UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
MESTRADO EM BIOQUÍMICA E FISIOLOGIA

Efeitos neurofisiológicos da dipirona sobre a depressão alastrante cortical em ratos jovens nutridos e desnutridos

ANA PAULA BARBOSA DO AMARAL

Recife, 2009

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ANA PAULA BARBOSA DO AMARAL

"Efeitos neurofisiológicos da dipirona sobre a depressão alastrante cortical em ratos jovens nutridos e desnutridos"

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RESUMO

A eficácia da ação de certos fármacos no cérebro pode variar em função do seu estado nutricional durante o desenvolvimento. A dipirona é um fármaco muito utilizado em crianças, graças à sua ação analgésica e antitérmica. Em ratos, a dipirona apresenta também ação anti-convulsivante. Considerando-se as relações entre a epilepsia e o fenômeno da depressão alastrante cortical (DAC), bem como o uso freqüente da dipirona em crianças, neste trabalho investigou-se, em ratos nutridos e desnutridos, o impacto, sobre a DAC, do tratamento com dipirona, por sub-períodos de 7 dias dentro do período crítico de desenvolvimento cerebral. Ratos Wistar foram amamentados em condições favoráveis (ninhadas compostas por 6 filhotes; grupo nutrido - N; n=76) ou desfavoráveis de lactação (ninhadas de 12 filhotes; grupo desnutrido - D; n=69). Em cada condição nutricional, parte dos animais (N, n=36; D, n=37) foi tratada, por gavagem, com dipirona na 2ª (N, n=10; D, n=12), 3ª (N, n=13; D, n=11), ou 4ª (N, n=13; D, n=11) semanas de vida. Os respectivos controles foram igualmente tratados com solução salina. Aos 35-45 dias, os animais foram anestesiados, submetidos à trepanação seguida do registro da DAC, na superfície do córtex cerebral, por 4 horas. Nos controles N e D, tratados com solução salina na 2ª, 3ª ou 4ª semanas, as velocidades da DAC (em mm/min) foram respectivamente 3.70±0.11, 3.77±0.16 e 3.78±0.13 (grupo N), e 4.13±0.10, 4.16±0.10 e 4.14±0.09 (grupo D). Nos grupos tratados com dipirona, esses valores foram 3.99±0.14, 4.03±0.16 e 4.30±0.19 (grupo N) e 4.47±0.17, 4.70±0.31 e 5.01±0.28 (grupo D). Conclui-se que os resultados confirmaram a hipótese de que a dipirona, ao atuar durante o período de rápido desenvolvimento neural, facilita a propagação da DAC, sendo esse efeito mais evidente no grupo tratado na 4ª semana de vida. A desnutrição precoce pareceu acentuar os efeitos da dipirona sobre a DAC. Este trabalho pioneiro levanta um alerta para o uso abusivo de certos fármacos em organismos humanos em desenvolvimento.

Palavras-chave: Dipirona, desenvolvimento cerebral, depressão alastrante cortical, interação fármacos-nutrição, desnutrição precoce.

ABSTRACT

The effectiveness of the action of certain drugs in the brain can vary as a function of its nutritional status during the development. Dipyrone is a drug that is very used in children, due to its analgesic and antipyretic effects. In rats, dipyrone also presents anticonvulsant effect. Considering the relationships between the epilepsy and the phenomenon known as cortical spreading depression (CSD), as well as the frequent use of dipyrone in children, in this work we investigated, in well-nourished- and early-malnourished rats, the impact, on CSD, of the treatment with dipyrone, for short periods (7 days) within the critical period of cerebral development. Wistar rats were suckled in lactation conditions considered favorable (litters with 6 pups, well-nourished group, n=76) or unfavorable (litters with 12 pups, malnourished group, n=69). Both nutritional groups received per gavage, during 7 days, either dipyrone (300mg/kg/d) or saline, during the 2nd, 3rd, or 4th week of life. At 35-45 days, CSD was recorded for 4h at 2 cortical points in the parietal region. In both W- and M-groups, dipyrone increased ($P<0.05$) the CSD-velocities, as compared to the respective saline-controls. This effect was more intense when dipyrone was applied at the fourth week of life, as compared to the other two weeks. For the W and M saline-treated groups, the mean±sd velocities (in mm/min) were 3.70±0.11, 3.77±0.16 and 3.78±0.13 (W-groups) and 4.13±0.10, 4.16±0.10 and 4.14±0.09 (M-groups), for the animals treated in the 2nd, 3rd and 4th week of life. For the dipyrone-treated groups, the values were 3.99±0.14, 4.03±0.16 and 4.30±0.19 (W-groups) and 4.47±0.17, 4.70±0.31 and 5.01±0.28 (M-groups). Results support the hypothesis of a CSD facilitating effect of dipyrone, which is more intense at a late stage within the brain development period and is facilitated by early-malnutrition. This pioneer work represents a warning on the abusive use of certain therapeutical drugs in human developing organisms.

Key words: dipyrone, brain development, cortical spreading depression, drug-nutrition interaction, early malnutrition.

1 INTRODUÇÃO

1.1 Desenvolvimento do sistema nervoso: ação de fatores medicamentosos e nutricionais

Nas fases iniciais da vida, o crescimento e o desenvolvimento do Sistema Nervoso Central (SNC) ocorrem com grande intensidade, através dos processos de hiperplasia, hipertrofia e mielinização. A gliogênese, a neurogênese, e a migração neuronal realizam-se, então, com velocidade máxima (DOBBING, 1968). Portanto, essa fase é denominada de período crítico ou período de crescimento rápido do cérebro. Nela as áreas cerebrais tendem a desenvolver-se em "saltos" rápidos (MORGANE et al., 1993), e o cérebro tem seu peso aumentado de maneira particularmente acelerada (DOBBING; SMART, 1974). Por essa razão, o cérebro torna-se mais vulnerável a fatores externos, tais como o uso de medicamentos (AMÂNCIO-DOS-SANTOS et al., 2006) ou a ingestão inadequada de nutrientes, que pode levar à desnutrição (DOBBING, 1968).

1.2 O Sistema Nervoso e a Dipirona

A dipirona faz parte do grupo dos derivados pirazolônicos com a presença de um grupo metassulfônico na estrutura (figura 1), um conjunto de drogas antiinflamatórias não esteroidais (DAINES), que possuem atividades analgésicas, antipiréticas e antiinflamatórias, usada largamente na rotina de terapias clínicas, tanto em adultos quanto em crianças. Tem sido largamente usada em crianças de vários países como antitpirético (CERASO, 1994; ERGUN et al., 2000). Esse controle medicamentoso representa um procedimento importante na preservação da saúde da criança, pois, quando não é adequadamente controlada, a hipertermia pode levar a crises convulsivas (GIACHETTO et al., 2001). Estas, quando repetidas, podem tornar o cérebro permanentemente epiléptico (CÍLIO et al., 2003). Embora a maioria dos DAINES tenham mecanismos de ação principalmente baseado na inibição da enzima ciclooxigenase (COX; ABBOTT; HELLEMANS, 2000; ALVES; DUARTE, 2002), o mecanismo envolvido nos efeitos da dipirona são pobremente conhecidos e há controversia sobre os locais de ação da droga (COLLARES; VINAGRE, 2003).

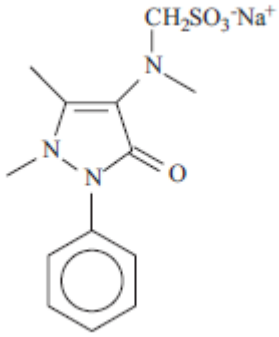


Figura 1 – Estrutura química da dipirona
(MARCOLINO JÚNIOR et al, 2005).

A incidência da epilepsia é variável segundo as diversas regiões do mundo. Nos países mais desenvolvidos, a incidência é de aproximadamente 1-2%, tornando-se o dobro em países menos desenvolvidos (LIGA BRASILEIRA DE EPILEPSIA, 2006). Além disso, a incidência pode ocorrer com maior freqüência associada, dentre outros fatores, à predisposição hereditária, às enfermidades infecciosas (muitas delas indutoras de crises de hipertermia), e à atenção médica insuficiente. Há também evidências clínicas e experimentais de que a desnutrição seria um fator predisponente à epilepsia. A hipótese da relação estreita entre a desnutrição e a epilepsia foi estudada por Nelson e Dean (1959) em 46 crianças sulafricanas desnutridas. Estes pesquisadores encontraram descargas eletroencefalográficas anormais focais em 36% dos casos, sugerindo um aumento da excitabilidade cerebral, semelhante ao que ocorre na epilepsia. No entanto, em humanos, a relação entre a deficiência nutricional e a susceptibilidade a uma ou mais formas de epilepsia não tem sido muito investigada nas últimas décadas.

1.3 O Sistema Nervoso e a Nutrição

Uma nutrição equilibrada decorre da ingestão de alimentos adequados capazes de assegurar as necessidades nutricionais do organismo. Esses alimentos devem ser ingeridos em quantidades suficientes para atender as sínteses orgânicas, e estar adequado à situação biológica do indivíduo, no que se refere à idade, tipo de trabalho e atividade física, etc. (OMS, 1984).

