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INFLUÊNCIA DA OVARIECTOMIA SOBRE O DESENVOLVIMENTO NEURAL: ANÁLISE ELETROFISIOLÓGICA

NORANEGE EPIFÂNIO ACCIOLY

Orientador:

Prof . Dr Rubem Carlos Araújo Guedes

Co-orientadora:

Prof. Dra Belmira Lara Silveira Andrade da Costa

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Noranogo Epifânio Accioly

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Aprovado por:

Prof. Dr. Rubem Carlos Araújo Guedes
Presidente

Prof. Dr. Luiz Bezerra de Carvalho Júnior

Profa. Dra. Maria Teresa Jansem de Almeida Catanho

Profa. Dra. Maria Inês Wanderley

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DEDICATÓRIA

Ao prof. Dr. Rubem Carlos Araújo Guedes que com exemplo, dedicação e profissionalismo faz do seu trabalho uma missão tornando o mundo um lugar melhor pra viver!

ÍNDICE ANALÍTICO

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“Bendize, ó minha alma, ao Senhor e não te esqueças de nenhum só de seus benefícios.” Salmo 103:2.

Sou imensamente grata, sobretudo a Jesus que morreu e ressuscitou para que pudéssemos ter livre acesso ao Pai e vida em abundância. Conhecê-lo mudou toda minha vida!!

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RESUMO

Nos mamíferos, evidências experimentais e clínicas demonstram que os hormônios ovarianos influenciam o cérebro, desde o seu desenvolvimento até a idade adulta. Durante o desenvolvimento, essa influência hormonal inclui vários mecanismos com importantes repercussões nas propriedades eletrofisiológicas do cérebro adulto, muitas delas influenciadas pela sua atividade sináptica. Neste trabalho, nós caracterizamos, em ratas adultas ovariectomizadas durante o desenvolvimento, a habilidade cerebral em propagar o fenômeno da depressão alastrante cortical (DAC) como indicador dos efeitos da ausência dos hormônios ovarianos no cérebro eletrofisiologicamente desenvolvido. Ratas wistar recém-nascidas (7 dias de idade) foram submetidas à ovariectomia (grupo Ovx), ou à cirurgia fictícia (grupo Sham), ou deixadas sem cirurgia (grupo "Intacto", ou ingênuo). Quando atingiram a idade de 90-130 dias, foram submetidas ao registro da DAC (eletrocorticograma e variação lenta de voltagem – DC) em dois pontos da superfície cortical durante 4h. Ovariectomia bilateral aos 7 dias de vida resultou em pesos corporais maiores (de 50-65 dias em diante). Houve também redução dos pesos uterinos e da

velocidade de propagação da DAC, em comparação com ambos os grupos controle (Intacto e Sham). Conclui-se que a ovariectomia durante o período do desenvolvimento cerebral está associada, de forma causal, com a redução da propagação da DAC no cérebro adulto, indicando um efeito de longo prazo. Sugere-se que esse efeito está relacionado com a supressão duradoura da ação dos hormônios ovarianos sobre a transmissão sináptica cerebral.

Palavras-chave: Desenvolvimento cerebral; hormônios ovarianos; depressão alastrante cortical; ratas.

ABSTRACT

The brain of mammals is one important target organ for the action of gonadal steroids and, when occurring during development, this hormonal influence may result in important repercussion on the brain electrophysiological properties at adulthood, some of which depending on the synaptic activity. Here we have characterized in early ovariectomized adult rats the brain ability to propagate cortical spreading depression (CSD), as an index of the cerebral electrophysiological effects of the early-induced absence of the ovarian hormones. Wistar female rat pups (7 days old) underwent bilateral ovariectomy (Ovx group; n=21) or sham surgery (Sham group; n=22), or no surgery (Naive group; n=22). When the pups became adult (90-130 days), they were anesthetized and submitted to the recording of CSD (electrocorticogram and slow DC voltage variation) in two points of the cortical surface during 4h. Compared with both Naïve and Sham controls, bilateral ovariectomy early in life resulted in significantly higher body weights (from day 50-65 onwards) and severely reduced uterus weights at adulthood. Furthermore, in the Ovx

animals the amplitudes and durations of the DC potential changes of CSD were higher, and the CSD propagation velocities were reduced. It is concluded that ovariectomy during the period of brain development is causally associated with the impairment of CSD propagation in the adult brain, indicating a long-lasting effect, which we suggest as being related to the long-term suppression of the action of the ovarian hormones on synaptic transmission.

Keywords: Brain development, Ovarian hormones, Cortical spreading depression, Rats

INTRODUÇÃO

O sistema nervoso central é um órgão-alvo importante para as ações dos hormônios esteróides gonadais, desde o desenvolvimento à vida adulta (Genazzani et al., 2005, Kawata, 1995). O cérebro é susceptível às variações sistêmicas dos hormônios ovarianos, pois eles podem atravessar a barreira hematoencefálica e exercer efeitos profundos sobre a função cerebral (Eikermann-Haerter et al., 2007; Kawata, 1995).

No sistema nervoso embrionário, os processos de neurogênese, gliogênese e migração neuronal ocorrem mais intensamente na fase chamada ―período de crescimento rápido do cérebro ou simplesmente período crítico. Essa fase é considerada crítica para o perfeito desenvolvimento e funcionamento neurológico, sendo uma etapa de grande vulnerabilidade do cérebro a agressões internas e/ou externas (Dobbing, 1968). Tal fase acha-se compreendida entre o terceiro trimestre gestacional e o segundo ano de vida, no homem, e corresponde ao período de aleitamento, no rato (Fig. 1; Morgane et al., 1993).

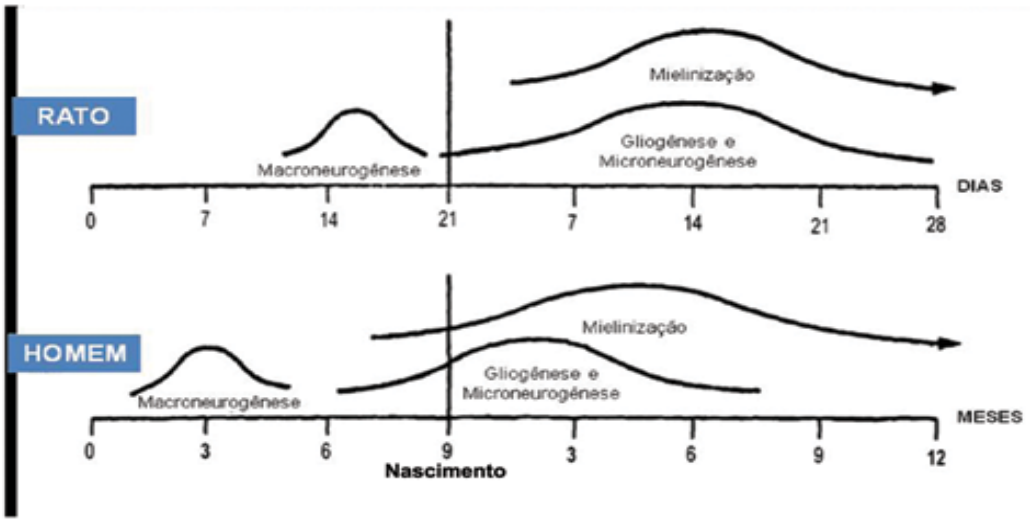

Figura 01: Comparação entre as etapas de desenvolvimento do sistema nervoso no homem e no rato. Adaptada de Morgane et al., 1993.

Nos últimos anos, o conhecimento de como os hormônios sexuais interferem no desenvolvimento das funções cerebrais dos mamíferos tem aumentado substancialmente. O ciclo de vida normal do sexo feminino está associado a uma série de variações hormonais, incluindo a menarca, a gravidez e a menopausa e freqüentemente uso de contraceptivos ou reposição hormonal. Todos esses eventos e intervenções alteram os níveis e ciclos de hormônios sexuais e podem influenciar o sistema nervoso.

No cérebro em desenvolvimento o estradiol atenua a excitotoxicidade mediada pelo glutamato diminuindo a regulação dos receptores metabotrópicos tipo 1 e diminuindo a quantidade de cálcio liberada do retículo endoplasmático (Hilton et al., 2006). Além disso, o estradiol marcadamente reforça as respostas GABAérgicas despolarizantes, específicas do período de desenvolvolvimento cerebral (Nunez et al., 2008; Perrot-Sinal et al., 2001).

