RITA DE CÁSSIA FARIAS SANTOS

EXPOSIÇÃO À HIPERTERMIA AMBIENTAL DE RATOS EM DESENVOLVIMENTO BEM NUTRIDOS E DESNUTRIDOS FACILITA A DEPRESSÃO ALASTRANTE CORTICAL NA IDADE ADULTA

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Dissertação apresentada ao Departamento de Bioquímica do Centro de Ciências Biológicas da Universidade Federal de Pernambuco para obtenção do grau de Mestre em Bioquímica e Fisiologia, Área de concentração em Neurofisiologia.
Orientador: Prof. Dr. Rubem Carlos Araújo Guedes

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Rita de Cássia Farias Santos

"Exposição à Hipertermia Ambiental de Ratos em Desenvolvimento Bem Nutridos e Desnutridos Facilita a Depressão Alastrante Cortical na Idade Adulta"

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

Aprovado por:

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DEDICATÓRIA

Aos meus pais, José dos Santos (*in memoriam*) e Margarida Ferreira de Farias Santos, ao meu tio Graciliano Ferreira de Farias, à minha irmã Rizomar Farias Santos e aos meus sobrinhos Werter Andrade Leite Júnior e a Raissa Farias de Oliveira.

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RESUMO

A Depressão Alastrante Cortical (DAC) é uma resposta cerebral relacionada á excitabilidade neural e a doenças como epilepsia e enxaqueca. A exposição de organismos em desenvolvimento a diversas condições como a desnutrição e ambientes quentes, pode permanentemente alterar a excitabilidade neural, mudando as características eletrofisiológicas cerebrais que podem ser relevantes na gênese de doenças como a epilepsia. Neste trabalho, foram investigados os efeitos duradouros, sobre a susceptibilidade à DAC, da exposição de ratos normonutridos (mães alimentadas no aleitamento com a dieta comercial do biotério, com 23% de proteína) e desnutridos (mães alimentadas com a "dieta básica regional", com 8% de proteínas) no período de desenvolvimento cerebral (10º ao 29° dia posnatal) a 15 sessões diárias (5 sessões por semana durante 3 semanas) a um ambiente quente (40±2°C). Na idade de 30-40d e 90-120d de vida (jovens e adultos respectivamente), eles foram anestesiados (uretana+cloralose; 1,000+40mg/kg ip) e o EcoG, bem como a variação lenta de voltagem que acompanha a DAC foram registrados em 2 pontos parietais por 4 horas. Comparado aos controles (mantidos à temperatura ambiente), os ratos expostos ao aquecimento apresentaram velocidades de propagação da DAC mais altas ($P<0,05$) em ambas as idades de registro e em ambas as condições nutricionais. As média±dp das velocidades da DAC (em mm/min) foram: para os ratos bem nutridos controles e submetidos ao aquecimento, respectivamente 3,75±0,15 e 4,17±0,19 (grupo jovem), e 3,33±0,06 e 3,88±0,26 (adultos); para as mesmas condições nos ratos desnutridos, 4,30±0,22 e 5,31±0,46 (jovens), e 4,18±0,20 e 4,88±0,35 (adultos). Em contraste à desnutrição precoce, e exposição ao ambiente quente não afetou os pesos corporais e cerebrais. Conclui-se que a exposição ao aquecimento durante o desenvolvimento cerebral aumenta de forma duradoura a susceptibilidade à DAC e este efeito não é modificado pela desnutrição precoce.

Palavras-chave: desenvolvimento neural, desnutrição, exposição ao calor, ativação térmica sensorial, depressão alastrante cortical

ABSTRACT

Cortical spreading depression (CSD) is a brain response related to neural excitability and to diseases like migraine and epilepsy. Exposure of developing organisms to adverse conditions like malnutrition and a warm environment can permanently alter the neural excitability, changing brain electrophysiological features, which may be relevant to epilepsy genesis. Here we investigated the lasting effects, on brain CSD susceptibility, of exposing well-nourished and malnourished developing rats (from postnatal day 10 to 29) to 15 daily sessions (5 sessions per week during 3 weeks) of a warm environment (40±2°C). At 30-40d and 90-120d of life (young and adult age, respectively), they were anesthetized (urethane+chloralose; 1,000+40mg/kg ip) and the ECoG plus slow potential change accompanying CSD were recorded on 2 parietal points for 4h. Compared to controls (maintained on the normal environment temperature), hyperthermia-treated rats displayed higher CSD-velocities ($P<0.05$) at both CSD-recording ages and nutritional statuses. The mean±sd CSD-velocities (in mm/min) were: for control- and hyperthermia well-nourished rats, 3.75±0.15 and 4.17±0.19 (young groups), and 3.33±0.06 and 3.88±0.26 (adult); for the same conditions in the malnourished rats, 4.30±0.22 and 5.31±0.46 (young), and 4.18±0.20 and 4.88±0.35 (adult). In contrast to early malnutrition, hyperthermia treatment did not affect body- and brain weights. It is concluded that early hyperthermia treatment long lastingly increased brain CSD-susceptibility and this effect is not modified by early malnutrition.

Keywords: Neural development, malnutrition, heat exposure, sensory thermal activation, cortical spreading depression

SUMÁRIO

Pág.

I. INTRODUÇÃO

I.1- Estimulação Ambiental e Função Neural

A hipertermia corpórea durante o desenvolvimento cerebral pode afetar diversos mecanismos celulares tanto em humanos como em animais. Em mamíferos, a estabilidade da temperatura corpórea em torno de 37ºC é regulada pelo sistema integrador hipotalâmico. A manutenção aproximada desse ponto de ajuste é importante para garantir uma perfeita execução das reações enzimáticas (Lent, 2002). Quando a temperatura corpórea tende a se elevar acima de 37ºC, o hipotálamo aciona de forma mais efetiva sua capacidade termorreguladora, tentando evitar a hipertermia. O organismo pode sofrer intermação, alterando os processos fisiológicos (Guyton e Hall, 1997). Em humanos, a hipertermia ambiental durante o desenvolvimento neural tem sido implicada na gênese de alterações do sistema nervoso central (SNC; Edwards et al., 1995).

Durante o desenvolvimento e maturação neural de mamíferos, a estimulação ambiental pode influenciar o desenvolvimento e a função cerebral, e tais efeitos são em parte devidos à ativação das várias vias sensoriais que ocorrem durante a estimulação (ver Renner & Rosenzweig, 1987, para uma revisão).

Tem sido demonstrado que a estimulação sensorial ajuda organismos em desenvolvimento na recuperação de condições deletérias para o desenvolvimento do cérebro e do comportamento, como por exemplo, a desnutrição (Carughi et al., 1989; Grantham McGregor et al., 1991) e doenças neurológicas (Fraser et al., 2002; Chen et al., 2005).