Um desequilíbrio e/ou uma deficiência de nutrientes no organismo é caracterizado como desnutrição (MARTORELLI, 2001). Apesar da indicação de que a prevalência de desnutrição em crianças de países em desenvolvimento vem declinando progressivamente nas duas últimas décadas (ONIS et al., 2000), e de que existe uma rápida elevação da prevalência de sobrepeso/obesidade (KOHN; BOOTH, 2003; OKEN; GILLMAN, 2003), a desnutrição infantil ainda permanece como um sério problema de saúde pública no Brasil (BATISTA-FILHO; RISSIN, 2003).

Os efeitos da desnutrição sobre o desenvolvimento do SNC têm sido estudados, principalmente, porque é preocupante a prevalência de desnutrição infantil ainda relativamente alta em várias partes do mundo, uma vez que existem evidências convincentes de seus efeitos neurais. (GRANTHAM-MCGREGOR, 1990; MORGANE et al., 1993). Muitos desses efeitos são de longa duração, ou mesmo permanentes, e podem ser observados na vida adulta (VASCONCELOS et al., 2004).

A nutrição adequada, especialmente no início da vida, é essencial para um bom desenvolvimento do cérebro em todos os níveis, incluindo o estrutural, o químico, o farmacológico e o funcional (PRASAD, 1998; FERNSTROM, 2000; GUEDES, 2005). No início da vida, esse "período crítico" para o desenvolvimento cerebral varia um pouco nas diferentes espécies de mamíferos. No caso do homem, considera-se que tal período começa no terceiro trimestre de gestação e vai até o segundo ou terceiro anos de vida, ou seja, inclui um período pré e outro pós-natal (figura 2). O mesmo padrão temporal perinatal ocorre também no porco. No rato, compreende apenas o período de aleitamento, que corresponde às três primeiras semanas de vida pós-natal, quando o leite materno é a única fonte de alimento para os filhotes (DOBBING, 1968; MORGANE et al., 1978; 1993). Em animais previamente desnutridos neste período, tem sido descritos: (1) retardo na maturação reflexa e somática (SMART; DOBBING, 1971), (2) alterações do número e tamanho de células nervosas (KRIGMAN; HOGAN, 1976; DOBBING; SMART, 1974; DOBBING, 1970), (3) diminuição do peso do cerebelo, do hipocampo e do córtex cerebral (CHASE et al., 1969; FISH; WINICK, 1969; DOBBING et al., 1971), (4) modificações na arquitetura sináptica (MORGANE et al., 1993) e (5) alterações na disponibilidade e eficácia de neurotransmissores (WIGGINS et al., 1984). Todas essas alterações são frequentemente mencionadas como principais causas de efeitos comportamentais e eletrofisiológicos duradouros em organismos desnutridos precocemente (MORGANE et al., 1978; 1993; RESNICK et al., 1979; FULLER;

WIGGINS, 1984; RESNICK; MORGANE, 1984; RUIZ et al., 1985; PRASAD, 1991; GUEDES et al., 1996; ALMEIDA et al., 2002).

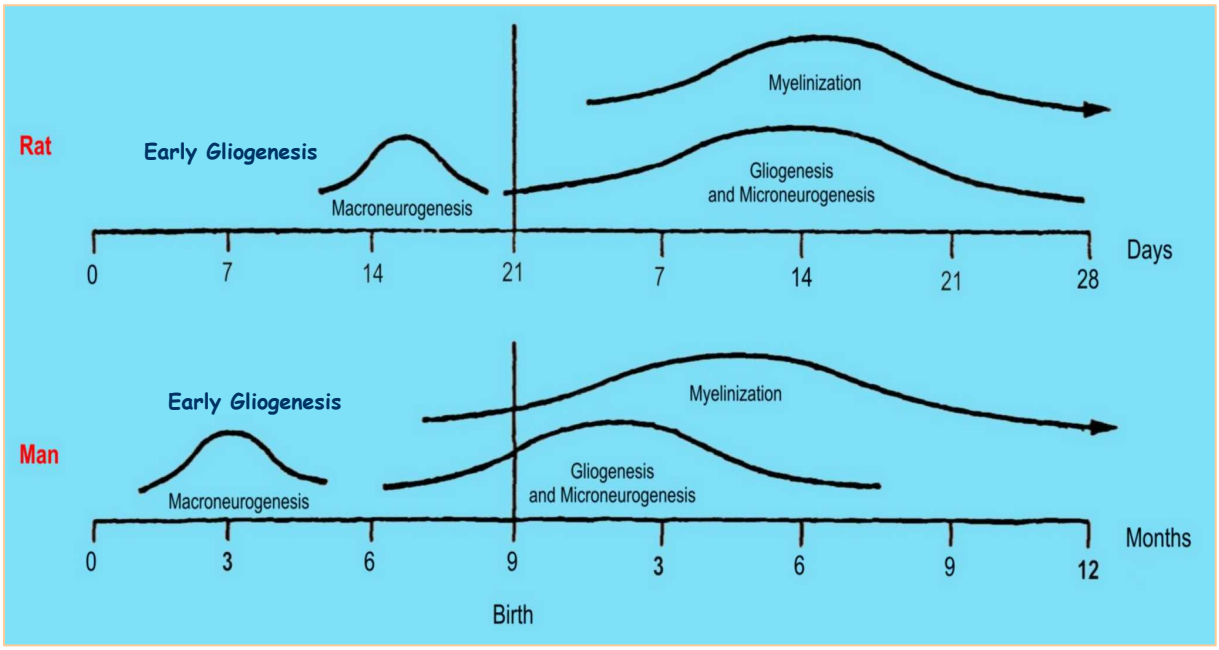


Figura 2 – Curso temporal dos principais eventos do desenvolvimento cerebral: comparação entre homem e rato (Adaptada de Morgane et al., 1993).

Além disso, o cérebro como um todo parece não se desenvolver homogeneamente, apresentando ritmos de crescimento diferenciado para suas distintas partes (MORGANE et al., 1992; 1993; LEVITSKY; STRUPP, 1995). Já se comprovou, por exemplo, que no hipocampo do rato a divisão celular está completa por volta do sexto dia de vida, enquanto no cerebelo do mesmo animal tal processo só deixa de existir em torno do 16º ou 17º dia. Já se verificou também que as células cerebrais migram de uma região para outra em períodos bastante específicos, o que fortalece a idéia de que o crescimento e a organização estrutural do órgão seguem um "cronograma" pré-determinado e inflexível, durante o qual a perda de cada oportunidade para o desenvolvimento normal pode se tornar irreversível (MORGANE et al., 1978; 1993). Assim, é possível que a ação de fatores externos, como certas drogas medicamentosas, administradas durante curtos períodos dentro da fase de lactação, afete algumas estruturas e processos neurais mais efetivamente do que outros, à semelhança do que já foi demonstrado para a desnutrição (ROCHA-DE-MELO; GUEDES, 1997).

1.4 Um método simples para induzir desnutrição

Como neste trabalho serão combinados os fatores descritos acima (fármacos, condições nutricionais e diferentes semanas do desenvolvimento cerebral), julgou-se pertinente comentar também a técnica presentemente utilizada para produzir desnutrição no rato. Trata-se de uma forma experimental muito simples e bastante eficiente de produzir desnutrição durante o período de amamentação. Consiste em se formar ninhadas maiores que as do grupo controle, ou seja, aumenta-se o número de filhotes amamentados por uma mesma nutriz (PLAGEMANN et al., 1998; 1999). O tamanho das ninhadas do grupo controle (doravante denominado "Nutrido") foi fixado em 6 filhotes porque, segundo Fishbeck e Rasmussen (1987) esse número de filhotes em uma ninhada parece conferir o maior potencial lactotrófico. Ao contrário, o aumento do número de filhotes gera competição pelo leite materno e resulta em desnutrição (FULLER; WIGGINS, 1984; ROCHA-DE-MELO et al, 2004; 2006).

1.5 Um método eletrofisiológico para avaliar a atividade cerebral: a depressão alastrante cortical (DAC)

O estudo de alterações eletrofisiológicas cerebrais decorrentes do uso de fármacos ou de condições nutricionais adversas requer métodos e técnicas específicas. O desenvolvimento tecnológico no campo da eletrônica permitiu a construção de equipamentos que propiciam o registro e a análise da atividade elétrica cerebral, a principal característica fisiológica do sistema nervoso. Tais estudos podem fornecer informações importantes tanto em condições normais quanto patológicas (GUEDES, 2005). Nesse contexto, o "Laboratório de Fisiologia da Nutrição Naíde Teodósio" (LAFINNT) tem utilizado, para tais estudos, o fenômeno conhecido como "depressão alastrante cortical" (DAC; GUEDES et al., 1987; 1992; GUEDES; BARRETO, 1992; COSTA-CRUZ; GUEDES, 2001), que será brevemente descrito a seguir.

A DAC foi primeiramente observada e descrita pelo pesquisador brasileiro Aristides Leão, durante estudos sobre epilepsia experimental, utilizando registros da atividade elétrica cortical cerebral em coelhos anestesiados (LEÃO, 1944).