No cérebro adulto, o estrógeno exerce efeitos sobre a excitabilidade cerebral regulando “para cima” a expressão do gene da subunidade do receptor excitatório N-metil-d-aspartato (NMDA: Eikermann-Haerter e al., 2007; Martin and Behbehani, 2006) e diminuindo a atividade inibitória dos neurônios GABAérgicos (Eikermann-Haerter et al.,

2007). Tanto o β-estradiol quanto a progesterona podem aumentar a potenciação de longo termo (LTP) nos tecidos neocorticais (Sachs et al., 2007).

Estados hipoestrogênicos no cérebro adulto podem causar mudanças neuroendócrinas em diferentes áreas cerebrais. A ausência de produção hormonal ovariana gera sintomas específicos devido ao desarranjo do sistema nervoso central, no hipotálamo, por exemplo, pode originar sintomas vasomotores, bem como distúrbios do comportamento alimentar e controle alterado da pressão arterial (Genazzani et al., 1999).

Estudos a partir de modelos animais indicam que o cérebro é realmente sensível à progesterona durante períodos críticos de desenvolvimento e maturação (López and Wagner, 2009). Receptores de progesterona (PR) são transitoriamente expressos durante desenvolvimento fetal e neonatal (Wagner, 2008). A progesterona é capaz de promover crescimento dendrítico, spinogênese e sinaptogênense nas células de Purkinje em desenvolvimento (Tsutsui, 2008).

Um modelo interessante para o estudo das relações entre hormônios gonadais femininos e excitabilidade cerebral constitui-se no fenômeno da depressão alastrante cortical (DAC), que foi empregado no presente trabalho. A DAC é um fenômeno eletrofisiológico caracterizado por uma onda de excitação neuronal seguida de inibição. O fenômeno se auto-propaga como uma onda de despolarização com características iônicas, metabólicas e hemodinâmicas peculiares, plenamente reversíveis ao cabo de alguns minutos, acompanhada por supressão transitória da atividade neuronal (Leão, 1944a,b).

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.

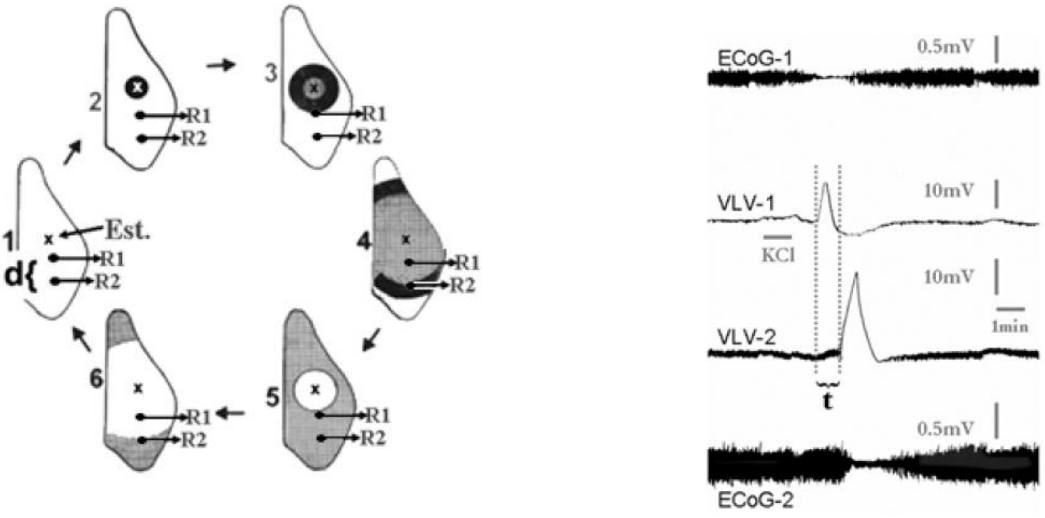

Figura 02: À esquerda observa-se a seqüência temporal cíclica dos eventos que ocorrem durante a propagação da DAC. R1 e R2 indicam pontos de registro. Um estímulo externo (x) deu início ao fenômeno (etapa 1) que se propaga de forma concêntrica (etapas 2-4). As áreas escuras (etapas 2, 3 e 4) representam 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 nos pontos R1 e R2. Observe a recuperação do ECoG após a passagem do fenômeno (Guedes et al., 2004).

Estudos experimentais prévios 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 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., 2001
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	Ximenes-da-Silva e Guedes,1991

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

Estudos prévios têm mostrado que alterações hormonais durante o desenvolvimento influenciam a propagação da DAC (Guedes e Pereira-da-Silva, 1993; Santos, 2000). Um estudo *in vitro* em ratos adultos sugere uma possível influência, dose-dependente, dos hormônios ovarianos sobre a DAC (Sachs et al., 2007). Entretanto, pouca atenção tem sido dada aos efeitos sobre a DAC dos hormônios ovarianos, ou da sua ausência, in vivo, quando atuando no cérebro em desenvolvimento.

Frente ao exposto, o presente trabalho se propôs a estudar o efeito da deficiência ovariana durante o desenvolvimento cerebral sobre a DAC em ratas adultas. Este trabalho é a continuação de uma linha de pesquisa do "Laboratório de Fisiologia da Nutrição Naíde Teodósio" (LAFINNT) que utiliza o fenômeno da DAC para estudar o efeito de fatores nutricionais, ambientais, farmacológicos e hormonais sobre o cérebro em desenvolvimento (Guedes, 2011).

OBJETIVOS

Geral

Avaliar, em ratas, o impacto da ovariectomia aos sete dias de vida sobre o desenvolvimento eletrofisiológico do sistema nervoso através da susceptibilidade cortical à DAC, na idade adulta.

Específicos

- Avaliar a eficácia da ovariectomia, analisando-se, nos animais castrados, alterações da evolução ponderal ao longo do desenvolvimento e, na idade adulta, a presença de

padrão atrófico do epitélio vaginal e do peso uterino, em comparação com os dois grupos controle.

- Quando os filhotes se tornarem adultos, avaliar, nos grupos estudados 1) Grupo Ovx (ratas submetidas à ovariectomia; 2) Grupo Sham (ratas submetidas à cirurgia fictícia à ovariectomia) e 3) Grupo Intacto (ratas mantidas sem tratamento), a incidência e propagação da DAC, por meio do seu registro eletrofisiológico (eletrocorticograma – EcoG - e variação lenta de voltagem), quantificando-se a velocidade de propagação, bem como a amplitude e a duração da variação lenta de voltagem que caracterizam o fenômeno.

ARTIGO CIENTÍFICO

Title: Ovariectomy in the developing rat decelerates cortical spreading depression in adult brain

Authors: Noranege Epifânio Accioly[2], Regina de Deus Lira Benevides[2], Belmira Lara da Silveira Andrade da Costa[1], Rubem Carlos Araujo Guedes[2CA]

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Affiliation: Departamento de Fisiologia e Farmacologia; [2]Departamento de Nutrição, Universidade Federal de Pernambuco, 50670901, Recife, PE, Brazil[CA]= Corresponding author; address, please see address #2 above

E-mail: rguedes@ufpe.br or guedes.rca@gmail.com

Telephone: +55-81-21268936 Fax: +55-81-21268473

Abstract

The brain of mammals is one important target organ for the action of gonadal steroids and, when occurring during development, this hormonal influence may result in important repercussion on the brain electrophysiological properties at adulthood, some of which depending on the synaptic activity. Here we have characterized in early ovariectomized adult rats the brain ability to propagate cortical spreading depression (CSD), as an index of the cerebral electrophysiological effects of the early-induced absence of the ovarian hormones. Wistar female rat pups (7 days old) underwent bilateral ovariectomy (Ovx group; n=21) or sham surgery (Sham group; n=22), or no surgery (Naive group; n=22). When the pups became adult (90-130 days), they were anesthetized and submitted to the recording of CSD (electrocorticogram and slow DC voltage variation) in two points of the cortical surface during 4h. Compared with both Naïve and Sham controls,

bilateral ovariectomy early in life resulted in significantly higher body weights (from day 50-65 onwards) and severely reduced uterus weights at adulthood. Furthermore, in the Ovx animals the amplitudes and durations of the DC potential changes of CSD were higher, and the CSD propagation velocities were reduced. It is concluded that ovariectomy during the period of brain development is causally associated with the impairment of CSD propagation in the adult brain, indicating a long-lasting effect, which we suggest as being related to the long-term suppression of the action of the ovarian hormones on synaptic transmission.

