spatial memory function checked in a radial arm maze Neurosc. 2008; 152(4): 859-866.

Rebec, G.V.; Pierce, R.C. A vitamin as neuromodulator: ascorbate release into the extracellular fluid of the brain regulates dopaminergic and glutamatergic transmission. Prog. Neurobiol. 1994;43:537–565.

Retsky, K.L.; Freeman, M.W.; Frei B. Ascorbic acid oxidation product(s) protect human low density lipoprotein against atherogenic modification. Anti-rather than prooxidant activity of vitamin C in the presence of transition metal ions, J Biol Chem 1993;268:1304–1309.

Rice, M.E., Ascorbate regulation and its neuroprotective role in the brain. Trends Neurosci. 2000;23: 209–216.

Richter, F.; Lehmenkühler, A.; Schaible, H.G. Voltage-gated calcium channels are not involved in generation and propagation of spreading depression (SD) in the brainstem of immature rats. Neurosci Lett. 2005;16;390(1):15-20.

Rocha-de-Melo, A.P.; Cavalcanti, J.B.; Barros, A.S.; Guedes, R.C.A. Manipulation of rat litter size during suckling influences cortical spreading depression after weaning and at adulthood. Nut. Neurosc. 2006; 9:155-160.

Rocha-de-Melo, A.P.; Guedes, R.C.A. Spreading depression is facilitated in adult rats previously submitted to short episodes of malnutrition within the lactation period. Braz. J. Med. Biol. Res. 1997;30: 663-670.

Sakagami, H.; Satoh, K. Modulating factors of radical intensity and cytotoxic activity of ascorbate (review). Anticancer Res. 1997 17(5A):3513-3520.

Santos, A.A., Pinheiro, P.C.F., Lima, D.S.C., Ozias, M.G., Oliveira, M.B., Guimaraes, N.X., Guedes, R.C.A. Fluoxetine inhibits cortical spreading depression in weaned and adult rats suckled under favorable and unfavorable lactation conditions. Exp. Neurol. 2006;200: 275-282.

Santos-Monteiro, J.S., Teodósio, N.R.T.; Guedes, R.C.A. Long-lasting effects of early environmental stimulation on cortical spreading depression in normal and early malnourished adult rats. Nutritional Neuroscience 2000; 3(1):29-40.

Schenk, J.O.; Miller, E.; Gaddis, R.; Adams, R.N. Homeostatic control as ascorbate concentration in the CNS extracellular fluid, Brain Res. 1982;253: 353-356.

Schweigert, I.D.; Souza, D.O.G.; Perry, M.L.S. Malnutrition, central nervous system maturation and neuropsychiatric diseases. Rev. Nutr. Campinas 2009: 22(2) 271-281.

Seregi, A.; Schaefer, A.; Komlós, M. Protective role of brain ascorbic acid content against lipid peroxidation. Experientia 1978;34:1056–1057.

Shahidi, S.; Komaki, A.; Mahmoodi, M.; Atrvash, N.; Ghodrati, M. Ascorbic acid supplementation could affect passive avoidance learning and memory in rat. Brain Res. Bull. 2008;76:109–113.

Shin, D.M.; Ahn, J.I.; Lee, K.H.; Lee, Y.S.. Ascorbic acid responsive genes during neuronal differentiation of embryonic stem cells. Neuroreport 2004;15:1959–1963.

Siesjö, B.K., Bengtsson, F. Calcium fluxes, calcium antagonists and calcium-related pathology in brain ischemia, hypoglycemia and spreading depression: a unifying hypothesis. J. Cereb. Blood Flow Metabol. 1989;9:127–140.

Song J.H., Shin S.H., Ross G.M., Oxidative stress induced by ascorbate causes neuronal damage in an in vitro system, Brain Res. 2001;895: 66–72.

Strupp, B.J., Levitsky, D.A. Enduring cognitive effects of early malnutrition: a theoretical reappraisal. J Nutr. 1995;125(8):2221S-2232S.

Susser E.; Neugebauer, R.; Hoek, H.W.; Brown, A.S.; Lin, S.; Labovitz, D. et al. Schizophrenia after prenatal famine. Further evidence. Arch Gen Psychiatry. 1996; 53(1):25-31.

Szent-Gyorgy, A. Observations on the function of peroxidase systems and the chemistry of the adrenal cortex: description of a new carbohydrate derivative, Biochem. J. 1928;22: 1387- 1409.

Tapiero H., Townsend, D.M., Tew, K.D. The role of carotenoids in the prevention of human pathologies. Biomed. Pharmacother. 2004;58: 100–110.

Tenorio, A.S.; Oliveira, I.D.V.A.; Guedes, R.C.A. Early vibrissae removal facilitates cortical spreading depression propagation in the brain of well-nourished and malnourished developing rats. Int. J. Develop. Neurosc. 2009;27:431-437.

Teodósio, N. R.; Lago, E.S.; Romani, S.A.M.; Guedes, R.C.A. A Regional Basic Diet From Northeast Brazil As A Dietary Model Of Experimental Malnutrition. Archivos Latinoamericanos de Nutrición. 1990; 40(4): 533-547

Udani, P.M. Protein energy malnutrition (PEM), brain and various facets of child development. Indian J. Ped. 1992; 59(2): 165-186.