Dentre os efeitos da estimulação ambiental térmica excessiva sobre o sistema nervoso central, o delírio, a convulsão e o coma são relevantes (ver Bouchama & Knochel, 2002; Sharma, 2005a, b). Uma convulsão febril é definida como uma convulsão que ocorre na infância após a idade de 1 mês, associada com uma doença febril na ausência de uma infecção do SNC e sem nenhuma convulsão febril prévia (Ilae, 1993).

Em humanos, a convulsão febril constitui um dos distúrbios neurais de maior prevalência durante a infância afetando aproximadamente 3-5% das crianças em todo o mundo, sendo comum na idade entre 6 meses e 6 anos (Kolfen et al., 1998; Shinnar & Glauser, 2002; Dubé et al., 2007; Chen et al., 1999; Herrgard et al., 2006; Dube et al., 2000; Verity et al., 1985**;** Hauser, 1994). O prognóstico é geralmente muito bom; uma simples convulsão febril leva somente a pouco risco de desenvolvimento de epilepsia (Verity e Golding, 1991). Contudo, se prolongadas e/ou repetidas, as convulsões podem alterar os circuitos hipocampais levando a mudanças moleculares e estruturais que promovem uma rede neuronal hiperexcitável (hiperexcitabilidade hipocampal). Assim, podem reduzir o limiar para convulsões subsequentes e contribuir para ocasionar disfunções neurológicas, como a paralisia cerebral e a epilepsia (Chen et al., 1999; Stokes, 2000; Wu et al., 2000; Dube et al., 2000; Bender et al., 2003; Chen et al., 1999; Hesdorffer & Hauser, 2002; Stokes, 2000; Trinka et al., 2002).

Muitos estudos têm reconhecido a associação entre convulsões febris complexas e um risco subseqüente aumentado de epilepsia do lobo temporal (ELT) (Trinka et al., 2002**;** Abou-Khalil et al., 1993; French et al., 1993; Cendes et al., 1995; Mathern et al., 1995)**.** Esta é definida como uma síndrome caracterizada principalmente por mudanças patológicas envolvendo o hipocampo (Engel, 2001). Porém, ainda não se tem claro se estas convulsões provocam epilepsia de início tardio, como tem sido suspeitado, em humanos (Dubé, 2006).

O aquecimento de animais com banhos de água ou ar quente pode levar a convulsões febris com comportamentos e características do EEG que relembram convulsões febris clínicas com efeitos a longo prazo sobre a excitabilidade neural (Chen et al., 1999).

Em ratos, um estudo com a técnica de “patch clamp” demonstrou que convulsões febris induzidas no aleitamento, provocam um aumento pré-sináptico seletivo na transmissão sináptica inibitória na idade adulta (Chen et al., 1999). De acordo com Sarkisian et al. (1999), roedores jovens e adultos submetidos à elevação da temperatura corpórea para 40º durante 45 minutos, associada à estimulação hipocampal contínua mostraram-se resistentes a danos cerebrais. As

convulsões hipertérmicas podem favorecer um aumento da excitabilidade límbica e da permeabilidade cerebrovascular (Ilbay et al., 2003; Dube et al., 2000). Em cobaias recém nascidas, foi observada uma diminuição do peso encefálico em conseqüência da exposição à hipertermia ambiental (Edwards, 1969; Edwards et al., 1971; Upfold et al., 1989).

I.2- Desnutrição e Maturação Neural

Durante o desenvolvimento, o cérebro está mais susceptível a sofrer alterações ambientais e nutricionais (Dobbing, 1968a,b; Guedes, 1984). A deficiência nutricional precoce pode alterar o desenvolvimento cerebral, podendo prejudicar a organização e as funções dos órgãos e tecidos neurais em animais e humanos (Morgane et al., 1978; Barker, 1997). É bem conhecido que a desnutrição é mais severa quando ocorre no período crítico de desenvolvimento, sendo que alguns dos efeitos podem se tornar permanentes (Hack et al., 1991; Borba et al., 2000; Rocha-de-Melo et al., 2004).

No rato, este período corresponde às primeiras semanas da vida pós-natal; por exemplo, o período de lactação é o tempo em que o cérebro apresenta a sua máxima vulnerabilidade a muitos tipos de injúrias, incluindo a desnutrição (Dobbing et al., 1971). Durante este período, o cérebro está em máximo desenvolvimento, seu peso aumenta com a maior velocidade. Uma dieta materna inadequada, imposta às mães durante este período inteiro, pode causar deficiência nutricional nos filhotes e os efeitos de tal condição nutricional, sobre o sistema nervoso central, dependerá da severidade e duração da desnutrição (Morgane et al., 1978). Relatos sobre a desnutrição associada a alterações duradouras do SNC incluem distúrbios morfológicos, bioquímicos, fisiológicos, e comportamentais (Resnick et al., 1979; Borba et al., 2000; Maia et al., 2006). Em ratos, a desnutrição precoce pode causar, dentre outros danos, retardo do desenvolvimento locomotor, redução do peso encefálico e do número de sinapses por neurônio (Morgane et al, 1978). Em

crianças, a desnutrição retarda o crescimento e o desenvolvimento, prejudica a concentração e a capacidade de aprendizado (Dastur et al., 1977; Monckeberg, 1988).

Evidências experimentais indicam que animais desnutridos apresentam elevada susceptibilidade a processos relacionados à excitabilidade neural, tais como reatividade aumentada a estímulos aversivos e facilitação para se obter crises convulsivas induzidas experimentalmente. O estudo da excitabilidade cerebral pode ser feito registrando-se e analisando-se a atividade elétrica produzida pelo cérebro. Este, enquanto está vivo, produz espontaneamente (isto é, sem qualquer estímulo intencional aplicado pelo pesquisador) um padrão de ondas elétricas de caráter oscilatório, que constitui o que se chama eletroencefalograma (abreviadamente, EEG), (Guedes et al, 2004).

Dados atuais sobre ratos em desenvolvimento têm indicado que a temperatura ambiental pode influenciar o desenvolvimento da morfologia corporal e o comportamento de preferência térmica (Vilarreal et al., 2007). A estimulação termal periférica pode modificar a excitabilidade no SNC (Liebregts et al., 2002). A implicação é que a estimulação periférica de uma única via sensorial, tal como a que conduz a informação da temperatura ambiental ao cérebro, poderia fornecer informações valiosas sobre a participação dessa via nos efeitos dependentes da excitabilidade mencionados acima.