Keywords: Brain development, Ovarian hormones, Cortical spreading depression, Rats

Introduction

In mammals, the fetal environment is rich in estradiol and progesterone derived from the maternal organism (McCarthy, 2009; Sanyal, 1978). In the female fetal brain of the rat, α-fetoprotein (AFP), a steroid binding globulin, sequesters circulating estrogens to avoid their brain masculinizing effects early in life (Bakker et al., 2006, Gillies and MCarthur, 2010) and selectively deliver estradiol to specific neuronal populations (Bakker and Baum, 2008; Bakker et al., 2006). As a result, the female fetal brain is exposed to lower levels of estradiol, as compared with the male fetal brain. Nevertheless, there is evidence that estradiol can be *de novo* synthesized (locally from cholesterol) directly in fetal and neonatal neurons during the female developing brain (Amateau et al., 2004; Bakker et al., 2002; McCarthy, 2008; Mellon and Vaudry, 2001). This hormonal scenario extends

postnatally and in the offspring it influences the sexual differentiation of the developing brain (McCarthy and Konkle, 2005; Amateau et al., 2004).

The brain enzyme aromatase, that synthesizes estradiol, presents its highest activity in the immature brain as compared to the mature brain (McCarthy, 2009) and the AFP activity no longer plays a significant role postnatally when the ovaries start to produce estrogens (Bakker and Baum, 2008). During this initial period of life, ovarian hormones can influence developmental processes in the brain (Bakker et al., 2002). Estradiol is capable of modulating brain development by enhancing depolarizing GABA responses, which are specific of the neonatal period, causing a trophic effect (Perrot-Sinal et al., 2003) and preventing glutamate-induced cell death (Hilton et al., 2006). In addition, progesterone is capable of promoting dendritic growth, spinogenesis, and synaptogenesis in the developing Purkinje cell (Tsutsui, 2008).

In the fully developed brain, estrogen can exert effects on excitability by upregulating the gene expression of excitatory N-metil-D-aspartate (NMDA) receptor subunit (Eikermann-Haerter et al., 2007; Martin and Behbehani, 2006) and by decreasing the inhibitory activity of γ-aminobutyric acid (GABA)-ergic neurons (Eikermann-Haerter et al., 2007). Both β-estradiol and progesterone may enhance long-term potentiation (LTP) induction in neocortical tissues (Sachs et al., 2007). These data indicate a relationship between ovarian hormones and neuronal excitability, which can be experimentally explored by using the electrophysiological phenomenon denominated as cortical spreading depression (CSD). CSD is characterized by a wave of self-propagating depolarization with characteristic ionic, metabolic, and hemodynamic changes followed by transient suppression of neuronal activity (Leao, 1944a,b). In one *in vitro* study, ovarian hormones applied to neocortical slices obtained from adult rats facilitated CSD (Sachs et al., 2007).

However, little attention has been paid to the effects of ovarian deficiency during brain development on CSD features in the cerebral cortex of adult rats.

The present study aimed to address these issues in female rats that had been previously ovariectomized early in life. Our hypothesis is that ovariectomy during the period of brain development is causally associated in adulthood with impairment of CSD propagation.

Material and methods

Animals

Wistar female newborn rats (n=65) from the colony of Departamento de Nutrição of Universidade Federal de Pernambuco (Brazil) were randomly distributed to three groups, submitted respectively on the postnatal day 7 to the following treatments: a) bilateral ovariectomy (OVX group; n=21); b) sham surgery (n=22); c) no surgery (naïve group; n=22).

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). The experimental design was approved by the University Committee on Ethics in animal research, which complies 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.

Bilateral ovariectomy

Under deep surgical cryoanesthesia (Phifer and Terry, 1986; see also Tenório et al., 2009), the ovaries of the 7-days old rat pups were removed through a dorsal midline incision on the lumbar region, as described elsewhere (Brouwer et al, 1980). In the Sham group, all rat pups received the same incisions as the OVX animals; the ovaries were identified and palpated, but not removed. Suture procedures were the same in both groups. After recovering from anesthesia, the pups were returned to the maternal cage. After weaning, they were housed in cages similar to the maternal ones (3-4 rats per cage). Total surgery time was 10-15 min and the post-surgery mortality was very low (3 out of 46 operated pups).

The effectiveness of the early ovariectomy was histologically confirmed on two occasions: at 60-90 days of life (by the atrophic pattern of the genital epithelium as well as by the delayed vaginal opening, as compared to the Sham and Naïve controls) and on the day of CSD recording (90-130 days of life), when the animal was killed and the uterus was removed, showing severe atrophy.

CSD elicitation and recording

When the pups were 90 to 130 days old, they were submitted to the CSD recording for a 4-hour period. In the sham- and naïve groups, the CSD recordings were performed

only when the animals were in the proestrus phase of the estrous cycle, which was histologically confirmed on the day of the CSD recording.

Under anesthesia (1 g/kg urethane plus 40 mg/kg chloralose, ip), three trephine holes (2–3 mm in diameter) were drilled on the right side of the skull. The first hole (on the frontal bone) was used to apply the stimulus (KCl solution) to elicit CSD. The propagating CSD wave was then recorded on two points of the parietal cortex surface through the other two holes, drilled on the parietal bone. Rectal temperature was continuously monitored and maintained at 37 ± 1ºC by a heating blanket. CSD was elicited at 20 min intervals by applying 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 for 1 min. The electrocorticogram (ECoG) and the slow DC potential change accompanying CSD were recorded simultaneously at the two parietal points on the cortical surface by using a pair of Ag-AgCl agar-Ringer electrodes. These electrodes consisted of plastic conic pipettes (5 cm length, 0.5 mm tip inner diameter), filled with Ringer solution and solidified with the addition of 0.5% agar, into which a chlorided silver wire was inserted. The pipettes were fixed together pair-wise with cyanoacrylate glue, so that the interelectrode distance was kept constant for each pair (range: 4–5.5 mm). Each pair of electrodes was connected to a lever that could be vertically moved by turning around a screw, so that the recording electrodes could be gently placed on the intact dura-mater, under low-power microscope control, without any excessive pressure on the cortical surface. A third electrode, of the same type, placed on the nasal bones, served as common reference electrode. The velocity of CSD propagation was calculated based on the time required for a CSD wave to cross the distance between the two recording electrodes. In the measurement of CSD velocities, the initial point of each DC negative rising phase was used as the reference point.

Statistics

Body-, uterus-, adrenals- and brain weights and 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

Body weights

Fig. 1 shows the body weights of the three groups of rats, measured at the following age-intervals: 40-46d, 50-65d, 74-85d and 100-120d. Compared to the two control groups, bilateral ovariectomy early in life resulted in significantly higher body weights from day 50-65 onwards (P=0.006). The mean values (in g) ranged from 114.9±22,2 to 224.0±26.5 for the Nv group, from 119.2±11.9 to 226.3±26.1 for the Sham group and from 127.1±12.7 to 262.8±34.8 for the Ovx rats.

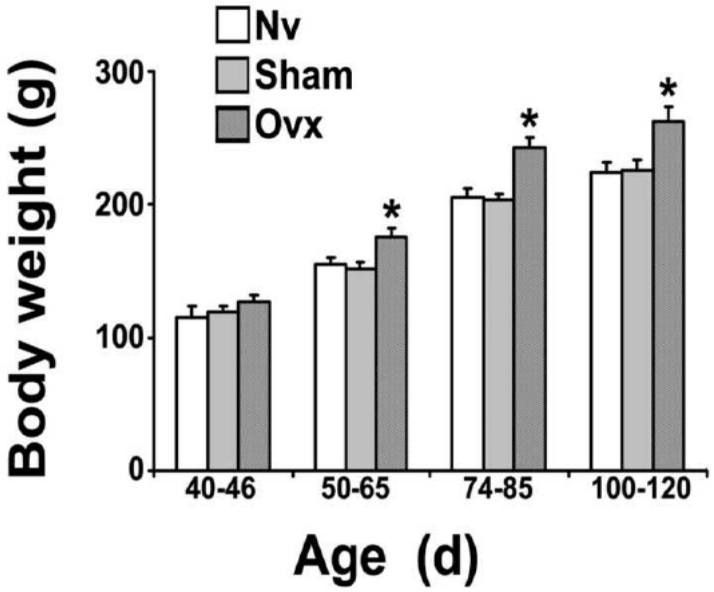

Fig 1. Body weights (mean±EPM) of rats previously submitted (at 7 days of life) to bilateral ovariectomy (Ovx group), or to sham operation (Sham group), or not submitted to any surgery (Nv; Naïve group). The weights were measured at the following ageintervals: 40-46d, 50-65d, 74-85d and 100-120d. The asterisks (*) indicate the OVX body weights that are significantly higher than the corresponding Sham and Naïve values (p=0.006; ANOVA followed by Tukey test).