O fenômeno consiste de uma "onda" de redução (depressão) reversível da atividade elétrica do tecido cortical, caracterizando uma resposta local à estimulação elétrica, mecânica ou química de um ponto da superfície cerebral. A reação prossegue na sua marcha mesmo depois de cessado o estímulo aplicado para iniciá-la (LEÃO, 1944) de forma lenta e concêntrica por todo o córtex. A DAC tem sido demonstrada em inúmeras espécies animais, desde peixes até primatas (BURES et al, 1974); mais recentemente, foi evidenciada também no cérebro humano, tanto "in vitro" (GORJI et al, 2001), como "in vivo" (MAYEVSKY et al, 1996). Em todas essas espécies, propaga-se usualmente com velocidade de 2 a 5 mm/min (no rato adulto normal, essa velocidade oscila entre 3 e 4 mm/min). A velocidade com que a DAC se propaga é altamente contrastante com aquela do potencial de ação neuronal, que é da ordem de m/s. À medida que a DAC se propaga para regiões cada vez mais afastadas, a atividade elétrica começa a se recuperar a partir do ponto estimulado. Antes de se recuperar, a região estimulada permanece, por um tempo variável, eletricamente inativa, devido às alterações na voltagem e na impedância cortical que ocorrem na frente da onda de DAC e que impedem que outra DAC seja iniciada (LEÃO; MARTINS-FERREIRA, 1953; BURES et al., 1975; KORELOVA; BURES, 1979; 1980). Ao final de 10 a 15 min, o tecido cortical acha-se totalmente recuperado (LEÃO, 1944; LEÃO, 1947). A seqüência de eventos cíclicos que ocorrem durante a DAC está representada na Figura 1.

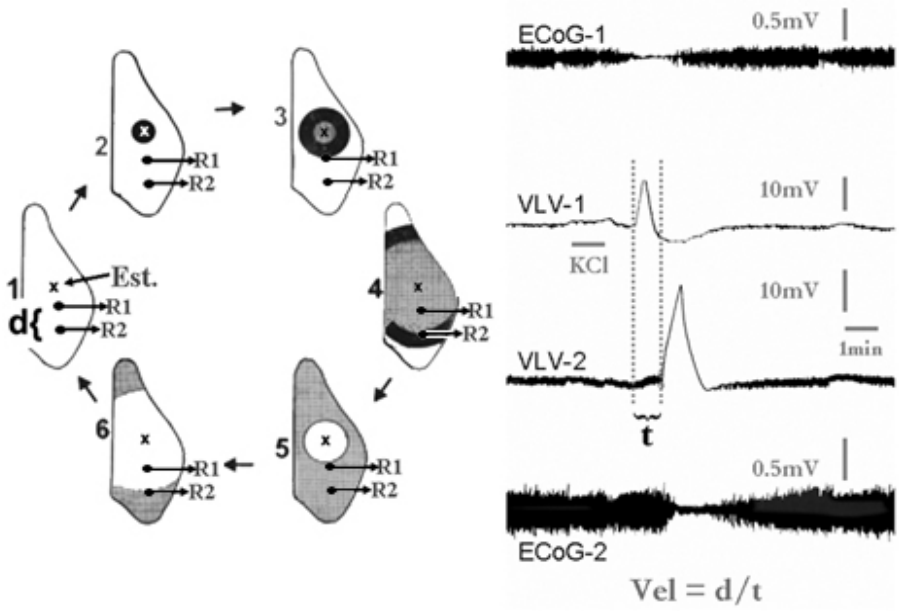


Figura 3: À esquerda, esquema (adaptado de Martins-Ferreira, 1954), mostrando a seqüência temporal cíclica de eventos da depressão alastrante cortical (DAC). Na etapa 1, um ponto cortical (x) foi estimulado (Est.), iniciando a DAC. Dois pontos de registro (R1 e R2) são igualmente mostrados nesta e em todas as outras etapas. A propagação concêntrica da DAC está ilustrada nas etapas 2 a 4, nas quais as áreas escuras representam porções do tecido cortical invadidas pelo fenômeno em tempos sucessivos. As áreas quadriculadas (em cinza) indicam regiões que já sofreram a DAC e agora estão se recuperando (estão, portanto, refratárias a uma nova estimulação). Nas etapas 5 e 6, observa-se que a recuperação (áreas claras) também se dá de forma concêntrica, sendo o ponto onde a DAC se originou primeiro a se recuperar totalmente. Finalmente, todo o tecido se recupera, retornando à condição inicial (etapa 1). À direita, mostram-se o eletrocorticograma (ECoG) e a variação lenta de voltagem (VLV), a qual sempre aparece durante a DAC, quando o ECoG diminui sua amplitude. Esses eventos foram registrados simultaneamente nos pontos R1 e R2. Neste exemplo, a depressão do ECoG recupera-se totalmente após cerca de 3 minutos (registros obtidos em nosso laboratório).

A depressão da atividade elétrica espontânea ou evocada é sempre acompanhada do aparecimento de uma variação lenta de voltagem (VLV) na mesma região cortical, em relação a um local de potencial fixo, como o tecido ósseo do crânio. Ao contrário do eletrocorticograma (ECoG), a VLV tem características do tipo "tudo ou nada", ou seja, a sua presença, com uma "forma de onda" característica e bem definida, com início e fim fáceis de identificar, sempre indica a existência da DAC (LEÃO, 1947; 1951). Por isso, a VLV é muito usada para calcular a velocidade com que o fenômeno se propaga pelo tecido nervoso (GUEDES et al, 2004).

Coincidindo com a VLV, pode surgir dilatação transitória dos vasos sanguíneos da pia-máter (LEÃO, 1944), variação da quantidade de água e das concentrações de íons no espaço

intersticial (HANSEN; OLSEN, 1980), aumento da impedância elétrica do tecido (LEÃO; MARTINS-FERREIRA, 1953), diminuição parcial de O2 (LUKYANOVÁ; BURES, 1967), dentre outros.

Durante a DAC, a presença de "ondas epileptiformes", similares às encontradas no ECoG de pacientes epilépticos, enquanto a atividade elétrica é deprimida, levou à idéia de que a DAC teria algo em comum com o fenômeno epiléptico, em termos de mecanismo. Essa natureza epileptiforme da reação é revelada devido à presença de fenômenos elétricos ativos sob a forma de ondas lentas de grande amplitude, ondas rápidas espiculares (com forma de espiga) e de alterações de calibre nos vasos cerebrais (LEÃO, 1944; 1972; GUEDES; DO CARMO, 1980).

Além da epilepsia, a DAC também pode ser usada como um modelo experimental no intuito de melhorar a compreensão dos processos que levam a outras doenças neurais, como a enxaqueca com aura. Ambos os fenômenos apresentam alterações vasculares semelhantes e as velocidades de propagação da DAC são parecidas com as de expansão, no campo visual, dos escotomas cintilantes relatados pelos doentes, pouco antes de sofrer um episódio de doença (LAURITZEN, 1987; LEHMENKUHLER et al, 1993). Finalmente, por uma lógica semelhante, alguns autores têm postulado um importante papel para a DAC na fisiopatologia da isquemia cerebral (TAKANO et al, 1996). Em todos os casos, as discussões atuais do tema freqüentemente mencionam o possível envolvimento de certos íons (GUEDES; DO CARMO, 1980; SIESJÖ; BENGTSSON, 1989), ou de radicais livres produzidos no tecido nervoso (NETTO; MARTINS-FERREIRA, 1989; GUEDES et al, 1996; EL-BACHA et al, 1998), ou da atividade de neurotransmissores. No entanto, o mecanismo final da DAC ainda está para ser definitivamente esclarecido, embora um grande volume de informações sobre o fenômeno venha sendo publicado nas seis décadas transcorridas desde o seu descobrimento.

Inúmeras condições de importância clínica, que sabidamente podem modificar a excitabilidade neural, quando prevalentes no organismo, podem influenciar a susceptibilidade cortical à DAC. Em alguns casos, o córtex pode se tornar mais vulnerável ao fenômeno, a julgar pelas velocidades de propagação mais altas, e, em outros, se tornar mais resistentes, a julgar pelas velocidades mais baixas (GUEDES, 2005; MONTE-SILVA et al, 2007). Elas incluem tratamentos não apenas locais, como aplicação tópica de fármacos (AMÂNCIO-DOS-SANTOS et al, 2006), como também tratamentos sistêmicos, *in vivo*, (VASCONCELOS et al., 2004; GUEDES; VASCONCELOS, 2008). Dentre as condições que

dificultam a propagação da DAC pode-se mencionar o envelhecimento (GUEDES et al., 1996), o uso de anestésicos (GUEDES; BARRETO, 1992), o hipotiroidismo (GUEDES; PEREIRA-DA-SILVA, 1993), dietas hiperlipídicas (PAIXÃO et al, 2007), a manipulação do sistema serotoninérgico através de dietas (VERÇOSA, 1997) e de drogas (CABRAL-FILHO et al., 1995; TRINDADE-FILHO, 1995; ARAÚJO, 1997; GUEDES et al., 2002; AMÂNCIO-DOS-SANTOS et al., 2006). Por outro lado, a hipoglicemia (XIMENES-DA-SILVA; GUEDES, 1991), a privação de sono paradoxal (GUEDES, 1984), o tratamento com agonistas do ácido gama-amino-butírico (GUEDES et al., 1992), a desnutrição (GUEDES, 1984) e dietas hipolipídicas (PAIXÃO et al., 2007) facilitam a velocidade de propagação deste fenômeno.