Neste trabalho, foi testada esta possibilidade pelo estudo, em ratos previamente submetidos a repetidos episódios de hipertermia ambiental, do fenômeno relacionado à excitabilidade neural conhecido como depressão alastrante cortical (DAC).

I.4- Características da Depressão Alastrante Cortical

Esse fenômeno foi primeiro descrito por Aristides Azevedo Pacheco Leão (Leão, 1944). A DAC é caracterizada por alterações maciças na homeostase iônica cerebrocortical em resposta à estimulação elétrica, química ou mecânica de um ponto sobre a superfície cortical (Martins-Ferreira et al. 2000; Somjen, 2001). Tais alterações iônicas resultam em uma "onda" de despolarização neural que se propaga com uma velocidade de 2–5 $mm.min^{-1,}$ através da superfície cortical, acompanhada por supressão reversível da atividade neuronal espontânea e evocada que se propaga lentamente através da superfície cortical (Teive et al., 2005). A DAC se propaga de forma concêntrica a partir do ponto estimulado. Alterações nas velocidades de propagação são interpretadas como indicação de que o tecido cortical está alterado, seja na sua estrutura, seja na sua atividade eletrofisiológica. Velocidades significantemente mais altas ou mais baixas do que aquela de animais normais (grupo controle) indicam, respectivamente, susceptibilidade do tecido cortical à DAC aumentada ou diminuída, sugerindo as variações correspondentes na excitabilidade cortical Guedes et al., 2004).

A recuperação do córtex deprimido pela DAC começa do ponto inicialmente estimulado e leva em torno de 5 a 10 minutos para se recuperar. Uma vez recuperado, o tecido é novamente capaz para gerar uma outra DAC, o que indica que é um fenômeno totalmente reversível. Simultaneamente à depressão do eletrocorticograma, tem sido também descrito o surgimento de uma variação lenta de voltagem da superfície cortical (Leão, 1947), bem como translocações de água e ions entre os espaços extra- e intra-celular (Kraig e Nicholson, 1978; Phillips e Nicholson, 1979). Em trabalhos anteriores foi observado que as DACs provocadas por acetato de sódio apareciam primeiro em ratos no período de aleitamento (12-15 dias de idade) (Richter et al., 1998). A DAC pode ocorrer em ratos adultos, mas a sua velocidade de propagação declina em função da idade, conforme testado em ratos na faixa de 2.5 a 24 meses (Guedes et al., 1996).

A respeito dos mecanismos responsáveis pela geração e propagação deste interessante

fenômeno, discussões atuais frequentemente mencionam o papel da atividade de neurotransmissores, em alguns casos facilitando a DAC (Guedes et al., 1992), e em outros casos dificultando-a (Gorelova et al., 1987; Guedes et al., 1987; Guedes et al., 1988).

Não há estudos *in vivo* disponíveis entre a relação: estimulação sensorial térmica e DAC. Contudo, um relato tem documentado evidências experimentais *in vitro* (fatias hipocampais de animais adultos) de efeitos da DAC produzidos pelo aquecimento da preparação. Nessas condições, a DAC ocorria quando a temperatura era aumentada de 34° para 40°C (Wu & Fischer, 2000). Estes novos dados são considerados interessantes tendo-se em vista uma condição patológica humana que pode afetar o cérebro em desenvolvimento de crianças e é conhecida como convulsão febril (Chen et al., 1999; Hesdorffer et al., 2002; Knudsen, 1996; Shinar e Glauser, 2002; Trinka et al., 2002).

A deficiência nutricional no início da vida, que pode alterar a exicitabilidade neural (Morgane et al., 1978; Palencia et al., 1996), pode também afetar a incidência e a propagação da DAC (De Luca et al., 1977; Rocha-de-Melo et al., 2006). O cálculo da velocidade de propagação deste fenômeno é relevante para a sua interpretação. No que se refere à desnutrição precoce, é conhecido que ela exerce um efeito facilitador sobre a propagação da DAC, a julgar pelas suas velocidades de propagação, mais altas nos animais adultos que foram precocemente desnutridos, em comparação com animais controle, bem-nutridos durante toda a vida (Guedes, 2005).

A suplementação, com proteínas, de uma dieta na qual esse nutriente era deficiente, tanto em quantidade quanto em qualidade, levou a resultados diversos, dependendo da qualidade da proteína usada na suplementação. Quando se suplementou a dieta carente com uma proteína de baixa qualidade (de origem vegetal), os efeitos sobre a DAC não foram revertidos. A reversão só foi conseguida quando a proteína usada na suplementação era a caseína, a proteína animal de excelência para os mamíferos (Andrade et al., 1990). Com base nessas observações pode-se concluir que os efeitos da desnutrição no início da vida sobre o desenvolvimento e as funções cerebrais não podem ser completamente evitados, se a alimentação deficiente for suplementada

apenas com proteínas de baixo valor biológico, isto é, de baixa qualidade, definida pela falta de alguns aminoácidos essenciais.

No rato, descobriu-se que mesmo episódios curtos de desnutrição (apenas uma das semanas do aleitamento) são capazes de alterar, de forma duradoura, a susceptibilidade cortical à DAC. O maior impacto ocorre quando esse episódio curto de desnutrição acontece na terceira semana do aleitamento, sugerindo que os eventos de desenvolvimento cerebral que ocorrem nessa semana têm grande importância para o estabelecimento das características da DAC, no cérebro adulto (Rocha-de-Melo e Guedes, 1997). Esses resultados indicam também que o cérebro não parece ser tão homogêneo quanto inicialmente se pensava, em termos de desenvolvimento, uma vez que diferentes estruturas cerebrais desenvolvem-se em sub-períodos diversos durante o aleitamento, de forma que mesmo episódios curtos de desnutrição podem ter conseqüências funcionais importantes, conforme a fase do desenvolvimento em que ocorram e a estrutura cerebral que afetem.

A desnutrição continua sendo um sério problema de saúde pública, principalmente (mas não exclusivamente) em países em desenvolvimento, apesar da diminuição global da prevalência da desnutrição grave, nas últimas décadas.

Apesar dos múltiplos estudos a respeito das possíveis causas da DAC, os mecanismos que determinam a sua deflagração e propagação ainda aguardam por um esclarecimento definitivo. Nesse contexto, a exposição de organismos em desenvolvimento a variações da temperatura ambiental, como um fator que pode influenciar a ocorrência da DAC, não tem sido estudada de forma sistemática e constitui um tema relevante. Do mesmo modo, também não há relatos de investigações sobre a relação entre nutrição, estimulação ambiental térmica e DAC. O presente trabalho teve como objetivo investigar esta relação.