Uterus and adrenal weights

As can be seen in Figure 2 (upper-left panel), the uterus weights were severely decreased (P<0.001) in the OVX group (80±27 mg), as compared with both Naïve and Sham controls (490±88mg and 447±88 mg, respectively). The adrenal weights of the Ovx group (71±27 mg) were slightly higher than the Sham (56±12 mg) and the Nv group (62±19 mg), but the difference did not reach statistical significance (upper-right panel of figure 2). The two photographs in the lower part of Figure 2 illustrate the easily recognizable uterus atrophy in the Ovx condition (right photograph), as compared to the Sham control (left photograph). The adrenal glands (also shown in the two photographs) were macroscopically comparable in the two groups.

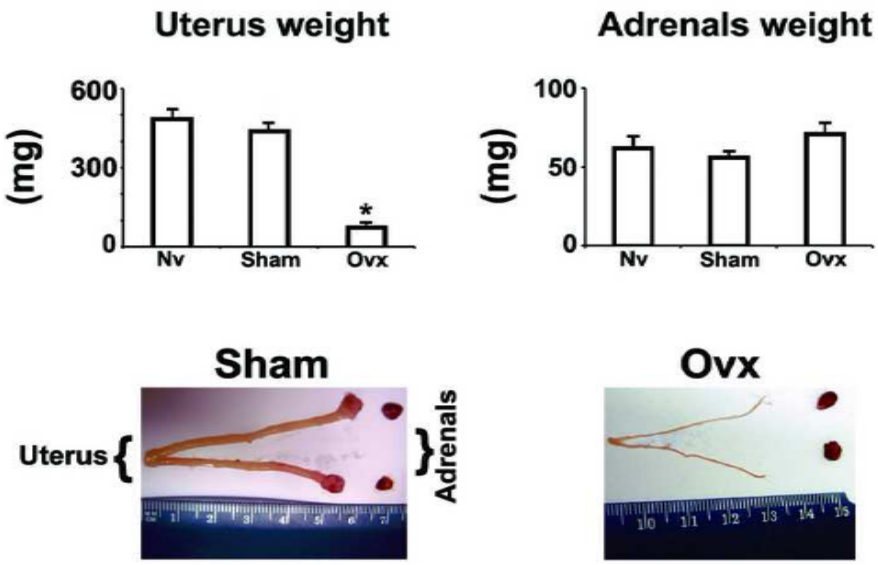

Fig 2. Uterus and adrenal weights (mean± EPM) of the Nv, Sham and Ovx groups, as defined in figure 1. The asterisk (*) in the upper-left panel indicates that the Ovx group presents uterus weights significantly lower ($p<0.001$) than those of the Sham and Naïve controls. The mean adrenal weights of the three groups were comparable (upper-right panel). The two photographs of the lower part of the figure illustrate the uterus atrophy in the Ovx condition (right photograph), as compared to the Sham control (left photograph). The adrenal glands are also shown in the two photographs, and no macroscopically appreciable intergroup difference could be detected. (ANOVA followed by Tukey test).

CSD propagation

Figure 3 shows a typical electrophysiological recording (slow DC-potential change and ECoG) in one Nv, one Sham and one Ovx rat. In all groups, the 1-min stimulation with 2% KCl at one point of the frontal cortex elicited a single CSD wave that propagated without interruption and was recorded by the two electrodes located more posterior in the parietal cortex (see stimulation- and recording points in the inset of the figure). One can notice that the ECoG depression and the slow potential change confirmed the presence of CSD, after KCl application.

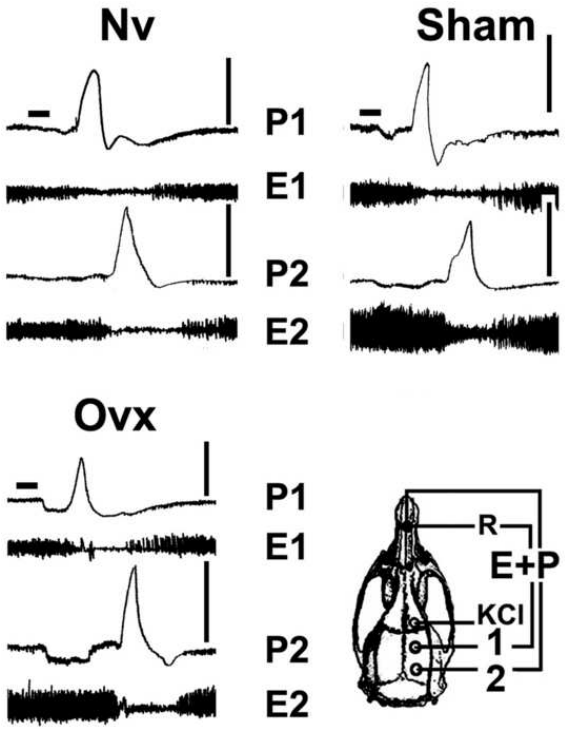

Fig. 3 - Electrocorticogram (E) and slow DC-potential variation (P) recorded during the passage of cortical spreading depression (CSD) at two points (designated as 1 and 2) in the parietal cortex. The horizontal black bars above the P1 traces indicate the period (1 minute) in which the chemical stimulus (2% KCl) was applied to trigger CSD, in the frontal region. The inset of the figure shows the position of the reference electrode (R), common to the two recording electrodes, as well as the point of KCl stimulation and the two recording sites. Vertical bars indicate -10 mV for P and -1 mV for ECoG.

In the Ovx rats, CSD propagated with significantly lower velocities (mean ± standard deviation: 2.72 ± 0.24 mm/min), as compared to the sham and to the naïve control groups (respectively 3.36 ± 0.09 mm/min and 3.36 ± 0.10 mm/min; $P<0.001$, ANOVA followed by Tukey test). Measurement of the amplitudes and durations of the CSD DC-potential change revealed intergroup significant differences (Ovx > Nv = Sham). Figure 4 (lower panel) and Table 1 show these CSD findings, as well as the brain weights (upper panel of Figure 4), which were comparable between the three groups: the mean brain

weights (in g) for the Nv, Sham and Ovx groups were respectively 1.703±0.088, 1.633±0.084 and 1.642±0.091.

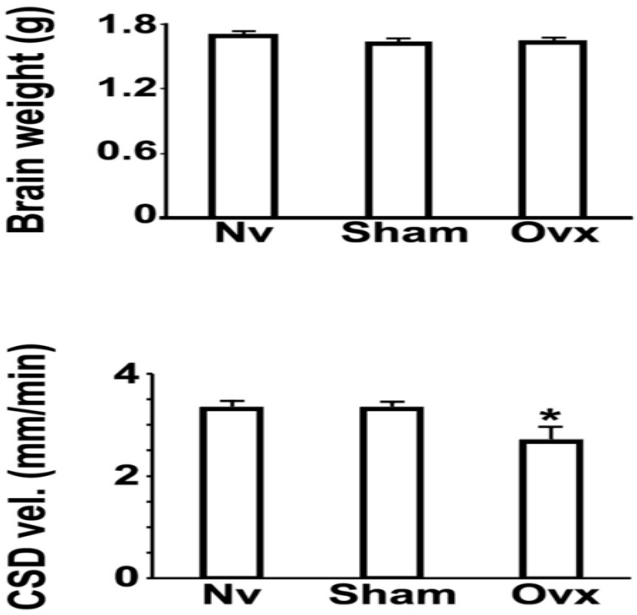

Fig 4 - Mean (± EPM) brain weights (upper panel) and CSD velocity of propagation (lower panel) in Nv, Sham and previously ovariectomized adult rats (group Ovx). The brain weights were comparable between the three groups. In the lower panel, the asterisk indicates that the propagation of CSD in the OVX group is significantly lower ($P < 0.001$) than the values of the two control groups, which didn't differ from each other (ANOVA followed by Tukey test).

Table 1

Amplitudes and durations of the CSD slow potential shifts in the Ovx, Sham and Naïve (Nv) groups. A and P refers to the anterior and posterior recording points respectively.