No que se refere à desnutrição precoce, estudos realizados no LAFFINT demonstraram que ela exerce um efeito facilitador sobre a susceptibilidade cortical à DAC, a julgar pelas velocidades de propagação, mais altas nos animais adultos que foram desnutridos no início da vida, em comparação com animais controles, bem-nutridos durante toda a vida (GUEDES, 1984; ANDRADE et al., 1990; XIMENES-DA-SILVA; GUEDES, 1991; ROCHA-DE-MELO et al., 2006). No entanto, se a desnutrição é imposta apenas na idade adulta, não há alterações sobre a DA (GUEDES et al., 1987). A eficácia de alguns fármacos no ser humano, em função do seu estado nutricional, tem sido objeto de estudo há alguns anos. Segundo a especulação de alguns autores, a redução da resposta terapêutica em alguns pacientes frente a certos fármacos, pode estar associada a episódios de deficiência nutricional precoce, a exemplo do que tem sido demonstrado em animais de laboratório. Nesses estudos experimentais, observou-se que a administração de substâncias como o diazepam (GUEDES et al., 1992), ou a glicose (COSTA-CRUZ; GUEDES, 2001) modifica as características da DAC no cérebro de ratos adultos normais, porém têm pouco efeito naqueles animais que foram desnutridos no aleitamento. Informações dessa natureza inexistem, com relação ao uso da dipirona durante o desenvolvimento cerebral.

O uso continuado de determinados fármacos por crianças e adolescentes pode levar a alterações imprevisíveis na química e na estrutura cerebrais, interferindo assim no desenvolvimento e maturação do cérebro infantil (RAEBURN, 2007).

Levando-se em consideração que alguns anti-inflamatórios não-esteroidais atuam no limiar da convulsão ou produzem mudanças eletrocorticográficas (STEINHAUER; HERTING, 1981), foi postulado que a dipirona poderia apresentar propriedades

anticonvulsivantes. De fato, a dipirona exibiu ação anticonvulsivante em três modelos de epilepsia experimental (DORETTO et al., 1998). Essa ação anticonvulsivante da dipirona foi depois confirmada em outros dois modelos experimentais de convulsão (ERGÜN et al., 2001). Considerando-se que o fenômeno epiléptico e a DAC podem ter pontos em comum nos seus mecanismos, decidiu-se testar, neste estudo, se a administração de dipirona, por períodos curtos durante o desenvolvimento cerebral, poderia influenciar as características da DAC. Adicionalmente, investigou-se se esse efeito, caso existisse, seria modificado pelo estado nutricional.

Assim, a finalidade deste trabalho foi investigar a ação da dipirona, no SNC em desenvolvimento, sobre a DAC, em ratos submetidos a diferentes estados nutricionais.

2 OBJETIVOS

2.1 Objetivo Geral

Estudar o efeito do tratamento com dipirona sobre a atividade eletrofisiológica cerebral, por meio da DAC, em ratos Wistar em desenvolvimento, nutridos e previamente desnutridos.

2.2 Objetivos Específicos

- Avaliar a evolução ponderal, em função do estado nutricional e do tratamento com dipirona, como um indicador do desenvolvimento;

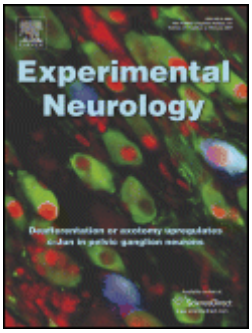
- Investigar os efeitos de períodos curtos de administração de dipirona, em distintas fases do desenvolvimento neural, sobre a DAC em ratos jovens;

- Determinar se existe uma fase do desenvolvimento em que os efeitos acima investigados sejam mais evidentes;

- Avaliar a influência do estado nutricional sobre os efeitos do tratamento com dipirona, na DAC

3. INTERACTION DRUG-NUTRITION IN THE DEVELOPING BRAIN: DIPYRONE ENHANCES CORTICAL SPREADING DEPRESSION IN RATS.

ESTE ARTIGO FOI SUBMETIDO À REVISTA EXPERIMENTAL NEUROLOGY



Title:

Interaction drug-nutrition in the developing brain: dipyrone enhances cortical spreading depression in rats.

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Abstract

The abusive use of pharmacological drugs and the inadequate ingestion of nutrients constitute external factors that, either individually or combined, can change brain development. Here, we used cortical spreading depression (CSD) as a neurophysiological parameter to investigate in the developing brain the effects of the antipyretic/analgesic/anti-inflammatory drug, dipyrone combined with malnutrition (M). Suckling malnourished rats (n=69; malnourished by increasing the litter size to 12 pups) and 76 well-nourished controls (W; litters with 6 pups) received per gavage, during 7 days, either dipyrone (300mg/kg/d) or saline during the 2nd, 3rd, or 4th week of life. At 35-45 days, CSD was recorded at 2 points in the parietal region. In both W- and M-groups, dipyrone increased ($P<0.05$) CSD propagation velocities, as compared to the respective saline-controls. This effect was more intense when dipyrone was applied at the fourth week of life, as compared to the other two weeks. For the W and M saline-treated groups, the mean±sd velocities (in mm/min) were 3.70±0.11, 3.77±0.16 and 3.78±0.13 (W-groups) and 4.13±0.10, 4.16±0.10 and 4.14±0.09 (M-groups), for the animals treated in the 2nd, 3rd and 4th week of life. For the dipyrone-treated groups, the values were 3.99±0.14, 4.03±0.16 and 4.30±0.19 (W-groups) and 4.47±0.17, 4.70±0.31 and 5.01±0.28 (M-groups). Results support the hypothesis of a CSD facilitating effect of dipyrone, which is more intense at a late brain developmental stage and is facilitated by malnutrition. Data might help in understanding brain excitability changes that are associated with pharmacological and nutritional factors acting on the developing brain.

Key words: Dipyrone; Brain development; Cortical spreading depression; Early malnutrition; Drug-nutrition interaction.

Introduction

Dipyrone is a pyrazolone-derived, non-steroidal antipyretic/analgesic/anti-inflammatory drug, which is largely used in the routine of clinical therapeutics, both in adults and in children (Mao et al., 2006; Reis et al., 2003). It has particularly been used in children as antipyretic, helping to prevent seizures induced by fever (Doretto et al., 1998). Its use is largely disseminated in children in several countries (Ergün et al., 2000; Vasquez et al., 2005), despite the possibility of deleterious side-effects in the developing brain. Although most of the non-steroidal antipyretic/anti-inflammatory drugs have their mechanism of action mainly based on the inhibition of the enzyme cyclooxygenase (COX; Abbott and Hellemans, 2000; Alves and Duarte, 2002), the mechanisms involved in the effects of dipyrone are poorly understood and there is controversy about the sites of action of the drug (Collares and Vinagre, 2003).

The insufficient intake of nutrients can lead to malnutrition, whose effects on the brain are more severe when nutrition-deficiency occurs early in life during the "brain growth spurt period" (Dobbing, 1968). In the rat, this phase corresponds to the suckling period (first three weeks of postnatal life). At this "time-window" the brain presents high vulnerability not only to nutritional deficiency (Dobbing and Smart, 1974), but also to other external factors like pharmacological agents (Amâncio-dos-Santos et al., 2006). However, the electrophysiological effects of dipyrone on the developing brain, either under normal or deficient nutritional conditions, have not been object of much systematic study.

There is evidence that the time-window represented by the above-mentioned "critical period" for the brain development is non-homogeneous, regarding the distinct neural structures and developmental processes (Levitsky and Strupp, 1995; Morgane et al., 1993). Concerning the brain electrical activity, the implication is that a deleterious factor like malnutrition would have the most important impact when acting at a certain time-point within

the critical period (Rocha-de-Melo and Guedes, 1997). This evidence allows one to predict a similar heterogeneous pattern of brain developmental effects for drugs like dipyrone.

Experimental models devoted to the study of developmental consequences of pharmacological and nutritional factors on the brain function may improve the understanding about the developing processes of the nervous system, as well as its strategies for adaptation to external insults, and this can be clinically relevant. When occurring early in life, such insults can modify the patterns of developmental processes in the brain, influencing its functions and mechanisms of neural plasticity (Buonomano and Merzenich, 1998; Guedes et al., 1996; Morgane et al., 1978; 1993; Rema et al., 2006).

In order to analyze the possible influence of dipyrone on neuronal excitability, we investigated, in the developing rat, the electrophysiological effects of dipyrone treatment on the propagation of the phenomenon known as cortical spreading depression (CSD). CSD is a slow-propagating excitability-related neural response that has been electrophysiologically demonstrated on the cortical tissue of experimental animals (Bures et al., 1974; Leão, 1944), and also in the human brain (Berger et al., 2008; Fabricius et al., 2008; Gorji and Speckmann, 2004; Mayevsky et al., 1996). It is a fully reversible phenomenon produced by electrical, mechanical or chemical stimulation of one point on brain tissue, from which it spreads concentrically to remote cortical regions (Leão 1944).

As indicated by experimental evidence, the neural tissue normally offers a certain degree of resistance to CSD propagation. This resistance can be increased or decreased by some kind of experimental treatment, resulting respectively in lower or higher CSD velocities of propagation (Guedes and Do Carmo, 1980; Rocha-de-Melo et al., 2006). So, the determination of the CSD velocity along the cortical tissue is a sound and easy way of estimating the brain CSD susceptibility. Experimental conditions that either facilitate or impair the brain ability to produce and propagate CSD may be helpful to the understanding of

the phenomenon and of the diseases related to them, such as epilepsy (Guedes and Cavalheiro, 1997; Guedes et al., 2009; Leão, 1944; 1972).