Valadares, C.T.; Almeida, S. Early protein malnutrition changes learning and memory in spaced but not in condensed trials in the Morris water-maze. Nutr Neurosc. 2005;8(1):39-47.

Vasconcelos, C.A.C.; Oliveira, J.A.F.; Costa, L.A.O.; Guedes, R.C.A. Malnutrition and REM-sleep deprivation modulate in rats the impairment of spreading depression by a single sub-convulsing dose of pilocarpine. Nut. Neurosc. 2004; 7(3):163-170.

Vieira-Filho, L.D.; Lara, L.S.; Silva, P.A.; Luzardo, R.; Einicker-Lamas, M.; Cardoso, H.D.; Paixão, A.D.; Vieyra, A. Placental oxidative stress in malnourished rats and changes in kidney proximal tubule sodium ATPases in offspring. Clin Exp Pharmacol Physiol. 2009;36(12):1157-1163.

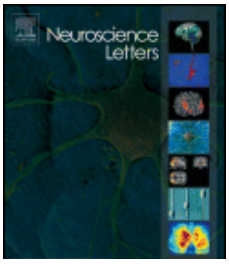
Winick, M., Brasel, J.A., Rosso, P. Nutrition and cell growth. Nutr. Devel. 1972: 49-98.

Ximenes-da-Silva, A., Guedes, R.C.A. Differential effect of changes in blood glucose levels on the velocity of propagation of cortical spreading depression in normal and malnourished rats. Braz. J. Med. Biol. Res. 1991;24: 1277-1281.

Ximenes-da-Silva, A.; Guedes, R.C.A. Differential Effect Of Changes In Blood Glucose Levels On The Velocity Of Propagation Of Cortical Spreading Depression In Normal And Malnourished Rats.. Braz. J. Med.Biol. Res. 1991; 24(12): 1277-1281.

Young, A.J., Lowe, G.M. Antioxidant and prooxidant properties of carotenoids. Arch Biochem Biophys. 2001;385(1):20-27.

Zalani, S., Rajalakshmi, R., Parekh, L.J. Ascorbic acid concentration of human fetal tissues in relation to fetal size and gestational age. Br. J. Nutr. 1989;61: 601-606.



ANEXO 01: Guia para autores

Neuroscience Letters

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Article structure

Introduction
Material and methods
Results
Discussion
Conclusions

Essential title page information

Title.
Author names and affiliations.
Corresponding author.
Present/permanent address.

Abstract

Keywords

Abbreviations

Acknowledgements

References

Examples:
Reference to a journal publication:
[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2000) 51–59.
Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, third ed., Macmillan, New York, 1979.
Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 1999, pp. 281–304.

ANEXO 02: Parecer do Comitê de ética em pesquisa

Universidade Federal de Pernambuco
Centro de Ciências Biológicas

Av. Prof. Nelson Chaves, s/n
50670-420 / Recife - PE - Brasil
fones: (55 81) 2126 8840 | 2126 8351
fax: (55 81) 2126 8350
www.ccb.ufpe.br

Recife, 17 de agosto de 2009.

Ofício nº 190/09

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: **Prof°: Rubem Carlos Araújo Guedes**
Departamento de Nutrição- CCS
Universidade Federal de Pernambuco
Processo nº 23076. 003614/2009-10

Os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) avaliaram seu projeto de pesquisa intitulado ***"Vitamina C e desenvolvimento cerebral: influencia Da suplementação de ácido ascórbico sobre a depressão alastrante cortical em ratos jovens em distintos estado nutricionais".***

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEEA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 9.605 – art. 32 e Decreto 3.179-art 17, de 21/09/1999, que trata da questão do uso de animais para fins científicos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais realizados.

Atenciosamente,

Observação:Mestranda Cinthia Karla Rodrigues Vasconcelos
Origem dos animais: Biotério do Departamento de Nutrição;
Animais: Ratos Wistar; Sexo: Machos e Fêmeas; Idade: 7 - 40 dias; Nº de Animais: 60

Profa. Maria Teresa Jansem
Presidente do CEEA
UFPE

CCB: Integrar para desenvolver

ANEXO 03: Comprovante de submissão do artigo

From: "Neuroscience Letters" nsl@elsevier.com
To: rc.guedes@terra.com.br
Sent: Qua 12/01/11 19:35
Subject: Neuroscience Letters Submission Confirmation

Dear Professor Guedes,

Your submission entitled "Chronic treatment with ascorbic acid enhances cortical spreading depression in developing well-nourished and malnourished rats." has been received for consideration in Neuroscience Letters.

You will be able to check on the progress of your manuscript by logging on to the Elsevier Editorial System as an author:
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Your username is: Rubem Guedes
If you need to retrieve password details, please go to:
http://ees.elsevier.com/nsl/automail_query.asp.

Your paper will be given a manuscript number shortly and you will then receive an e-mail with this number for your reference.

Thank you for submitting your manuscript to Neuroscience Letters. Should you have any questions, please feel free to contact our office.

Kind regards,
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Email: nsl@elsevier.com