Do acima exposto, foram formuladas as seguintes hipóteses:

(1) Durante o desenvolvimento cerebral, a estimulação ambiental térmica diária seria transmitida ao cérebro por meio das vias sensoriais que conduzem informações sobre a temperatura do ambiente e influenciariam a susceptibilidade cortical à DAC;

(2) Esse efeito sobre a DAC seria de longa duração;

(3) Tal efeito seria influenciado pela deficiência nutricional provocada no início da vida.

II. OBJETIVOS

II.1. Objetivo Geral

Estudar o fenômeno da DAC no córtex cerebral de ratos adultos previamente submetidos, no aleitamento, a repetidos episódios de hipertermia ambiental associada à desnutrição.

II.2. Objetivos Específicos

1- Analisar o desenvolvimento dos filhotes por meio da evolução dos seus pesos corporais (do aleitamento até a idade adulta) e encefálicos (na idade adulta).

2- Avaliar a susceptibilidade cortical à DAC, quando os filhotes se tornarem adultos, por meio da sua velocidade de propagação.

III. ARTIGO SUBMETIDO À REVISTA "NEUROSCIENCE LETTERS"

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Title:

Exposure of developing well-nourished and malnourished rats to environmental hyperthermia facilitates cortical spreading depression at adulthood

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Abstract

Cortical spreading depression (CSD) is a brain electrical response related to neural activity and to diseases like migraine and epilepsy. Adverse conditions like malnutrition and a warm environment exposure during brain development can permanently alter the neural excitability, changing electrophysiological features of the brain responses. Here we investigated the lasting effects of heat exposure on brain CSD susceptibility in well-nourished and malnourished developing rats. From postnatal day 10 to 29, rats were exposed to daily sessions (one session per day, 5 sessions per week during 3 weeks; total of 15 sessions) of a warm environment (40±2°C). At 30-40d and 90-120d of life (young and adult ages, respectively), they were anesthetized (urethane+chloralose; 1,000+40mg/kg ip) and the electrocorticogram plus the slow potential change accompanying CSD were recorded on 2 parietal points for 4h. Compared to controls (maintained on the normal environment temperature, 23±2°C), heat-exposed rats displayed higher CSD-velocities ($P<0.05$; ANOVA plus Tukey test) at both CSD-recording ages and nutritional statuses. The mean±sd CSD-velocities (in mm/min) were: for control- and heated well-nourished rats, 3.75±0.15 and 4.17±0.19 (young groups), and 3.33±0.06 and 3.88±0.26 (adult); for the same conditions in the malnourished rats, 4.30±0.22 and 5.31±0.46 (young), and 4.18±0.20 and 4.88±0.35 (adult). In contrast to early malnutrition, heat-exposure did not affect body- and brain weights. It is concluded that early heat exposure treatment long lastingly increased brain CSD-susceptibility and this effect is not modified by early malnutrition.

O que foi usado?

Keywords: Neural development, malnutrition, heat exposure, sensory thermal activation, cortical spreading depression.

Introduction

During the mammalian neural development and maturation, environmental stimulation can influence brain development and function, and such effect is at least in part due to the activation of the various sensory pathways, which occurs during environmental stimulation (see [43], for a review). Sensory stimulation has been shown to help in recovering developing organisms suffering from brain- and behavior-impairing conditions as, for example, malnutrition [3, 15] and neurological diseases [5, 11].

Recent data on developing rats have indicated that environmental temperature can influence the development of body morphology and thermal preference behavior [50]. Peripheral thermal stimulation may modulate neural excitability [35]. The implication is that peripheral stimulation of a single sensory pathway, such as that carrying the environmental temperature information to the developing brain, could provide valuable cues on the participation of this pathway in the above-mentioned excitability-dependent effects. We have presently tested this possibility in rats previously submitted to repeated episodes of heat exposure, by studying the neural activity-related phenomenon known as cortical spreading depression (CSD).

CSD has been characterized as a reversible “wave” of suppression of the evoked and spontaneous neuronal activity that slowly propagates across the cortical surface in response to electrical, mechanical or chemical stimulation of one point on brain tissue [31]. The recovery of CSD-depressed cortex starts from the initially stimulated point, and lasts about 5 to 10 minutes. Once recovered, the tissue is again capable to generate another CSD, what indicates that it is a fully reversible phenomenon. A slow negative DC-potential change of the cortical surface [32], as well as water and ion translocations [26, 41], have been reported to occur simultaneously to the ECoG-depression.

Nutritional deficiencies early-in-life, which can alter neural excitability [37, 39], can also affect the CSD incidence and propagation [8, 44]. Malnutrition still continues being a serious

public health problem, mainly (but not exclusively) in developing countries, despite the overall decrease of malnutrition prevalence in the last decades.

Concerning the relationship between sensory stimulation and CSD, no *in vivo* studies on the effects of environmental thermal stimulation are available. However, a report has documented *in vitro* experimental evidence of CSD-effects produced by hyperthermia [52]. These novel data are considered interesting in view of a human pathological condition, which can affect the children's developing brain, and is known as febrile seizure [4, 23, 24, 46, 48]. In humans, the febrile convulsion constitutes a highly prevalent neural disorder during infancy, affecting approximately 3-5% of children in the whole world, being common in the age-range of 6 months to 6 years [4, 9, 22, 25, 46]. The prognosis is generally very good, but if prolonged, it can cause neurological disturbances, as the cerebral paralysis and the epilepsy [4, 23, 48]

From the above, it was here hypothesized that (1) during brain development, daily heat-stimulation of the peripheral sensory pathway that carries information on environment temperature would alter the cortical CSD susceptibility, (2) this effect would be long lasting, and (3) it could be influenced by early malnutrition. An abstract with some of the present results has appeared [10].

Materials and methods

Experimental animals and Diets

Developing Wistar rats (n=82) from the colony of Department of Nutrition of Universidade Federal de Pernambuco (UFPE), Brazil, were employed in these experiments. The litters were formed by pooling 24-h old newborn rats from several dams and thereafter distributing them randomly to form litters with 6 pups per lactating dam and assigned to two nutritional groups according to the mother's dietary conditions: (a) Well-nourished group (W, n=39), suckled by dams fed a commercial laboratory chow diet (Purina do Brazil Ltd) containing 23% protein, and (b) Malnourished group (M, n=43), suckled by dams fed a deficient diet with only 8 % protein

called “regional basic diet” (RBD) of low-income human populations of Northeastern Brazil [47]. The RBD has been largely employed in previous CNS-studies as an experimental model of malnutrition (see [42]). After weaning (21days), the pups were housed in groups of 4-5 per cage (51 x 35.5 x 18.5 cm), and kept on the control diet until the day of the electrophysiological recording (30-40 days for the young groups or 90-120 days for the adult groups). Housing conditions included controlled temperature (23±2°C) and a standard 12/12h light/dark cycle (lights on at 7:00 am). All experiments were carried out in accordance with the “Principles of Laboratory Animal Care” (National Institutes of Health, Bethesda, USA) and were approved by the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco, Brazil.