In three models of experimental epilepsy, an anticonvulsive action has been demonstrated for dipyrone (Doretto et al., 1998). Considering that perhaps the CSD- and epilepsy mechanisms share some common features (Guedes and Do Carmo, 1980; Leão, 1944; 1972), we considered reasonable to postulate that the brain CSD susceptibility could be changed by dipyrone treatment early in life. By using electrophysiological recording of CSD, three questions in the brain of weaned young rats, either well-nourished or previously subjected to malnutrition during lactation, have been presently addressed: (1) How does daily administration of dipyrone at three distinct "sub-periods" of 7 days within the critical period of brain development affect CSD propagation; (2) in which of the three sub-periods of treatment would dipyrone be more impacting in terms of changing the brain CSD susceptibility; (3) if so, how would this effect be influenced by the previous nutritional condition of the developing brain.

Materials and Methods

Animals

Wistar rat pups of both genders (n=145, of which 75 were males) were at birth randomly distributed in two groups, according to the nutritional status consequent to the lactation conditions: well-nourished and malnourished (respectively W- and M-group). The W-group originated from litters with six pups whereas in the M-condition the litters were formed by twelve pups during the entire lactation (0-25 days of life), as described previously (Rocha-de-Melo et al. 2006). They were reared in polyethylene cages (51cm x 35.5cm x 18.5cm) in a

room maintained at 22 ± 2ºC with a 12:12-h light-dark cycle (lights on at 7h a.m.). During the suckling period, their mothers were fed a lab chow diet (Purina do Brasil Ltd.), with 23% protein. After weaning, pups were housed 4-6 per cage, and fed the same maternal lab chow diet until the day of the electrophysiological recording of CSD (between postnatal days 35-45). The body weights were determined on postnatal days 7, 14, 21, 28 and 38. The animals of this study were handled in accordance with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA) and with the norms of the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco, Brazil.

Dipyrone treatment

The pups of both nutritional conditions were treated by gavage, for 7 days, with 300mg/kg/day of dipyrone (D, from Sigma Co.; 36 W- and 34 M-rats) or with an equivalent volume of saline solution (S; 40 W- and 35 M-rats). Both D- and S-treated pups, in each of the two nutritional conditions, were subdivided in three groups, according to the week of life in which the gavage was carried out: 2nd (21 W- and 23 M-rats), 3rd (26 W- and 22 M-rats), or 4th week (29 W- and 24 M-rats). So, a final number of 12 groups were studied (Table 1). The dipyrone was dissolved in distilled water immediately before the administration. The volume of the gavage solutions ranged from 0.5 ml/d (in the second week of life) to 1.0 ml/d (in the third and fourth week of life). The gavage was always performed between 12:00 and 14:00 h. The dose of dipyrone presently used was chosen based on the work of Doretto et al. (1998). In each group, the proportion of males was approximately equal to that of females.

CSD Recording

When the animals were 35-45 days old, they were submitted to the recording of CSD for a period of 4-h according to the following protocol: under anesthesia (1g/kg urethane plus

40mg/kg chloralose, *ip*), three trephine holes (2-3 mm in diameter) were drilled on the right side of the skull (two at the parietal- and one at the frontal bones). The three holes were aligned in the anteroposterior direction and were also parallel to the midline.

CSD was elicited at 20 min intervals by applying to the anterior hole drilled at the frontal region, for 1 min, a cotton ball (1–2 mm diameter), soaked in 2% KCl solution (approximately 0.27 M). The 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 5cm long plastic conic pipettes (0.5mm tip inner diameter), filled with Ringer solution, solidified with the addition of 0.5% agar, into which a chlorided silver wire was inserted. The pipettes were pair-wise fixed together with cyanoacrylate glue, so that the inter-electrode distance was kept constant for each pair (range: 4 - 5.5 mm). Each pair of electrodes was connected to a lever which 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 common reference electrode, of the same type, was placed on the nasal bones. The velocity of CSD propagation was calculated based on the time required for a CSD wave to cross the distance between the 2 recording electrodes. In the measurement of CSD velocities, the initial point of each DC negative rising phase was used as reference point. During the recording session, rectal temperature was maintained at 37+1ºC by means of a heating blanket. After the recording session was terminated, the animal, while anesthetized, was submitted to euthanasia by bulbar injury. This was carried out by introducing a sharp needle into the cisterna magna, provoking immediate cardio-respiratory arrest.

Statistics

Intergroup differences were compared by using an ANOVA three-way including as factors: nutritional status (W and M), drug treatment (saline and dipyrone) and age at the treatment (2nd, 3rd and 4th weeks of life) followed by a post hoc (Tukey–Kramer) test, where indicated. Differences were considered significant when $P \leq 0.05$.

Results

Interaction nutrition-dipyrone and Body weight changes

The body weights of the 12 groups are presented in Figure 1. From day 7 to 38, all M-groups presented lower ($P<0.05$) body weights, as compared with those of the corresponding W-groups. In the well-nourished condition, the mean (± standard deviation in g) body weights at the 7^{th}, 14^{th}, 21^{st}, 28^{th} and 38^{th} days of life were respectively (a) for the saline-treated rats: 17.6±1.8, 31.6±3.1, 50.9±5.1, 79.4±8.4 and 128.4±20.1 for the group treated at the second week of life, 17.4±1.7, 31.1±1.6, 45.8±2.6, 73.2±5.2 and 127.9±14.4 for the group treated at the third week of life and 16.5±0.8, 29.9±1.0, 45.3±3.5, 68.5±5.8 and 126.7±8.8 for the group treated at the fourth week of life; (b) for the dipyrone-treated rats: 18.0±2.6, 31.1±3.8, 50.5±8.7, 77.8±9.7 and 142.4±23.0 for the group treated at the second week of life, 16.1±1.5, 30.2±1.8, 44.3±3.6, 71.1±4.6 and 118.2±11.7 for the group treated at the third week of life and 16.8±1.4, 30.4±2.7, 47.2±4.7, 67.3±6.2 and 125.2±15.4 for the group treated at the fourth week of life. In the malnourished condition, the following mean body weights were obtained (a) for the saline-treated rats: 14.1±2.6, 23.3±2.6, 37.4±4.9, 61.4±8.5 and 116.4±27.8 for the group treated at the second week of life, 12.2±2.0, 21.3±2.2, 32.7±2.6, 53.8±5.1 and 109.1±20.2 for the group treated at the third week of life and 11.1±2.8, 19.7±4.6, 31.6±7.9, 49.3±10.1 and 100.3±30.6 for the group treated at the fourth week of life; (b) for the

dipyrone-treated rats: 13.8±2.4, 21.5±3.2, 34.6±6.3, 56.7±10.6 and 111.1±31.1 for the group treated at the second week of life, 12.22±2.4, 21.5±2.9, 30.7±5.5, 51.8±9.3 and 101.2±18.6 for the group treated at the third week of life and 12.4±1.3, 20.9±1.6, 33.1±2.2, 47.0±5.6 and 101.3±12.5 for the group treated at the fourth week of life. The treatment with dipyrone did not affect body weights in neither of the nutritional conditions

Interaction nutrition-dipyrone and CSD propagation

The 1-min stimulation with 2% KCl was very effective in eliciting, in all groups, a single CSD wave, which propagated and was recorded by the two electrodes located more posteriorly in the stimulated hemisphere. Figures 2-4 present electrophysiological recordings obtained in 6 W- and 6 M-rats that were treated with saline solution or dipyrone (3 W and 3 M rats in each case) during the second (Figure 2), the third (Figure 3) or the fourth (Figure 4) week of life. The ECoG depression and the slow potential change confirmed the presence of CSD after each KCl-stimulation.

In the saline-treated rats CSD velocities in the M groups were higher ($P<0.05$) than in the corresponding W groups, characterizing the already known effect of early malnutrition on CSD propagation (see figure 5).

In both W- and M-groups, the 7d dipyrone-treatment at the three time-points increased ($P<0.05$) the CSD-velocities of propagation, as compared to the respective saline-treated controls. This effect was significantly ($P<0.05$) more intense in the groups treated at the fourth week of life, as compared to the groups treated at the other two weeks. In these two weeks, in the W-condition, the CSD facilitating effects were comparable. However, in the M-animals the dipyrone CSD-effects were greater in the group treated in the third week than in the group that received dipyrone at the second week. For the W and M saline-treated groups, the mean±sd velocities (in mm/min) were 3.70±0.11, 3.77±0.16 and 3.78±0.13 (W-groups)

and 4.13±0.10, 4.16±0.10 and 4.14±0.09 (M-groups), for the animals treated in the 2nd, 3rd and 4th week of life. For the dipyrone-treated groups, the corresponding mean velocities were 3.99±0.14, 4.03±0.16 and 4.30±0.19 (W-groups) and 4.47±0.17, 4.70±0.31 and 5.01±0.28 (M-groups). These data are illustrated for all groups in Figure 5.

Discussion

The present findings constitute the first report on CSD-effects of dipyrone, produced in the developing rat by administering this drug during short periods (7 days) within the lactation phase. Data on this novel electrophysiological action of dipyrone indicate that the treatment with this drug increased CSD susceptibility in the developing brain, as indexed by its CSD velocity of propagation, and early malnutrition facilitated this effect. It is unlikely that the here described CSD-action of the dipyrone treatment has been caused by the stress eventually produced in the gavage procedure, since the control groups have been equally submitted to the same procedure (treated per gavage with saline solution), and did not present those CSD alterations.