Group	**Amplitude (mV)**		**Duration (s)**	
	A	**P**	**A**	**P**
Nv	10,2 ± 4,1	12,1 ± 5,8	61,8 ± 11,4	76,5 ± 20,2
Sham	10,1 ± 4,7	11,4 ± 4,6	62,2 ± 12,1	75,1 ± 12,6
Ovx	18,2 ± 7,7*	21,8 ± 10,6*	77,3 ± 10,4*	76,2 ± 11,4

Data are expressed as mean ± standard deviation. The asterisks indicate values significantly different from the corresponding Sham and Naïve group.

Discussion

Ovariectomy in developing rats

In this study we observed important developmental systemic and localized alterations resulting from bilateral ovariectomy, which was performed in the developing rats as early as the postnatal day 7. We have chosen this age because it corresponds to the time-point in which the ovaries become capable of secreting significant amounts of their hormones (Lamprecht et al, 1976). In accordance with a previous report (Gitlin, 1974), the effectiveness of the ovariectomy was presently evidenced by the severe atrophic patterns of uterus- and genital epithelium, delayed vaginal opening (as compared to the controls), as well as body weight increment. Regarding the ovariectomy-induced increase in body weights, it is known that the hypoestrogenic status participates in the increased food intake and the resulting higher body weight gain, as compared to the controls (Wade, 1975; Roesch, 2006). The post-ovariectomy deficiency in the uterus development would be expected, since it is well described in the literature; ovaries participate in the uterine growth observed between the second and the fourth postnatal weeks (Branham and Sheehan, 1995). Concerning adrenal weights the lack of intergroup statistically significant difference is in agreement with data of (Ramaley (1973). Taken together, our data on the systemic and localized developmental effects seen in early ovariectomized rats, besides assuring the effectiveness of the ovariectomy procedure also supports the causal link between ovarian hormones deficiency and the here described brain CSD effects.

CSD propagation

The main electrophysiological finding of the present study was that chronic ovarian hormones deficiency that was provoked during development reduced brain capability to propagate CSD in adulthood, as indexed by its lower velocities in comparison to the velocities of the sham and naïve controls. The alterations in CSD amplitude and duration also reinforce this conclusion. It is well established that the gonadal steroids exert some of its action on the central nervous system, which is one important target organ for their actions, both during development and in adult life (Genazzani et al., 2005; Kawata, 1995). In contrast to its role on the adult brain, in the developing brain GABA is the predominant source of excitation via membrane depolarization, and estradiol markedly enhance depolarizing GABA responses in neonatal neurons (Nunez et al., 2008; Perrot-Sinal et al., 2001). Furthermore, in the developing brain estradiol dampens glutamate-mediated excitotoxicity by downregulating their receptors (mGluR1 and mGluR5) and by decreasing the amount of calcium released from the endoplasmic reticulum (Hilton et al., 2006). Therefore, it appears to us unequivocal accepting that ovariectomy early in life changes excitability of the developing brain, and our CSD findings support that.

Considering that CSD is influenced by changes in the brain excitability (Leão, 1944; 1972; Guedes and Cavalheiro, 1997; Guedes et al, 2009; Guedes, 2011), it is reasonable to raise the question of if, and how early ovarian deficiency (or ovarian absence, as in the ovariectomy paradigm) would modulate brain excitability during development, and therefore influencing phenomena like seizures and CSD at adulthood. One possibility to explain the effects of ovariectomy on the excitability of the adult brain would be based on the influence of ovarian hormones on the glutamatergic neurotransmitter system. Estrogens can increase neuronal excitability via upregulating the gene expression of NMDA receptor

subunit and by decreasing the inhibitory action of GABAergic neurons (Eikermann-Haerter et al, 2007; Martin and Behbehani, 2006). Estrogen also can inhibit L-glutamate uptake by astrocytes (Sato et al, 2003), and increases the number of dendritic spines, which are densely populated with NMDA receptors (Woolley et al., 1997). As a rule, higher levels of estrogen are associated with an increased seizure frequency in females (Klein and Herzog, 1998) and seizure thresholds are decreased during peak estrogen levels (Woolley and Timiras, 1962), while progesterone is associated with seizure control in catamenial epilepsy (Herzog, 2009). Application of exogenous estrogen to the cerebellum of female rats significantly potentiates the excitatory neuronal response of Purkinje cells to glutamate applied by iontophoresis (Smith et al, 1988). Besides the glutamatergic, the GABAergic neurons also are strongly modulated by ovarian steroids. Estrogen can increase GABA release and upregulate the number of GABA receptors (Shughrue and Merchenthaler, 2000). Progesterone has been shown to have depressant effects on CNS excitability via modulation of the gamma-aminobutiric acid type A ($GABA_A$; Lambert et al., 2003). These, and several other pieces of evidence showing that gonadal hormones can affect brain excitability (Eikermann-Haerter et al., 2007; 2009; Sachs et al., 2007; Woolley et al., 1997; Martin and Behbehani, 2006; Scharfman and MacLusky, 2006; Scharfman et al., 2005), collectively suggest that ovarian hormones could have a critical role in epilepsy, and perhaps could also play a role in epilepsy treatment (Herzog, 2009).

Concerning the ovarian hormones/CSD relationship, several findings from others deserve comment. The CSD susceptibility in familial hemiplegic migraine type 1 (FHM1) knockin mice is higher in females than in males; ovariectomy reverses this gender difference, which is partially restored by estradiol replacement, suggesting that actually estrogens can modulate CSD susceptibility (Eikermann-Haerter et al. 2009). The thresholds

for CSD elicitation with KCl- and electrical stimulation are lower in female mice compared to males (Brennan et al. 2007). In rat neocortical slices, application of both β-estradiol and progesterone enhances CSD features (Sachs et al. 2007). This compelling evidence from the literature favors our initial hypothesis that ovariectomy during the period of brain development is causally associated in adulthood with CSD impairment.

In conclusion we demonstrated for the first time that the brains of adult rats that had been ovariectomized during their development are more resistant (or less sensible) to CSD propagation and we suggest that this effect is related to the long-term suppression of the physiological action of the ovarian hormones on synaptic transmission. Our findings document the importance of further searching for the molecular mechanisms underlying the role of the ovarian hormones on the brain development and their electrophysiological properties.

Acknowledgments

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References

Amateau, S.K., Alt, J.J., Stamps, C.L., McCarthy, M.M., 2004. Brain estradiol content in newborn rats: sex differences, regional heterogeneity, and possible de novo synthesis by the female telencephalon. Endocrinol. 145, 2906–2917.

Bakker, J., Honda, S., Harada, N., Balthazart, J., 2002. The aromatase knock-out mouse provides new evidence that estradiol is required during development in the female for the expression of sociosexual behaviors in adulthood. J. Neurosci. 22, 9104-9112.

Bakker, J., Baum, M.J., 2008. Role for estradiol in female-typical brain and behavioral sexual differentiation. Front. Neuroendocrinol. 29, 1–16.

Bakker, J., De Mees, C., Douhard, Q., Balthazart, J., Gabant, P., Szpirer, J., Szpirer, C., 2006. Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens. Nat. Neurosci. 9, 220–226.

Branham, W.S., Sheehan, D.M., 1995. Ovarian and Adrenal Contributions to Postnatal Growth and Differentiation of the Rat Uterus. Biol. Reprod. 53, 863-872.

Brennan, K.C., Reyes, M. R., Valdés, H.E.L., Arnold, A.P., Charles, A.C., 2007. Reduced threshold for cortical spreading depression in female mice. Ann. Neurol. 61, 603–606.

Brouwer, E.A., Dailey, R., Brouwer, J.B., 1980. Ovariectomy of newborn rats: a descriptive surgical procedure. Lab. An. Sci. 30, 546-548.

Eikermann-Haerter, K., Kudo, C., Moskowitz, M.A., 2007. Cortical spreading depression and estrogen. Headache. 47[suppl 2], S79-S85.

Eikermann-Haerter, K., Dileköz, E., Kudo, C., Savitz, S.I., Waeber, C., Baum, M.J., Ferrari, M.D., van den Maagdenberg, A.M.J.M., Moskowitz, M.A., Ayata, C., 2009. Genetic and hormonal factors modulate spreading depression and transient hemiparesis in mouse models of familial hemiplegic migraine type 1. J. Clin. Invest. 119, 99-109.

Genazzani, A.R., Bernardi, F., Pluchino, N., Begliuomini, S., Lenzi, E., Casarosa, E., Luisi, M., 2005. Endocrinology of menopausal transition and its brain implications. CNS Spectr. 6, 449-57.