Exposure to a warm environment

Starting on postnatal day 10 and finishing on day 29, half of each litter was submitted to daily sessions of environmental heating (EH; one session per day, 5 days per week, during 3 weeks; total of 15 sessions). During such sessions, the pups were placed in a 18x13x11 cm plastic box, which remained in a warm environment (a water-heater apparatus at 40±2°C), for 15, 20 and 30 min in the 1^{st} , 2^{nd} and 3^{rd} week respectively, between 12 am and 15 pm of each day. The floor of the plastic box was covered with a 1cm-thick layer of soft-paper strips to avoid the direct contact of the animals with the plastic floor during the heating session. The lateral walls of the plastic box had several holes to allow the air exchange between the inner environment of the box and the warm-room atmosphere of the water-heater apparatus. The control group was formed with the other half of each litter, which was submitted to the same conditions, but with the water-heater apparatus turned off (i.e., these control animals remained at the normal environment temperature of the testing room, 23±2°C).

CSD recording

When the pups were 30-40 days old (young groups) or 90-120 days (adult groups), they were intraperitoneally anesthetized with a mixture of 1g/kg urethane plus 40 mg/kg chloralose (both from Sigma Co., USA). This anesthetic mixture provides a very stable anesthesia, which lasts for several hours, being very convenient for CSD recording in acute experiments (in which the recovery of the animal from anesthesia is not required). It is also suitable for CSD-recording, because it does not block CSD, in contrast to other anesthetics, like Ketamine, which does block it [13]. The animal's head was secured in a stereotaxic apparatus and three trephine holes (2-4 mm diameter) were drilled in the right side of the skull. The holes were aligned in the anteroposterior direction and parallel to the midiline. One hole, drilled in the frontal bone, was used for KCl stimulation to elicit CSD. The other two holes, drilled on the parietal bone, were used to record the propagating CSDs, by means of Ag-AgCl agar-Ringer electrodes. Simultaneous recordings of the spontaneous cortical electrical activity (electrocorticogram; ECoG) and the slow potential change accompanying CSD were performed continuously for 4h, against a third electrode of the same type, placed on the nasal bones and used as a common reference electrode. Rectal temperature was continuously monitored and kept at 37 ± 1°C, by means of a heating blanket. CSD was elicited at 20min intervals by applying a cotton ball (1-2mm diameter) soaked with 2% KCl solution (approximately 0.28 M) to a point of the frontal cortical surface (dura mater intact) during 1 min. CSD propagation velocities were calculated based on the recording interelectrode distance and on the time spent for a CSD-wave to cross that distance. This time was measured using the beginning of the rising phase of the negative slow DC-potential change as the initial point.

Body and brain weight

Body weights were measured at postnatal days 10, 25, 45 and 90. At the end of the CSD recording session, the animal, while still anesthetized, was killed by lesioning the bulbar region with a sharp

needle, promptly provoking cardio-respiratory arrest. After that, its brain was immediately removed and weighed (wet-brain weight) and thereafter it was kept in a stove at 100°C and weighed each other day, until it reached a constant weight (dry-brain weight).

Statistical analysis

Body and brain weight-, as well as CSD velocity intergroup differences, were compared by using ANOVA followed by a post hoc (Tukey) test, where indicated. Differences were considered significant when $p \leq 0.05$.

Results

Body and brain weights

The two M-groups presented body- and brain weights significantly lower ($p < 0.05$) than those of the respective W-animals, indicating that the procedure used to induce malnutrition early in life was effective. In the same nutritional condition, weights were not influenced by the early heat exposure (Figure 1).

PLEASE INSERT FIGURE 1 ABOUT HERE

CSD propagation

The KCl stimulation at the frontal cortex for 1 min consistently elicited a single CSD wave, which was recorded at the two electrodes located on the parietal region of the stimulated hemisphere. The panels A and B of Figure 2 show electrophysiological recordings representative of W and M rats from the control and heated groups, both at the young (panel A) and adult (panel B) ages. When compared to the well-nourished groups, the corresponding previously malnourished animals displayed higher CSD velocities of propagation ($p<0.05$). In both nutritional conditions, CSD propagation in the cortical tissue of the groups submitted to environmental hyperthermia was

faster than in the corresponding controls submitted to the normal environment temperature. The mean±sd CSD velocities (in mm/min) in the well-nourished young groups were: 3.75±0.15 and 4.17±0.19, for the control- and heated groups, respectively; in the malnourished young groups, the corresponding velocities for the two temperature conditions were 4.30±0.22 (control temperature group) and 5.31±0.46 (previously heated group). In the well-nourished adult animals, the CSD velocities were 3.33±0.06 and 3.88±0.26 for the control- and heated groups, respectively, whereas in the malnourished adults the corresponding velocities were 4.18±0.20 and 4.88±0.35. This is shown in Figure 2, panels C and D, for the young and adult groups, respectively.

PLEASE INSERT FIGURE 2 ABOUT HERE

Discussion

The present results show that, like other conditions that change brain excitability, early environmental heat exposure also enhanced CSD propagation in adult rats. The daily exposure of developing rats to environmental heating resulted in a significant increase in the CSD velocity, both in the well-nourished and in the malnourished condition. Furthermore, the effects of peripheral thermal stimulation on CSD propagation lasted for at least 90 days, i.e., until adulthood. Importantly, these effects cannot be attributed neither to the confining environment (water-heater apparatus), nor to the anesthesia, as control animals that underwent thermal sham stimulation (i.e., that were put in the water-heater apparatus turned off) and the same anesthesia conditions did not show any change in the CSD propagation.

Various mechanisms could explain the present CSD changes. Repetitive peripheral thermal stimulation may have altered brain excitability as indexed by changes in CSD. CSD does provide a measure of cortical excitability. Environmental, pharmacological and nutritional manipulations, which influence brain development and excitability, have also been shown to modify CSD susceptibility [8, 16, 1, 7, 12]. For example sleep deprivation, which is known to enhance brain

excitability, can cause an increase in CSD propagation [49, 21]. Therefore, the findings of our study show that heat exposure might have increased cortical excitability.