The convulsive threshold (Steinhauser and Herting, 1981), and the amplitude of the cortical electrical activity (Wallenstein, 1985) are neural parameters that are modified by some non-steroidal anti-inflammatory drugs. The mechanisms by which dipyrone exerts its action on the peripheral and central nervous system are not yet fully understood. Actions on prostaglandin synthesis (Campos et al., 1999; Vane, 1971; Weithmann and Alpermann, 1985), on the system of endogenous opioids (Reis et al., 2003; Vasquez and Vanegas, 2000), on $GABA_A$ receptors (Halliwell et al., 1999), on nitric oxide systems (Lorenzetti and Ferreira, 1996) and on ATP-sensitive potassium channels (Alves and Duarte, 2002) have been postulated, but still need experimental confirmation.

The anticonvulsive action of dipyrone, first demonstrated in the rat by Doretto et al. (1998) in the electroconvulsive- and audiogenic seizure models, has been later confirmed by Ergün et al. (2001) on rats submitted to ethanol-withdrawal seizures, as well as to seizures induced by pentylenetetrazol. The anticonvulsant effect seems to be specific for dipyrone, since other non-steroidal anti-inflammatory drugs did not present it (Doretto et al., 1998). Concerning the mechanisms for the anticonvulsant effects of dipyrone, one of the two existing studies on this subject (Reis et al, 2003) provides evidence for an opioid-based mechanism; the other one (Ergün et al., 2001) discuss several possibilities, namely the dipyrone-dependent actions (1) on the prostaglandin synthesis, (2) on the adenosine reuptake, (3) on the interaction with the GABAergic system and (4) on the inhibition of the central excitatory glutamate action. The confirmation of the hypotheses about the mechanisms for the protective role of dipyrone against convulsions requires additional controlled studies. It must be kept in mind however, that at this stage we cannot discard the possibility that the mechanisms may vary depending on the neural structures involved in the production of seizures in the different experimental models, as previously suggested for other drugs (Amâncio-dos-Santos et al., 2006; Guedes et al., 1992).

Although the mechanisms by which dipyrone acts as a CSD-enhancer is not known, we can speculate from data on CSD experiments involving the mechanisms proposed for some of the neural dipyrone action. Interestingly, all the above mentioned mechanisms, proposed as participating in the anticonvulsive action of dipyrone, also influence to a more or less extent the production of the CSD features in the brain: opioid system manipulation (Guedes et al., 1987a), prostaglandin synthesis and release (Cui et al., 2008), adenosine system (Schock et al., 2007), GABAergic system (Guedes et al., 1992); Nitric oxide (Meng et al., 1995), glutamatergic system (Guedes et al., 1988; Marrannes et al., 1988) and free radicals counteraction by antioxidant substances (Abadie-Guedes et al., 2008; Bezerra et al., 2005).

Brain structural and functional maturation is programmed to occur in the rat mainly during the lactation period. This intense and extense synaptogenic period can be considered equivalent to the human synaptogenic stage, which starts at the third trimester of prenatal development and continues during the first year of postnatal life (Dobbing, 1968; Morgane et al., 1978; 1993). Two basic reasons prompted us to chose the lactation period to treat the animals with dipyrone: first, because in this period dipyrone would be more likely to produce structural and physiological effects on the developing brain, as demonstrated for other drugs (Amâncio-dos-Santos et al., 2006); second, because this period corresponds to a phase in the human's life in which the therapeutic use of dipyrone is very frequent (Ergün et al., 2000). A relatively short period (7 days) of dipyrone-treatment was presently shown to be effective in enhancing CSD propagation and this effect was still present up to 45 days of life, suggesting that the dipyrone action on CSD is long lasting. If one assumes that any compensatory mechanism had been activated between the dipyrone treatment and the CSD-recording period, it certainly was not sufficient to decrease the CSD-velocities to the levels of the control group. A longer follow-up (i.e., recording CSD at adulthood, at a longer time-interval after dipyrone treatment) would be required in the future to confirm that the CSD-effects are long lasting. However, it is pertinent to mention in favor of this assumption, that a long lasting neuroprotective effect of dipyrone has been demonstrated in rats after experimental ischemia (Coimbra et al., 1996). The confirmation of a long lasting CSD-effect of dipyrone would be compatible with the hypothesis of alterations in the developing brain structure lastingly affecting processes that mediates CSD propagation. In this respect, a reasonable process to be investigated would be the dipyrone action on the brain structures containing prostaglandin receptors. These receptors are involved in the regulation of vascular physiology (Wright et al., 2001), and also influence brain vascular changes occurring during CSD (Cui et al., 2008; Meng et al., 1995).

Another important finding of the present study was that the dipyrone treatment in the fourth week of life resulted in higher CSD-velocities, as compared to the treatment in the second and third weeks. Two interpretations might originate from this: (1) concerning the CSD propagation, the later stage of brain development is more vulnerable to the insult represented by dipyrone, as compared to the earlier stages; (2) the greater CSD-effect in the group treated at the later stage simply reflected the insufficiency of the time necessary for the brain to recover at this later time-point. The first interpretation is favored by data from others, demonstrating greater vulnerability at later stages in the brain development period, for pathological processes like the pilocarpine-induced epilepsy (Cavalheiro et al., 1987; Cilio et al., 2003). Also, for homeostatic processes such as the thyroid function programming, the fourth week of life is reported as being more susceptible (Toste et al., 2006). In addition, long lasting CSD-effects of short (7days) episodes of malnutrition early in life have been shown to be more conspicuous in adult rats in which the nutritional insult had occurred at late stages of the rat brain developmental period, in comparison to the groups malnourished at earlier stages (Rocha-de-Melo and Guedes, 1997).

It has been convincingly demonstrated that malnutrition early in life enhances CSD propagation (De Lucca et al., 1977; Guedes et al., 1987b; Rocha-de-Melo et al., 2006), and this has been presently confirmed. In comparison to well-nourished rats, early-malnourished animals present diminished (Guedes et al., 1992; Ximenes-da-Silva and Guedes, 1991), equal (Amâncio-dos-Santos et al., 2006; Guedes et al., 2002), or enhanced (Vasconcelos et al., 2004) changes of brain CSD reaction in response to the administration of distinct substances like glucose and diazepam (diminished CSD responses in the malnourished rats), citalopram and fluoxetine (equal CSD responses) and pilocarpine (enhanced CSD responses). Concerning the CSD effects of dipyrone, we presently found that early malnutrition enhanced the brain CSD-reaction to that drug. These data collectively indicate that cortical CSD-responsiveness

to distinct pharmacological agents may not be the same for all the classes of pharmacological drugs. If the CSD-effects could be extrapolated, in the human species, to other neural actions of distinct drugs, used with therapeutical purposes, the possible clinical implication would be that such drugs could present different degrees of effectiveness, depending on the early nutritional status of the patient. We consider strongly recommended the investigation of this possibility in the medical clinic.

In summary, this study documented a novel electrophysiological action of dipyrone on CSD propagation in well-nourished and early-malnourished developing rats, allowing us to draw the following three main conclusions: first, treatment with dipyrone for short periods (of only 7 days) within the critical period of brain development enhances CSD propagation; second, the later treatment-period corresponding to the fourth week of life is the most susceptible to that dipyrone CSD-effect, in comparison to the earlier periods; third, malnutrition early in life intensified the dipyrone action, as evaluated for the higher CSD velocities of propagation. These findings might help in the understanding of the interaction between pharmacological and nutritional factors in the developing brain.

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TABLE 1 – Distribution of the 12 experimental groups according to the nutritional condition (W, well-nourished; M, early malnourished), to the gavage treatment (D and S, respectively treatment with dipyrone and saline) and to the week of life in which the gavage was applied (respectively 2nd, 3rd and 4th week of life). Numbers in parentheses at the right column indicate number of rats studied.

Groups	**Nutritional Condition**	**Gavage Treatment**	**Week of Life**
1	W (76)	S (40)	2nd (11)
2			3rd (13)
3			4th (16)
4		D (36)	2nd (10)
5			3rd (13)
6			4th (13)
	M (69)	S (35)	2nd (11)
8			3rd (11)
9			4th (13)
10		D (34)	2nd (12)
11			3rd (11)
12			4th (11)

Legends of figures

Figure 1 – Body weights (mean±s.d.) of well-nourished (W) and malnourished (M) Wistar rats treated per gavage in the 2nd, 3rd and 4th week of life, with saline solution (S) or dipyrone (D). Body weights were measured on days 7, 14, 21, 28 and 38. Asterisks indicate that all M-values are significantly different from the corresponding W-ones ($P<0.05$; ANOVA plus Tukey test).

Figure 2 – Eletrocorticogram (E) and slow potential change (P) recordings during cortical spreading depression (CSD) in the right hemisphere of two 35-45days-old well-nourished (W) and two malnourished (M) rats, which were treated per gavage during the 2nd week of life with dipyrone (300mg/kg/d) or saline solution. The upper drawing shows the stimulation point (where 2% KCl was applied to elicit CSD), the recording positions 1 and 2, as well as the position of the common reference electrode (R). The horizontal bars in the P1-traces indicate the period (1 min) in which KCl stimulation was applied to the frontal region of the same hemisphere. Vertical bars correspond to 10mV in P and 1mV in E (negative upwards). The velocity of CSD propagation was calculated based on the time required for a CSD wave to cross the distance between the 2 recording electrodes, which in this case was 4.0 and 4.8 mm for the W- and M-animals, respectively.