Gillies, G.E., MCarthur, S., 2010. Estrogen Actions in the Brain and the Basis for Differential Action in Men and Women: A Case for Sex-Specific Med. Pharmacol. Rev. 62, 155–198.

Gitlin, G., 1974. Vaginal opening and vaginal epithelium following ovariectomy in newborn rats. Acta Anat. 90, 117-132.

Guedes, R.C.A., 2011. Cortical Spreading Depression: A Model for Studying Brain Consequences of Malnutrition. in: Preedy, V., Watson, R., Martin, C. (Eds.), Handbook of Behavior, Food and Nutrition. Springer, Berlin, pp. 2343-2355.

Guedes, R.C.A., Cabral-Filho, J.E., Teodósio, N.R., 1992. 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). Springer, Berlin, pp 17-26.

Guedes, R.C.A., Cavalheiro, E.A., 1997. Blockade of spreading depression in chronic epileptic rats: reversion by diazepam. Epil. Res. 27, 33-40.

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

Guedes, R.C.A., Oliveira, J.A.C., Amâncio-dos-Santos, A., García-Cairasco, N., 2009. Sexual Differentiation of Cortical Spreading Depression propagation after Acute and Kindled Audiogenic Seizures in the Wistar Audiogenic Rat (WAR). Epil. Res. 83, 207-214.

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

Herzog, A.G., 2009. Hormonal Therapies: Progesterone. Neurotherapeutics. 6, 383–391.

Hilton, G.D., Nunez, J.L., Bambrick, L., Thompson, S.M., McCarthy, M.M., 2006. Glutamate-mediated excitotoxicity in neonatal hippocampal neurons is mediated by mGluR-induced release of Ca++ from intracellular stores and is prevented by estradiol. Eur. J. Neurosci. 24, 3008–3016.

Klein, P., Herzog, A.G., 1998. Hormonal effects on epilepsy in women. Epilepsia. 39, S9–S16.

Kawata, M., 1995. Roles of steroid hormones and their receptors in organization in the nervous system structural. Neurosci. Res. 24, 1-46.

Lambert, J.J., Belelli. D., Peden. D.R., Vardy, A.W., Peters, J.A., 2003. Neurosteroid Modulation of GABAA receptors. Progr. Neurobiol. 71, 67–80.

Lamprecht, S.A., Kohen, F., Ausher, J., Zor, U., Lindner, H.R., 1976. Hormonal stimulation of estradiol-17 beta release from the rat ovary during early postnatal development. J. Endocrinol. 68, 343–344.

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

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

Leão, A.A.P., 1972. Spreading depression. in: Purpura, D.P., Penry, K., Tower, D.B., Woodbury, D.M.,Walter, R.D. (Eds.), Experimental Models of epilepsy. Raven Press, N. York, pp. 173-195.

Martin, V.T., Behbehani, M., 2006. Ovarian hormones and migraine headache: understanding mechanisms and pathogenesis-part I. Headache. 46, 3-23.

McCarthy, M.M., 2009. The two faces of estradiol: effects on the developing brain. Neuroscientist. 15, 599–610.

McCarthy, M.M., 2008. Estradiol and the developing brain. Physiol. Rev. 88, 91–124.

McCarthy, M.M., Konkle, A.T.M., 2005. When is a sex difference not a sex difference? Front. Neuroendocrinol. 26, 85–102.

Mellon, S.H., Vaudry, H., 2001. Biosynthesis of neurosteroids and regulation of their synthesis. Internat. Rev. Neurobiol. 46, 33-78.

Nunez, J.L., Aberdeen, G.W., Albrecht, E.D., McCarthy, M.M., 2008. Impact of estradiol on GABA- and glutamate-mediated calcium responses of fetal baboon (papio anubis) hippocampal and cortical neurons. Endocrinology. 149, 6433–6443.

Perrot-Sinal, T.S., Auger, A.P., McCarthy, M.M., 2003. Excitatory GABA-induced pCREB in developing brain is mediated by L-type Ca+2 channels and dependent on age, sex and brain region. Neuroscience. 116, 995–1003.

Phifer, C.B., Terry, L.M., 1986. Use of hypothermia for general anesthesia in preweanling rodents. Physiol. Behav. 38, 887-890.

Ramaley, J.A., 1973. Adrenal function in female rats before puberty: The effects of gonadectomy. Steroids. 22, 597-608.

Roesch, D.M., 2006. Effects of selective estrogen receptor agonists on food intake and body weight gain in rats. Physiol. Behav. 87, 39–44.

Sachs, M., Pape, H.C., Seckmann, E.J., Gorji, A., 2007. The effect of estrogen and progesterone on spreading depression in rat neocortical tissues. Neurobiol. Dis. 25, 27-34.

Sanyal, M.K., 1978. Secretion of progesterone during gestation in the rat. J. Endocrinol. 79, 179 –190.

Sato, K., Matsuki, N., Ohno, Y., Nakazawa, K., 2003. Estrogens inhibit l-glutamate uptake activity of astrocytes via membrane estrogen receptor alpha. J. Neurochem. 86, 1498–1505.

Scharfman, H.E., Goodman, J.H., Rigoulot, M.A., Berger, R.E., Walling, S.G., Mercurio, T.C., Stormes, K., Maclusky, N.J., 2005.Seizure susceptibility in intact and ovariectomized female rats treated with the convulsant pilocarpine. Exp. Neurol. 196, 73-86.

Scharfman, H.E., MacLusky, N.J., 2006. The influence of gonadal hormones on neuronal excitability, seizures, and epilepsy in the female. Epilepsia. 47, 1423-1440.

Shughrue, P.J., Merchenthaler, I., 2000. Estrogen is more than just a “sex hormone”: novel sites for estrogen action in the hippocampus and cerebral cortex. Front. Neuroendocrinol. 21, 95-101.

Smith, S.S., Waterhouse, B.D., Woodward, D.J., 1988. Locally applied estrogens potentiate glutamate-evoked excitation of cerebellar Purkinje cells. Brain Res. 475, 272-282.

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

Tsutsui, K., 2008. Minireview: Progesterone Biosynthesis and Action in the Developing Neuron. Endocrinology. 149, 2757–2761.

Wade, G.N., 1975. Some effects of ovarian hormones on food intake and body weight in female rats. J. Comp. Physiol. Psychol. 88, 183–193.

Woolley, C.S., Weiland, N.G., McEwen, B.S., Schwartzkroin, P.A., 1997. Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. J. Neurosci. 17, 1848–1859.

Woolley, D.E., Timiras, P.S., 1962. Estrous and circadian periodicity and electroshock convulsions in rats. Am. J. Physiol. 202, 379–382

CONCLUSÕES

A análise dos resultados desta dissertação permite as duas seguintes conclusões e uma sugestão:

- A técnica presentemente utilizada, de remoção cirúrgica dos ovários, em ratas com 7 dias de vida, foi eficaz em alterar o desenvolvimento das suas estruturas genitais e reprodutoras.

- Tal efeito é baseado na análise macroscópica do padrão atrófico vaginal e uterino.

- Esta conclusão é também reforçada pelo ganho de peso corporal, significante a partir de 50-65 dias de vida.

- Os cérebros das ratas adultas que foram ovariectomizadas durante seu desenvolvimento tornaram-se mais resistentes (ou menos sensíveis) à propagação da DAC.

- Sugere-se que este efeito da ovariectomia precoce sobre a DAC seja devido à supressão da ação dos hormônios ovarianos sobre mecanismos sinápticos cerebrais, o que deverá ser investigado em futuros experimentos.

REFERÊNCIAS BIBLIOGRÁFICAS

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

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

Amateau, S.K., Alt, J.J., Stamps, C.L., McCarthy, M.M., 2004. Brain estradiol content in newborn rats: sex differences, regional heterogeneity, and possible de novo synthesis by the female telencephalon. Endocrinol. 145, 2906–2917.

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., 2006. Fluoxetine inhibits cortical spreading depression in weaned and adult rats suckled under favorable and unfavorable lactation conditions. Exp. Neurol. 200,275-282.

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

Bakker, J., Honda, S., Harada, N., Balthazart, J., 2002. The aromatase knock-out mouse provides new evidence that estradiol is required during development in the female for the expression of sociosexual behaviors in adulthood. J. Neurosci. 22, 9104-9112.

Bakker, J., Baum, M.J., 2008. Role for estradiol in female-typical brain and behavioral sexual differentiation. Front. Neuroendocrinol. 29, 1–16.