Several lines of evidence additionally support the association of CSD to brain excitability, as for example the postulated relationship between CSD and excitability-related diseases like epilepsy [33, 19] and migraine [30]. Concerning to migraine, evidence includes similar ranges of propagation velocity of migraine aura and CSD [29, 40], as well as the demonstration, by TMS and magnetoencephalogram techniques, that migraine is associated with an increase in brain excitability [2, 27] and that migraine patients also respond to anticonvulsive therapy [45]. Furthermore, conditions that are capable to trigger migraine, like hypoglycemia, are also effective in increasing CSD propagation [7], and serotoninergic drugs that have anti-depressive action, like citalopram [17] and fluoxetine [1], or that are therapeutically employed against migraine, such as sumatriptan [36], counteract CSD. Therefore, it is tempting to speculate that peripheral stimulation by heat exposure can lead the developing brain to a state of increased excitability similar to the increase in brain excitability that is found in certain migraine cases.

Given that heat exposure effects on CSD might be due to an increase in cortical excitability, it is important to discuss the mechanisms underlying this increase in the cortical excitability. One possible explanation is that environmental heat exposure could have changed cortical synaptic effectiveness, and this could have increased CSD propagation by a kind of a potentiation effect. Such a potentiation effect has been demonstrated *in vivo,* in the frog optic tectum [20] and in the rat spinal cord [14], respectively, after induction of CSD, suggesting a CSD-associated facilitation of synaptic transmission. If one assumes that neuronal activity can be changed by peripheral heat stimulation, then it is reasonable to consider that this can ultimately alter glial cells. Of note, reactive glial changes have been reported, in association to the reorganization of synaptic functioning that constitutes a response to certain patterns of neuronal activity [51].

Another important aspect of the present study was the long-lasting effect of heat exposure on CSD propagation. The effect lasted until 90 days of life and may have lasted more if animals

had been followed for longer time. If any compensatory mechanism had been activated during this post-heat exposure period, it was not sufficient to decrease the CSD velocities to the control levels. This long-lasting effect of environmental heating may provide useful cues into the biological mechanisms to explain long lasting behavioral and clinical effects of temperature stimulation in febrile seizures [9, 22].

Nutritional status deficiencies early-in-life can be demonstrated by a very easily obtained indicator, the measurement of the body weight [37, 47]. In the present study, we could conclude that the maternal deficient diet (RBD) was effective in producing malnutrition in the pups, as judged by their reduced body weights, as compared to the well-nourished animals. When the nutritional deficiency occurs during the “brain growth spurt period”, malnutrition-induced body weight reduction usually is accompanied by decrease in brain weight [37]. In line with these authors, the present study also revealed a brain weight decrease in the malnourished animals, when compared with the corresponding well-nourished ones. Morphological studies indicate that such malnutrition-related brain weight reduction probably results from the reduced number and/or size of cell elements, as well as from alterations in the events that cause neuronal maturation. Processes like myelination, synapse formation and development of dendrites and of glial cells are reduced when the developing organism is affected by early-malnutrition [37, 38, 42]. We speculate that such nutrition-dependent developmental changes in brain structure could be involved, at least in part, in the presently demonstrated CSD effects of malnutrition.

Several authors have demonstrated that in the malnourished and in the overnourished rat, CSD propagation is respectively increased and decreased, in comparison to the normally nourished controls [8, 18, 44]. The facilitating action of malnutrition on CSD has been confirmed in the present study, as indexed by the CSD velocities in the control-temperature condition, which were higher in the malnourished group, as compared to the corresponding well-nourished one (see Figure 2, panels C and D). As commented above, early malnutrition also affects glial function and myelination [37]. It has been proposed that glia can play a role in promoting the interaction

between neurons and so, it could contribute to synaptic development, as well as to the CSD, whose most essential constituent would be a slowly propagating, regenerative event in the neuroglia compartment [34]. As previously postulated, glia-dependent physiological processes [28] and nutrition-dependent myelination alterations [8] could have played a role in the presently described CSD facilitation in the malnourished condition, as compared to the corresponding CSD values for the well-nourished animals.

In summary, our study showed that peripheral sensory stimulation through environmental heat exposure can facilitate CSD propagation and this effect is long lasting, if not permanent, and is not appreciably influenced by early malnutrition. Further physiopathological implications of such results remain to be clarified, but it is tempting to postulate that it may be important to support the clinical management of pathological conditions that might be triggered by heating stimulation such as what happens in febrile seizures in children.

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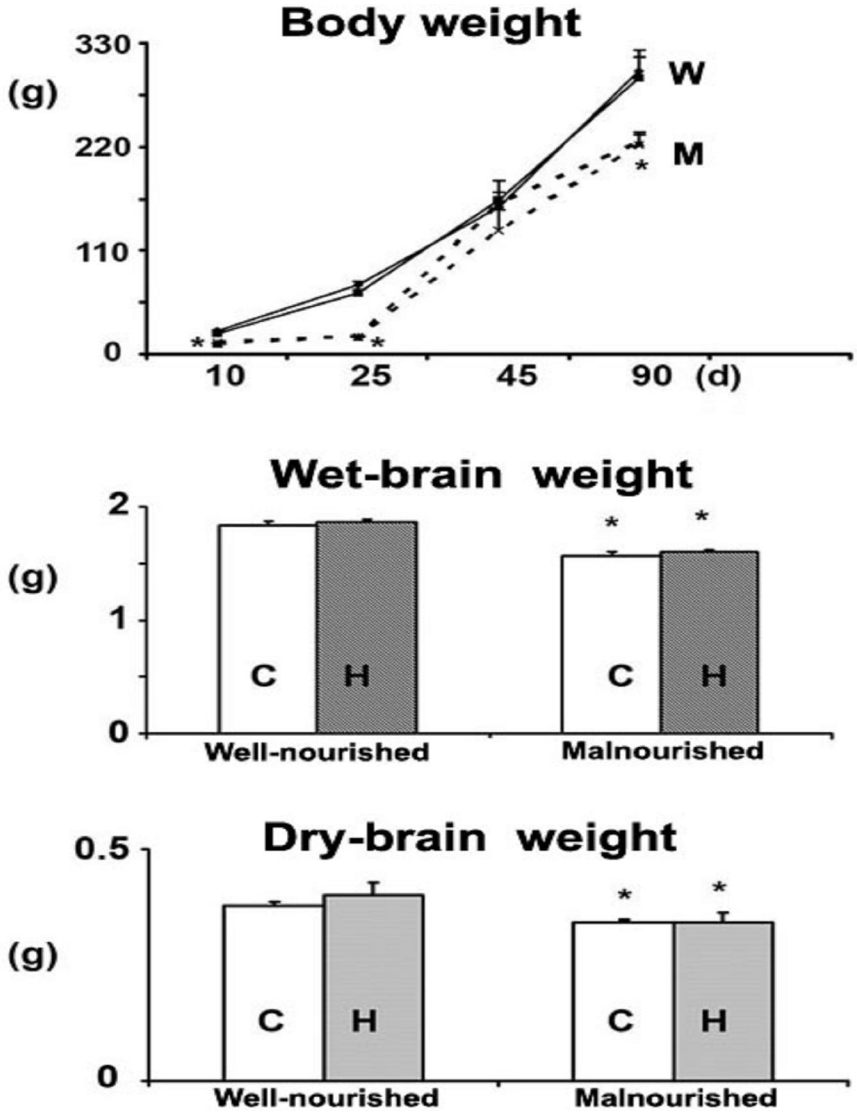