Figure 3 - Eletrocorticogram (E) and slow potential change (P) recordings during cortical spreading depression (CSD) in the right hemisphere of two 35-45days-old well-

nourished (W) and two malnourished (M) rats, which were treated per gavage during the 3rd week of life with dipyrone (300mg/kg/d) or saline solution. All other details as described in Figure 2, except the distance between the 2 recording electrodes, which in this case was 4.0 mm for the W- and M-animals.

Figure 4 -Eletrocorticogram (E) and slow potential change (P) recordings during cortical spreading depression (CSD) in the right hemisphere of two 35-45days-old well-nourished (W) and two malnourished (M) rats, which were treated per gavage during the 4th week of life with dipyrone (300mg/kg/d) or saline solution. All other details as described in Figure 2, except the distance between the 2 recording electrodes, which in this case was 4.0 and 5.3 mm for the W- and M-animals, respectively.

Figure 5 – CSD velocities of propagation (mean ±s.d.), calculated in mm/min in well-nourished and malnourished 35-45 days-old rats. In both nutritional conditions, gavage treatment early-in-life with 300 mg/kg/d of dipyrone (D) was associated with higher CSD velocities, as compared with the corresponding saline solution- (S) treatment. Data are expressed as the average velocities along the 4-h recording period. All M-values are significantly higher than the corresponding W-ones. * = significantly different from the corresponding S-group; # = different from the D2 and D3 groups; @ = different from the D2 and D4 groups ($P<0.05$; ANOVA plus Tukey test).

Figure 1

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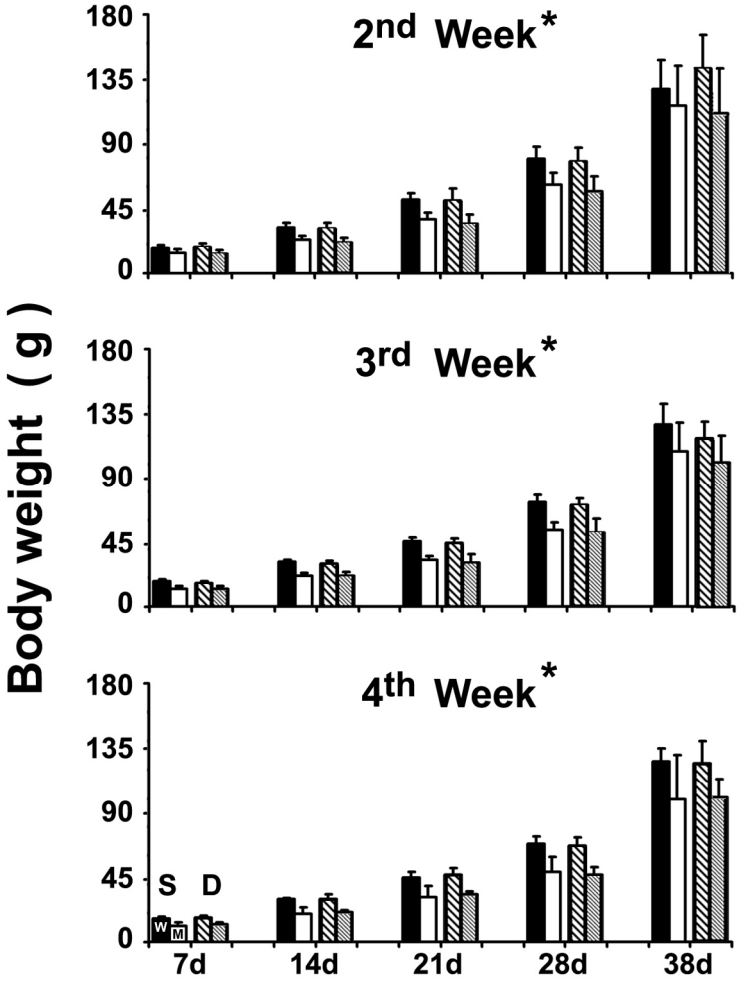

Figure 2

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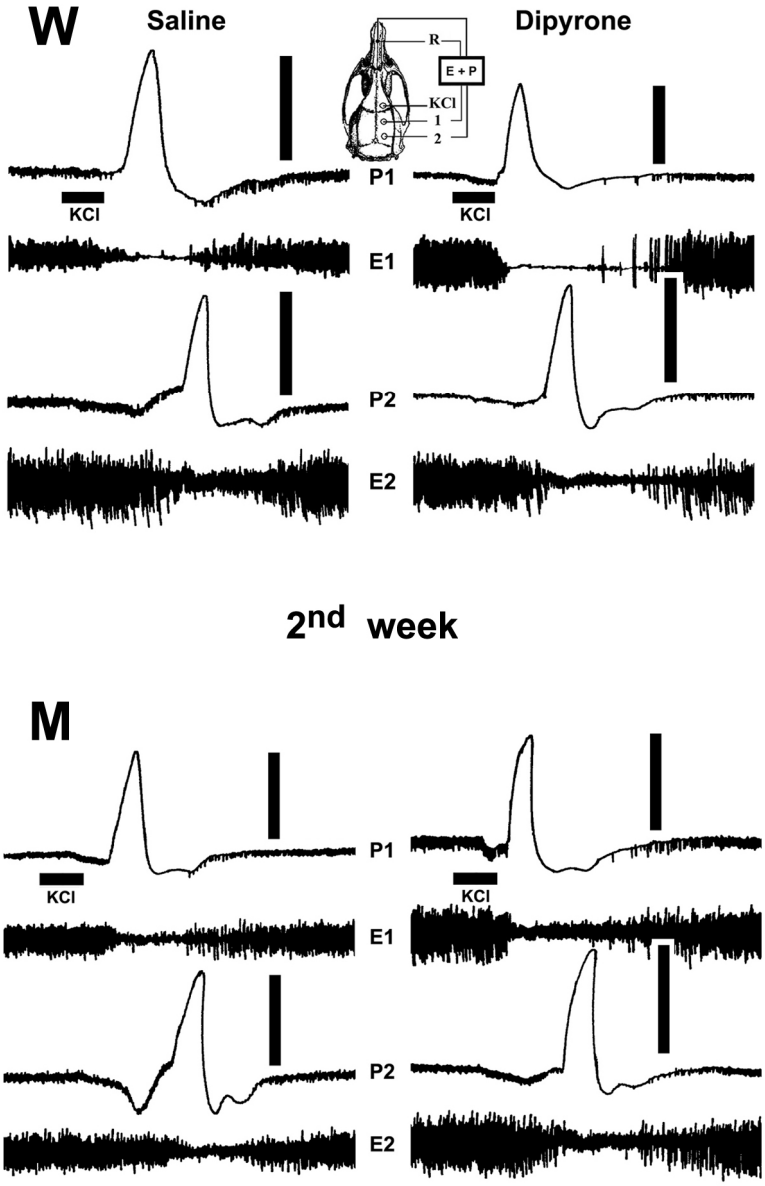

Figure 3

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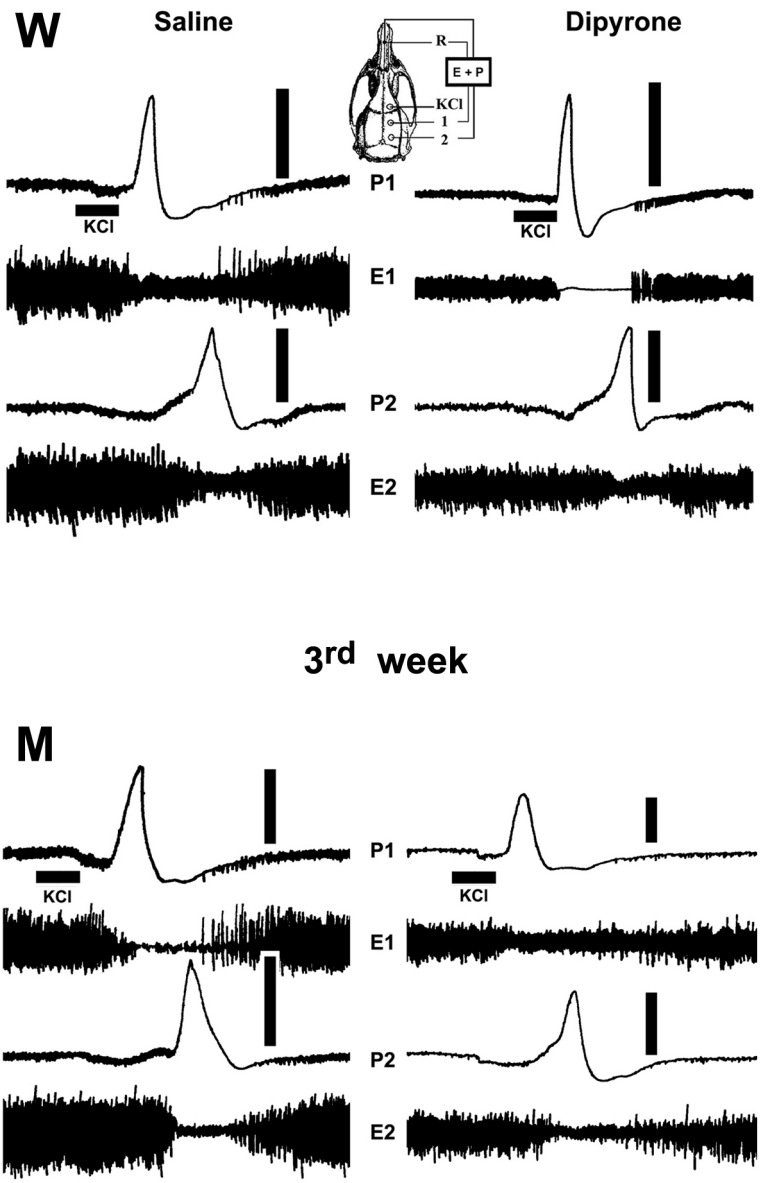