Bakker, J., De Mees, C., Douhard, Q., Balthazart, J., Gabant, P., Szpirer, J., Szpirer, C., 2006. Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens. Nat. Neurosci. 9, 220–226.

Bezerra, R.S., Abadie-Guedes, R., Melo, F.R.M., Paiva, A.M.A., Santos, A.A., Guedes, R.C.A., 2005. Shrimp carotenoids protect the developing rat cerebral cortex agains. the effects of ethanol on cortical spreading depression. Neurosc. Let. 391, 51-55.

Branham, W.S., Sheehan, D.M., 1995. Ovarian and adrenal contributions to postnatal growth and differentiation of the rat uterus. Biol. Reprod. 53, 863-872.

Brennan, K.C., Reyes, M. R., Valdés, H.E.L., Arnold, A.P., Charles, A.C., 2007. Reduced threshold for cortical spreading depression in female mice. Ann. Neurol. 61, 603–606.

Brouwer, E.A., Dailey, R., Brouwer, J.B., 1980. Ovariectomy of newborn rats: a descriptive surgical procedure. Lab. An. Sci. 30, 546-548.

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

Costa-Cruz, R.R.G., Santos, A.A., Guedes, R.C.A., 2006. 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. 401, 271-275.

Dobbing ,J., 1969. Vulnerable periods in developing brain. in: Davison, A.N., Dobbing, J. (Eds), Applied Neurochemistry, Blackwell. Oxford, pp 287–316.

Eikermann-Haerter, K., Kudo, C., Moskowitz, M.A., 2007. Cortical spreading depression and estrogen. Headache. 47[suppl 2], S79-S85.

Eikermann-Haerter, K., Dileköz, E., Kudo, C., Savitz, S.I., Waeber, C., Baum, M.J., Ferrari, M.D., van den Maagdenberg, A.M.J.M., Moskowitz, M.A., Ayata, C., 2009. Genetic and hormonal factors modulate spreading depression and transient hemiparesis in mouse models of familial hemiplegic migraine type 1. J. Clin. Invest. 119, 99-109.

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., 2009. Exposure of developing well-nourished and malnourished rats to environmental heating facilitates cortical spreading depression propagation at adulthood. Neurosc. Let. 454, 218-222.

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., 2007. Effects of transcranial direct current stimulation coupled with repetitive electrical stimulation on cortical spreading depression. Exp. Neurol. 204, 462-466.

Fregni, F., Monte-Silva, K.K., Oliveira, M.B., Freedman, S., Pascual-Leone, A., Guedes, R.C.A., 2005. 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. 21, 2278-2284.

Genazzani, A.R., Bernardi, F., Pluchino, N., Begliuomini, S., Lenzi, E., Casarosa, E., Luisi, M., 2005. Endocrinology of menopausal transition and its brain implications. CNS Spectr. 6, 449-57.

Genazzani, A.R., Spinetti, A., Bernardi, F., 1999. Menopause and the central nervous system: intervention options. Maturitas, 31, 103-110.

Gillies, G.E., MCarthur, S., 2010. Estrogen Actions in the Brain and the Basis for Differential Action in Men and Women: A Case for Sex-Specific Med. Pharmacol. Rev. 62, 155–198.

Gitlin, G., 1974. Vaginal opening and vaginal epithelium following ovariectomy in newborn rats. Acta Anat. 90, 117-132.

Guedes, R.C.A., 1984. On some conditions that influence cortical spreading depression. Anais da Academia Brasileira de Ciências. 56, 445-455.

Guedes, R.C.A., 2011. Cortical spreading depression: a model for studying brain consequences of malnutrition. in: Preedy, V., Watson, R., Martin, C. (Eds.), Handbook of Behavior, Food and Nutrition. Springer, Berlin, pp. 2343-2355.

Guedes, R.C.A., Amorim, L.F., Teodósio, N.R., 1996. Effect of aging on cortical spreading depression. Braz. J. Med. Biol. Res. 29, 1407-1412.

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

Guedes, R.C.A., Barreto, J., 1992. Effect of anesthesia on the propagation of cortical spreading depression. Braz. J. Med. Biol. Res. 25, 393-397.

Guedes, R.C.A., Cabral-Filho, J.E., Teodósio, N.R., 1992. Gabaergic mechanisms involved in cortical spreading depression in normal and malnourished rats. in: Do Carmo, R.J. (Ed.), Spreading depression. experimental brain research series. Springer, Berlin, pp 17-26.

Guedes, R.C.A., Cavalheiro, E.A., 1997. Blockade of spreading depression in chronic epileptic rats: reversion by diazepam. Epil. Res. 27, 33-40.

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

Guedes, R.C.A., Frade, S.F., 1993. Effect of ethanol on cortical spreading depression. Braz. J. Med. Biol. Res. 26, 1241-1244.

Guedes, R.C.A., Oliveira, J.A.C., Amâncio-dos-Santos, A., García-Cairasco, N., 2009. sexual differentiation of cortical spreading depression propagation after acute and kindled audiogenic seizures in the wistar audiogenic rat (WAR). Epil. Res. 83, 207-214.

Guedes, R.C.A., Pereira-da-Silva, M., 1993. Effect of pre- and postnatal propylthiouracil administration on the propagation of cortical spreading depression of adult rats. Braz. J. Med. Biol. Res. 26, 1123-1128.

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

Guedes, R.C.A., Silva, A. T. Teodósio, N.R., Amorim, L.F., 1989. Effect of dietary lithium on cortical spreading depression. Braz. J. Med. Biol. Res.. 22, 923-925.

Herzog, A.G., 2009. Hormonal Therapies: Progesterone. Neurotherapeutics. 6, 383–391.

Hilton, G.D., Nunez, J.L., Bambrick, L., Thompson, S.M., McCarthy, M.M., 2006. Glutamate-mediated excitotoxicity in neonatal hippocampal neurons is mediated by

mGluR-induced release of Ca++ from intracellular stores and is prevented by estradiol. Eur. J. Neurosci. 24, 3008–3016.

Klein, P., Herzog, A.G., 1998. Hormonal effects on epilepsy in women. Epilepsia. 39, S9–S16.

Kawata, M., 1995. Roles of steroid hormones and their receptors in organization in the nervous system structural. Neurosci. Res. 24, 1-46.

Lambert, J.J., Belelli. D., Peden. D.R., Vardy, A.W., Peters, J.A., 2003. Neurosteroid Modulation of GABAA receptors. Progr. Neurobiol. 71, 67–80.

Lamprecht, S.A., Kohen, F., Ausher, J., Zor, U., Lindner, H.R., 1976. Hormonal stimulation of estradiol-17 beta release from the rat ovary during early postnatal development. J. Endocrinol. 68, 343–344.

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

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

Leão, A.A.P., 1972. Spreading depression. in: Purpura, D.P., Penry, K., Tower, D.B., Woodbury, D.M.,Walter, R.D. (Eds.), Experimental Models of epilepsy. Raven Press, N. York, pp. 173-195.

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

López, V., Wagner, C.K., Progestin receptor is transiently expressed perinatally in neurons of rat isocortex. J. Comp. Neurol. 512, 124–139.

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., 2009. L-Arginine administration during rat brain development facilitates spreading depression propagation: evidence for a dose- and nutrition-dependent effect. Nut. Neurosc. 12, 73-80.

Martin, V.T., Behbehani, M., 2006. Ovarian hormones and migraine headache: understanding mechanisms and pathogenesis-part I. Headache. 46, 3-23.

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

McCarthy, M.M., 2009. The two faces of estradiol: effects on the developing brain. Neuroscientist. 15, 599–610.

McCarthy, M.M., 2008. Estradiol and the developing brain. Physiol. Rev. 88, 91–124.

McCarthy, M.M., Konkle, A.T.M., 2005. When is a sex difference not a sex difference? Front. Neuroendocrinol. 26, 85–102.

Mellon, S.H., Vaudry, H., 2001. Biosynthesis of neurosteroids and regulation of their synthesis. Internat. Rev. Neurobiol. 46, 33-78.

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

Nunez, J.L., Aberdeen, G.W., Albrecht, E.D., McCarthy, M.M., 2008. Impact of estradiol on GABA- and glutamate-mediated calcium responses of fetal baboon (*Papio anubis*) hippocampal and cortical neurons. Endocrinol. 149, 6433–6443.

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., 2007. Impact of two early malnutrition models on renal and neural functions in rats. in: Vesler LW (Ed) Malnutrition in the 21st Century. Nova Science Publishers, Inc., N.York, Chapter 13, pp 239-263.