Figure 1 – Body and brain weights of well-nourished (W) and early-malnourished (M) rats submitted from postnatal day 10 to 29 to 15 episodes of heat exposure (H; 40±2°C). Control rats (C) remained at the normal environment temperature (23±2°C). Data are expressed as mean±s.e.m. Both wet- and dry brain weights are from 90 days old rats. Asterisks indicate malnourished values that are significantly lower than the corresponding well-nourished group ($P<0.05$; ANOVA plus Tukey test).

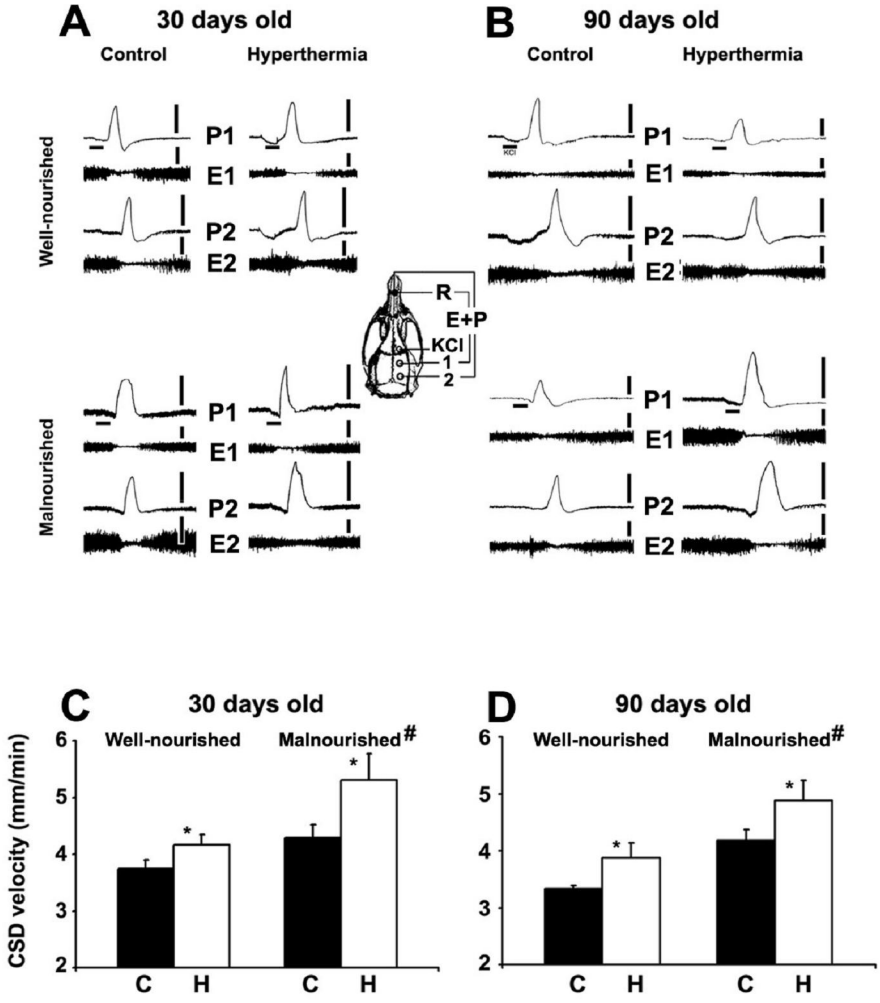

Figure 2 – A and B, electrophysiological recordings of spontaneous cortical activity (ECoG) – (E) and slow potential change (P) during cortical spreading depression (CSD) in two young (30 days old; panel A) and two adult (90 days old; panel B) rats. At each age are shown one control and one animal previously submitted during lactation to 15 heat exposure episodes from postnatal day 10 to 29. CSD was elicited by applying 2% KCl for 1 min on the right frontal cortex (see inset at center). The horizontal bars in the upper traces indicate the time (1 min) of KCl stimulation to elicit the CSD. Vertical bars represent -10mV for (P) and -1mV for (E). Numbers 1 and 2 at the inset indicate the recording points, from which the traces marked with the same numbers were obtained. Inset also shows the position on of reference electrode (R), on the nasal bones. C and D, mean+s.e.m. CSD velocities in the groups recorded at 30 days (panel C) and at 90 days (panel D). The # symbol indicates that all malnourished groups are different ($P<0.05$) from the corresponding control groups. Asterisks indicate that the heat-exposed groups (H) are significantly different ($P<0.05$) from the corresponding control (C) groups.

IV. CONCLUSÕES

A hipertermia ambiental durante o período de aleitamento facilita a propagação da DAC de ratos jovens e adultos, como julgado pelas suas velocidades mais altas, sugerindo que os episódios de aquecimento ambiental durante o desenvolvimento cerebral afetaram a susceptibilidade cortical à DAC.

A facilitação da propagação da DAC, induzida pela hipertermia ambiental em ratos desnutridos, foi similar àquela previamente observada em animais nutridos. Indicando que a desnutrição precoce não influencia esse efeito sobre a DAC.

Nosso estudo mostrou que o efeito facilitador da hipertermia ambiental sobre a DAC é duradouro, se não permanente, e não é apreciavelmente influenciado pela desnutrição.

Muitas implicações fisiopatológicas de tais resultados permanecem a ser esclarecidas, mas é tentador postular que esses achados podem ser importantes para auxiliar no tratamento clínico de condições patológicas que podem ser desencadeadas pela estimulação por aquecimento tal como acontece em convulsões febris em crianças.

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VI. ANEXOS

VI. 1 PRIMEIRO RESUMO PUBLICADO EM ANAIS DE CONGRESSO

EVENTO: 41ST CONGRESS OF THE BRAZILIAN PHYSIOLOGY SOCIETY & JOINT MEETING WITH THE PHYSIOLOGICAL SOCIETY, 2006, RIBEIRÃO PRETO /SP

SPREADING DEPRESSION PROPAGATION IN ADULT RATS SUBMITTED EARLY IN LIFE TO HYPERTHERMIA EPISODES.