Figure 4

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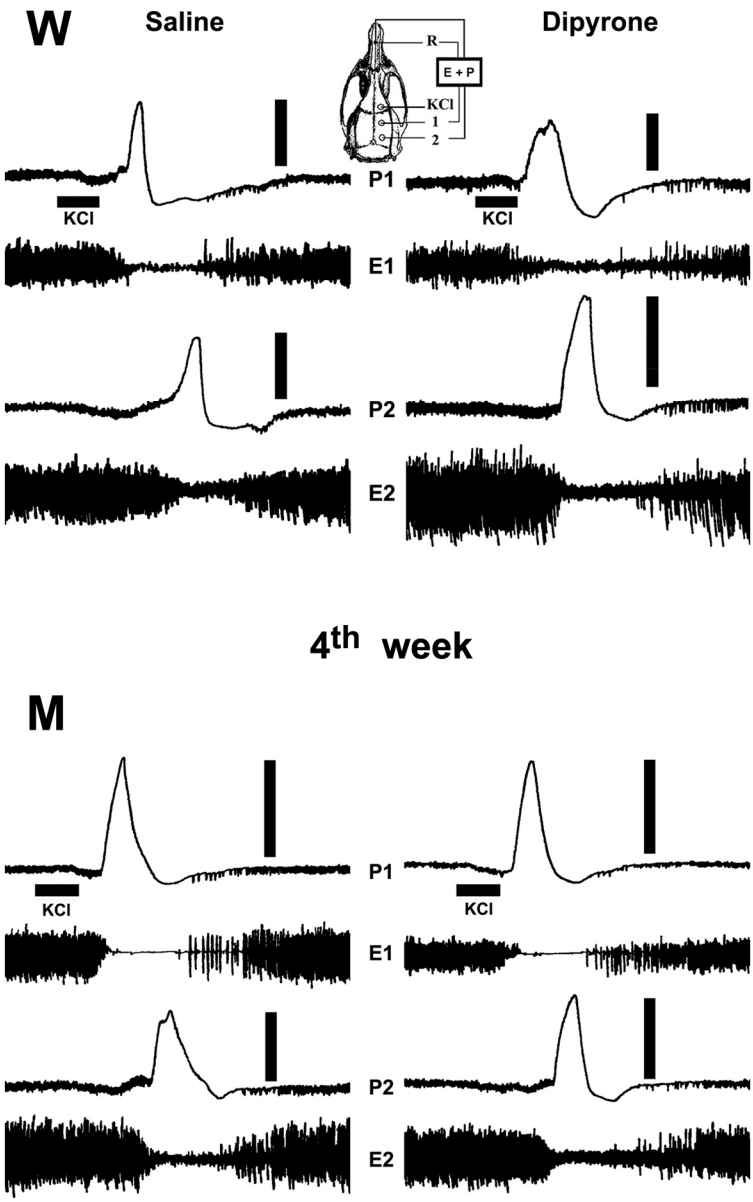

Figure 5

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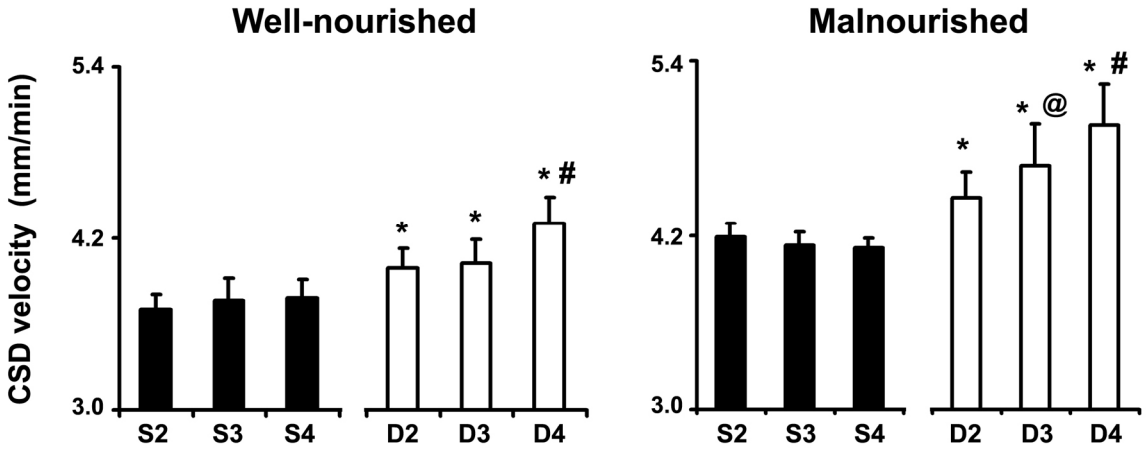

4. CONCLUSÕES

O presente trabalho constitui o primeiro relato sobre os efeitos neurofisiológicos da dipirona sobre a depressão alastrante cortical, efeito este produzido durante o desenvolvimento em ratos nutridos e desnutridos. Chama a atenção o fato de que apenas 7 dias de tratamento foram suficientes para produzir os efeitos ora descritos. Os dados acerca desta nova ação eletrofisiológica da dipirona indicam que o tratamento com esse fármaco aumentou a susceptibilidade cortical à depressão alastrante no cérebro em desenvolvimento, a julgar pela velocidade de propagação do fenômeno. Eles indicam também que a desnutrição no início da vida acentuou o efeito da dipirona. Considera-se pouco provável que tal efeito seja devido ao estresse, produzido eventualmente durante o procedimento de gavagem, pois o grupo tratado com salina foi igualmente submetido ao mesmo procedimento de gavagem e não apresentou alterações na depressão alastrante.

O uso abusivo de fármacos, bem como a ingestão inadequada de nutrientes, constituem fatores externos que, seja individualmente, seja combinados, podem alterar o desenvolvimento cerebral. A interação entre o tratamento com dipirona e a desnutrição parece ser um exemplo de combinação de fatores que influencia parâmetros eletrofisiológicos cerebrais, como é o caso da depressão alastrante.

De acordo com os resultados deste trabalho, três conclusões principais podem ser extraídas: primeiro, que o tratamento com dipirona por curto período (7 dias), dentro da fase de desenvolvimento rápido do cérebro facilita a propagação da depressão alastrante; segundo, que o cérebro, no período mais tardío do desenvolvimento (correspondente à quarta semana de vida), parece ser o mais susceptível à presente ação da dipirona, em comparação com o tratamento em períodos mais precoces; terceiro, a desnutrição intensificou a ação da dipirona, a julgar pelas mais altas velocidades da depressão alastrante nessa condição nutricional. Os presentes resultados eletrofisiológicos podem ser úteis para a compreensão da interação entre fatores farmacológicos e nutricionais no cérebro em desenvolvimento.

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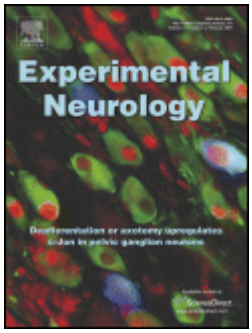
6. ANEXOS

6.1 Indicadores de produção 2008

6.1.1. Normas da revista Experimental Neurology



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6.1.2. Resumos em congressos

Resumo na FeSBE Regional 2008

02.004 – AMARAL, A.P.B.; RAMOS, I.L.T.; BARBOSA, M.S.S.; SOUZA, V.C.; SANTOS, R.C.F.; GUEDES, R.C.A. Tratamento, de ratos em desenvolvimento, com dipirona facilita a propagação da depressão alastrante cortical. In: III Reunião Regional da FeSBE, 2008, Fortaleza. Reusmos da III Reunião Regional da FeSBE, 2008. v. único.
Palavras-chave: Dipirona, desenvolvimento cerebral, depressão alastrante cortical.
Referências adicionais: Classificação do evento: Nacional; Brasil/ Português; Meio de divulgação: Vários.

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02.008 - AMARAL, A.P.B.; RAMOS, I.L.T.; BARBOSA, M.S.S.; SOUZA, V.C.; SANTOS, R.C.F.; GUEDES, R.C.A. Efeito diferencial da dipirona, sobre a depressão alastrante cortical, em distintas fases do desenvolvimento do rato. In: XXIII Reunião Anual da FeSBE, 2008, Águas de Lindóia. Reusmos da XXIII Reunião Anual da FeSBE, 2008. v. único.
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Resumo no Neurolatam 2008

C.03.004 (RS25850B) – AMARAL, A.P.B.; RAMOS, I.L.T.; BARBOSA, M.S.S.; SOUZA, V.C.; SANTOS, R.C.F.; GUEDES, R.C.A. Interaction nutrition-drug in the brain: neurophysiologic effects of dipyrone on cortical spreading depression in early-malnourished

adult rats. In: I Congresso IBRO/LARC de Neurociências da América Latina, Caribe e Península Ibérica (Neurolatam), 2008, Búzios. Resumos do I Neurolatam, 2008. v. único. Palavras-chave: Dipyrone; Cerebral developmental; Cortical spreading depression. Referências adicionais: Classificação do evento: Latino-amaricano; Brasil/ Português; Meio de divulgação: Vários.