Perrot-Sinal, T.S., Auger, A.P., McCarthy, M.M., 2003. Excitatory GABA-induced pCREB in developing brain is mediated by L-type Ca+2 channels and dependent on age, sex and brain region. Neuroscience. 116, 995–1003.

Perrot-Sinal,T.S., Davis, A.M., Gregerson, K.A., Kao, J.P., McCarthy, M.M., 2001. Estradiol enhances excitatory gamma-aminobutyric [corrected] acid-mediated calcium signaling in neonatal hypothalamic neurons. Endocrinol. 142, 2238-2243.

Phifer, C.B., Terry, L.M., 1986. Use of hypothermia for general anesthesia in preweanling rodents. Physiol. Behav. 38, 887-890.

Ramaley, J.A., 1973. Adrenal function in female rats before puberty: the effects of gonadectomy. Steroids. 22, 597-608.

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

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

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

Roesch, D.M., 2006. Effects of selective estrogen receptor agonists on food intake and body weight gain in rats. Physiol. Behav. 87, 39–44.

Sachs, M., Pape, H.C., Seckmann, E.J., Gorji, A., 2007. The effect of estrogen and progesterone on spreading depression in rat neocortical tissues. Neurobiol. Dis. 25, 27-34.

Santos-Monteiro, J.S., Teodósio, N.R.T., Guedes, R.C.A., 2000. Long-lasting effects of early environmental stimulation on cortical spreading depression in normal and early malnourished adult rats. Nut. Neurosc. 3, 29-40.

Santos, R.S., 2000. Nutrição, hipertireoidismo precoce e desenvolvimento cerebral: estudo em ratos recém-desmamados. Dissertação de Mestrado. Depto. De Nutrição, UFPE.

Sanyal, M.K., 1978. Secretion of progesterone during gestation in the rat. J. Endocrinol. 79, 179 –190.

Sato, K., Matsuki, N., Ohno, Y., Nakazawa, K., 2003. Estrogens inhibit l-glutamate uptake activity of astrocytes via membrane estrogen receptor alpha. J. Neurochem. 86, 1498–1505.

Scharfman, H.E., Goodman, J.H., Rigoulot, M.A., Berger, R.E., Walling, S.G., Mercurio, T.C., Stormes, K., Maclusky, N.J., 2005.Seizure susceptibility in intact and ovariectomized female rats treated with the convulsant pilocarpine. Exp. Neurol. 196, 73-86.

Scharfman, H.E., MacLusky, N.J., 2006. The influence of gonadal hormones on neuronal excitability, seizures, and epilepsy in the female. Epilepsia. 47, 1423-1440.

Shughrue, P.J., Merchenthaler, I., 2000. Estrogen is more than just a "sex hormone": novel sites for estrogen action in the hippocampus and cerebral cortex. Front. Neuroendocrinol. 21, 95-101.

Smith, S.S., Waterhouse, B.D., Woodward, D.J., 1988. Locally applied estrogens potentiate glutamate-evoked excitation of cerebellar Purkinje cells. Brain Res. 475, 272-282.

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

Tsutsui, K., 2008. Minireview: Progesterone Biosynthesis and Action in the Developing Neuron. Endocrinology. 149, 2757–2761.

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

Wade, G.N., 1975. Some effects of ovarian hormones on food intake and body weight in female rats. J. Comp. Physiol. Psychol. 88, 183–193.

Wagner, C.K., 2008. Progesterone Receptors and Neural Development: A Gap between Bench and Bedside? Endocrinol. 149, 2743-2749.

Woolley, C.S., Weiland, N.G., McEwen, B.S., Schwartzkroin, P.A., 1997. Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. J. Neurosci. 17, 1848–1859.

Woolley, D.E., Timiras, P.S., 1962. Estrous and circadian periodicity and electroshock convulsions in rats. Am. J. Physiol. 202, 379–382

Ximenes-da-Silva, A., Guedes, R.C.A., 1991. 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, 1277-1281.

ANEXO 1: Guia para autores

INTERNATIONAL JOURNAL OF DEVELOPMENTAL NEUROSCIENCE

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

Acknowledgements

References

Examples:

- Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2000. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.

- Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 1999. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

ANEXO 2: 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, 31 de março de 2009

Ofício nº 126/09

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: **Profº.:Rubem Carlos Araújo Guedes**
Departamento de Nutrição
Processo nº 23076.002005/2009 -35

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 ***"Influência da ovariectomia em ratas lactentes sobre o desenvolvimento e eletrofisiologia do sistema nervoso ."***

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. Maria Teresa Jansem
Presidente do CEEA

Observação: Aluna: Noranege Epifânio accioly
Origem dos animais: Biotério do Departamento de Nutrição;
Animal: Ratos; Sexo: Fêmeas; Idade: Entre 01 a 120 dias;
Número de animais previsto no protocolo: 30 animais.

ANEXO 3: Apresentação de trabalho em congresso

SBNeC 2010

XXXIV Congresso Anual da Sociedade Brasileira de Neurociências e Comportamento

Certificado

Certificamos que o trabalho CARACTERIZAÇÃO DA DEPRESSÃO ALASTRANTE CORTICAL EM RATAS ADULTAS PREVIAMENTE SUBMETIDAS À OVARIECTOMIA DURANTE O DESENVOLVIMENTO CEREBRAL., NORANEGE EPIFÂNIO ACCIOLY, REGINA DE DEUS LIRA, RUBEM CARLOS ARAUJO GUEDES foi apresentado sob a forma de painel durante o XXXIV Congresso Anual da Sociedade Brasileira de Neurociências e Comportamento - SBNeC 2010, realizado de 8 a 11 de setembro de 2010, no Hotel Glória e Palace Hotel, Caxambu, MG.

Prof. Dr. Marcus Vinícius Chrysóstomo Baldo
Presidente da SBNeC (2008 - 2011)

ANEXO 4: Trabalho a ser apresentado em congresso

XXVI REUNIÃO ANUAL DA FESBE - FESBE 2011
Centro de Convenções Sulamérica
24 a 27 de agosto de 2011

[Menu Principal]

Sr(a) **Noranege Epifânio Accioly**

São Paulo, 23 de maio de 2011.

Comunicamos que o seu resumo intitulado **EFFECTS OF CHRONIC OVARIAN HORMONES DEFICIENCY DURING BRAIN DEVELOPMENT ON FATTY ACID COMPOSITION AND SPREADING DEPRESSION FEATURES IN THE CEREBRAL CORTEX OF ADULT RATS** de autoria **ACCIOLY, N. E. ; BENEVIDES, R. D. D. L. ; NAVARRO, D. A. F. ; SANTOS, G. K. N. ; COSTA, B. L. D. S. A. D. ; GUEDES, R. C. A. Depto. de Nutrição, UFPE**foi aceito para apresentação sob a forma de poster na XXVI Reunião Anual da FeSBE, que será realizada no Centro de Convenções Sulamerica -Rio de Janeiro - RJ - Brasil, de 24/08/2011 a 27/08/2011.

Número de Apresentação: **21.058**

Data da Apresentação: **25 / 8 / 2011**

Horário da Apresentação: **horario inicio 13h30 e horario fim 15h30**

Local: Salão Principal

Atenciosamente,

Comissão Organizadora.

FeSBE - Federação de Sociedades de Biologia Experimental

ANEXO 5: Comprovante de concessão de bolsa de iniciação científica (PIBIC) da Facepe à aluna Regina de Deus Lira Benevides (Processo BIC-0065-2.07/11), para dar continuidade à linha de pesquisa desta dissertação.

PROCESSO	SUBÁREA	TÍTULO	INSTITUIÇÃO	ORIENTADOR	CANDIDATO
BIC-0065-2.07/11	Neurofisiologia	INFLUÊNCIA DOS HORMÔNIOS OVARIANOS SOBRE O DESENVOLVIMENTO E ELETROFISIOLOGIA DO SISTEMA NERVOSO.	UFPE - RECIFE	RUBEM CARLOS ARAÚJO GUEDES	REGINA DE DEUS LIRA BENEVIDES

Edital FACEPE 02/2011-

Programa de Bolsas de Iniciação Científica PIBIC/FACEPE/CNPq-2011

(BIC-0065-2.07/11)

ANEXO 6: Comprovante de submissão do artigo

From: "Int. J. Developmental Neuroscience" regino.perez-polo@utmb.edu

To: rc.guedes@terra.com.br

Sent: Sex 29/07/11 23:17

Subject: Fwd: Submission Confirmation

Dear Rubem,

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