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Introduction: Spreading depression (SD) is a neural response that has been related to neural excitability changes and to phatologies like epilepsy and migraine. Hyperthermia in developing children and animals can alter brain electrical activity, leading to seizures. *In vitro*, hyperthermia elicits SD (Wu, J. Neurophysiol. 84: 1355-1360, 2000). Here we characterized SD in the cerebral cortex of adult rats submitted during lactation to hyperthermia episodes. **Methods:** Wistar suckling rats (n=9) were submitted to a 40±2ºC environment (15 daily sessions; 5d per week). When the pups became adults (90-120 d), they were anesthetized (urethane+chloralose; 1,000+40mg/kg ip) and EcoG- plus slow potential changes accompanying SD were recorded on 2 parietal points for 4h. **Results:** Early hyperthermia resulted, in adulthood, to higher SD propagation velocities ($P<0.05$), as compared to control rats (n=11) submitted to similar sessions at room temperature (25±2ºC). Mean SD-velocities (in mm/min) ranged from 3.64±0.19 to 3.99±0.44 (experimental group) and from 3.28±0.05 to 3.33±0.09 (control group). Body- and brain weights were not affected by the early treatment. **Discussion and conclusions:** A hypertermia-induced SD propagation facilitation is suggested, as judged by SD velocity enhancement. This effect was qualitatively similar to that previously observed *in vitro* (see Introduction), suggesting common mechanisms in both conditions. Hyperthermia during suckling is sufficient to lastingly alter SD-susceptibility in the adult rat brain.

Finantial support: CNPq, Capes.

VI. 2 SEGUNDO RESUMO PUBLICADO EM ANAIS DE CONGRESSO

Evento: II Simpósio do Instituto Internacional de Neurociências de Natal IINN
23 a 25 de Fevereiro de 2007. Natal/Brasil

EXPOSURE TO ENVIRONMENTAL HYPERTHERMIA EARLY IN LIFE LASTINGLY FACILITATES CORTICAL SPREADING DEPRESSION IN PREVIOUSLY MALNOURISHED ADULT RATS.

Santos, R.C.F.; Araújo, L.L.; Lira, M.C.A.; Pereira, D.S.; Sá, I.R.; Pimentel, M.R.F. Guedes, R.C.A.
Dept. of Nutrition, Federal Univ.of Pernambuco State, 50670-901, Recife, PE, Brasil.

Cortical spreading depression (CSD) is a neural response related to neural excitability changes and to pathologies like epilepsy and migraine. Exposure to a heated environment can alter the neural excitability, changing the brain electrophysiological features. We have previously demonstrated in rats that 1) CSD is facilitated by early malnutrition and 2) by hyperthermia exposure during lactation. However, the CSD-effects of the association of these two conditions have not been investigated. Here we have extended our previous observations, by analyzing CSD features in adult rats previously submitted to the association between hyperthermia and malnutrition. Wistar rat pups, suckled by mothers fed the “regional basic diet” (RBD, with 8% of protein, instead of 23%, as in the control diet), were placed, during the suckling period, in a hot environment (40±2ºC; 15 daily sessions; 5 per week). When the pups became adults (90-120 d), they were anesthetized (urethane+chloralose; 1,000+40mg/kg ip) and the ECoG- plus the slow potential changes accompanying SD were recorded on 2 parietal points for 4h. Early hyperthermia was associated, in adulthood, to higher SD propagation velocities (mean SD-velocities, in mm/min per recording hour ranging from 4.68±0.34 to 5.06±0.43; n=16), as compared to control rats (from 4.08±0.18 to 4.24±0.22; n=14) submitted to similar sessions at room temperature (25±2ºC). Body- and brain weights were not affected by the early hyperthermia treatment. The results, suggesting a hyperthermia-induced SD propagation facilitation, are similar to those observed in well-nourished animals, indicating that early malnutrition did not influence the effect of hyperthermia on SD. **Finantial support:** CNPq, Capes.

VI. 3 TERCEIRO RESUMO PUBLICADO EM ANAIS DE CONGRESSO

Evento: I Congresso Ibro/Larc de Neurociências da América Latina, Caribe e Península Ibérica – Búzios/RG_Brasil

EXPOSURE OF DEVELOPING RATS TO ENVIRONMENTAL HYPERTHERMIA FACILITATES THE PROPAGATION OF CORTICAL SPREADING DEPRESSION.

Farias-Santos, R. C.; Gomes, K. B. A; Queiroz, P. M. A; Guedes, R. C. A.
Dept. of Nutrition, Federal Univ.of Pernambuco State, 50670-901, Recife, PE, Brasil.

Aims: Cortical spreading depression (CSD) is a brain response related to neural excitability changes and to diseases like epilepsy and migraine. Exposure to a warm environment can alter the neural excitability, changing brain eletrophysiological features, which may be relevant to epilepsy genesis. Here we investigated the effects, on brain CSD susceptibility, of exposing developing rats to a warm environment.
Methods: Wistar suckling rats (n=6), suckled by mothers fed a commercial diet with 23% protein, were submitted from day 10 st to 29 st day of life to 15 daily sessions (5 sessions per week during 3 weeks) of a warm environment (40±2°C), for 15, 20 and 30 min (in the 1st, 2nd and 3rd week, respectively). At 30-40 d of life, they were anesthetized (urethane+chloralose; 1,000+40mg/kg ip) and the ECoG plus slow potential change accompanying CSD were recorded on 2 parietal points for 4h.
Results: Early environmental hyperthermia resulted in higher CSD propagation velocities ($P<0.05$), as compared to control rats (n=6) submitted to similar sessions at room temperature (25±2°C). Mean CSDvelocities (in nm/min) ranged from 4.08±0.14 to 4.28±0.18 (experimental group) and from 3.74±0.16 to 3.75±0.16 (control group).
Conclusion: Environmental Hyperthermia during the suckling period facilitates CSD propagation in the brain of young weaned rats, as judged by its high velocities, suggesting that the warming episodes occurring during brain development effected cortical susceptibility to CSD. The possible relationship with brain alterations due to febrile seizures in children justify further investigation.

Parecer do comitê de Ética em Experimentação Animal

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Ofício nº 62/05

Recife, 26 de dezembro de 2005

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: Prof. Rubem Carlos Araújo Guedes
Departamento de Nutrição - UFPE

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 **"Caracterização da depressão alastrante cortical em ratos adultos previamente submetidos, no aleitamento, à associação entre hipertermia e desnutrição".**

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,

Profª. Silene Carneiro do Nascimento
UFPE
Presidente CEEA